# ABSTRACT

BOWEN, BLAKE. Genetic and Cultural Management Studies of the Production of Industrial Sweetpotatoes from "Cut Root Pieces". (Under the direction of Dr. G. Craig Yencho.)

Sweetpotato has potential to be used as a biofuel feedstock in the corn deficit southeastern US, but is limited by its high cost of production. This thesis focuses on a root piece planting technique that has been tested by previous researchers. In an effort to reduce planting costs for industrial sweetpotatoes to be used as a biofuel feedstock, it was revisited with modern lines currently grown in the southeastern US. A series of three cultural management experiments, and a trial designed to estimate the heritability of traits associated with root piece production were performed to measure different aspects of this planting technique. The cultural management experiments included: 1) a greenhouse test to measure the effect of storage root cutting on proximal dominance and sprout production; 2) a field test to measure differences between cut root sections and whole roots on storage root partitioning index, daughter and enlarged seed root yield; and 3) a field experiment to measure differences between conventional and root piece planting techniques and their effects on partitioning index, defined as, [(daughter yield – seed root enlargement yield) / total yield], average enlarged seed roots, number seed roots remaining, daughter, enlarged seed, number 1, canner, jumbo, and cull root yields.

The greenhouse test showed significantly different amounts of sprouts produced per root section ($p<0.05$) in uncut roots, but showed no differences in total sprouts produced between cut and uncut roots ($p>0.5$). In uncut roots, proximal root sections produced the most sprouts, but cutting was found to reduce sprouting in the proximal section and increase sprouting in the distal and middle sections.

The field study designed to test root piece sections, showed that root piece section had a significant effect on daughter, enlarged seed, and total root yield (*p*<0.05) in 2008. The proximal treatment had the least seed root enlargement both years with a calculated 1.8 and 5.9 T/ha respectively. Daughter root yield was highest in the proximal treatment and total yield varied in 2007, but was not significantly different across treatments in 2008. Partitioning index was shown to be highest in proximal end treatments and lowest in whole root treatments.

Field study two, which compared conventional and root piece planting showed significant differences between conventional and seed root planting treatments for appearance, partitioning index, daughter, enlarged seed, total, number 1, and jumbo root yield (*p*<0.05) across all years, but showed no differences between canner and cull yield. Clonal variation across years was significant except in a few cases.

Experiments to estimate heritability were conducted in two locations, Clinton and Kinston, NC. Parent offspring heritability estimates for dry matter, partitioning index, daughter root, seed root enlargement, root piece survival, and total yield were 0.54, 0.64, 0.12, 0.36, -0.04, and 0.27 in Clinton and 0.63, 0.37, -0.1, 0.76, -0.09, and 0.18 in Kinston, respectively. Half sib family variance component analysis on family means of the same traits showed estimates of 1.2, 0.62, 0.45, 0.82, 0.54, and 0.60 respectively. Genetic correlation analysis showed no correlation between daughter root yield and dry matter content, seed root enlargement, and root piece survival (*r* = -0.03, -0,04, and -0.06), but a positive correlation was observed between daughter and total yield and the partitioning index (*r* = 0.76 and 0.57, respectively). Partitioning index correlated with daughter yield, seed root enlargement, and root piece survival with values of 0.57, -0.70, -0.54 respectively. Partitioning index showed no correlation to total yield or dry matter (-0.01 and -0.08). Lastly, root piece survival was correlated with seed root enlargement (0.72), total yield (0.42), and partitioning index (-0.54) and showed no correlation with daughter yield and dry matter (-0.06 and -0.01).

Genetic and Cultural Management Studies of the Production of Industrial Sweetpotatoes from “Cut Root Pieces”

by
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# DEDICATION

This research is dedicated to God, my wife, and my family. All of which without their help I would not be where I am today. I am truly thankful for all the prayers and support you all have given me all my life.

## BIOGRAPHY

Blake Douglas Bowen was born on May 19, 1985 in Lincoln, Nebraska. He was the eldest of three kids from his parents Michael and Stacy Bowen. At a young age his family moved to Richmond, Virginia where he began to attend school. He spent many of his summers and holiday breaks in Nebraska where he enjoyed spending time with his grandparents and extended family. While in Virginia he played many sports, but most notably football, basketball, and soccer. In high school he competed in both football and soccer for James River High School and thoroughly enjoyed the competition and hard work that went into playing sports.

In 2003 he graduated from James River High School with their advanced degree and left home to attend his undergraduate at North Carolina State University to major in Biology. North Carolina State University was a major time of transition in Blake's life. Blake's career goals originally involved genetic research in cancer (preferably in humans), but that changed when he started working with Dr. Bryon Sosinski and with the then PhD student Ramon Molina-Bravo and his advisor Dr. Gina Fernandez. Suddenly plant research and plant breeding became very interesting to Blake and led to his decision to apply to graduate school at North Carolina State University.

In May 2007 Blake graduated from his undergraduate studies from North Carolina State University with a B.S. in biology and a concentration in genetics. That same year he was accepted to graduate school at North Carolina State University for his Masters. After speaking to many professors in Horticulture Dr. Craig Yencho and Blake decided to team up to further Blake's education. Blake worked with Dr. Craig Yencho and his right hand man Ken Pecota for the next three years eating, breathing, and learning about sweetpotatoes and research. It was with their help that he learned how to perform his own research and navigate towards graduation.

After graduate school, Blake will join the crop science department at North Carolina State University working in cotton breeding under the guidance of Dr. Vasu Kuraparthy.

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First and foremost I thank God for all that he is done in my life and helping me through this thesis. Without his help, not only would I not be graduating, but also I would not have had the opportunity. During the late nights of studying, writing and editing he gave me strength to succeed and persevere. It truly amazes me the love, acceptance, and strength that I found in him for which I am truly thankful in helping me to complete this work.

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To my wife and her patience and perseverance I am truly grateful. Her kind spirit and encouragement has brought me through many a rough day. Her willingness to spend late nights in the graduate office keeping me company will never be forgotten. Thank you so much Olivia for working with me as I learned and pushed through graduate school. It means more to me than I will ever be able to express.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# Chapter 1: Background and Literature Review

Blake Bowen

### 1.1 Introduction to Sweetpotato:

Sweetpotatoes (*Ipomoea batatas*) are a dicotyledenous plant in the Convolvulaceae or morning glory family. It has long been hypothesized that the crop was originally domesticated in tropical Central America at least 5000 years ago (Loebenstein, 2009). Recent genetic work using a diverse set of sweetpotato germplasm found that sweetpotato from areas south of the "Meso America" region had little genetic association to sweetpotatoes found in other parts of the world (notably the Oceania region)(Rossel et al., 2000). This work provides strong evidence that sweetpotato originates from the "Meso America" region of Central America, which roughly equates to the region from the Yucatán Peninsula of Mexico and the Orinoco River in Venezuela.

Sweetpotatoes are widely grown around the world and serve as an important food source for many people in developing counties. The United Nations Food and Agriculture Organization estimated that 110 million tons of sweetpotatoes were produced globally in 2008 (FAOstat, 2009). Sweetpotato therefore ranks as the 13th most important crop in the world in terms of production tonnage.

The top twenty sweetpotato producing countries, which account for over 95% of global production, consist primarily of Asian and African nations, and most are considered developing countries. The majority of the world's sweetpotatoes are grown by China. In

2008, China produced an estimated 85 million tonnes or 77 % of total world production (FAOstat, 2009). Uganda and Nigeria are tied for second place, with approximately 3 million tonnes of annual production each (FAOstat, 2009).

The reason for the widespread of use of sweetpotato can be attributed to its broad climatic adaptability, ease of cultivation and favorable nutritional characteristics. Although sweetpotato is a perennial species adapted to a tropical climate it can be grown successfully in a wide variety of climates (Bouwkamp, 1985). Sweetpotatoes have relatively simple cultural techniques, including: ease of propagation, tolerance of different soils, relatively low input requirements, fast growth rates, wide harvest windows and flexibility of production systems (Bouwkamp, 1985; Edmond and Ammerman, 1971; GCPES, 1943). The nutritional value of sweetpotato is dependent on the variety grown but they are a good source of starch, protein, and contain varying amounts of beta-carotene, ascorbic acid, thiamin, riboflavin, niacin, pantothenic acid, pyridoxine and its derivatives, folic acid, tocopherol, various amounts of amino acids, iron, potassium, dietary fiber, and even antioxidants (Oomen, 1971; Woolfe, 1992).

There are significant regional differences in commonly grown sweetpotato types. Farmers in many developing countries tend to grow white-fleshed varieties that are high in starch, whilst low dry matter orange-fleshed varieties are typically grown in the United States. However, this tendency is changing due to the superior nutritional value of the orange-fleshed varieties (Stapleton, 2008).

The United States is the only non-Asian or African country in the top twenty sweetpotato producing countries. In 2008, the United States ranked 12$^{th}$ in terms of total global production (FAOstat, 2009). In the United States annual sweetpotato production fluctuated around 550 thousand tonnes over the thirty years from 1968 to 1998, but since that time its production has subsequently increased to over 800 thousand tonnes (FAOstat, 2009). The southeastern United States accounts for nearly 75 % of the nations total sweetpotato production (NASS, 2010) with North Carolina being the largest sweetpotato producing state. North Carolina accounted for 45% (ca. 46,000 acres) of the total United States acreage planted in 2008 (USDA, 2008). In 2007, three counties (Nash, Johnson, and Sampson counties) contributed 52% of North Carolina's sweetpotato production (NCSPC 2008). The farm-gate value of the North Carolina sweetpotato crop was approximately $160 million in 2008.

### 1.2 Sweetpotatoes as a Biofuel Feedstock:

In addition to being a food item, sweetpotatoes have potential as a source of biomass for the bio-ethanol and bio-processing industries (Van Der Maarel et al., 2002). Several studies have shown that high starch "industrial-type" sweetpotatoes (ISP) can produce large yields of starchy biomass, which can be readily converted into ethanol (Dangler et al., 1989; Lee et al., 2008). It has been shown that conventional table-stock sweetpotato clones can produce 23%-30% more ethanol per hectare than corn (Table 1.1) (Lee et al., 2008; Wang, 1984; Ziska et al., 2009).

Fuel bioethanol in the United States is produced almost exclusively from corn from the Midwest region of the country. Currently, various states in the United States are attempting to increase the production of bioethanol from local feedstocks sources. Given that corn supplies can be limited in some regions this creates a need for more locally suitable feedstocks. The southeastern United States is a corn deficit region, producing less than 2.5 % of the nations total corn crop (USDA, 2008). Given the suitability of the southeast for sweetpotato production, and the large pre-existing sweetpotato industry, sweetpotato represents a potential candidate as a biofuel feedstock crop for the region.

A major impediment to the use of industrial sweetpotatoes as a biofuel feedstock is related to the cost of production. The production cost of sweetpotato is almost four times higher than corn, at $4102/ha compared to $1011/ha (Estes and Schultheis, 2006; Bullon and Weddington, 2010). One of the larger cost factors in sweetpotato is crop establishment, which accounts for roughly 15% of sweetpotato production costs (Estes and Schultheis, 2006). New sweetpotato crops are established using plants, which must be both cut from beds and transplanted into fields by hand, resulting in high labor costs. For comparison, the cost to produce sweetpotato plants for planting is $690/ha (Estes and Schultheis, 2006), but for corn crop establishment is only around $173/ha (Bullon and Weddington, 2010). To reduce the cost of sweetpotato production it would be desirable to develop a planting system than is more amenable to mechanization. Potato, which is a superficially similar crop to sweetpotato, is produced from cut root pieces (CRP). This process is amenable to mechanization and suggests that a similar planting method could be used for sweetpotato.

### 1.3 Other uses for sweetpotato:

While biofuel use was the focus of this research sweetpotatoes are not limited to biofuel uses alone. Some lines of sweetpotato are purple fleshed and high starch. These industrial sweetpotatoes have use not only in biofuels due to their high starch nature, but also as a source of antioxidants from anthocyanins in these sweetpotatoes (Bridgers et al., 2010). The ability to extract anthocyanins from sweetpotato has been proven by Bridgers et al. (2010) and can be used as industrial colorants and dyes. Also, after the anthocyanins have been extracted there are still fermentable sugars available.

Researchers have also learned to extract from biomass substances to produce bioplastics. One such substance, polyhydroxyalkanoates (PHA), is described by Noda and Schechtman (1999) is a process to extract this from viable bioplastic producing crops such as sweetpotato.

Lastly, it should also be mentioned that sweetpotato can be used to produce butanol from biomass feedstocks (Qureshi et al., 2010). There are still improvements that can be made to increase the concentration of butanol, but it is possible and an alternative to bio-ethanol production using the renewable sugars from industrial sweetpotatoes.

### 1.4 Cut Root Pieces in Sweetpotato:

Given the potential economic and practical advantages of growing sweetpotato from cut root pieces (CRP), various researchers have investigated the technique over the past sixty years. The earliest published work on planting sweetpotatoes from cut root pieces originated from the southeastern United States in the mid-1940s (GCPES, 1943; Lutz et al., 1946). These studies reported mixed success. It was found that the planted root pieces would act as a photoassimilate sink and they enlarged to form large misshapen roots, termed *mother roots*, and failed to produce new storage roots, termed *daughter roots*. This tendency, however, could be minimized if appropriate cultural practices were used. The Georgia Coastal Plain Experiment Station (GCPES, 1943) concluded that although root piece planting could be used in sweetpotato it better suited the production of material for livestock feed because of low yields, poor stands, and the misshaped roots produced due to root piece enlargement.

In the 1950's and 1960's, Japanese scientists began work on CRP planting of sweetpotato. One group looked mainly at cultural aspects of CRP planting including growth habit, root development, and comparison of direct planting versus traditional planting (Kodoma, 1962; Kodoma and Kobayashi, 1952; Kodoma and Kobayashi, 1954, Kodoma et al., 1954; Kodoma and Nomoto, 1955; Kodoma et al., 1958). Notably, this work found an inverse relationship between the enlargement of mother roots and daughter root production. It was also found that certain practices could be used to reduce mother root enlargement. These included shallow planting, planting in sandy soil and selecting appropriate varieties

(Kodoma, 1962). These findings were similar to those of the Georgia Costal Plain Experiment Station.

A second group of Japanese researchers focused primarily on the selection of sweetpotato cultivars suited to direct planting (Akita and Kobayashi, 1962; Akita and Kobayashi, 1965; Akita et al., 1962). This group found that CRP planting ability is a rare trait, but they did identify a number of promising clones. This group also discovered that most varieties could be planted as root pieces if the pieces were planted, allowed to sprout, and then exposed to air and light just after planting (Akita et al., 1962).

Later, research by Kobayashi, who was affiliated with Kodoma's group, briefly reviewed the research done by he and his colleagues (Kobayashi, 1968). This review concluded that:

- The success of root piece planting ultimately relied on a restriction of mother root growth.
- There were two different classes of daughter root, those originating from mother roots (direct daughter roots) and those originating from stems (indirect daughter roots).
- Indirect daughter roots were typically ranked as being morphologically superior to direct daughter roots.
- Seasonal variability in lines that produced primarily indirect daughter roots was significantly less than lines that produced primarily direct daughter roots.

- Deep planting (10 cm) was more effective at restricting mother root growth compared to shallow planting.
- Lines bred from low daughter root parents showed high skewness for low daughter root yield, unless a parent with high daughter root yield from cut seed pieces was used.
- The indirect daughter root characteristic of a top line (Chugoku 25) was a simply inherited and partially dominant trait.

The Japanese research on sweetpotato cut root piece planting lasted for ~20 years before largely ending in the 1970's. The success of Japanese root piece research renewed efforts in the United States. A number of researchers investigated root piece planting in sweetpotato, with most of the work conducted by a group in Maryland headed by John Bouwkamp (Bouwkamp, 1982; Bouwkamp, 1985; Bouwkamp and Scott, 1972; Bouwkamp et al., 1971). This research addressed the problem of poor stand establishment in sweetpotato by experimenting with different pre-planting treatments of the root pieces

A key finding from the work conducted in the United States was that the fungicide 2,6-dichloro-4-nitroaniline (Botran) will protect against *Rhizopus stolonifer,* a common soft rot that can cause serious damage to sweetpotatoes in storage (Bouwkamp et al., 1971). It was also found that pre-sprouting could be used to promote root piece sprouting, but too much increased the incidence of rot. The studies in the United States also expanded on the three CRP growth types indentified by Kobayashi (Kobayashi, 1968). The new system recognized five different growth types (Table 1.2).

The recognition of these growth type categories were important because the success of a variety depends on its ability to put energy in producing new “normal daughter roots” or what is referred to as “indirect daughter roots” and the root piece not becoming an energy sink. However, many of the “root piece disappearing” lines were some of the poorer sprouting lines. This was interpreted to mean that poor sprouting results in fewer surviving hills. Understanding the different responses to planting with CRP gives insight into how useful a variety can be since more yield in normal daughter roots is preferred over enlarged root piece yield (Bouwkamp, 1982).

Other work in the United States on CRP was conducted by researchers from the Tuskegee Institute, Alabama (Allen and Phillis, 1979; Phillis and Allen, 1979). The Tuskegee work was summarized only in conference abstracts so details regarding the work are not available, but it involved variety screening and cultural management work. Of particular relevance was a study that looked at the outcome of planting with root pieces cut from different parts of the sweetpotato root. This work found that proximal root sections produced more yield than distal or middle sections of cut root pieces, and that middle pieces had the highest mortality, but also enlarged the mother piece the least.

There was little other ongoing CRP research from the early 1980s to the 2000s. There was a release of a Japanese variety specifically bred to be used in cut root piece production, but the variety was never put into commercial production (Shikata et al., 1975). Hosokawa (1998) experimented with the development of a self propelled mechanized seed piece planter. The machine functioned successfully but had the drawback of requiring root pieces to be fed into the planter by hand. The workers estimated the speed of the machine to be roughly double the speed of conventional transplanting techniques using plants. However the figures of 0.19 to 0.33 m/sec quoted by the paper equate to only 0.2 to 0.4 hectares per hour. Yamashita (2000) investigated using plantlets transplanted with a small root piece. They sought to solve the mother piece sizing issue in using CRP and thought it would be more amenable to mechanized transplantation. While they were successful and found their method to be superior to using cut sprouts by generating plantlets using small root pieces as small as 10g, they still had a low shooting rate, low productivity, and a lack of suitable planting machines.

### 1.5 Heritability:

Currently, most research that has been done on cut root pieces has been cultural in nature. Some studies have screened germplasm for lines that are amenable to CRP planting (Allen and Phillis, 1979; Bouwkamp and Scott, 1972; Harmon, 1970; Kobayashi et al., 1969; Kusuhara et al., 1972; Nakazawa, 1973; Phillis and Allen, 1979; Shikata et al, 1975; Tompkins and Horton 1974). Each study did identify clones that performed similar to slips at their location.  Almost no active breeding work has been done do develop lines for CRP planting and it is still not currently understood how best to breed for CRP planting ability.

Of particular value to breeders would be a quantitative measure of the heritability of cut root piece planting ability. Heritability is the measure of the degree to which a phenotype is genetically influenced and can be modified by selection (Schlegel, 2003). Falconer (1989) and Nyquist (1991) state that there are two distinct meanings of heritability and they can be described as (1) being heritable as determined by genotype [degree of genetic determination (Falconer, 1989)] or (2) being heritable as transmitted from parent to offspring (Nyquist, 1991). Type 1 heritability is known as Broad sense heritability and is generally used as a descriptive measure in vegetatively or apomictically propagated species. Broad sense heritability is the ratio of the genotypic variance over the phenotypic variance or $\sigma^2_G / \sigma^2_P = \sigma^2_G / \sigma^2_G + \sigma^2_{GE} + \sigma^2$(NYQUIST, 1991). Type 2 heritability is known as narrow sense heritability and is used in species that sexually reproduce, and is a way to predict the outcome from parent to offspring (Hanson 1963). Narrow sense heritability is the ratio of additive genetic variance to the phenotypic variance or $\sigma^2_A / \sigma^2_P = \sigma^2_A / \sigma^2_G + \sigma^2_{GE} + \sigma^2$ (Nyquist, 1991).

There are multiple ways to test for heritability, but there are three common methods: 1) testing for broad sense heritability (H); 2) testing by variance-component techniques ($h^2$); and 3) testing by parent-offspring regression ($h^2$) (Jones, 1969; Jones, 1977; Jones, 1986; Jones et al., 1976). According to Jones (1986), sweetpotatoes exhibit a quantitative inheritance pattern and out of 72 estimates of H and 207 $h^2$ using three tests of root weight yield, growth cracks, flesh color, and *Fusarium* wilt resistance he found that H averaged 0.85, $h^2$ by variance-covariance technique averages 0.57, and $h^2$ by parent-offspring regression averaged 0.48. Due to the differences in heritability it can be seen that experience with a crop should be relied on to determine the best way to estimate $h^2$ (Jones, 1986). In sweetpotato, parent-offspring regression techniques generally provide a more conservative estimate for heritability in all traits tested (root weight yield, growth cracks, flesh color, and Fusarium wilt) (Jones, 1986).

When using heritability estimates it is important to remember that heritability estimates apply only to the population studied, using the same experimental techniques, and under similar environmental conditions (Falconer, 1989). Similarly, it is important to remember that high heritability is not to be confused with a positive trait, but that it has higher chance to pass on the trait if it has a higher heritability (Jones, 1986).

### 1.6 Research Objectives:

An understanding of the heritability of cut root piece planting ability in sweetpotato will help to inform breeding programs that aim to develop varieties suited to this planting technique. Most importantly, it will determine to what extent root piece planting ability can be improved via breeding and selection. Currently, no research has been focused on this area of root piece planting. The only research to date was done by Kusuhara et al. (1972) on root piece types (indirect, direct, mother root enlarging, and intermediate). The goal of this research was therefore to estimate the narrow sense heritability of multiple cut root piece traits using parent-offspring regression of parent means on the means of their offspring.

Along with estimating heritability of root piece planting ability, this work also investigated a number of cultural management methods related to root piece planting. Although previous work in the United States and Japan has examined cultural management practices needed to optimize the success of root piece planting in sweetpotato, it is important to confirm these findings under different environmental conditions, with current and developing varieties, and using new industrial sweetpotato varieties. My research included experiments designed to: 1) evaluate how cutting affects sprouting; 2) determine how root piece types affect daughter root production and sized root piece enlargement; and 3) the performance of industrial clones planted as CRP versus coventional planting techniques using unrooted plants.

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**Table 1.1** Comparison of corn and sweetpotato ethanol yield (Liters/Tonne) assuming equal fresh and dry weight for each crop (Dien et al., 2002; Lee et al., 2008; Li and Chan-Halbrendt, 2009).

| | Ethanol Production Yield (L/T) | |
|---|---|---|
| Crop | Fresh Weight | Dry Weight |
| Corn | 360.5 | 403.2 |
| Sweetpotato | 154.4 | 497.2 |

**Table 1.2:** Response types of sweetpotato planted as cut root pieces (Bouwkamp et al 1972).

| **Type** | **Normal Daughter Roots** | **Enlarged Root Piece** |
|---|---|---|
| Indirect Daughter, Root Piece Enlarging(IDRE) | Yes | Enlarged, Sink |
| Indirect Daughter, root piece remaining(IDRR) | Yes | No sizing |
| Indirect Daughter, root piece disappearing | Yes | Rotted |
| Direct Daughter, root piece remaining | No | No sizing |
| Mother root piece enlarging | No | Enlarged, became Sink |

Table 1.3: Enterprise budgets for costs/ha from field preparation prior to planting through harvest for sweetpotato, corn and potato in North Carolina.

| | Sweetpotato | Corn | Potato[1] |
|---|---|---|---|
| Operating Cost | $3,484.85 | $897.88 | $3,913.93 |
| Fixed Cost | $617.76 | $113.42 | $1,297.30 |
| Total Cost | $4,102.61 | $1,011.30 | $5,211.23 |
| | | | |
| Cost of Plants | $691.90 | $172.97 | $568.34 |
| Cost of Labor | $92.66 | $22.81 | $222 |
| Cost of Feriliter, Herbicide, Inseciticide, Fungicide[2] | $428.28 | $505.80 | $2,357.90 |
| | | | |
| % Cost of seed | 17% | 17% | 11% |
| % Cost of chemicals | 10% | 50% | 45% |

[1]: Potato budget was from Pennsylvania since at time of publishing no budget was available for North Carolina
[2]: 'Chemicals' in this column include only fertilizer, herbicide, insecticide and fungicides used from planting to harvest (not post harvest treatment)

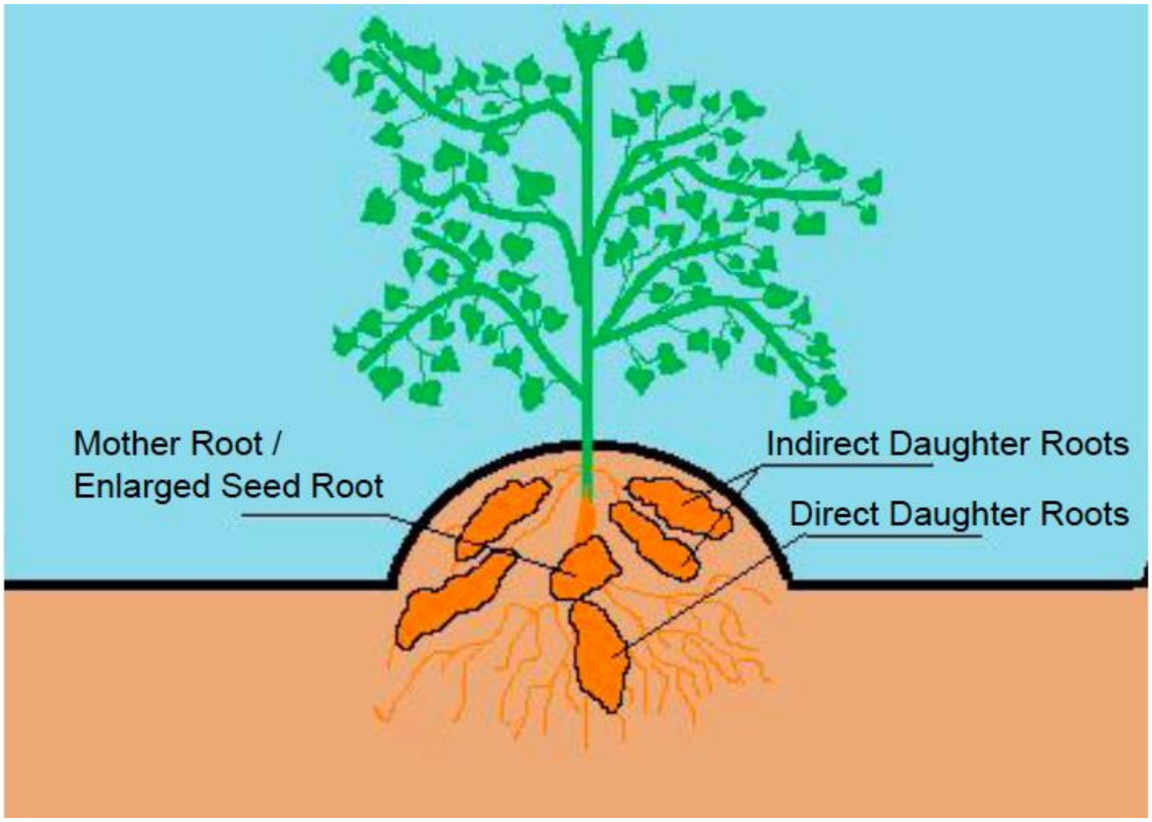


**Figure 1.1: A diagram depicting the different outcomes that can happen from cut root piece planting.**

Sweetpotatoes : Estimated Rev., Operating Expenses, Annual Ownership Expenses, and Net Return Per Acre

Budget 98-1
Jan-02

| OPERATING INPUTS | UNITS | PRICE | QUANTITY | VALUE | YOUR VALUE |
|---|---|---|---|---|---|
| Swpotato Harv./Sort | Crtn | 1.000 | 650.000 | 650.00 | |
| 40 lb. box | Each | 1.000 | 475.000 | 475.00 | |
| Sw.Potato Plants | Thsd | 20.000 | 14.000 | 280.00 | |
| Pre-Emrg. Herb. | ACRE | 18.830 | 1.000 | 18.83 | |
| Post-Emrg. Herb. | ACRE | 7.220 | 1.000 | 7.22 | |
| Pre-Plant Insect. | ACRE | 19.230 | 1.000 | 19.23 | |
| Contact Insect. | ACRE | 8.650 | 4.000 | 34.60 | |
| Nematicide | ACRE | 44.000 | 1.000 | 44.00 | |
| N | CWT | 38.250 | 0.650 | 24.86 | |
| P | CWT | 27.440 | 0.260 | 7.13 | |
| K | CWT | 10.240 | 1.660 | 17.00 | |
| Boron | LBS | 0.300 | 0.500 | 0.15 | |
| ANNUAL OPERATING CAPITAL | DOL. | 0.073 | 174.855 | 12.68 | |
| MACHINERY LABOR | HR. | 8.5 | 13.121 | 111.52 | |
| OTHER LABOR | HR. | 7.50 | 5.00 | 37.50 | |
| MACHINERY FUEL, LUBE, REPAIRS | DOL. | | | 145.55 | |
| TOTAL OPERATING COSTS | | | | 1885.27 | |

| FIXED COSTS | AMOUNT | VALUE | | YOUR VALUE |
|---|---|---|---|---|
| MACHINERY | | | | |
| INTEREST AT 8.00% | 743.65 | 59.49 | | |
| DEPR, TAXES, INSURANCE | | 87.83 | | |
| EQUIPMENT | | | | |
| INTEREST AT 8.00% | 135.00 | 10.80 | | |
| DEPR, TAXES, INSURANCE | | 92.43 | | |
| TOTAL FIXED COSTS | | | 250.55 | |

| PRODUCTION | UNITS | PRICE | QUANTITY | VALUE | YOUR VALUE |
|---|---|---|---|---|---|
| Sw. Potatoes,40 Lb. | Crtn | 5.00 | 475.00 | 2375.00 | |
| Canners | Cwt | 3.75 | 35.00 | 131.25 | |
| Jumbos | Cwt | 3.75 | 35.00 | 131.25 | |
| TOTAL RECEIPTS | | | | 2637.50 | |

| | |
|---|---|
| RETURNS ABOVE TOTAL OPERATING COST | 752.23 |
| RETURNS ABOVE ALL SPECIFIED COSTS | 501.68 |

Blend price reflects 3/4 of crop sold fresh and
1/4 sold to processors.
Excludes grading charge

E. Estes, Ext. Economist
515-4553
J. Schultheis, Ext. Hort.
515-1225
H. Sampson, Budget Processor

DEVELOPED AND PROCESSED BY DEPARTMENT OF AGRICULTURAL AND RESOURCE ECONOMICS
NORTH CAROLINA STATE UNIVERSITY

Figure 1.2: A copy of the sweetpotato enterprise budget produced by North Carolina State University.

# Chapter 2: Studies on Characteristics of Sweetpotato (*Ipomoea batatas)* Planted Using Cut Root Pieces

Blake Bowen, Nicholas George, Ken Pecota, and Craig Yencho

**Abstract**

Sweetpotato has potential to be used as a biofuel feedstock in the corn deficit southeastern United States, but is limited by its high cost of production. This manuscript focuses on a root piece planting technique that has been tested by previous researchers (Allen and Phillis, 1979; Bouwkamp and Scott, 1972; Kobayashi, 1968; Kodoma et al., 1958) and in an effort to reduce planting cost for industrial sweetpotatoes to be used as a biofuel feedstock was revisited with modern lines currently grown in the southeastern US. A series of three experiments were performed in order to measure different aspects of root piece production. These tests included a greenhouse test to measure the effect of cutting on proximal dominance and sprouting, a field test to measure differences between cut root sections and whole roots on daughter root yield, enlarged seed root (ESR or SRE) yield, and partitioning index (PI), defined as [(daughter root yield – enlarged seed root yield) / Total Yield], and lastly, another field test to measure the differences between traditional slip planting and root piece planting techniques and their effects on daughter root yield, enlarged seed root yield, partitioning index, average enlarged seed roots, number of seed roots remaining, and number 1, canner, jumbo, and cull yield. The greenhouse test showed significantly different amounts of sprouts per root section ($p<0.05$) in uncut roots, but showed no differences in total sprouts between cut and uncut roots ($p>0.05$). In uncut roots

proximal root sections produced the most roots, but cutting was found to reduce sprouting in the proximal section and increase sprouting in the distal and middle sections. The first field study showed that root piece type had a significant effect on daughter root yield and enlarged seed root yield ($p<0.05$) as well as total yield in 2008. The proximal treatment had the least seed root enlargement both years with 1.8 and 5.9 T/ha respectively. Daughter root yield was highest in the proximal treatment and total yield varied in 2007, but was not significantly different across treatments in 2008. Partitioning index was shown to be highest in the proximal end treatments and lowest in the whole root treatment. Field study two showed significant differences between the traditional slip planting and seed root planting treatments for appearance, partitioning index, daughter root, enlarged seed root, total, number 1, and jumbo yield ($p<0.05$) across all years, but showed no differences between canner and cull yield. Clonal variation across years was significant except for a few clones that were not different for a few traits. There were no differences between treatments for the following breeding lines and treatments: NC03-417 and partitioning index, NC03-395 and total yield/appearance, NCDM04-226 and seed root enlargement, and lastly NCDM02-105 and NCDM02-180 for total yield.

## 2.1 Introduction

The United States has recently began to increase the production and consumption of fuel ethanol from biomass, from 6.7 billion liters in 2001 to 26.31 billion liters in 2007 (EIA, 2009). The majority of this fuel ethanol is derived from corn produced in the mid-western states. North Carolina has recently established an ambitious goal of producing 10 percent of its liquid fuel needs from biofuels grown and produced within the state by 2017 (Burke et al., 2007). However, North Carolina produces less than 1% of the U.S. corn crop (USDA, 2008), and like most of the southeast, will therefore be unable to meet the demand for bioethanol without importing corn from other states. Alternative ethanol feedstocks are therefore required.

North Carolina is the largest producer of sweetpotatoes in the United States, producing 47.4% of the nation's crop in 2008 (USDA, 2008). Several studies have shown that sweetpotatoes may have the potential to produce substantially greater ethanol yields per hectare than corn (Dangler et al., 1989; Hall and Smittle, 1983; Lee et al., 2008; Ziska et al., 2009). Sweetpotato has been shown to produce 23 percent more starch per ton of dry weight than corn (Dien et al., 2002; Lee et al., 2008; Li and Chan-Halbrendt, 2009), making sweetpotatoes a good candidate as an ethanol feedstock alternative. Specifically, high dry matter white fleshed sweetpotatoes, called, industrial sweetpotatoes (ISP). Industrial sweetpotatoes have a greater threshold for ethanol production than the traditional low dry matter orange fleshed sweetpotato typically grown in the United States due to their higher starch content and fermentables (Hall and Smittle, 1983; Lee et al., 2008).

The main problem associated with using industrial sweetpotato as a biofuel feedstock source is the cost of production. The production costs of sweetpotato are almost four times higher than corn, at $4102/ha compared to $1011/ha (Estes and Schultheis, 2006; Bullon and Weddington, 2010). The major reason for this cost differential is that sweetpotato plant production, planting, and harvest is done primarily by hand with little mechanization (Estes and Schultheis, 2006). Current methods for sweetpotato production involve bedding stored roots of sweetpotatoes in the spring, sometimes mechanically but typically by hand. Sprouts from these bedded roots, termed slips, are cut and processed by hand. Slips are then transplanted into fields using a mechanical transplanter that is fed by hand. The crop is harvested using a specialized plow or mechanical digger, which exposes the roots on the soil surface, from where they are then collected by hand.

In an effort to reduce the costs of industrial sweetpotato production we have been conducting research to develop a seed root planting technique similar to that used for potato (*Solanum tuberosum)* (Bouwkamp and Scott, 1972; Hosokawa et al., 1998; Yamashita, 2000). This technique involves using whole or cut root pieces to establish a sweetpotato crop. Seed root planting should lend itself to mechanization and significantly reduce the labor involved in producing sweetpotato (Hosokawa et al., 1998). Several research groups, primarily in Japan and the United States, have investigated the cultural, genetic, and mechanization aspects of planting sweetpotato using seed roots (Akita et al., 1962; Bouwkamp, 1982; Bouwkamp and Scott, 1972; Hosokawa et al., 1998; Kobayashi, 1968; Kodoma, 1962; Yamashita, 2000). Despite this, the technique has never been fully commercialized and there are still a number of obstacles that inhibit its success, in particular a lack of information regarding appropriate cultural methods.

This study describes the results of a greenhouse experiment, and two field experiments, designed to address key questions relating to the use of the cut seed piece planting techniques in sweetpotato. Each experiment was designed to answer a different hypothesis related to seed root planting techniques in sweetpotato. For the greenhouse experiment, it was hypothesized that cutting storage roots would lead to more total sprouts and increased distal end sprouting. The root piece type study hypothesized that planting cut seed roots would result in higher daughter root and total root yield, and less seed piece sizing, when compared to planting whole roots. In the third experiment, it was hypothesized that planting a sweetpotato crop using seed root planting techniques would produce lower yield and poorer quality roots when compared to traditional slip planting techniques.

## 2.2 Materials & Methods

### Plant Material Selection and Preparation

*Greenhouse sprout production experiment* - The greenhouse experiment included five sweetpotato clones, representing table-stock and industrial lines, thought to have either high proximal dominance or low proximal dominance (NCDM01-158, NCFT4-89, Covington, NCDM04-226, and NC93-17). NC93-17 and NCDM04-226 exhibit low proximal dominance, while NCDM01-158, NCFT4-89, and Covington exhibit high proximal dominance. Each replicate included four different types of seed roots: 1) a seed root derived from the proximal end of the storage root; 2) a seed root derived from the middle portion of the storage root; 3) a seed root derived from the distal end of the storage root; and 4) a whole uncut root.

All seed roots were dipped in the fungicide Botran 75W (2,6-dichloro-4-nitroaniline, Gowan Company, Yuma, AZ) mixed at a rate of 2g/L. Whole roots were the marked into thirds representing the apical, middle and distal section using a permanent marker. All roots were placed longitudinally in 40 x 40 x 13 cm potting trays (Stuewe & Sons Inc, Corvallis, OR) filled three quarters full with Fafard 4P soil mix (Conrad Fafard Inc, Agawam, MA). Proximal ends of the roots were identified and oriented towards the back of the tray for quick identification of the proximal and distal ends. Roots were partially pressed into the soil, so that the top half of the root was exposed to the air and the bottom was imbedded in the soil. This was done so that sprouting would be clearly visible and to better facilitate the quantification of sprouts emerging in each region of the root. Trays were organized into a completely randomized block design and bi-weekly observations were taken on the number and location of sprouts on each root. The greenhouse temperature was maintained at approximately 30°C and pots were watered as needed.

*Field Study 1: Effect of clone and cut seed piece treatment on sweetpotato yield* - This field study, conducted in 2007 and 2008, comprised seven clones, three planting treatments (proximal, distal, and whole root pieces) and four replications. A fully factorial randomized block experimental design was used. Clones NC93-17, NC03-007, NC03-417, NC03-395, NCDM04-226 and MD810 were evaluated in 2007 and clones NC03-030, NC03-395, NC04-468, NCDM02-105, NCDM02-180, and NCDM04-226 were used in 2008. Due to rotting in storage and insufficient material of clones NC03-007, NC03-417, and MD810 they were excluded in both years so NC03-030, NC04-468, NCDM02-105, and NCDM02-180 were substituted. Clones were chosen to screen developing table stock and industrial lines that had been planted previously with some success for this technique.

For each replicate, forty-eight roots ranging from 110 to 230g were chosen at random for each clone. Twenty-four roots were then randomly selected and cut in half (forming root pieces weighing 55-115g) to form the proximal and distal end treatments. The remaining twenty-four roots were kept whole for the third treatment, but they were selected so that they were approximately the same weight as the individual cut pieces. All roots were dipped in a 2g/L solution of Botran 75W. Roots were then placed in a humidity and temperature controlled room as a pre-sprouting treatment for 7 to 10 days at 30°C and 90% relative humidity. Pre-sprouting conditions were varied slightly between years to control for soft rot caused by *Rhizopus stolonifer*. In 2007, after four days of pre-sprouting the temperature was reduced to 26°C and 50% relative humidity for the last four days. In 2008 after six days the temperature and relative humidity were reduced similarly to 2007 for the final six days. In 2009 pre-sprouting techniques were changed to a lower temperature and humidity, 24°C and 70%, to better control soft rot.

*Field Study 2: Effect of planting technique (conventional planting versus root piece planting) on sweetpotato yield* - This study included fifteen clones over the three years studied, two treatments (planting with slips versus cut seed piece), and four replications. Different clones were used in each year: NC03-030, NC03-417, NC03-395, NC03-417, NC93-17, Beauregard, NCDM02-105, NCDM02-180, NCDM04-226, NCFTA94 in 2007; NC03-007,

NC03-417, NC04-097, NC03-089, NC03-395, NC03-417, Beauregard, NCDM02-105, NCDM02-180, Covington, NCDM04-226, NCFTA94, and NCFT4-89 in 2008; and all clones in 2009). Clones were selected to study their performance of root piece planting compared to conventional planting. Table stock and industrial lines needed to be studied to see if the effects were similar or different using a direct comparison of each planting type and clone. While this type of experiment has been done previously elsewhere with these lines it is unique to North Carolina and its breeding and table stock lines. The experimental design consisted of a randomized split-plot. Main effects were replicates and treatment, with the sub plot being the individual clone by treatment interactions. In each replicate and for each clone, 24 slips and root pieces were planted. Roots sizes ranging from 110 to 170 g were cut in half to form cut root pieces that weighed from 55-85g. Cutting methods, fungicide treatments and pre-sprouting conditions were the same as for field study 1.

**Field Preparation and Planting**

Both field experiments were planted in Norfolk sandy loam soils in Kinston and Clinton, NC, and the fields were bedded into rows at a row spacing of 1.1 m. Experimental plots within a row were 7.6 m long, and slips and cut seed pieces were planted on 0.3 m spacing in the row. The sweetpotato plants (slips) were planted using a typical sweetpotato transplanter (Holland Model #900). In 2007, the seed pieces were planted using a mulch pot planter (Holland Model #1265). In 2008 and 2009 seed root pieces were planted using a small plot potato planter (Model RP-20M, Afiveplus Inc., Torrington, WY).

Standard fertilizer applications began at either one week prior to planting or at first cultivation. Fertilizer treatments comprised either 450 kg/ha 0-10-25(with boron), if soil tests showed phosphorus levels to be inadequate, otherwise 170 kg/ha 0-0-60 (with boron) was used During the second and third cultivations 170 kg/ha of 0-0-60 and 170-180 kg/ha of 34-0-0 prilled ammonium nitrate were applied as a side dressing and incorporated using rolling sweeps. Insect and weed control practices included Telone II (1,3-Dichloropropene, Dow Agrosciences, Indianapolis, IN) three weeks prior to planting for nematode control, Command 3ME (Clomazone, FMC Corporation, Philidelphia, PA) 3 L/ha + Eptam (EPTC, Gowan Company, Yuma, AZ) 4 L/ha PPI for weed control prior to planting in the formed beds, and for insect control Lorsban 15G or 4E (Chlorpyrifos, Dow Agrosciences, Indianapolis, IN) was broadcast sprayed and incorporated 10-15cm into the soil prior to planting.

### Harvest and Data Collection

For the greenhouse study, bi-weekly observations were recorded on the number and location of sprouts on each root for six weeks. For the field studies, stand counts were recorded starting two weeks after planting and continuing until six weeks after planting. At harvest, the field studies were dug using a chain digger, hand graded into size-classes and weighed.

For the first field study, two categories of data were collected, the total yield of daughter roots produced (shown in table as wet ton/ha) and the yield of enlarged seed roots (ESR). Daughter roots are defined as any new storage root formed from the cut seed piece planted at the beginning of the season. Enlarged seed roots are cut root pieces the have increased in size following planting.

For the second field study, the yield of daughter roots and yield of enlarged seed roots were recorded in 2007. In 2008 and 2009 the daughter roots were further graded into US No.1's, canners, jumbos, culls (based on NSCG standards), and enlarged seed roots and the yield of each class was recorded. Data was also recorded on the appearances of roots at harvest, number of remaining enlarged seed roots. In 2009, dry matter was obtained for all the plots. Sampling for this was done by selecting three no. 1 sized roots and blending them in a food processor to attain approximately 100 g of root material and drying it at 80°C.

From the data collected in the field studies two other traits were calculated. These traits were the partitioning index (PI) and average mass of enlarged seed roots per plot. The partitioning index is a measure from -1 to +1 of how yield is distributed between new daughter roots and enlarged seed roots. For example, if there were 100 percent daughter root yield, then that would be equivalent to +1. The partitioning index was calculated for each clone using the formula: Partitioning Index (PI) =*(Daughter Root Yield – Enlarged Seed Root Yield) / Total Yield.*

**Data Analysis and Statistics**

Replications (blocks) were placed according to field layout to account for field variation. Tests were analyzed each year individually due to differences in clones. Analysis of variance (ANOVA) was performed to test for significant differences between replications, treatments, clones, and clone by treatment interactions for daughter root yield, total yield, partitioning index and for the planting type study dry matter, overall root appearance, number one, canner, jumbo, and cull root production using SAS v. 9.1 (SAS Institute, Cary, NC). A Waller-Duncan K ratio T-test was performed in order to test for differences among averages scores for each trait measured at alpha =.05.

## 2.3 Results

*Greenhouse sprout production experiment –*

The clones NC93-17 and NCDM04-226 suffered severe rotting and pest damage, and therefore could not be included in the analysis leaving only three clones for the analysis of sprout production. Across both the cut and uncut roots, highly significant differences in the number of sprouts produced on the proximal, middle and distal cut seed root treatments were observed ($p$<0.05) in

Table 2.1. The middle and distal sections of a storage root showed significantly less sprouting, producing four and three sprouts respectively, compared to the proximal end, which averaged ten sprouts. The middle and distal end treatments were not significantly different from each other ($p$>0.05). Significant interactions between extent of sprouting in different locations and clone were also observed (Table 2.1).

Comparing the cut versus uncut treatments shows that cutting the seed piece did not significantly affect total sprout production ($p$>0.05)(Table 2.2). The number of sprouts produced per cut root was 18 versus 16 produced per uncut root. There were a number of significant interactions between treatments in the experiment. A significant interaction was found between the location where sprouts were produced and whether or not the roots were cut ($p$<0.05) (Table 2.1). While the total number of sprouts was the same for cut and uncut treatments, cutting reduced the number of proximal end sprouts and increased sprouting in the middle and distal portions. Figure 2.3 shows that in uncut roots 70% of the sprouts emerged from the proximal location, compared to only 36% in the cut roots.

*Field Study 1: Effect of clone and root piece treatment on sweetpotato yield –*

Root piece type (proximal vs. distal vs. whole) had a significant effect on the yield of daughter roots and sized seed pieces, although the trend was not consistent both years (Table 2.3).

In 2007 the whole root treatment produced the most daughter root yield, 13.1 T/ha, followed by the proximal piece treatment, which produced 11.9 T/ha. In 2008 the leading treatment for daughter root production was the proximal cut root piece treatment followed by the distal end cut root piece, with 18.7 and 14.4 ton/ha, respectively (Table 2.4). (Total yield was calculated by combining the daughter root yield and the sized seed piece yield (SSP). When this was done for both the whole root and proximal treatments we observed that the whole piece treatment had the highest total yield (17.3 tons/ha) in 2007 and the proximal piece treatment had the second highest, 13.7 ton/ha, which was significantly different ($p$=<0.05) from the total yield in the whole piece treatment (Table 2.4)**.** There were no significant differences between the treatments for total yield in 2008 (Table 2.4). However, contrasted with 2007, the proximal treatment produced the lowest yield of enlarged root pieces, and the distal piece treatment the second lowest producing 5.9 and 8.8 ton/ha, respectively, when comparing seed piece sizing.

The partitioning index of the clones both years was similar to the differences observed between years for yield. Higher values were generally observed for the proximal and distal treatments reflecting the lower enlarged seed root yield in 2007 (Figure 2.2). In 2007 every clone except NC03-007 had a positive partitioning index (PI) for all treatments. In 2007 the proximal treatment had more daughter root yield than enlarged seed root yield making it the best treatment for all clones except for MD810. In 2008, the proximal treatment the partitioning index across all clones and treatments was consistently greater (Figure 2.2).

Differences between clones were examined using ANOVA for three traits; daughter root yield, enlarged seed root yield and total yield (Table 2.4). In 2007 there were three clones that showed no significant differences for daughter root yield (NC03-395, NC93-17, and NCDM04-226). There were two clones that showed no significant difference for sized seed piece yield (NC03-417 and MD810) and one clone (NCDM04-226) that was not significant for all three traits (daughter root yield, enlarged seed root yield, and total yield). In 2008 three clones were not significant between the treatments for daughter root yield (NC03-030, NC03-395, NCDM04-226). Table 2.4 also shows that only one clone was not significant for enlarged seed root yield (NC04-468), and that NC04-468 is also the only clone that was not significantly different for total yield (Table 2.4).

*Field Study 2: Effect of planting technique (conventional planting versus root piece planting) on sweetpotato yield –*

Four common traits were measured in this experiment during all three years. These traits were enlarged seed root yield, daughter root yield, total yield, and partitioning index (PI). Significant differences in all four traits were observed when the two planting methods (conventional planting vs. seed root planting) were compared. Treatment differences (across all clones) were highly significant for the yield of number ones, jumbos, and overall appearance for both years **(**Table 2.5). The difference observed in the yield of canners between conventional planting and root piece planting was significant only in 2009. Cull yield did not differ significantly between treatments in any years. In 2009, dry matter percentage was measured and we found dry matter to be significantly different between the clones. Treatments by clone interactions were all highly significant for all traits except cull and dry matter. The planting method (conventional vs seed root) had a highly significant effect on daughter root yield across all years and all clones, except for clone NC03-395 in 2008 and clones NC04-097 and NCDM02-105 in 2009 (Table 2.6). The yield of enlarged seed roots was affected, primarily increasing, significantly across all years for all the clones except NC03-089, NC03-417, NCDM04-226, and MD810. The difference between total yield (daughter root and enlarged seed root yield together) and yield from plants was highly significant, with cut root pieces typically being lower. However, there were exceptions to this across all years. Notably, NC03-395 showed no significant difference between total yield from root pieces and plants in all years tested. Partitioning Index was measured for all three years and compared between treatments (Table 2.6). Only one clone showed no significant difference between treatments for all three years (NC03-417). Lastly, in table 2.7 all other clones showed a decrease in PI when planted as CSP.

During 2008 and 2009, appearance measurements were taken on each plot and compared between clones and treatments. Appearance measurements included shape, shape variability, eyes, lenticels, maturity, skin color, skin texture, flesh color, length/diameter, and overall appearance. There was no difference between eyes, lenticels, maturity, skin color, skin texture, flesh color, and length/diameter (data not shown), but there were differences in shape, shape variability, and overall appearance. Due to the complexity of defining shape and how shape / shape variability relate to overall appearance, overall appearance is used primarily to describe these clones. Of all clones tested, three clones, NC03-395, NCDM02-180, and NCFTA94, were found not to be significant for changes in appearance across both years (Table 2.6).

For the experiment in which the storage roots were graded into US No. 1s, canners, jumbos, and cull roots we observed significant levels of variation in the effect of treatment on the relative yield of the different size categories between years. Only No.1 yield was consistent across all clones. Most clones tended to show no significant difference between treatments in canner and jumbo yield. Treatment only had a significant effect on the yield of culls in two clones but these differences were only significant in one single different year each (Table 2.7).

Several clones performed well over the three years of this study. For example, NC03-417 consistently showed a high partitioning index across during all years, while NC03-395 showed little difference between total yield and root appearance when conventional planting and root piece planting were compared. Likewise, NCDM04-226 showed very little seed root enlargement and in 2009 produced similar total yield and similar appearance ratings when comparing conventional and cut root piece planting techniques. Lastly, NCDM02-105 and NCDM02-180 produced similar total yield as cut root piece compared to conventional planting.

## 2.4: Discussion

*Greenhouse sprouting study*

The goal of this experiment was to examine how cutting sweetpotato roots affects sprout production from the different sections of the root. We hypothesized that cutting the sweetpotato root would promote distal end sprouting and increase sprouting from the root overall. Cutting of the storage root does promote distal end sprouting. Total sprouts on the distal end increased from 5% to nearly 30% in the cutting treatment (Figure 2.3).

However, we also observed that cutting a storage root did not increase the total number of sprouts produced per root. This trend was consistent across all clones. It appears that cutting of the sweetpotato root breaks proximal dominance, therefore, reducing sprouting in the apical portion of the root and leading to an increase in sprouting in middle and distal portions. Other workers have also examined the effect of cutting on the sprouting of sweetpotato roots (Allen and Phillis, 1979; Bouwkamp, 1982; Edmond and Ammerman, 1971; Kobayashi, 1968; Phillis and Allen, 1979). These studies have also found that cutting will break proximal dominance, however they also report that cutting increased sprout production. These papers do not provide detail regarding methods so the reason for this difference is unclear. In the three clones listed in table 2.2 or figure 2.3, all have very strong proximal dominance. There are differences in the number of sprouts, but not in behavior. However, the behavior of NC93-17 or NCDM04-226 may have been different and further testing would be needed to confirm.

Given these findings, we would recommend that if seed roots were to be used for sweetpotato planting they should be cut to promote sprouting on both ends of the root. Cutting the storage roots has the advantage that it will produce more “seed roots” for the planting process and would still provide an equivalent amount of sprouting compared to not cutting This change is good because it increases the amount of seed roots available by increasing sprout production on the distal end. This may decrease the amount of nodes below ground which is responsible for indirect daughter root set, thus cultural tests such as maturity would need to be done for a promising line such as NCDM02-105. At this point the optimal size of a "seed root" is unclear. However, Hosokawa et al (1998) observed that optimal weight for CSP is between 25-99g and that cutting more than once led to a negative return on yield. However, it is possible that a greater area of cut surface could lead to greater mortality of pieces prior to sprouting and therefore poorer stand establishment as a result of pathogen attack (rotting) due to the increased surface area.

*Field Study 1: Effect of clone and cut seed piece treatment on sweetpotato yield*

This study examined the effect of planting either whole seed roots or apical or distal sections of cut root pieces on the yield and quality of sweetpotatoes grown in the field. In general, for all the clones studied, we observed that planting cut root pieces lead to greater yields of daughter roots and less sizing of the planted root pieces, also called mother roots (Bouwkamp and Scott, 1972). This observation is similar to that of the finding of other researches conducting similar investigations (Allen and Phillis, 1979; Phillis and Allen, 1979). The reason for the reduction in yield of mother root pieces, and greater production of daughter roots, when cut root pieces are planted is not clear. It was, however, not due to the cut pieces being initially smaller than the whole roots because this was controlled in the experimental design. It is also unlikely to be due to greater sprouting from the cut root pieces because, as was observed in the greenhouse study, cutting decreased sprouting in the apical section. We believe that the most likely explanation for this observation is that the cut seed roots have a greater surface area than whole roots and as a result they are at a greater risk of pathogen attack, and are therefore more prone to rot, leading to lower yields of mother roots as seen at harvest (data not shown). Phyllis and Allen (1979) conduced a similar experiment that also included a middle piece treatment and noticed that it had the highest mortality, but when they survived they yielded high daughter root yields with low seed or mother root sizing.

Even though the effect of cutting was significant for all clones the effect of root piece cutting varied between clones. For example, in 2007 three clones (NC03-007, NC03-417 and MD810) showed a significant difference in daughter root production between the treatments, whereas the other three clones did not. Only two clones were available in both years, NC03-395 and NCDM04-226. Clone NC03-395 showed a consistent trend across both years where as NCDM04-226 did not. NCDM04-226 exhibited significant sizing of the seed piece in the second year, but not the first.

The differences observed between years can possibly be explained due to environmental factors, namely higher rainfall in 2008. In 2007 there was a moderately severe drought throughout the entire season, from May to October. In 2008 there was drought shortly after planting, from May to July, but drought severity decreased for the remainder of the season (NCSCO 2009). This late season rain may explain the higher yields obtained in 2008. Given variability between years, and the fact that all the varieties were not available for both years, it is recommended that this study be repeated. With this caveat in mind, it is recommended that if root piece planting of sweetpotato is to be used then the seed root be cut in order to decrease the likelihood of mother piece sizing.

*Field Study 2: Effect of planting technique (conventional versus root pieces) on sweetpotato yield* –

This study compared the effect of traditional sweetpotato production using plants and cut root pieces on sweetpotato yield and quality. We hypothesized that the cut seed pieces would produce lower yield, a lower PI, and have a decrease in appearance quality, compared to the conventional planting technique. Overall, the findings of the study supported this hypothesis. In all three years of the study, conventional planting resulted in higher daughter root yields, partitioning indices, and appearance ratings. For example, the total yield of daughter roots from the conventionally planted sweetpotatoes was 34 T/ha compared to 16 T/ha for the crop produced from cut seed pieces. While the PI was positive for both plant and root piece planting, the cut seed piece treatment tended to be closer to zero, which equates to nearly 50% of the yield consisting of enlarged seed roots. The appearance ratings also differed significantly between the treatments, from 4.7 for slips to 3.1 for cut root pieces (Table 2.7). These findings agree with those of several other researchers who have performed similar experiments using other sweetpotato clones (Allen and Phyllis, 1979; Bouwkamp, 1982; GCPES, 1943; Kobayashi, 1968; Lutz et al., 1946; Phillis and Allen, 1979). Yield were graded into number 1, canner, jumbo, and culls which for industrial sweetpotato do not matter, but are important for other uses of sweetpotato such as for processing to produce chips and fries in which shapes and sizes do matter. There was a significant interaction between the planting method and clones for most measured traits. Interestingly, two clones (NC03-417, NC03-395) showed little difference between the conventional and seed root planting methods, and these were consistent across years. This is significant because it shows that some clones are better suited for seed root planting than others, and for breeders it is important to develop clones that are better suited/adapted to this method of planting.

*Conclusions*

This research examined three cultural aspects of planting sweetpotatoes using storage root pieces as "seed" for planting. First, in a greenhouse experiment, we examined the sprout production behavior of the different sections (apical, middle and distal) of a storage root and we determined how this behavior was impacted by cutting the storage root to break apical dominance. We then conducted two field experiments; the first was designed to compare the results of planting cut and uncut roots in on sweetpotato yield and quality. The second field study was designed to compare the outcome of planting using conventional plant-based techniques versus cut root pieces. We observed that cutting effectively breaks apical dominance of roots and leads to increased sprouting on distal pieces, increasing quantities of root piece planting material available. Proximal pieces showed in the first field study to be superior to distal end pieces by producing more daughter root yield and less enlarged seed root yield, but since only planting proximal pieces would reduce the seed amounts by half only using proximal pieces is not recommended, thus it would be better to have lines that produced similar daughter root yield regardless of planted section. We also found that cutting roots tends to reduce mother piece enlargement and increases daughter root yields. Planting a sweetpotato crop from seed roots was found to generally lead to lower yields of usable daughter roots and a reduction in root appearance. However, significant differences between years, and more importantly, between clones were observed. Two clones (NC03-417, NC03-395) showed little difference between the conventional and seed root planting methods, and these were consistent across years. Different clones sprout more readily than others and faster sprouting lines could become established quicker and thus produce more yield by harvest. This was looked into by stand counts taken over the course of the experiment (data not shown) and no significance was found due to how fast plants emerged from the ground, thus ruling out differences on yield by how fast they emerge post planting. This research therefore shows that both cultural management practices and breeding can be used to improve the success of root piece planting of sweetpotatoes, which is a conclusion that has been reached by other workers (Akita et al., 1962; Allen and Phillis, 1979; Bouwkamp, 1982; Bouwkamp and Scott, 1972; GCPES, 1943; Kobayashi, 1968; Kodoma, 1962; Lutz et al., 1946; Phillis and Allen, 1979). The variability between clones and years also shows that making conclusion based on limited clones and one year could be misleading and research work evaluating root piece planting should involve multiple clone over multiple years and sites.

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**Table 2.1: Analysis of variance table summarizing the effect of clone (NCDM01-158, NCFT4-89, and Covington), Treatment 1 and Treatment 2 and their interactions on the extent of sprouting from sweetpotato storage roots.**

| Source | DF | MS | P-value |
|---|---|---|---|
| Rep | 11 | 8.84 | 0.6517 |
| Clone | 2 | 109.75 | <0.0001 |
| Trt1 (Prox, Mid, Dist)[1] | 2 | 1024.65 | <0.0001 |
| Clone*Trt1 | 8 | 43.43 | 0.0049 |
| Trt2 (Whole vs Cut)[2] | 1 | 22.75 | 0.1562 |
| Trt1*Trt2 | 3 | 612.70 | <0.0001 |
| Clone*Trt2 | 4 | 4.34 | 0.6801 |
| Clone*Trt1*Trt2 | 8 | 212.55 | <0.0001 |

[1] Treatment 1 examined the effect of location of the cut portion (proximal, middle, distal) on sprouting.

[2] Treatment 2 examined the effect of uncut versus cut seed pieces on sprouting.

**Table 2.2. The mean number of plant sprouts produced by clone and root section in Treatment one and comparisons of average sprouts between cut and uncut (Treatment two). Comparisons done between the means by Waller Duncan test at the *p*=0.05 level represent the means for each root section averaged across all clones for cut (A, B, C) and uncut (D, E, F) seed pieces, respectively, and the mean number of plant sprouts averaged over clones and treatments (H,I, J).**

| **Clone** | **Cut** | | | **Cut Total** | **Uncut** | | | **Uncut Total** | **Combined Total Overall** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Prox** | **Mid** | **Dist** | | **Prox** | **Mid** | **Dist** | | **Prox** | **Mid** | **Dist** | Total |
| Covington | 5 | 4 | 6 | 15 | 10 | 1 | 2 | 13 | - | - | - | - |
| NCDM01-158 | 8 | 3 | 6 | 17 | 14 | 1 | 1 | 16 | - | - | - | - |
| NCFT4-89 | 9 | 6 | 8 | 23 | 16 | 3 | 1 | 20 | - | - | - | - |
| Mean | $7.3^{A}$ | $4.3^{B}$ | $6.7^{B}$ | $18.3^{J}$ | $13.3^{D}$ | $1.7^{E}$ | $1.3^{E}$ | $16.3^{J}$ | $10.6^{H}$ | $3.1^{I}$ | $4^{I}$ | $17.3^{J}$ |

**Table 2.3. Analysis of variance p-values and significance (*) at *p*=0.05 level between three traits (Daughter root yield, enlarged seed root yield, and total yield) between proximal, distal, and whole piece treatments, for ten sweetpotato clones, and their interaction.**

| | 2007 | | | 2008 | | |
|---|---|---|---|---|---|---|
| **Trait** | **Daughter** | **ESR[1]** | **Total** | **Daughter** | **ESR** | **Total** |
| Rep | .006* | .546 | .007* | .023* | .174 | .013 |
| Trt | .021* | .000* | .011* | .000* | .000* | .590 |
| Clone | .000* | .000* | .000* | .000* | .000* | .000* |
| Clone*Trt | .006* | .001* | .092 | .001* | .000* | .025* |

[1] Yield of enlarged seed roots (ESR)

**Table 2.4: Mean values of yield (wet tons/ha) and Analysis of Variance with *p* = 0.05 level of significance (denoted '*') showing the comparison of ten sweetpotato clones for all treatments (proximal, distal and whole) between traits (daughter, enlarged seed root, and total yield) and mean yield of treatment between traits with their respective Waller-Duncan grouping by column.**

| Clone | 2007 Daughter | 2007 ESR[1] | 2007 Total | 2008 Daughter | 2008 ESR | 2008 Total |
|---|---|---|---|---|---|---|
| NC03-007 | $17.3^{*A}$ | $10.8^{*A}$ | $28.1^{A}$ | - | - | - |
| NC03-030 | - | - | - | $13.2^{C}$ | $8.9^{*B}$ | $22.1^{B}$ |
| NC03-395 | $13.2^{B}$ | $3.0^{*C}$ | $16.2^{BC}$ | $17.2^{B}$ | $7.5^{*BC}$ | $24.7^{B}$ |
| NC03-417 | $7.5^{*C}$ | O.1$^{D}$ | $7.6^{*D}$ | - | - | - |
| NC04-468 | - | - | - | $14.2^{*BC}$ | $8.9^{B}$ | $23.1^{*B}$ |
| NC93-17 | $11.7^{B}$ | $6.4^{*B}$ | $18.1^{B}$ | - | - | - |
| NCDM02-105 | - | - | - | $20.6^{*A}$ | $16.9^{*A}$ | $37.6^{A}$ |
| NCDM02-180 | - | - | - | $20.9^{*A}$ | $15.3^{*A}$ | $36.2^{A}$ |
| NCDM04-226 | $13.5^{B}$ | $1.1^{D}$ | $14.6^{C}$ | $16.1^{BC}$ | $6.3^{*C}$ | $22.4^{B}$ |
| MD810 | $6.7^{*C}$ | $0.3^{D}$ | $7.0^{*D}$ | $0.4^{D}$ | $<0.1^{D}$ | $0.5^{C}$ |
| **Trt Averages** | | | | | | |
| Proximal | $11.9^{AB}$ | $1.8^{A}$ | $13.7^{B}$ | $18.7^{A}$ | $5.9^{A}$ | $24.6^{A}$ |
| Distal | $9.9^{B}$ | $4.9^{B}$ | $14.8^{B}$ | $14.4^{B}$ | $8.8^{B}$ | $23.2^{A}$ |
| Whole | $13.1^{A}$ | $4.3^{A}$ | $17.3^{A}$ | $10.9^{C}$ | $12.7^{C}$ | $23.6^{A}$ |

[1] Yield of enlarged seed roots (ESR)

**Table 2.5. Analysis of variance showing the main and split plot effects between conventional and cut root piece planting techniques for the advanced yield trials conducted during 2007-2009.**

| | 2007 | | | | 2008 | | | | | | | | | 2009 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Main Plot** | **ESR** | **DR** | **Tot** | **PI** | **#1** | **Can** | **Jum** | **Cull** | **ESR** | **DR** | **Total** | **PI** | **App** | **#1** | **Can** | **Jum** | **Cull** | **ESR** | **DR** | **Tot** | **PI** | **App** | **DM** |
| Rep | NS | ** | ** | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | ** | * | NS | NS | NS | ** | ** | NS | ** | ** |
| Trt | ** | ** | ** | ** | ** | NS | ** | NS | ** | ** | ** | ** | ** | ** | ** | ** | NS | ** | ** | ** | ** | ** | ** |
| Error | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | * |
| **Subplot** | | | | | | | | | | | | | | | | | | | | | | | |
| clone | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| Trt*Clone | ** | ** | ** | ** | ** | ** | ** | * | ** | ** | ** | ** | ** | ** | ** | ** | NS | ** | ** | ** | ** | ** | NS |

ESR: Enlarged Seed Roots (wet tons/ha), DR: Daughter Roots (wet tons/ha), Total Yield (wet tons/ha), PI: Partitioning index of roots shows the distribution of normal daughter roots to ESR from 1 to -1. With 1 being 100% daughter roots and 0% ESR, App: Overall appearance of roots (range from 0 – 8 possible), DM: Dry Matter (%).

*, **: significant at the $p$<0.05 level and $p$<.001 level respectively

#1's - Roots 2" to 3 1/2" diameter, length of 3" to 9", must be well
shaped and free of defects, Canners - Roots 1" to 2" diameter, 2" to 7" in length, Jumbos - Roots that exceed the diameter, length and weight requirements of the above two grades, but are of marketable quality. Culls - Roots must be 1" or larger in diameter and so misshapen or
unattractive that they could not fit as marketable roots in any of the above three grades.

**Table 2.6: Tests of significance for clonal variation using ANOVA for all traits measured for 2007, 2008, and 2009 for the advanced yield trial comparing slips to cut seed pieces.**

| | 2007 | | | | 2008 | | | | | | | | | 2009 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Clone** | **ESR** | **DR** | **Tot.** | **PI** | **#1** | **Can** | **Jum** | **Cull** | **ESR** | **DR** | **Tot.** | **PI** | **App** | **#1** | **Can** | **Jum** | **Cull** | **ESR** | **DR** | **Tot** | **PI** | **App** | **DM** |
| NC03-007 | - | - | - | - | ** | NS | NS | NS | ** | ** | NS | ** | * | ** | ** | ** | NS | ** | ** | ** | ** | ** | NS |
| NC03-030 | NS | ** | ** | NS | - | - | - | - | - | - | - | - | - | ** | ** | NS | NS | ** | ** | ** | ** | * | ** |
| NC03-089 | - | - | - | - | ** | ** | NS | NS | NS | ** | * | NS | * | ** | NS | NS | NS | NS | ** | ** | ** | ** | NS |
| NC03-395 | ** | * | NS | NS | * | NS | NS | NS | ** | NS | NS | ** | NS | * | ** | NS | NS | ** | * | NS | ** | NS | NS |
| NC03-417 | NS | ** | ** | NS | * | * | NS | ** | NS | ** | ** | NS | NS | ** | ** | NS | NS | NS | ** | ** | NS | ** | NS |
| NC04-097 | - | - | - | - | ** | ** | NS | NS | ** | ** | NS | ** | * | * | NS | NS | NS | ** | NS | NS | ** | ** | NS |
| NC93-17 | ** | ** | ** | ** | - | - | - | - | - | - | - | - | - | ** | NS | NS | ** | ** | ** | * | ** | * | NS |
| Beau | ** | ** | ** | ** | ** | NS | ** | NS | ** | ** | NS | ** | ** | ** | * | ** | * | ** | ** | ** | ** | ** | NS |
| Covington | - | - | - | - | ** | NS | ** | NS | ** | ** | ** | ** | NS | ** | NS | ** | NS | ** | ** | * | ** | ** | NS |
| NCDM02-105 | ** | * | NS | * | ** | NS | * | NS | ** | ** | * | ** | NS | NS | NS | NS | NS | ** | NS | NS | ** | NS | NS |
| NCDM02-180 | * | ** | ** | NS | NS | NS | ** | NS | ** | ** | NS | ** | NS | * | NS | NS | NS | ** | * | NS | ** | NS | * |
| NCDM04-226 | NS | ** | ** | NS | ** | NS | NS | NS | NS | ** | ** | ** | ** | ** | NS | NS | NS | NS | * | NS | ** | ** | NS |
| NCFT4-89 | - | - | - | - | ** | NS | NS | NS | ** | ** | NS | ** | ** | ** | NS | NS | NS | ** | ** | NS | ** | ** | ** |
| NCFTA94 | ** | ** | ** | ** | * | NS | ** | * | ** | ** | ** | ** | NS | ** | NS | * | NS | ** | ** | NS | ** | NS | ** |
| MD810 | NS | ** | ** | * | ** | ** | ** | NS | NS | ** | ** | ** | ** | - | - | - | - | - | - | - | - | - | - |

ESR: Enlarged Seed Roots (tons/ha), DR: Daughter Roots (tons/ha), Total Yield (tons/ha), PI: Partitioning index of roots shows the distribution of normal daughter roots to ESR from 1 to -1. With 1 being 100% daughter roots and 0% ESR, App: Overall appearance of roots (range from 0 – 8 possible), DM: Dry Matter (%)

*, **: Significant at the $p$<0.05 level and $p$<0.01 level respectively

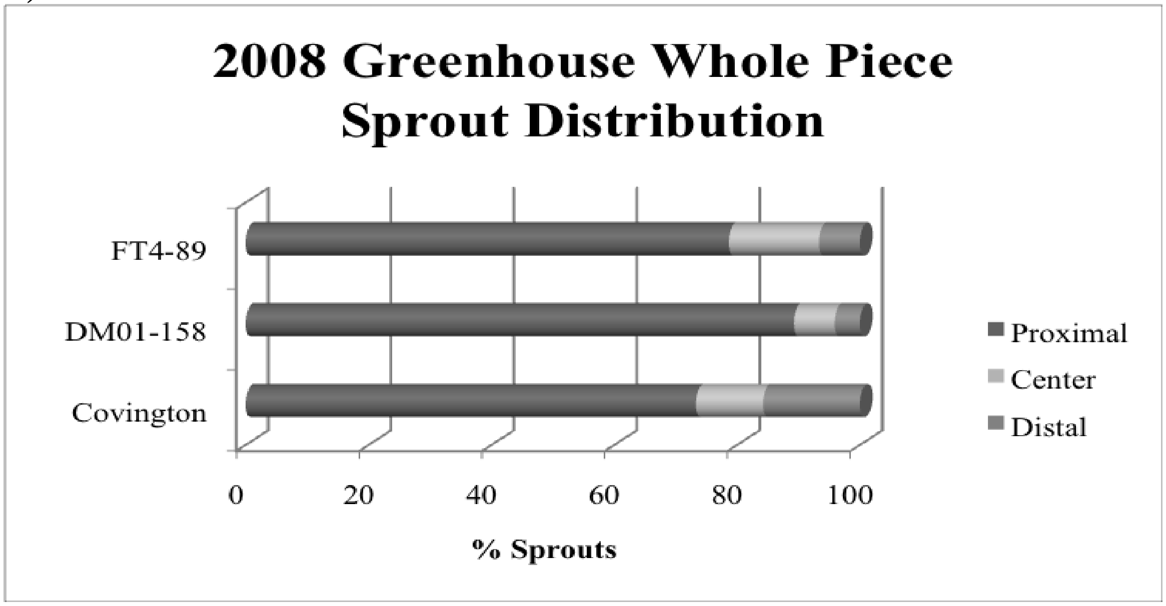
#1's - Roots 2" to 3 1/2" diameter, length of 3" to 9", must be well shaped and free of defects, Canners - Roots 1" to 2" diameter, 2" to 7" in length, Jumbos - Roots that exceed the diameter, length and weight requirements of the above two grades, but are of marketable quality. Culls - Roots must be 1" or larger in diameter and so misshapen or unattractive that they could not fit as marketable roots in any of the above three grades.

**Table 2.7: Mean values for all traits examined in sweetpotato for the advanced yield trial for slips and CSP treatments for 2007, 2008, and 2009.**

| | 2007 | | 2008 | | 2009 | |
|---|---|---|---|---|---|---|
| **Trait** | **Slip** | **CSP** | **Slip** | **CSP** | **Slip** | **CSP** |
| #1 | - | - | 17 | 5.1 | 19.4 | 7 |
| Canner | - | - | 5.9 | 6.1 | 7 | 4.4 |
| Jumbo | - | - | 6.8 | 1.2 | 5.4 | 2.3 |
| Cull | - | - | 6.3 | 4.7 | 7.9 | 6.6 |
| ESR | 0 | 3.6 | 0 | 9.4 | 0 | 8.7 |
| DR | 26.6 | 12.4 | 36 | 17 | 39.7 | 20.2 |
| Total | 26.6 | 16 | 36 | 26.5 | 39.7 | 28.8 |
| Ratio | 1 | 0.79 | 1 | 0.62 | 1 | 0.69 |
| PI | 1 | 0.6 | 1 | 0.31 | 1 | 0.39 |
| App | - | - | 4.9 | 3.4 | 4.5 | 2.8 |
| DM | - | - | - | - | 0.23 | 0.21 |

ESR: Enlarged Seed Roots (wet tons/ha), DR: Daughter Roots (wet tons/ha), Total Yield (wet tons/ha), Ratio : is the ratio of daughter roots to total yield (daughter/total) PI: Partitioning index of roots shows the distribution of normal daughter roots to ESR from 1 to -1. With 1 being 100% daughter roots and 0% ESR, App: Overall appearance of roots (range from 0 – 8 possible), DM: Dry Matter (%)

A)




B)

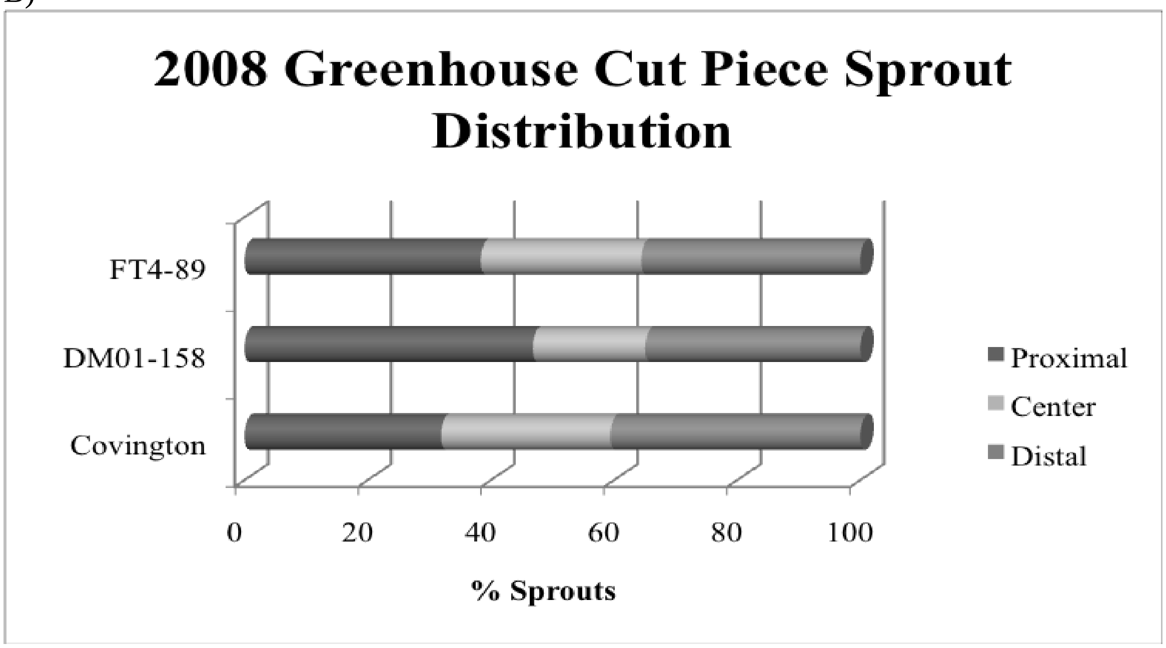



**Figure 2.1: Average percent of total plant sprouts observed in each clone in the 'whole' piece treatment (A) and the 'cut' piece treatment (B).**

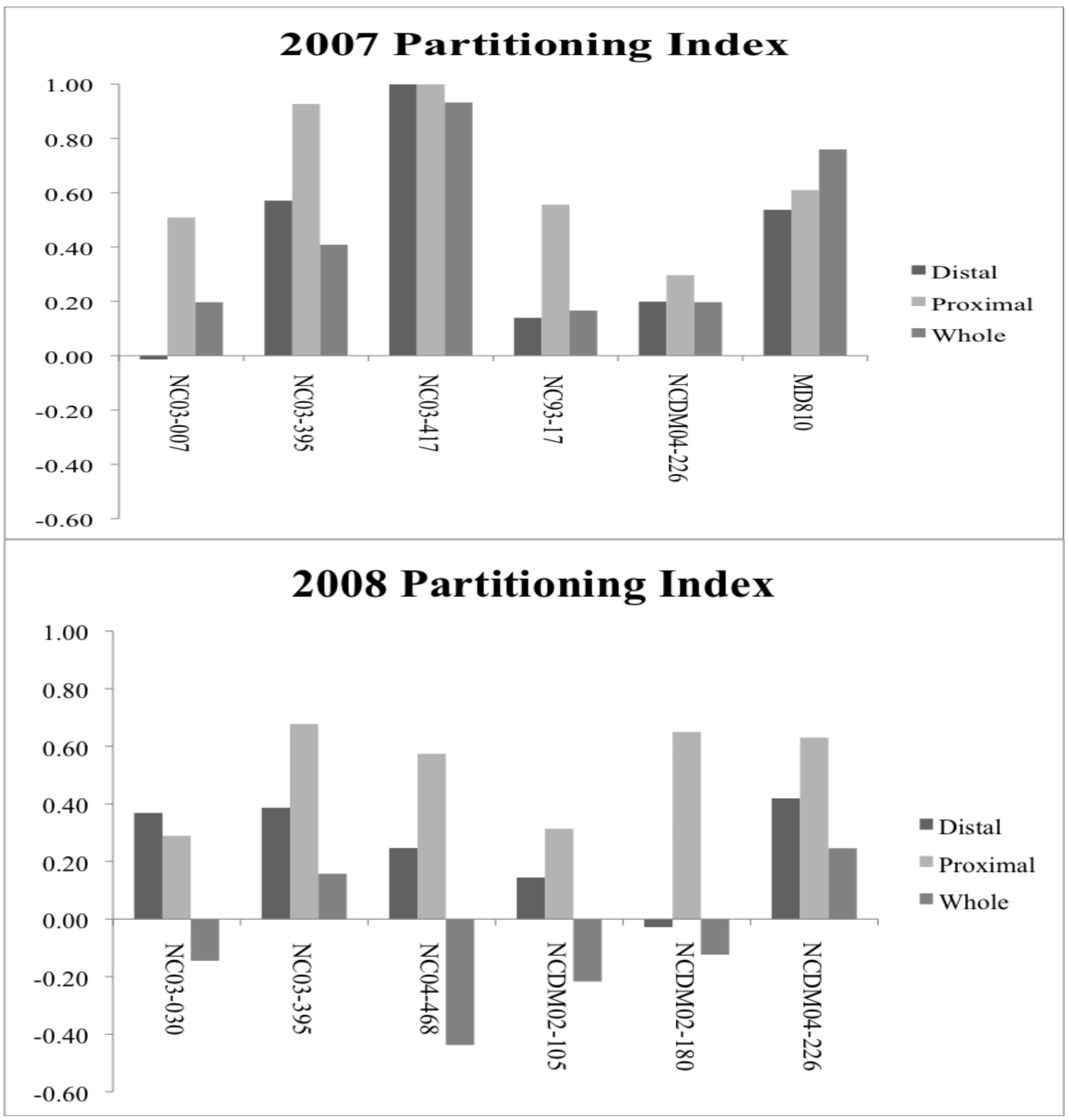



**Figure 2.2: Partitioning Index of clones in 2007 and 2008 showing the tendency towards daughter root production (1) and ESR production (-1) for each of the three cut seed piece treatments (Distal end, Proximal end, and Whole Pieces).**

# Chapter 3: Inheritance of DM content and traits associated with the production of sweetpotato (*Ipomoea batatas*) from cut root pieces (CRP)

Blake Bowen, Nicholas George, Ken Pecota, and Craig Yencho

## Abstract

Sweetpotato has shown promise as a feedstock for biofuels in the corn deficit region of the southeastern United States. However, high production costs due mainly to high labor inputs associated with the production of sweetpotatoes are major impediments to the development of this crop as a biofuel feedstock. Enterprise budgets indicate that sweetpotato planting costs account for roughly 15% of total production costs. Our group is conducting research to reduce these costs using industrial sweetpotatoes bred to produce a crop from direct-planted, root pieces [or cut root pieces (CRP)], which can be planted mechanically, significantly reducing production costs. Narrow sense heritability estimates for storage root dry matter content, daughter root yield, partitioning index (PI) defined as, [(daughter yield – seed root enlargement yield) / total yield], total yield, root piece survival (RPS), and seed root enlargement (SRE) were estimated by mid-parent offspring regression and half-sib variance component analysis. Experiments to estimate heritability were conducted in two locations, Clinton and Kinston, NC. Parent offspring heritability estimates for dry matter, partitioning index, daughter root yield, seed root enlargement, total root piece survival and total yield were 0.54, 0.64, 0.12, 0.36, -0.04, and 0.27 in Clinton and 0.63, 0.37, -0.1, 0.76, -0.09, and 0.18 in Kinston, respectively.

Half sib family variance component analysis on a family means of the same traits resulted in estimates of 1.2, 0.62, 0.45, 0.82, 0.54, and 0.60 respectively. Genetic correlation analysis showed no correlation between daughter root yield and dry matter content, seed root enlargement, and root piece survival (-0.03, -0,04, and -0.06), but a positive correlation was observed between daughter root yield and total yield and the partitioning index (0.76 and 0.57). PI was correlated with daughter root yield, seed root enlargement, and root piece survival with values as 0.57, -0.70, -0.54 respectively. Partitioning index showed no correlation to total yield or dry matter (-0.01 and -0.08). Lastly, root piece survival was correlated with seed root enlargement (0.72), total yield (0.42), and partitioning index (-0.54) and showed no correlation with daughter root yield and dry matter (-0.06 and -0.01).

## 3.1 Introduction

There has been a considerable increase in the production, demand, and consumption of fuel ethanol in the United States. Between 2001 and 2007, annual ethanol production increased from 6.7 billion liters to 26 billion liters (EIA, 2009). Most of the fuel ethanol in the United States is derived from corn; therefore, most of the nation's ethanol production facilities are located in the corn-growing states of the mid-west. States outside of this region seeking to increase ethanol production face a challenge finding adequate supplies of corn or other feedstocks to meet the growing biofuel demand. For example, North Carolina currently produces less than one percent of the nation's total corn crop, but is amongst the largest producers of hogs and poultry in the nation, both of which are raised on corn-based feedstocks. Because farmers in North Carolina cannot produce enough corn for these industries, they import corn from other states to meet local demand (USDA, 2008). Therefore, capacity for North Carolina to meet local demand for transportation fuels from corn-based ethanol is extremely limited. Many other states located outside the mid-west corn-belt face a similar corn-limited scenario, thus there is great need to develop alternative ethanol feedstocks.

Like corn, sweetpotatoes produce significant amounts of starch, which can easily be converted into fuel ethanol (Dangler et al., 1989). We are exploring the use of high starch, "industrial" sweetpotatoes as a potential biofuel feedstock for the southeastern United States. The United States is the 12$^{th}$ largest producer of sweetpotato in the world and the majority of the sweetpotato crop is produced in the southeast (EIA, 2009; USDA, 2008). Approximately half of all sweetpotato production in the United States occurs in North Carolina. Several factors make sweetpotato a good candidate as an ethanol feedstock alternative in the Southeast, including the capacity to produce substantially greater ethanol yields per hectare than corn (Dangler et al., 1989; Hall and Smittle, 1983; Lee et al., 2008; Ziska et al., 2009). Ethanol production from sweetpotato is, however, not currently economically viable because production costs for sweetpotato are almost five times higher than those of corn (Estes and Schultheis, 2006; Bullon and

Weddington, 2010). This acts as a major obstacle to the development of a biofuel industry based on sweetpotato.

A key reason for the high cost of sweetpotato production is the need to produce plants to propagate the crop (Schultheis et al., 2005). The current planting technique for sweetpotato involves "bedding" storage roots from the previous harvest season in the early spring. Storage roots are planted in the early spring in the field on roughly one-meter wide plant production “beds”. The plant production beds are then often covered with clear plastic to retain heat and promote growth of adventitious sprouts from the "bedded" storage roots. The resulting plants produced by the storage roots are cut above the soil surface and then planted as un-rooted, vegetative propagules into production fields using hand-fed mechanical transplanters. This multistep, labor-intensive, process is expensive requiring 15% of the total cost of sweetpotato production (Estes and Schultheis, 2006). A possible solution to reduce the cost of sweetpotato production is to develop sweetpotato cultivars that can be mechanically planted using root pieces, similar to potato (*Solanum tuberosum*) (Bouwkamp and Scott, 1972; Hosokawa et al., 1998).

It has been shown by several research groups in Japan and the United States that given suitable cultural practices and varieties, producing a crop from sweetpotato root pieces can be successful and easier to mechanize than current planting methods, thereby reducing the overall cost of sweetpotato production (Akita and Kobayashi, 1962; Allen and Phillis, 1979; Bouwkamp and Scott, 1972; GCPES, 1943; Hosokawa et al., 1998; Kobayashi, 1968; Kodoma, 1962; Kusuhara et al., 1972; Nakazawa, 1973; Phillis and Allen, 1979; Yamashita, 2000).

Previous research on the direct planting of sweetpotato has investigated the cultural, genetic, and mechanization aspects of the root piece planting method (Akita and Kobayashi, 1962; Allen and Phillis, 1979; Bouwkamp and Scott, 1972; GCPES, 1943; Hosokawa et al., 1998; Kobayashi, 1968; Kodoma, 1962; Kusuhara et al., 1972; Nakazawa, 1973; Phillis and Allen, 1979; Yamashita, 2000). However, the technique has never been fully commercialized and there are still a number of obstacles that inhibit the use of this new sweetpotato planting technology. One of the most important aspects is how to breed sweetpotatoes that produce high yields when planted using root pieces instead of plants. To date, only a few researchers have looked into the genetics and breeding of sweetpotatoes that produce a commercially viable crop from root pieces (Bouwkamp and Scott, 1972; Kobayashi, 1968; Kodoma, 1962; Kusuhara et al., 1972; Shikata et al., 1975). Research to estimate the heritability of factors related to root piece production of sweetpotato is limited and has only been reported by two groups (Kobayashi, 1968; Kusuhara et al., 1972).

Kobayashi (1968) suggested that a sweetpotato crop planted using the root piece technique generally produces a new crop two ways - via the formation of indirect daughter roots or direct daughter roots. Indirect daughter roots are formed from adventitious sprouts that grow from the root piece, while direct daughter roots are roots that form off the root piece directly. Kobayashi (1968) found evidence that the way the root piece grows after being planted is a simply inherited and partially dominant trait. He observed that after crossing at least one “good” yielding parental root piece line, the F1 lines produced a standard normal curve for daughter root yield and when no “good” CRP parents were present the progeny produced low daughter root yield. Kusuhara et al. (1972) found the indirect daughter root types had relatively high heritability ($h^2$=0.54), while direct daughter root lines were moderately heritable ($h^2$=0.29), and that earlier planting helped decrease root piece sizing, but there were no significant differences between the variances of each type.

This study used parent-offspring regression (due to its conservative estimation of $h^2$) and half sib family variance component heritability analysis to estimate the heritability of traits that are important to root piece planting, including dry matter production, partitioning index, seed root enlargement (where the planted root piece becomes a sink and gains additional size), daughter root yield, and the tendency of root pieces to rot post-planting and otherwise referred to as root piece survival, (RPS). Estimating the heritability of these important traits will provide sweetpotato breeder researchers information to aid in the development of breeding of lines for this planting system.

## 3.2 Materials & Methods

### Crosses

Twelve parents were selected based on prior testing for use in seed root planting. These materials included NC93-17, NCDM02-105, NCDM02-180, NCDM03-003, MD810, NC03-417, NC03-007, Beauregard, NC413, Evangeline, L258, and NCDM03-068. The clones represented breeding lines and commercial cultivars and they were chosen based on their ability to produce a crop from root pieces. Positive parents were those that produced 75 % or more of their yield as daughter roots, and negative parents were lines in which ≥ 50% produced yield as enlarged seed roots. All parents were tested for selfing. Self-compatible lines were emasculated prior to crossing to prevent self-fertilization (Table 3.1). Twenty families were used in the study based on seed availability from crossing.

**Plant and root production**

Nine parents were used based on crossing compatibility and thirty-six seed from each cross of the four females x five males were hand scarified using No. 100 sandpaper to breach the seed coat. The seeds were then planted in 72 cell trays filled with Fafard 4P soil mix (Conrad Fafard Inc, Agawam, MA) and placed in the greenhouse at ~27˚C with no supplementary lighting. After two months growth, seedlings were planted into the field on 60 cm spacing and grown for 20 days. Ten cuttings were then taken from each plant and transplanted at 23 cm spacing into a second field. After 90 days the roots were harvested, cured at 30˚C and 85% RH for one week, and stored in a cooler at 18˚C, 85% RH until the following planting season.

**Inheritance test**

Fifteen families remained after storage. Families 4, 9, 14, and 19 were not used because one of the parents (DM03-068) perished and family 17 did not produce enough seed to provide a good measure of lines to test. Of the remaining fifteen families, twenty progeny from each family were selected at random for use in the experiment.

Roots ranging from 30-150 g, with an average weight of 64 ± 15g, were cut in half to form root pieces. The seed pieces were treated with the fungicide Botran 75W (2,6-dichloro-4-nitroaniline, Gowan Company, Yuma, Az) mixture (2 g/L) using a pump-sprayer. Cut roots were pre-sprouted in 25˚C and 70% relative humidity for 6-7 days and then planted by hand at a depth of 5-10 cm in a randomized complete block design with three replications at two locations.

**Fertilization**

Standard fertilizer applications began one week prior to planting or at first cultivation. Fertilizer treatments comprised either 450 kg/ha 0-10-25 (with .56kg/ha boron), if soil tests showed low phosphorus levels, otherwise 170 kg/ha 0-0-60 (with 0.56 kg/ha boron) was used. During the second and third cultivations 170 kg/ha of 0-0-60 and 170-180 kg/ha of 34-0-0 prilled ammonium nitrate ($NH_4NO_3$) were applied as a side dressing and incorporated using rolling sweeps. Insect and weed control practices included Telone II (1,3-Dichloropropene, Dow AgroSciences, Indianapolis, IN) three weeks prior to planting for nematode control, Command 3ME (Clomazone, FMC Corporation, Philidelphia, PA) 3 L/ha + Eptam (EPTC, Gowan Company, Yuma, AZ) 4 L/ha PPI for weed control prior to planting in the formed beds, and for insect control Lorsban 15G or 4E (Chlorpyrifos, Dow AgroSciences, Indianapolis, IN) was broadcast sprayed and incorporated 10-15cm into the soil prior to planting.

### Data Collection and Analysis

At harvest, data was collected for weight of sized seed pieces, weight of daughter roots, number of seed pieces remaining, and samples were taken to determine dry matter content. A storage root partitioning index was calculated for each clone using the following formula: (daughter root yield – seed root enlargement yield) / total yield. The partitioning index, ranging from +1 to -1, was calculated to estimate how yield was partitioned between daughter roots and seed root enlargement. A partitioning index of -1, 0, and 1 would represent no daughter root yield, 50% daughter root yield and 100% daughter root yield, respectively.

ANOVA and PROC REG in SAS v. 9.1 (SAS Institute, Cary, NC) were used to provide two estimates of heritability for dry matter, partitioning index, daughter root yield, total yield, seed root enlargement yield, and root piece survival. PROC REG and mid-parent offspring regression analyses were used to calculate narrow-sense heritability using parental and offspring means and the formula $h^2 = b = V_a/V_p$; where $V_a$ = additive variance, and $V_p$ = total phenotypic variance for each family and trait. The narrow-sense heritability estimates were then averaged across all families to determine the mean heritability and standard error for each trait. PROC CORR in SAS v. 9.1 (SAS Institute, Cary, NC) was used to estimate Pearson's correlation coefficients for every trait measured.

Variance component heritability analyses based on half-sib families were conducted using ANOVA and the statistical model $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$ proposed by (Comstock and Robinson, 1948; Falconer and Mackay, 1996) where: $Y_{ij}$ = jth observation within the ith group; $\mu$ = overall mean; $\alpha_i$ = effect of *i*th group; and $\varepsilon_{ij}$ = residual error. The components of variance due to the differences between parental groups $\sigma_g^2$ were estimated as: $\sigma_g^2 = (MS_g – MS_w)/k$; where $MS_g$ and $MS_w$ were the mean squares for the between and within family groups; and k = the approximate number of progeny per parent group (Table 3.2). From this, narrow sense heritability was estimated using the formula: $h^2 = 4\sigma_g^2 / (\sigma_w^2 + \sigma_g^2)$, with $\sigma_g^2 = ¼ V_a$ ; where $V_a$ is the additive variance component and $\sigma_w^2$ = within group variance or error. This model assumes the progeny between families were half sibs, no inbreeding and random selection of parents and progeny from the population (Comstock and Robinston, 1948).

### 3.3 Results

Genetic correlation analysis showed no correlation between daughter root yield and dry matter content, seed root enlargement, and root piece survival (-0.03, -0,04, and -0.06), but a positive correlation was observed between daughter root yield and total yield and the partitioning index (0.76 and 0.57) (Table 3.3). Dry matter was not significantly correlated with any of the traits measured. Partitioning index was correlated with daughter root yield, seed root enlargement, and root piece survival with values as 0.57, -0.70, -0.54 respectively. Partitioning index also showed no correlation to total yield or dry matter (-0.01 and -0.08). As would be expected, seed root enlargement was highly correlated with average seed piece size (0.90). And, root piece survival was correlated with seed root enlargement (0.72), total yield (0.42), and partitioning index (-0.54) but showed no correlation with daughter root yield and dry matter (-0.06 and -0.01) (Table 3.3).

Family, parent, and offspring means are shown in table 3.4. Generally, parents performed better than the offspring for daughter root production, but this varied from family to family as shown in Figure 3.1. Min and max values are also shown in table 3.4 for the offspring in each family, and they exhibited a wide range depending on the trait analyzed. For example, yield had a range of 0 to ~30 kg/plot and traits like dry matter had a smaller range due to the nature of the trait. One example is family 3, in which the average daughter root yield for the parents was 59.5 kg/plot and the offspring only yielded 17.3kg/plot, whereas in four families (5, 12, 15, and 20) the offspring out-yielded the parents. Performance comparisons for parents vs offspring were highly variable for all other traits. Location did have an effect on daughter root yield, enlarged seed root yield, and partitioning index. In Figure 3.2 (a, b, c) direct comparisons for each of these traits are shown. Kinston was the favorable location to produce greater daughter root yields and higher partitioning index for all families. Enlarged seed root (ESR) yield varied between locations, but Kinston also had more families with higher ESR yield as well. Lastly, in Kinston partitioning index values were best for all families and in some cases as high as a 20% difference between the two locations (Figure 3.2).

Parent offspring heritability estimates separated by location were variable as shown on table 3.5. Clinton had better heritability for partitioning index ($h^2$ =0.64), daughter root yield ($h^2$ =0.12), and root piece survival ($h^2$ = 0.27) than Kinston ($h^2$ = 0.37, -0.1, and 0.18) respectively. However, dry matter and enlarged seed root yield in Kinston ($h^2$ = 0.63 and 0.76), performed better than the test in Clinton ($h^2$ = 0.54 and 0.36). Total yield was low for both locations at $h^2$ = -0.04 and -0.09 for Clinton and Kinston respectively. Standard errors were moderate and ranged from 0.11 to 0.28 across both locations.

Variance component heritability estimates also predicted moderately high heritability for all the traits studied (Table 3.5). Table 3.5 shows that estimates ranged from daughter root yield (0.45) as the lowest to dry matter (1.2) as the highest. Estimates for variance component heritability were in most cases 30% higher than the parent offspring component estimates. In a few cases such as daughter root yield, total yield and root piece survival variance component analysis the differences between parent-offspring and variance component was even higher than 30%. Errors were all low with the exception dry matter.

### 3.4 Discussion

Currently, there are cultural and genetic obstacles that inhibit the use of the cut root piece planting technique. Some important obstacles that must be overcome are, 1) decrease root piece enlargement by either cultural techniques or selection of clones that do not size the root piece, 2) increase daughter root yield by selecting for high partitioning index lines that still produce sufficient yield, and 3) select indirect daughter root types as these types are highly heritable (Kusuhara et al., 1972) and are more successful in producing daughter root yield with high partitioning index. In this study, the heritability of key traits associated with the production of ISP from cut root pieces were examined. Several groups in the US and Japan have observed loss of yield due to root piece enlargement (Bouwkamp and Scott, 1972; Kobayashi, 1968; Kodoma et al., 1958; Nakazawa, 1973). This is a large obstacle to be overcome, but prior research suggests that root piece production system may be feasible. Genetic analysis has shown root types to be heritable (Kusuhara et al., 1972) and further genetic research will aid researchers in determining how to breed for these sweetpotato types. Genetic research coupled with improved cultural techniques to maximize crop yield when planting a crop of sweetpotato using root pieces may significantly decrease planting costs, making biofuel production from sweetpotato more economically feasible.

Daughter root yield exhibited a high correlation with total yield ($r$ = 0.76) and a moderate correlation to partitioning index ($r$ = 0.57) (Table 3.3). These traits are correlated because daughter root yield is a component of both the total yield and the partitioning index that was calculated. Similarly, enlarged root piece yield is also a component of total yield, partitioning index, and average enlarged root pieces. The negative correlation for enlarged root piece yield and partitioning index was the result of how the partitioning index was calculated. When enlarged root piece yield increases, this decreased the percent of the yield that is daughter roots, thus giving a lower partitioning index score. Thus, selecting for partitioning index will result in the reduction of enlarged root piece yield and vice versa based off how these traits are estimated.

Enlarged root piece yield also exhibited a high correlation with root piece survival ($r$ = 0.72). This would be expected because when enlarged root pieces do not form, root piece survival is zero. Thus, selecting for enlarged root piece yield would positively select for root piece survival. Daughter root yield and partitioning index were also correlated ($r$ = 0.57) (Table 3.3) which allows for daughter root yield to be used to select for high partitioning index and vice versa. Since partitioning index was moderately heritable ($h^2$=0.64 in Clinton and 0.37 in Kinston) we expect that partitioning index could be improved by selecting plots with a higher distribution of daughter roots and/or lower distribution of seed root enlargement.

Four families (5, 12, 15, and 20) on average produced more daughter root yield as offspring than as parents (Table 3.4). Three of these families had L99-35 as a parent and experience with this line showed that when planted or bedded too deep (>5cm) the storage roots of this clone had a tendency to rot (data not shown). It is likely that the low yields and poor stands from this parent were responsible for the offspring out yielding the parents in these families. Overall, these four families all had improved partitioning indices and seed root surviving scores potentially representing the possibility of gains through breeding.  Interestingly, Kobayashi (1968) stated that root piece planting ability was a simply inherited and partially dominant trait. However, from table 3.4 and figure 3.3 it can be seen from the min/max data that there were offspring that outperformed the parents. This suggests that is not a single gene or simply inherited trait, but multiple genes are responsible.

Correlation studies are useful to help see how key traits are related and can be used to aid selection for low heritability traits. Based on our studies, the key traits deemed important for the production of ISP's from root pieces include partitioning index, root piece survival, daughter root yield, and enlarged root pieces. In this study, we estimated the narrow sense heritability of these traits in two locations using parent-offspring regression and variance component analysis. All traits in the parent-offspring regression except daughter root yield and total yield had a moderate heritability. Of the non yield components, root piece survival (RPS) had the lowest $h^2$ across both locations with 0.27 in Clinton and 0.18 in Kinston. Dry matter had the highest at $h^2 = 0.54$ and 0.63 respectively which was similar to what has been estimated by previous research on dry matter (Jones, 1977). Daughter root yield and total yield exhibited the lowest heritabilities at approximately zero. All traits except root piece survival ($h^2 = 0.27$ and 0.18 respectively), daughter root and total yield, had moderate heritability of around $h^2 = 0.5$ on average (Table 3.5).

Variance component heritability analysis of the same traits predicted high heritabilities. Comparison of variance components and parent offspring heritability analysis agreed with past research (Jones, 1986) in that variance component analysis was less conservative in estimating heritability. Large differences in heritability were shown in daughter root yield and total yield between the methods used to estimate $h^2$., These differences are believed to be the result of outliers of poor parents that were present in the parent-offspring regression causing a higher error variance. The same reasons are also likely responsible for the negative $h^2$ values of daughter and total yield and generally can be treated as zero.

Heritability by location showed large differences between certain traits and location. While the heritability of the yield traits remained low across both locations, Clinton held better yield heritabilities than Kinston. The most notable were the differences between enlarged seed root yield and partitioning index between locations because they varied by almost 33 and 40% (0.64 to 0.37 for PI and (0.36 to 0.76 for ESR) respectively for Clinton and Kinston. We expect the reasons for these differences to be related to the variable field conditions between locations since these traits were so different between each location. More testing would need to be done to see if there are consistent differences between locations for these two traits across years, but selection of lines by PI may be better in Clinton and selection of ESR may be better for Kinston. All other heritabilities tested (dry matter, daughter root yield, total yield and root piece survival) had narrow sense heritabilities that were within the standard errors of one another (Table 3.5) and can likely be tested effectively at either location.

While research has been conducted on root piece type, the heritability of partitioning index, root piece enlargement, daughter root yield, and root piece survival has not been estimated in the past. Since all but two traits had moderately high heritability we should be able to make effective breeding gains using mass selection based on the phenotypes (except daughter root yield) of the parents. It is not recommended to base selection off daughter root or total yield since their parent offspring

$h^2$ are around 0. Instead, selection based on high partitioning index or low root piece survival would be more successful for daughter root yield due to their correlations of $r$ = 0.57 and $r$ = -0.62. As previously mentioned, data showed that partitioning index had a higher heritability in Kinston and so Kinston would be a better location to select for this trait. According to Jones (1986), $h^2$ estimates of 0.4 or higher for variance component and 0.3 or higher for regression are suitable for improving a trait in sweetpotato. Similarly, to make progress in breeding (Hartl, 1988) states that $h^2$ of 0.2 or greater is good for individual plant selection, but lower heritability estimates would require other selection methods such as family or within-family selection. Thus, mass selection techniques are suitable to judge dry matter, partitioning index and seed root sizing in the field. For traits that are lower than the estimates mentioned above increased replication, more parents or widening the gene base might be considered (Jones, 1986).

Previous research by Kusuhara et al. (1972) estimated narrow sense heritability on four root yield types (direct daughter root, indirect daughter root, seed root enlarging, and an intermediate type). These root types had moderately high heritability ranging from 0.29 to 0.54 with an average of 0.44 across the four root types (i.e. direct daughter root, indirect daughter root, seed root enlarging, and an intermediate type). Indirect daughter type was the highest with $h^2$=0.54 and seed root enlarging the lowest with $h^2$ = 0.29. Kusuhara et al., (1972) observed that heritability estimates were greatest for indirect daughter type roots. This information supports our research because we found that partitioning index heritability was highly heritable and could be used to aid in the selection of indirect daughter types.

In conclusion, the results of our experiments demonstrate that gains can be made through breeding for the root piece planting technique when partitioning index and seed root sizing traits are observed. This research coupled, with the findings of Kusuhara et al. (1972) supports efforts to breed for high dry matter indirect daughter root type sweetpotatoes with high partitioning index in an effort to reduce planting costs for industrial sweetpotatoes and make sweetpotato an economical biofuel source for North Carolina. Improvements still need to be made by breeding better varieties adapted to this planting style, but there are promising lines such as NCDM02-105, NCDM02-180, NCDM04-226, and NC03-417. Then cultural techniques can be modified to improve production for that specific line. Additionally, further research into ways to prevent seed root enlargement will help to maximize daughter root yield. This research addresses planting sweetpotato to reduce cost another side of the equation is harvest. We estimate that an additional ten percent (Estes and Schultheis, 2006) of total sweetpotato production cost could be saved by a mechanized system of harvest. A potential system of harvest would use potato harvest as a model. This system is completely mechanized and is able to be harvested significantly faster. Since sweetpotato is an expensive crop to grow, addressing both sides of planting and harvest is a must for ISP to be profitable for growers.

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**Table 3.1: The compatibility between female parents (columns) and male parents (rows) used in this study, with "Y" denoting compatible, "UNK" meaning unknown, and "N' denoting incompatible crosses. Compatibility was determined by crossing each combination 20 times. If no seeds formed after 20 crosses the cross was deemed incompatible.**

| | | FEMALE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Clone[1]** | **NC93-17** | **NC03-417** | **NCDM02-105** | **NCDM02-180** | **MD810** | **BEAU** | **NCDM03-003** | **NCDM03-068** | **L99-35** | **L258** | **NC413** | **NC03-007** |
| | **NC93-17** | N | Y | Y | N | N | Y | N | Y | Y | Y | Y | Y |
| | **NC03-417** | Y | N | N | Y | Y | Y | Y | Y | Y | Y | Y | UNK |
| | **NCDM02-105** | Y | N | N | Y | Y | Y | Y | Y | Y | Y | Y | UNK |
| **M** | **NCDM02-180** | N | Y | Y | N | Y | Y | Y | Y | Y | Y | Y | UNK |
| **A** | **MD810** | N | Y | Y | Y | N | Y | N | Y | Y | Y | Y | Y |
| **L** | **BEAUREGARD** | Y | Y | Y | Y | Y | N | Y | N | Y | Y | Y | UNK |
| **E** | **NCDM03-003** | N | Y | Y | N | N | Y | N | Y | Y | Y | Y | UNK |
| | **NCDM03-068** | Y | Y | Y | Y | Y | N | Y | N | Y | Y | Y | Y |
| | **L99-35** | Y | Y | Y | Y | Y | Y | Y | Y | N | Y | Y | Y |
| | **L258** | Y | N | Y | Y | Y | Y | Y | Y | N | Y | Y | Y |
| | **NC413** | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | N | Y |
| | **NC03-007** | Y | UNK | N | Y | Y | UNK | Y | Y | UNK | Y | Y | N |

**[1]: Clones were determined to be 'root piece planting clones' for the traits of interest or 'non root piece planting clones' for the traits of interest prior to crossing as mentioned in the materials and methods section. 'Good' parents are as follows: NC93-17, NC03-417, NCDM02-105, NCDM02-180, MD810, and NC03-007**

**Table 3.2: ANOVA for estimating differences for between and within family groups and expected mean squares from (Falconer and Mackay, 1996)**

| Source of variation | Degrees of Freedom | Mean Square | Expected mean squares |
|---|---|---|---|
| Between family groups | g-1 | $MS_g$ | $\sigma_\omega^2 + \sigma_g^2$ |
| Within family groups | $\sum n_i - g$ | $MS_w$ | $\sigma_\omega^2$ |
| Total | $\sum n_i - 1$ | | |

$\sum n_i$ = sum of n for each I; g= number of families; $\sigma_\omega^2$ = within group component of variance or error; $\sigma_g^2$ = between group component of variance

**Table 3.3: Genetic correlations are shown between various traits measured in the parent-offspring regression test and their corresponding significance at the $p \leq 0.05$ level.**

| | DR | SRE | Total | DM | Avg SRE | PI | RPS |
|---|---|---|---|---|---|---|---|
| **DR** | 1 | -0.039 | 0.758* | -0.031 | -0.091 | 0.566* | -0.061 |
| **SRE** | -0.039 | 1 | 0.621* | 0.033 | 0.895* | -0.695* | 0.723* |
| **Total** | 0.7586* | 0.621* | 1 | -0.002 | 0.511* | -0.009 | 0.423* |
| **DM** | -0.031 | 0.033 | -0.003 | 1 | 0.012 | -.078*' | -0.01 |
| **Avg SRE** | -0.092 | .0.895* | 0.512* | 0.012 | 1 | 0-.629*' | 0.927* |
| **PI** | 0.566* | -0.695*' | -0.009 | -0.079*' | -0.679*' | 1 | -0.538* |
| **RPS** | -0.062*' | 0.723* | 0.423* | -0.01 | 0.927* | -0.538*' | 1 |

* : significant at the 5% level.
DR: daughter root yield (wet kg/plot) SRE: seed root enlargement yield (wet kg/plot), Total: total yield (wet kg/plot), DM: dry matter percent, Avg SRE: average size of an individual enlarged sized seed root/plot, PI: partitioning index RPS: post-harvest root piece survival

**Table 3.4: Family, parents, and means for parents (represented 'P') and offspring (represented 'O') for each trait measured (Daughter root yield, seed root enlargement yield, dry matter (%), partitioning index, and root piece survival in the parent-offspring regression. Minimum and maximum values (X, Y) are shown in each offspring "O" column for each family.**

| Family | Maternal Parent | Paternal Parent | Daughter Root Yield | | Seed Root Enlargement | | Dry Matter Content (%) | | Partitioning Index | | Root Piece Survival | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | P | O | P | O | P | O | | O | P | O |
| 1 | NC93-17 | NC03-417 | 19.895 | 13.075 (0,34.05) | 5.645 | 8.67 (0, 31.4) | 19.5 | 20.5 (.13,.29) | 0.56 | 0.178 (-1,1) | 1.21 | 2.276 (0,10.7) |
| 2 | NC93-17 | Beauregard | 15.805 | 11.944 (.55,45.8) | 14.255 | 4.9 (0,16.7) | 18.5 | 19.4 (.13,.32) | 0.065 | 0.399 (-.75,1) | 2.915 | 1.202 (0,8.3) |
| 3 | NC93-17 | NCDM02-105 | 26.985 | 7.869 (0, 23.9) | 8.295 | 4.286 (0,18.6) | 22.5 | 20.37 (.12,.31) | 0.485 | 0.3 (-1,1) | 1.675 | 1.35 (0,13.8) |
| 5 | NC93-17 | L99-35 | 12.99 | 19.057 (1.75,50.7) | 7.025 | 6.15 (0,24.7) | 19.5 | 19.89 (.14,.35) | 0.285 | 0.515 (-.8,1) | 2.685 | 1.419 (0,4.9) |
| 6 | NCDM02-180 | NC03-417 | 21.175 | 18.29 (0,48.7) | 5.695 | 5.925 (0,27.3) | 26.5 | 26.94 (.15,.37) | 0.555 | 0.466 (-1,1) | 1.275 | 1.757 (0,7.9) |
| 7 | NCDM02-180 | Beauregard | 17.085 | 12.9669 (.7,38.8) | 14.305 | 12.42 (.1,41.5) | 25.5 | 25.22 (.16,.35) | 0.06 | -0.01 (-.9,.9) | 2.98 | 2.89 (.08,12.4) |
| 8 | NCDM02-180 | NCDM02-105 | 28.265 | 13.053 (.4,37.05) | 8.345 | 6.52 (0,30.4) | 29.5 | 25.95 (.18,.36) | 0.48 | 0.327 (-.9,1) | 1.74 | 1.609 (0,9.1) |
| 10 | NCDM02-180 | L99-35 | 28.54 | 15.05 (.3,38.8) | 7.075 | 7.558 (0,30.9) | 26.5 | 26.9 (.15,.38) | 0.28 | 0.3319 (-.57,1) | 2.75 | 1.9117 (0,12.28) |
| 11 | NCDM03-003 | NC03-417 | 11.825 | 11.284 (.35, 35.9) | 6.73 | 8.363 (0,23.3) | 26 | 23.68 (.17,.31) | 0.125 | 0.1206 (-.88,1) | 1.49 | 2.299 (0,14.82) |
| 12 | NCDM03-003 | Beauregard | 7.735 | 10.2 (0,39.6) | 15.34 | 11.797 (1.45,29.4) | 20 | 22.9 (.15,.31) | -0.37 | -0.1367 (-1,.91) | 3.195 | 2.757 (.34,11.9) |
| 13 | NCDM03-003 | NCDM02-105 | 18.915 | 9.1796 (.15,38.4) | 9.38 | 9.236 (0,28.9) | 24 | 23.4 (.14,.34) | 0.05 | 0.0606 (-.98,1) | 1.955 | 2.353 (0,12.1) |
| 15 | NCDM03-003 | L99-35 | 4.92 | 12.04 (.55,41.8) | 8.11 | 9.37 (.35,30.5) | 21 | 23.69 (.15,.31) | -0.15 | 0.1204 (-.9,.96) | 2.965 | 2.03 (.25,9.7) |
| 16 | NC413 | NC03-417 | 16.66 | 15.78 (1,41.4) | 10.475 | 11.918 (0,32.1) | 24 | 25.55 (.17,.32) | 0.315 | 0.12808 (-.78,1) | 2.035 | 2.6794 (0,10) |
| 18 | NC413 | NCDM02-105 | 23.75 | 13.786 (1.2,46.9) | 13.125 | 16.224 (0,48.2) | 27 | 23.77 (.13,.34) | 0.24 | -0.0038 (-.95,1) | 2.5 | 3.76 (0,16.4) |
| 20 | NC413 | L99-35 | 9.755 | 12.074 (.4,39.8) | 11.855 | 12.989 (0,33.9) | 24 | 24.2 (.12,.34) | 0.04 | -0.0492 (-.96,1) | 3.51 | 2.74 (0,10) |

Daughter: daughter root yield (kg/plot) SSP: sized seed piece yield (kg/plot), DM: dry matter percent, PI: partitioning index, SRS: post-harvest seed root survivability

**Table 3.5: Narrow sense heritability scores ($h^2$) and standard error (±) are shown here using parent-offspring regression and variance component analysis of half sib family means. Parent Offspring (P-O) regression is separated into its two separate locations for each trait.**

| Trait | Parent Offspring Regression | | Variance Component |
|---|---|---|---|
| | Clinton | Kinston | |
| Dry matter | 0.54 ± 0.12 | 0.63 ± 0.14 | 1.20 ± 1.75 |
| PI[2] | 0.64 ± 0.16 | 0.37 ± 0.18 | 0.62 ± 0.16 |
| Daughter[3] | 0.12 ± 0.13 | -0.10 ± 0.11 | 0.45 ± 0.01 |
| Seed Root Enlargement | 0.36 ± 0.20 | 0.76 ± 0.28 | 0.82 ± 0.01 |
| Root Piece Survival | 0.27 ± 0.26 | 0.18 ± 0.22 | 0.54 ± 0.05 |
| Total Yield | -0.04 ± 0.19 | -0.09 ± 0.17 | 0 .60 ± 0.01 |

[1]$h^2$ estimates are from twice the parent offspring regression of offspring means on parent means
[2]PI = Partitioning index of roots shows the distribution of normal daughter roots to SRE from 1 to -1. With 1 being 100% daughter roots and 0% SRE
[3]Daughter = Daughter root yield

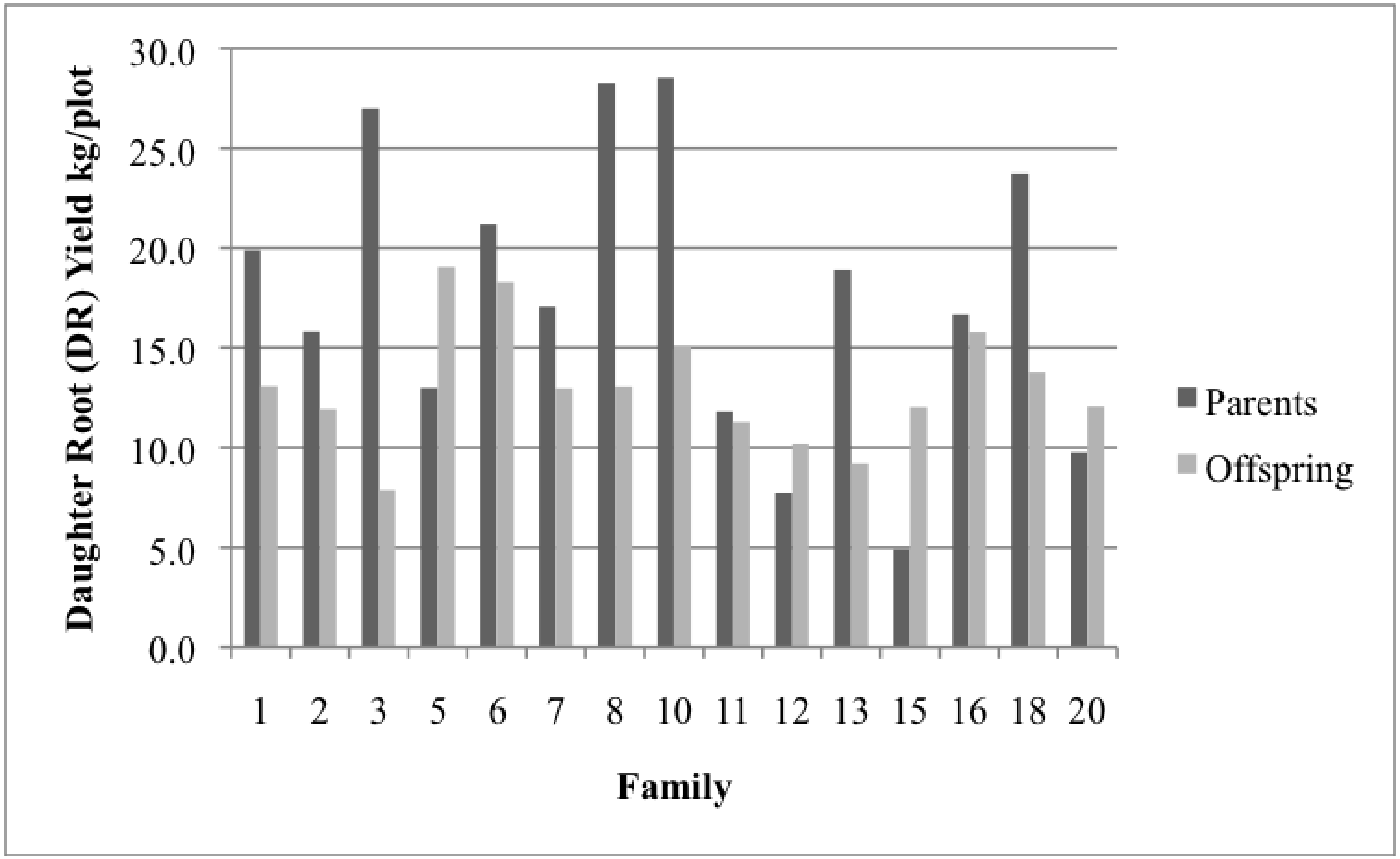
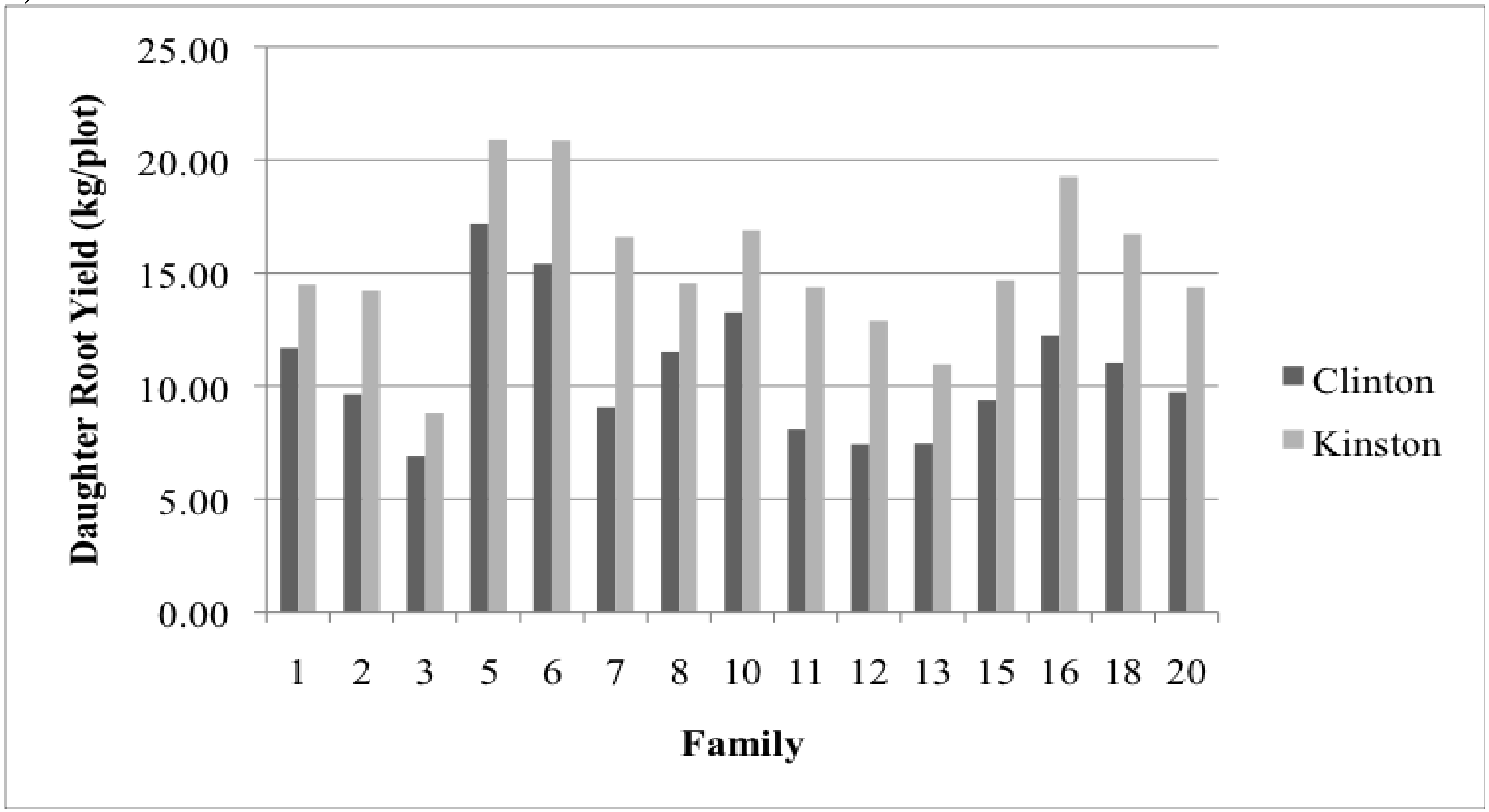



**Figure 3.1: Comparison of parents and offspring for average daughter root yield (kg/plot) for all families tested averaged across two experimental sites located in Kinston and Clinton, NC.**

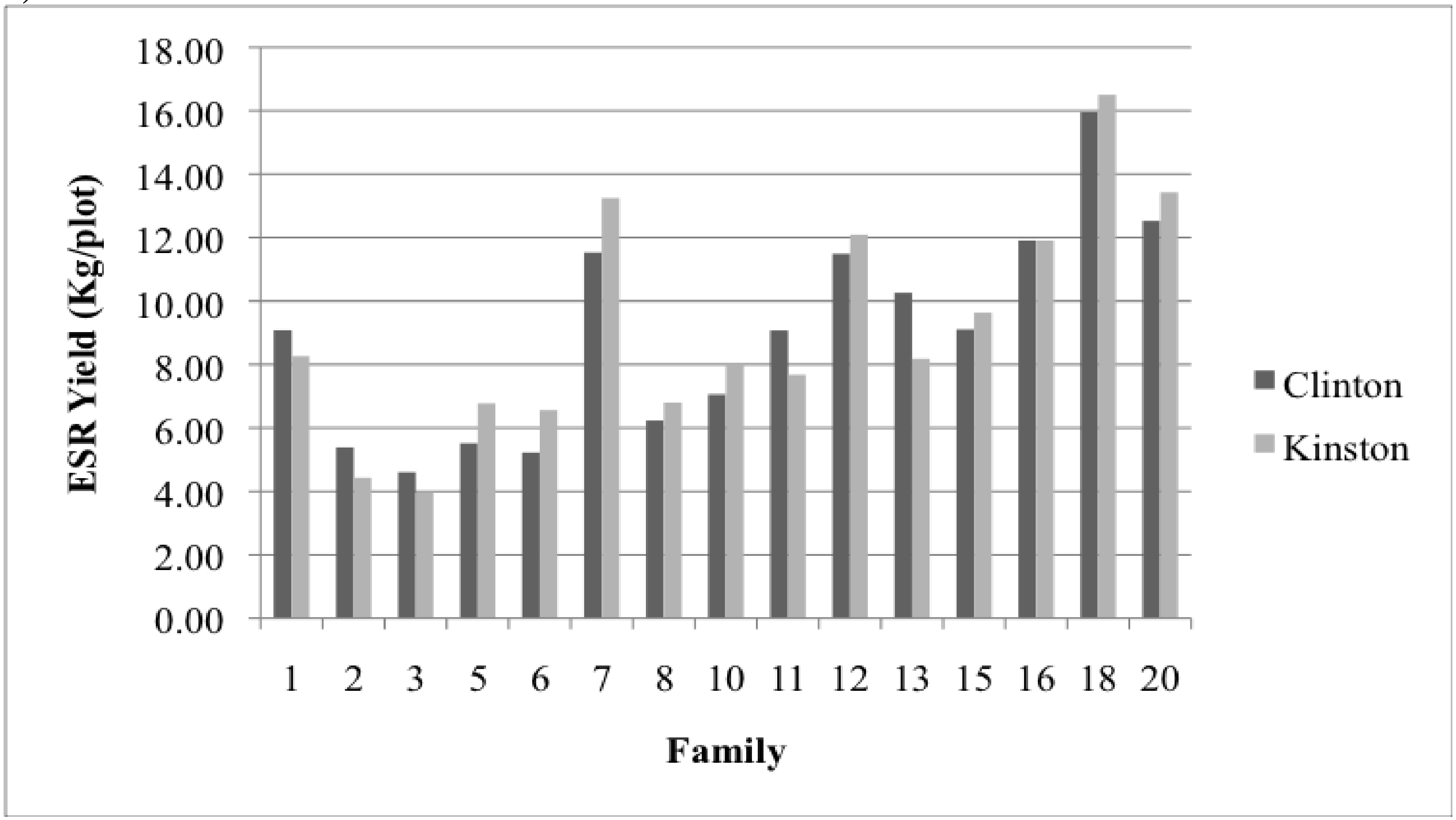
**Figures 3.2: Location and family differences for three important traits in root piece planting. These traits are daughter root yield (kg/plot) (a), enlarged seed root yield (kg/plot) (b), and partitioning index (c) and are shown in each figure averaged across progeny in each family for both locations Clinton and Kinston, NC.**

A)



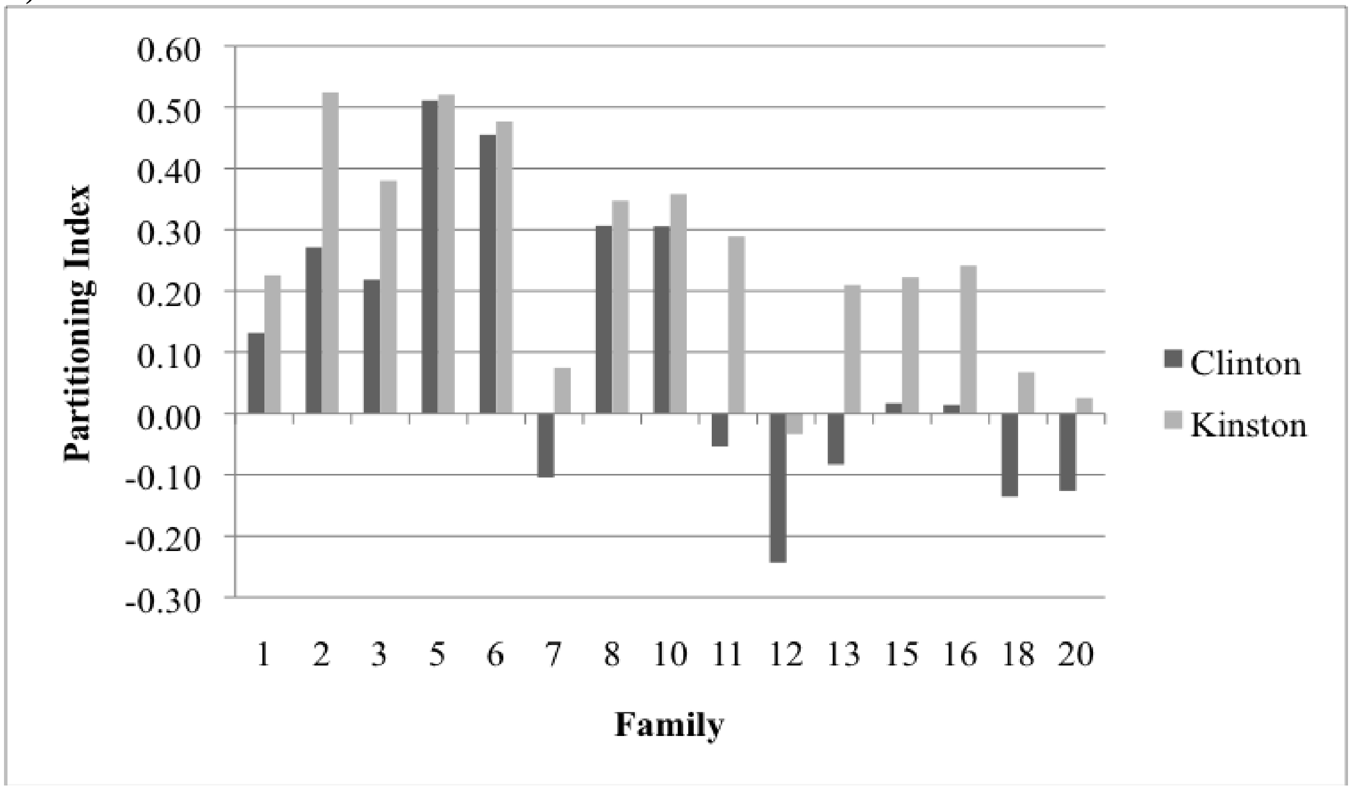
25.00
20.00
15.00
10.00
5.00
0.00
Daughter Root Yield (kg/plot)
1
2
3
5
6
7
8
10
11
12
13
15
16
18
20
Family
Clinton
Kinston

B)



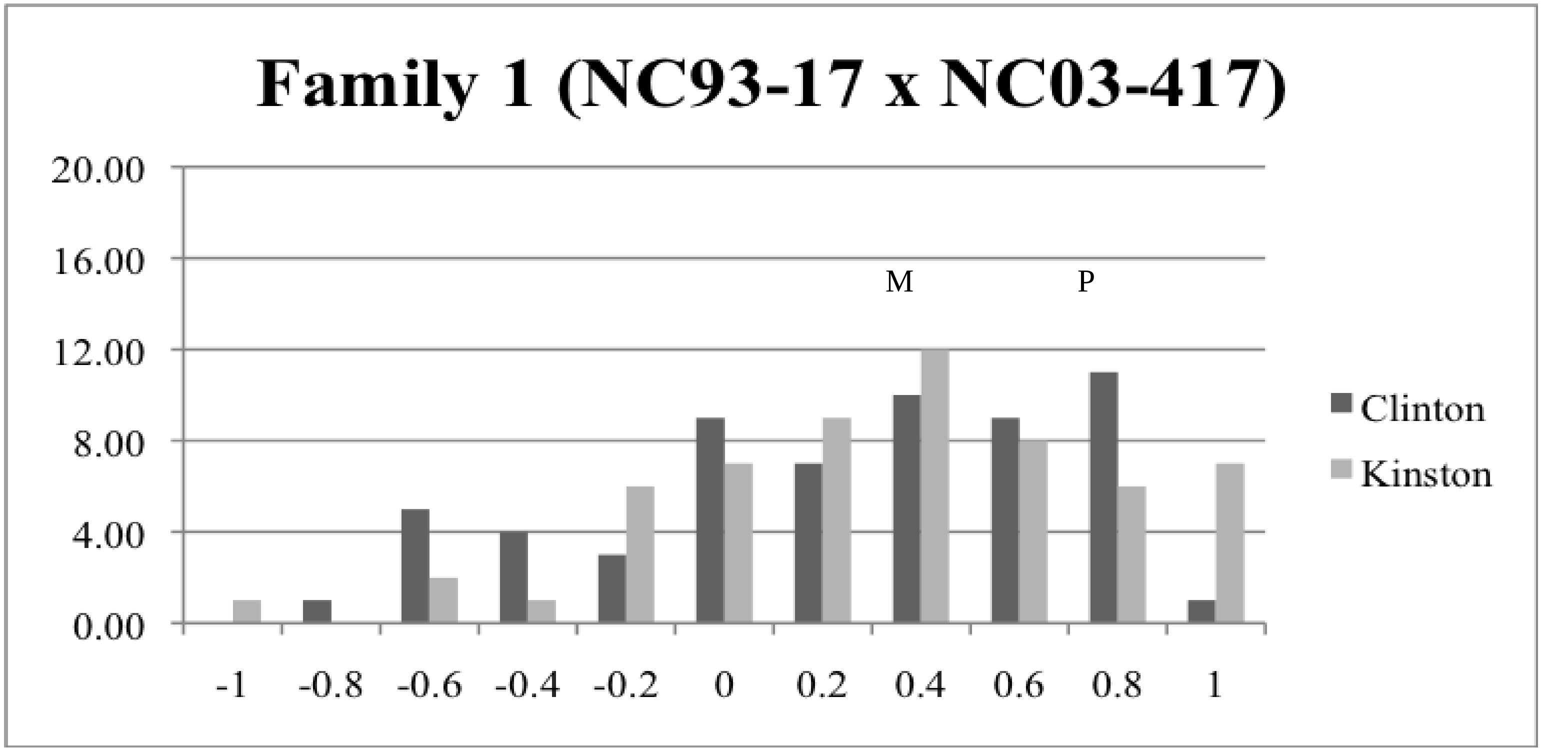
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
ESR Yield (Kg/plot)
1 2 3 5 6 7 8 10 11 12 13 15 16 18 20
Family
Clinton
Kinston

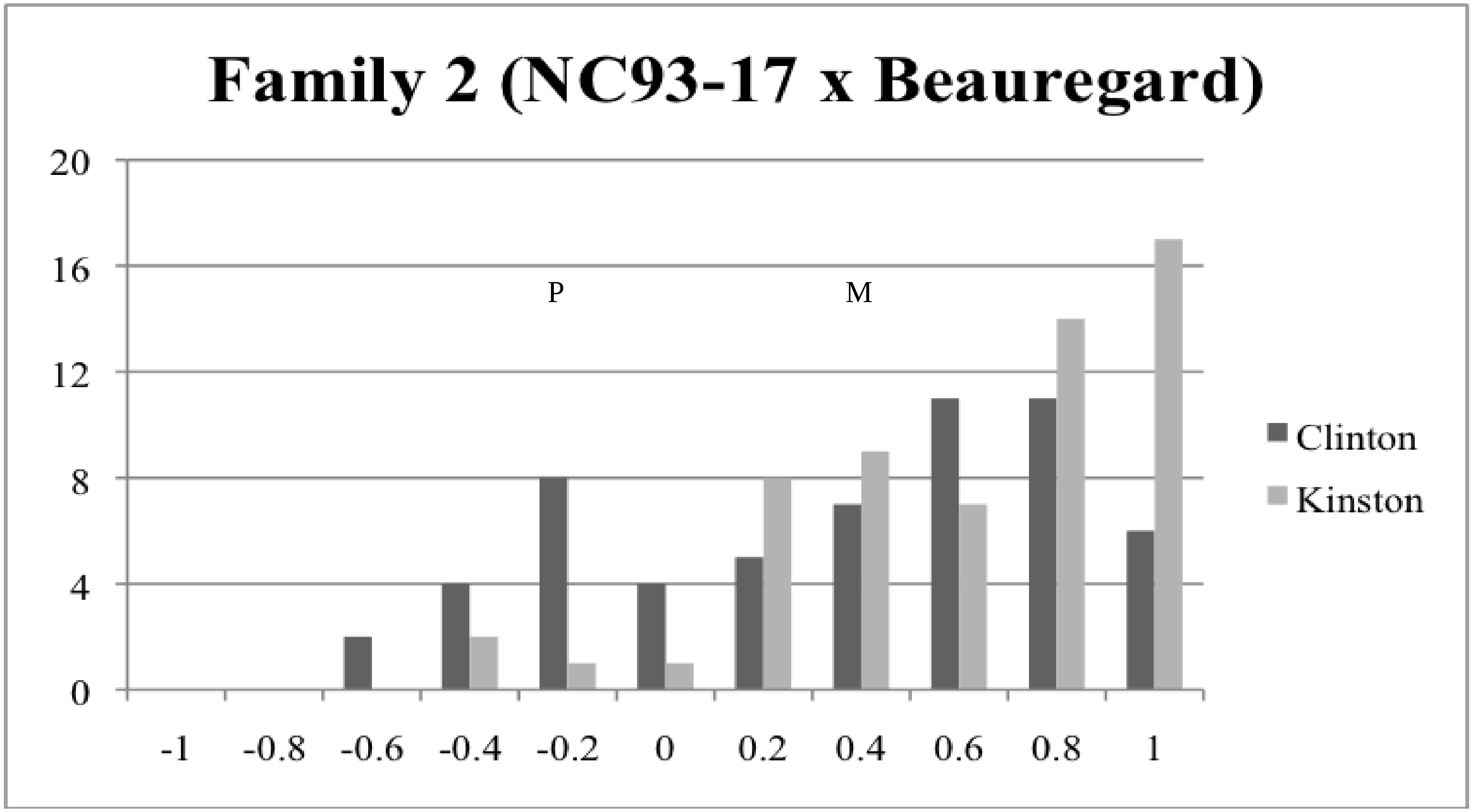
C)



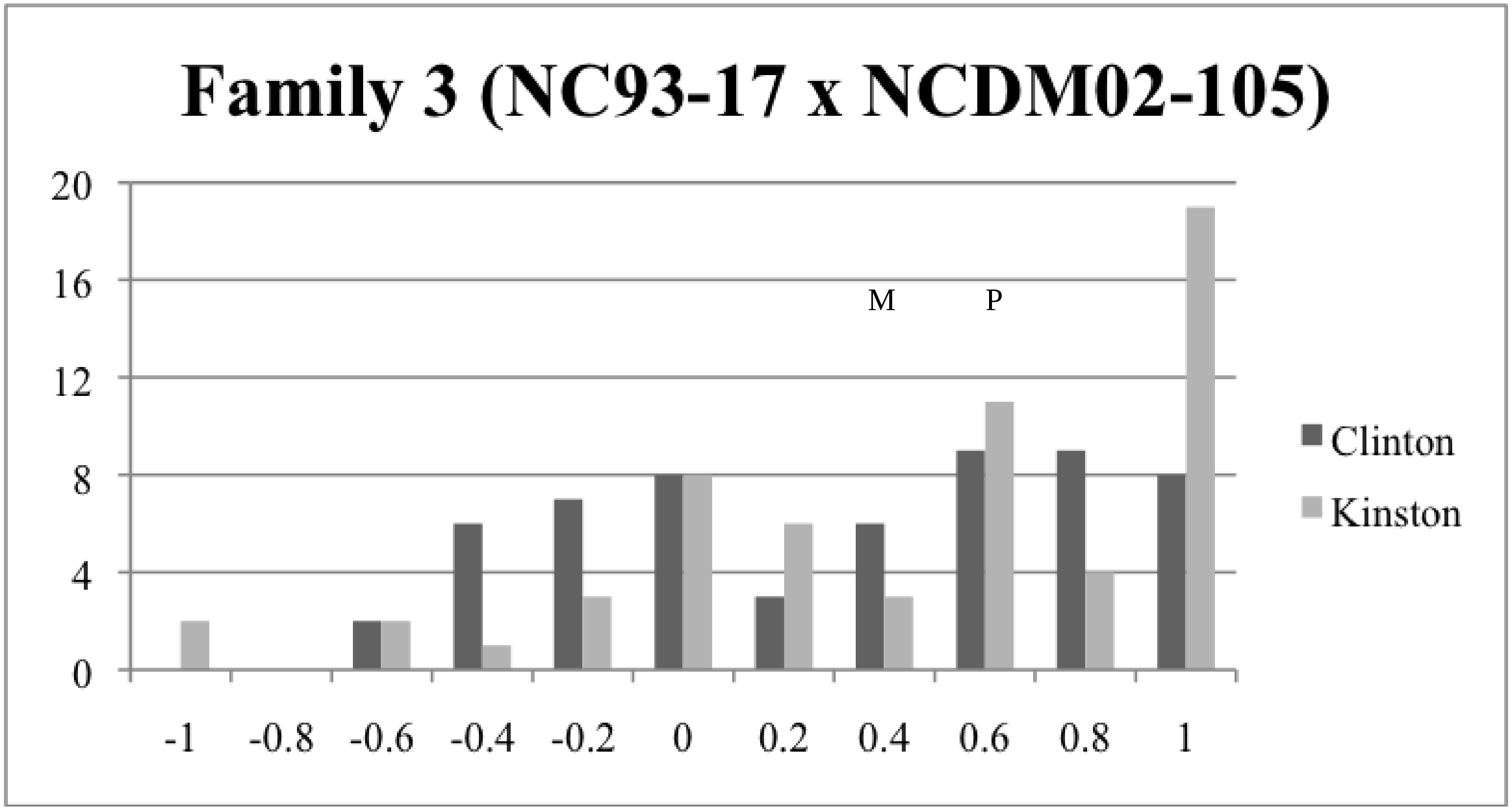
0.60
0.50
0.40
0.30
0.20
0.10
0.00
-0.10
-0.20
-0.30
Partitioning Index
1 2 3 5 6 7 8 10 11 12 13 15 16 18 20
Family
Clinton
Kinston

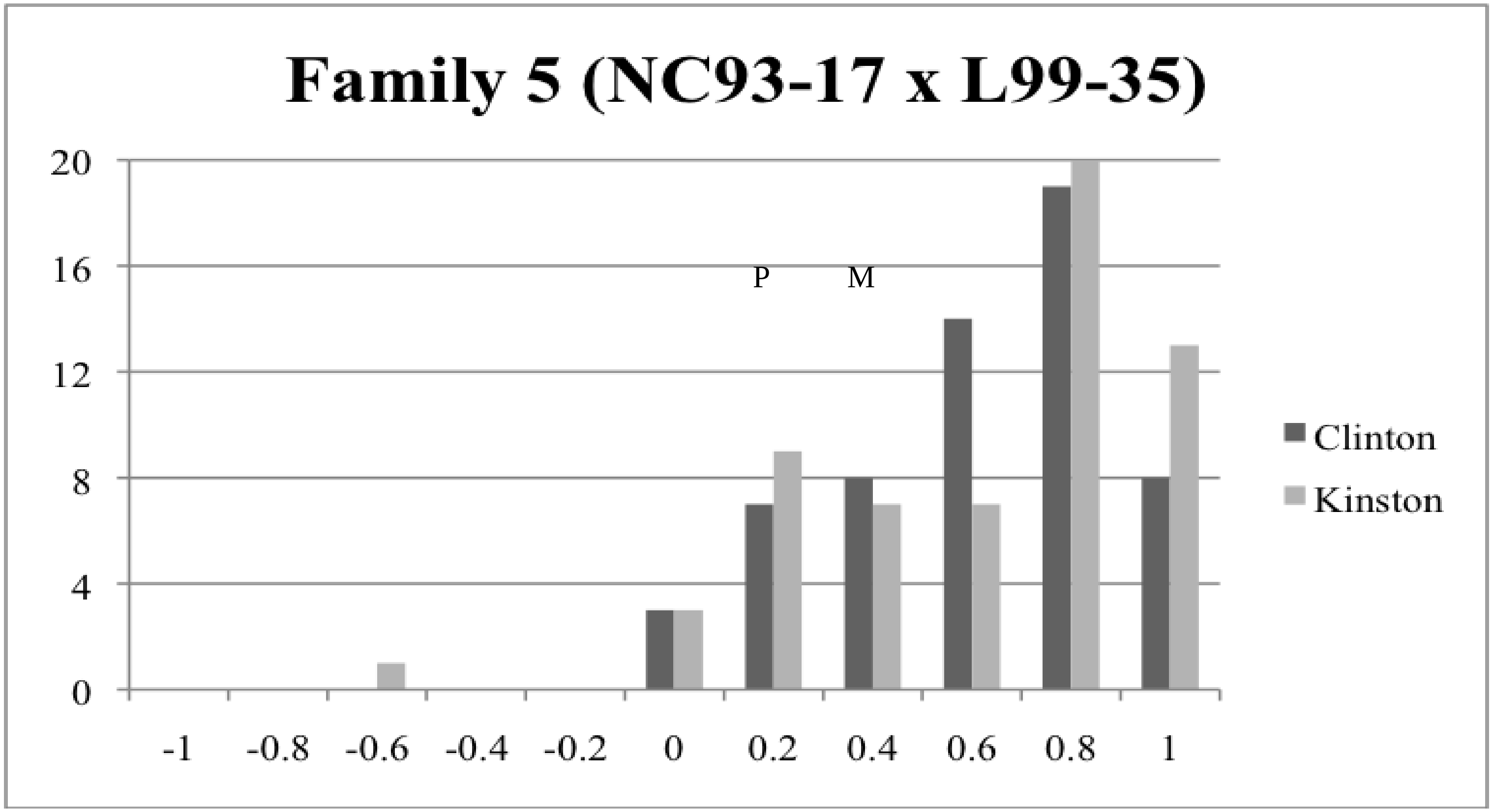
**Figures 3.3: Frequency histograms for progeny in every family separated by location, Clinton and Kinston NC, for partitioning index. 'M' and 'P' denote maternal and paternal parent averages.**

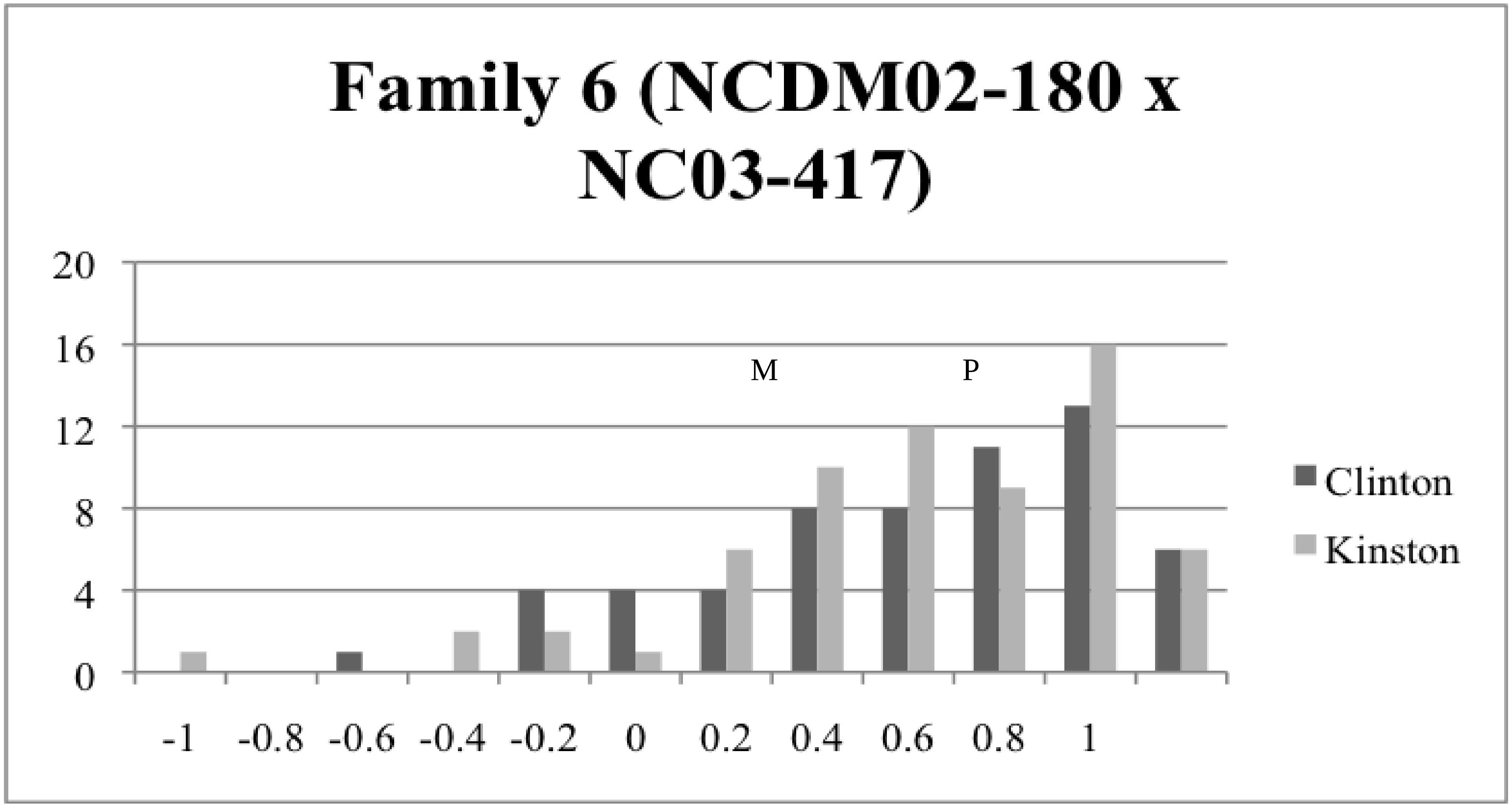

Family 1 (NC93-17 x NC03-417)
20.00
16.00
12.00
8.00
4.00
0.00
M
P
Clinton
Kinston
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1

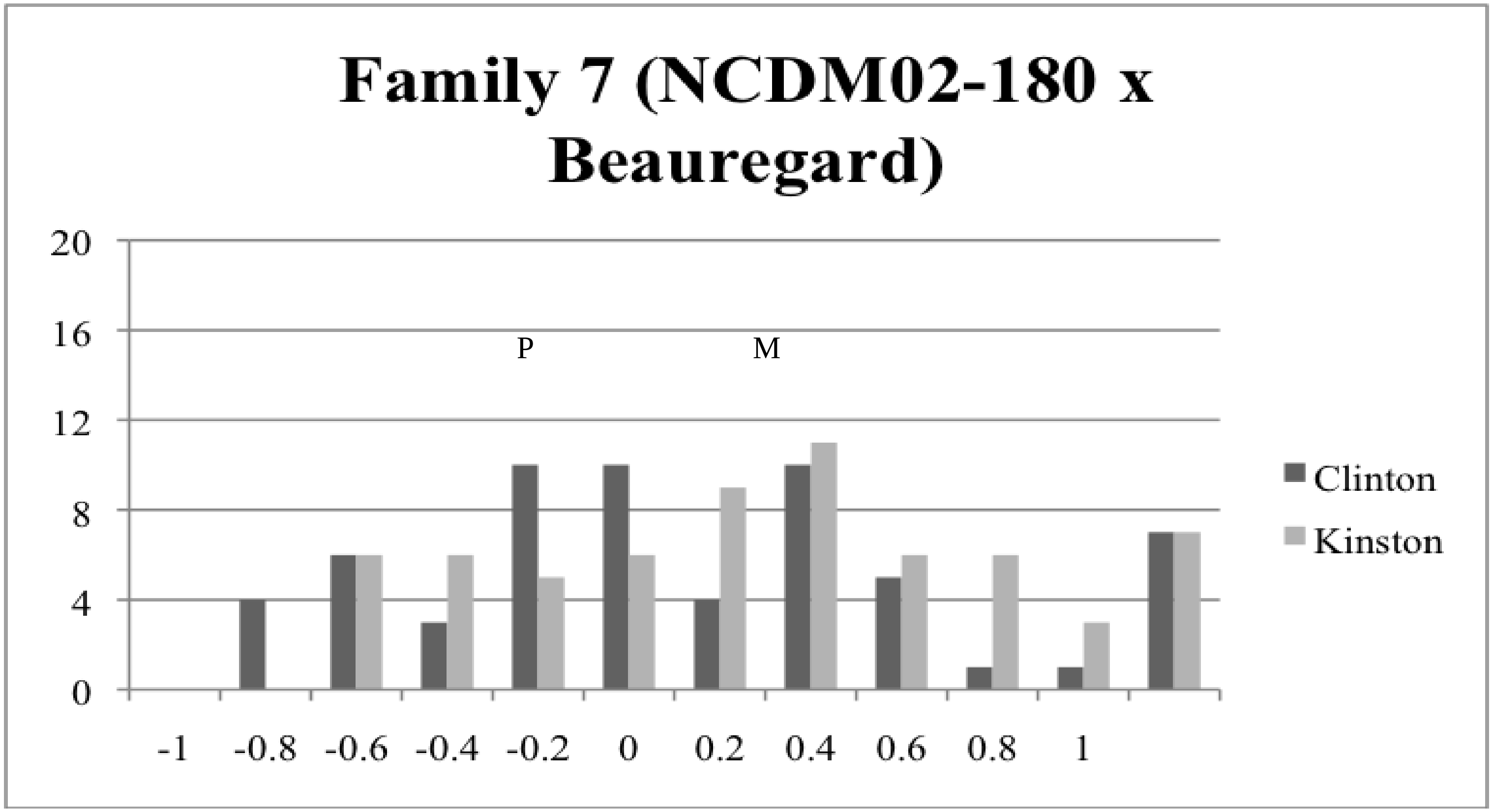


Family 2 (NC93-17 x Beauregard)
20
16
12
8
4
0
P
M
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Clinton
Kinston

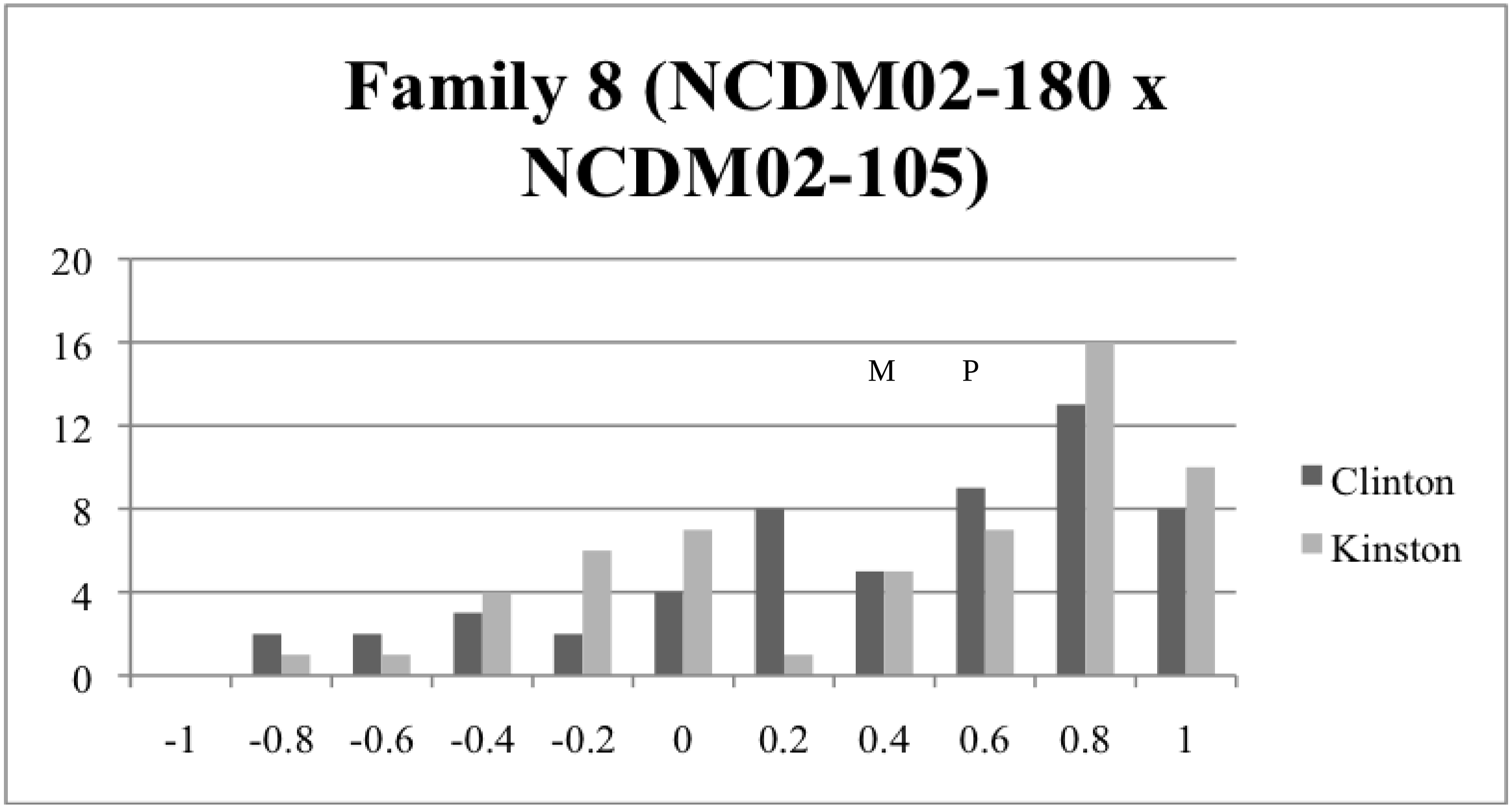

Family 3 (NC93-17 x NCDM02-105)
20
16
12
8
4
0
M
P
Clinton
Kinston
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1


Family 5 (NC93-17 x L99-35)
20
16
12
8
4
0
P
M
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Clinton
Kinston

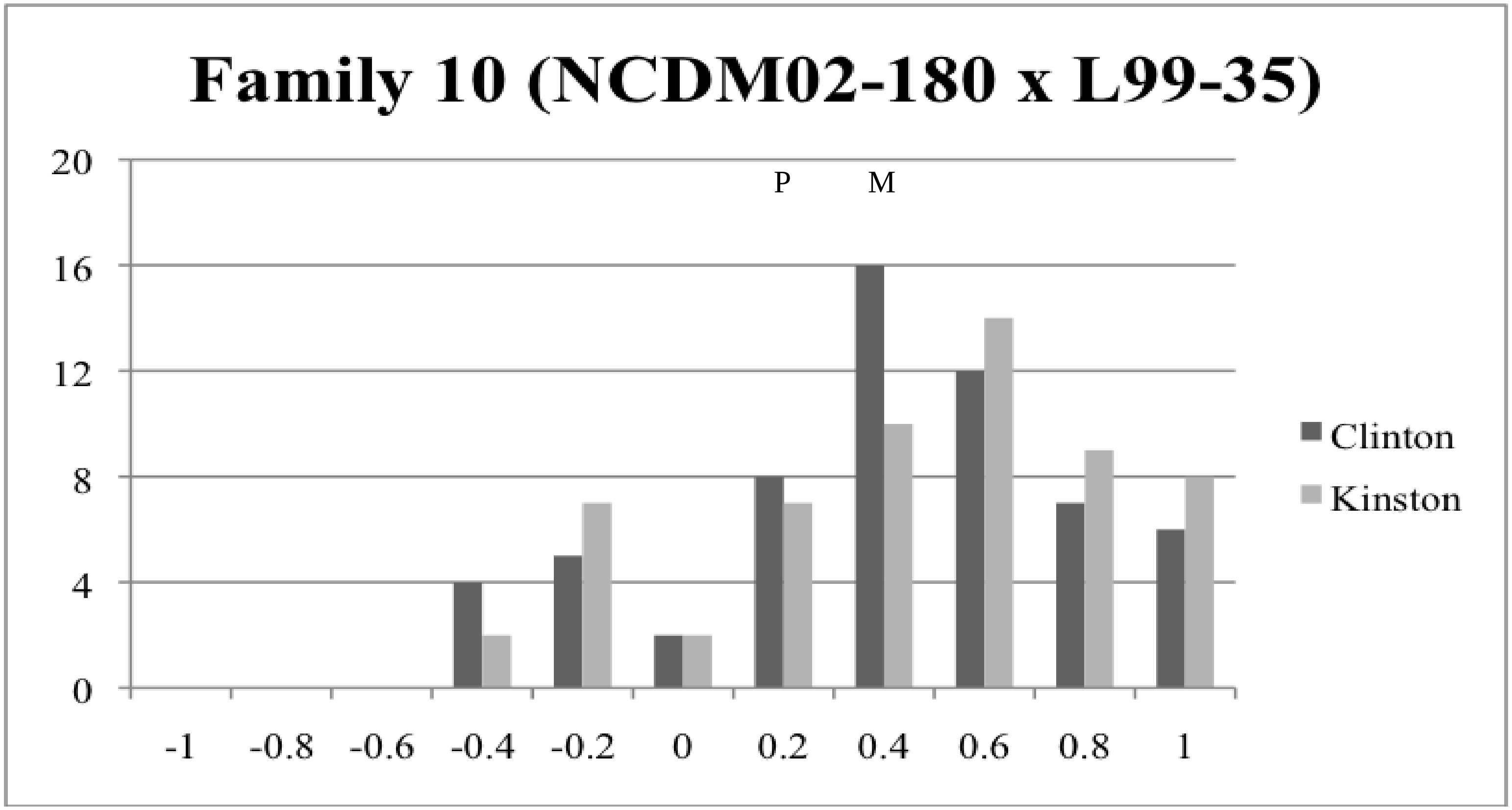

Family 6 (NCDM02-180 x NC03-417)
20
16
12
8
4
0
M
P
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Clinton
Kinston

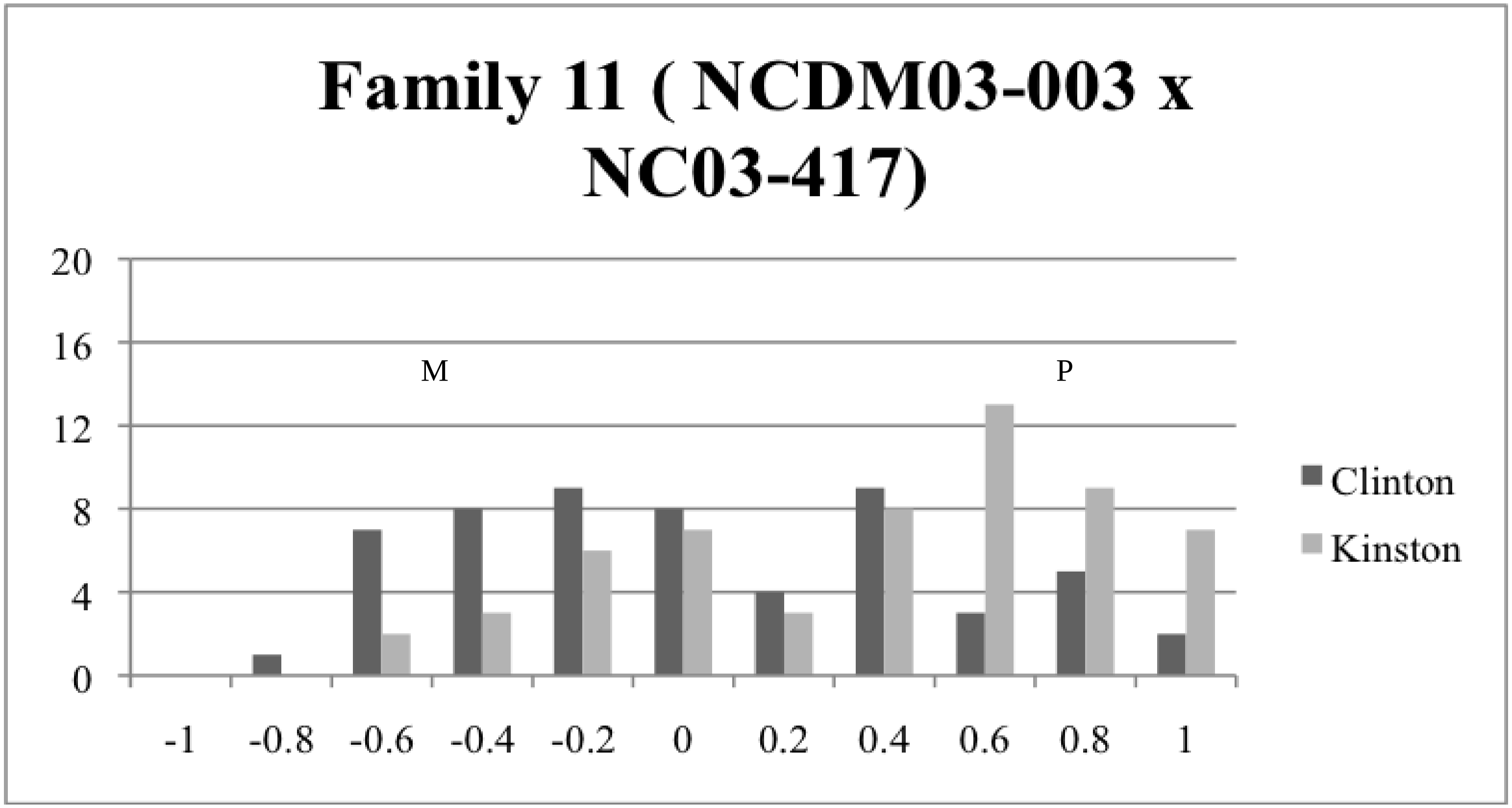


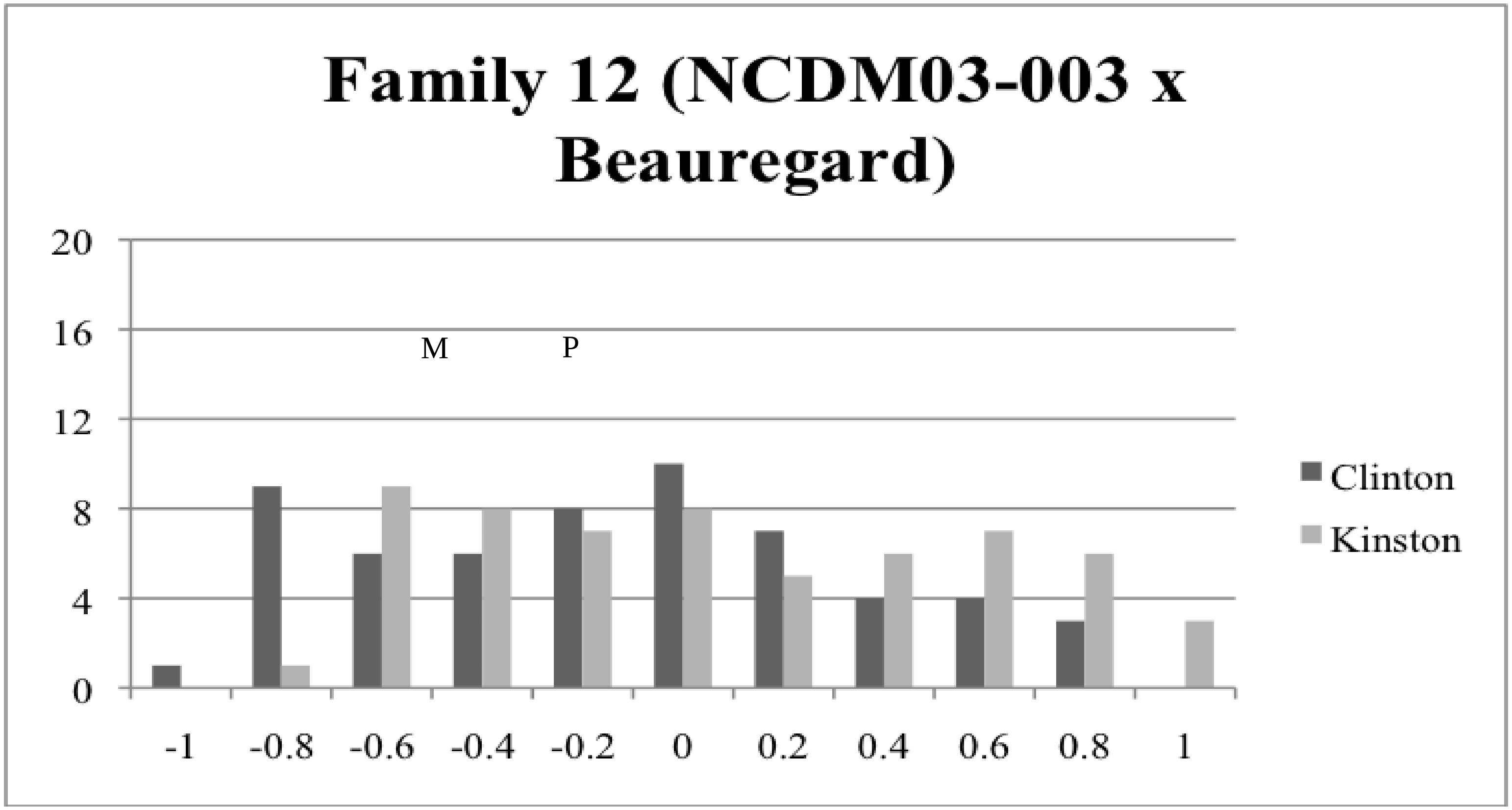
Family 7 (NCDM02-180 x Beauregard)
20
16
12
8
4
0
P
M
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Clinton
Kinston

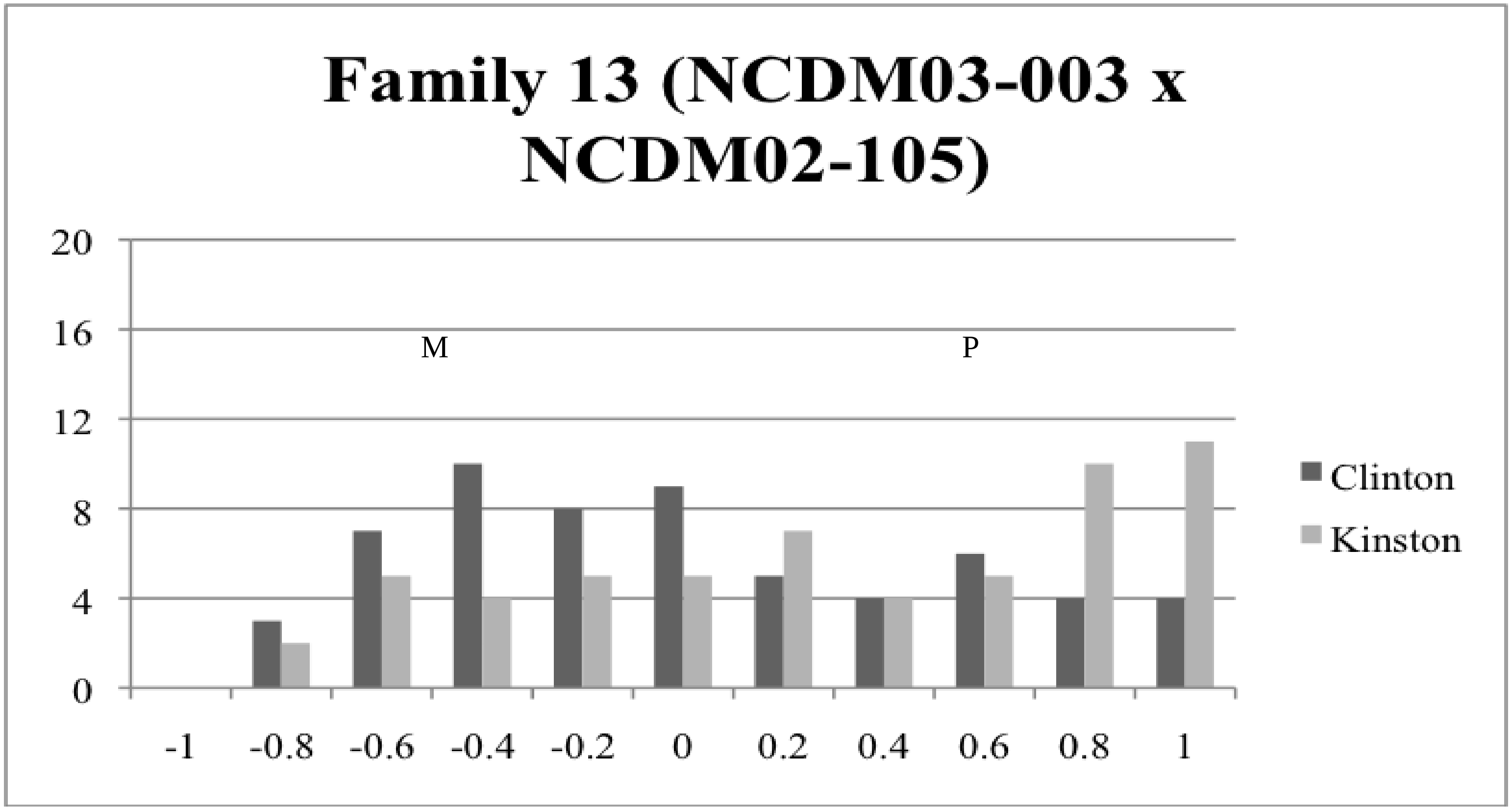


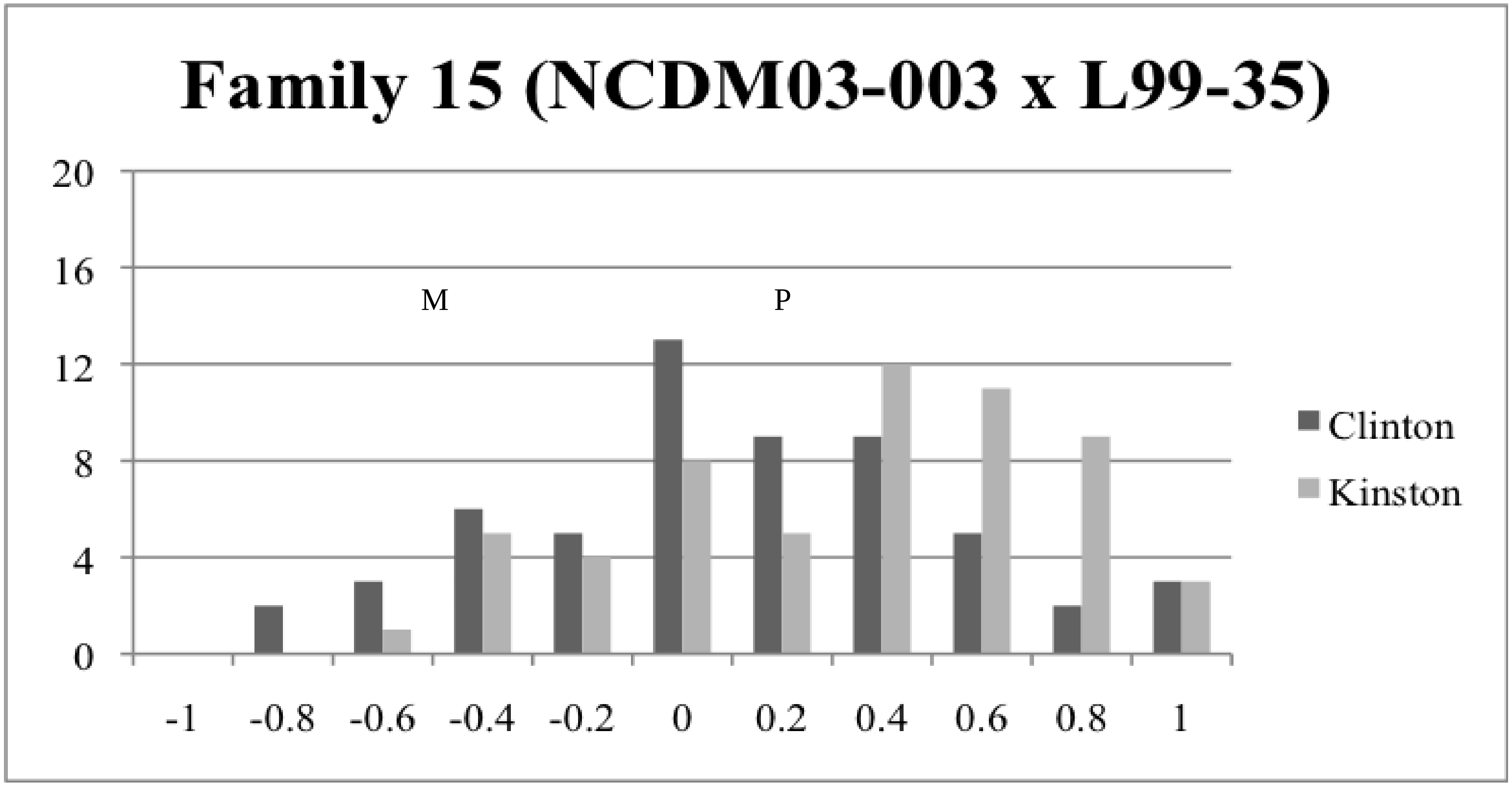
Family 8 (NCDM02-180 x NCDM02-105)
20
16
12
8
4
0
M
P
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Clinton
Kinston

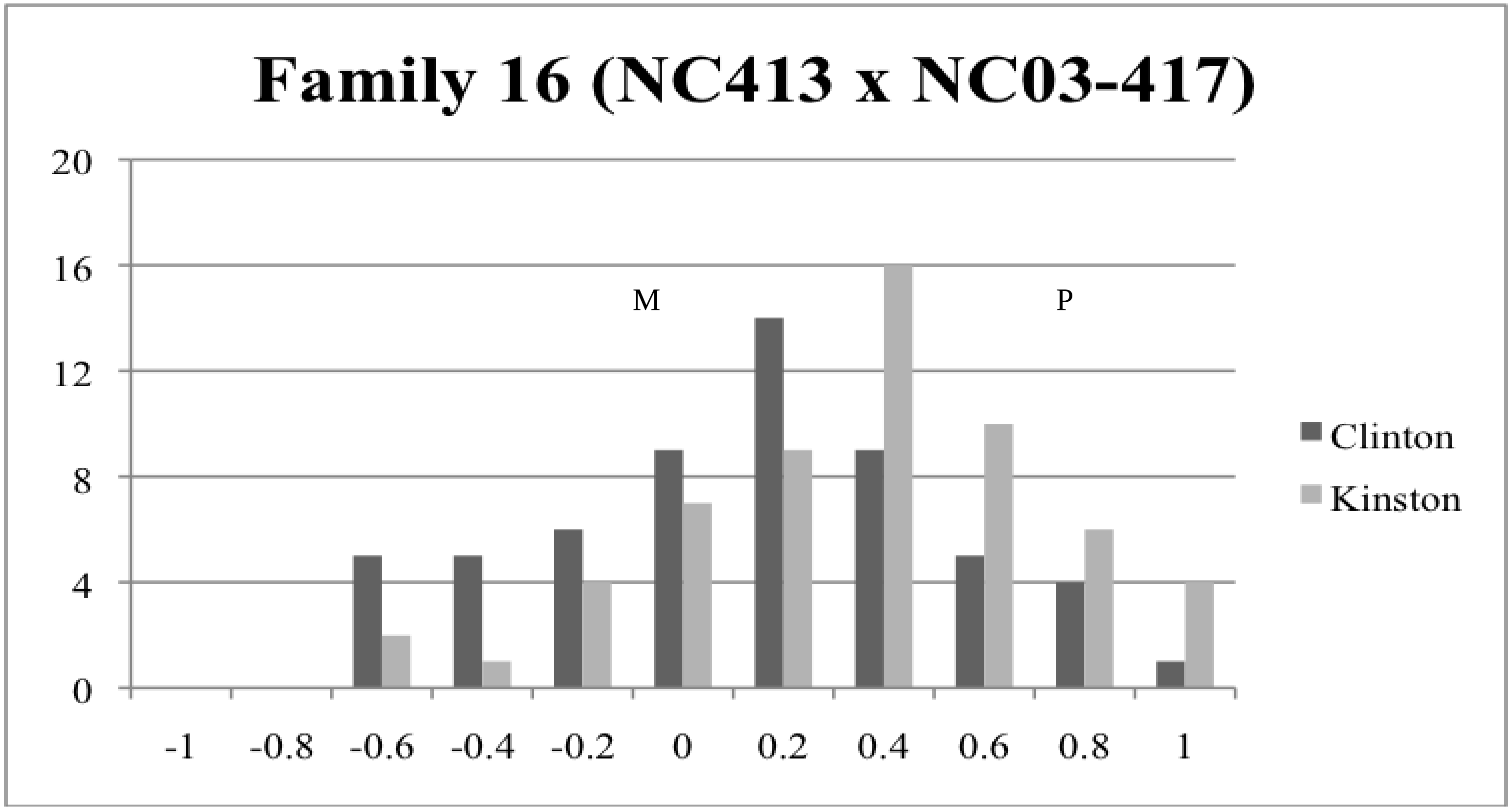

Family 10 (NCDM02-180 x L99-35)
20
16
12
8
4
0
P
M
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Clinton
Kinston


Family 11 ( NCDM03-003 x NC03-417)
20
16
12
8
4
0
M
P
Clinton
Kinston
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1

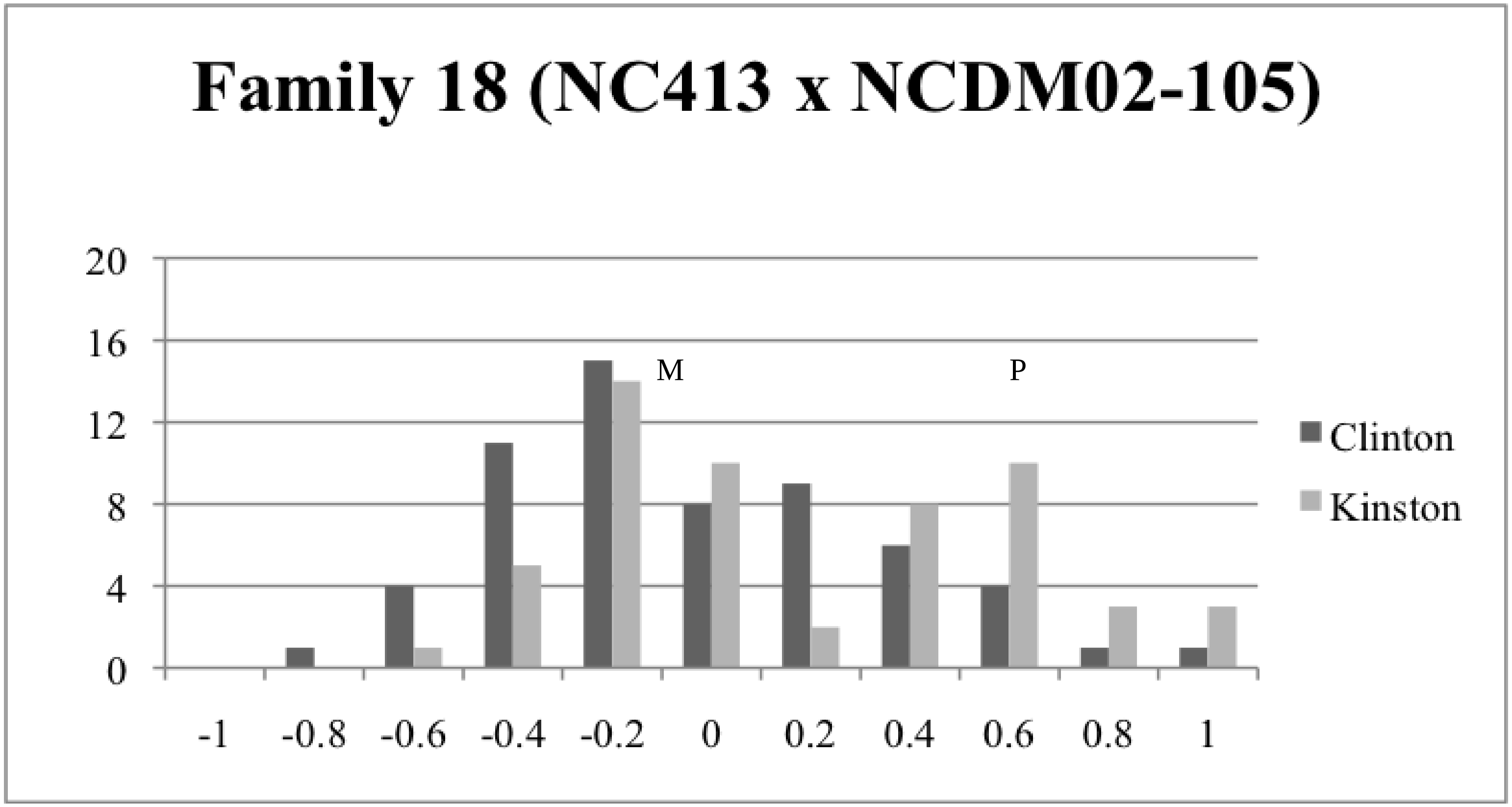

Family 12 (NCDM03-003 x Beauregard)
20
16
12
8
4
0
M
P
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Clinton
Kinston


Family 13 (NCDM03-003 x NCDM02-105)
20
16
12
8
4
0
M
P
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Clinton
Kinston

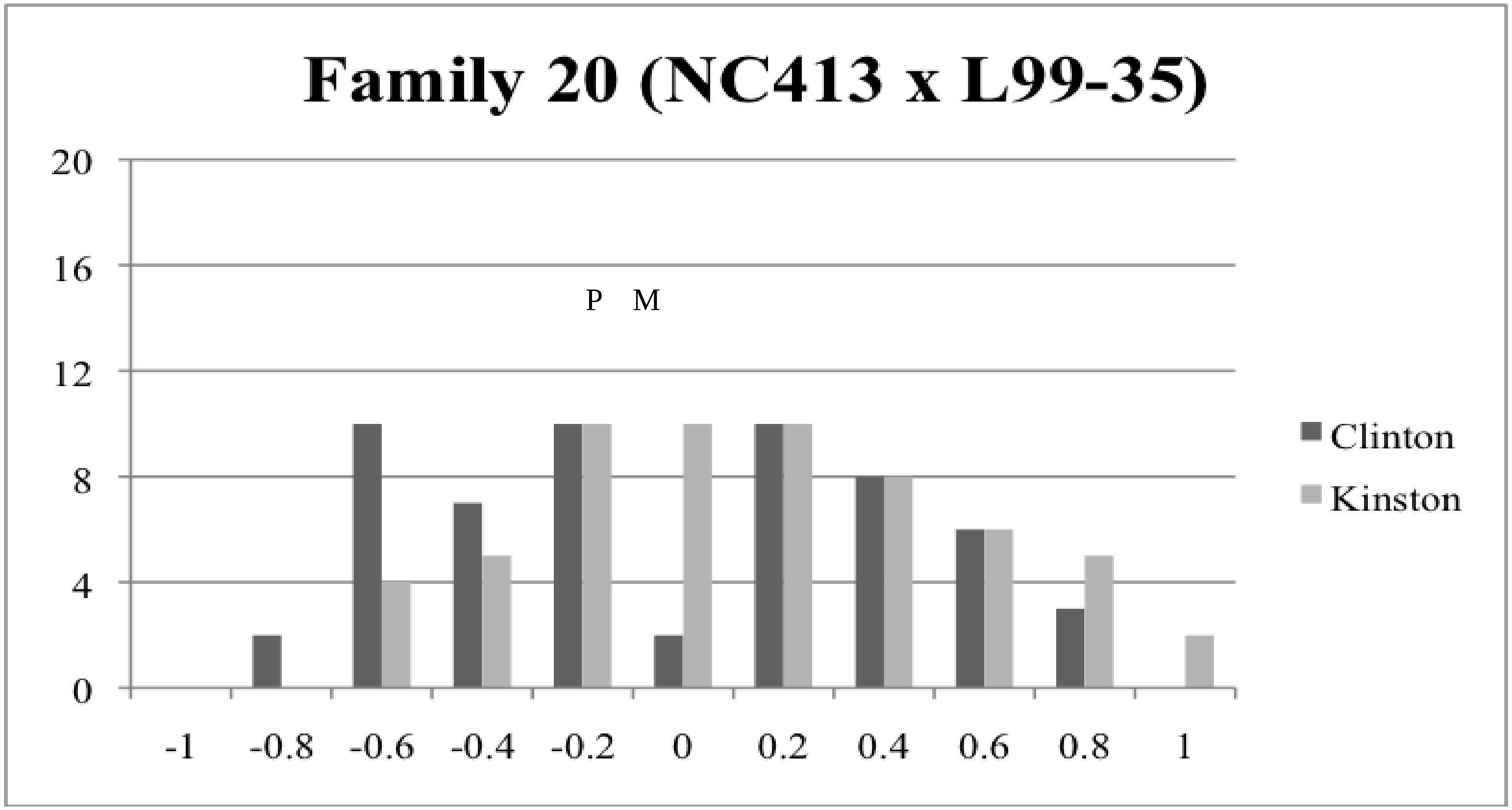

Family 15 (NCDM03-003 x L99-35)
20
16
12
8
4
0
M
P
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Clinton
Kinston



Family 16 (NC413 x NC03-417)
20
16
12
8
4
0
M
P
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Clinton
Kinston



Family 18 (NC413 x NCDM02-105)
20
16
12
8
4
0
M
P
Clinton
Kinston
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1


Family 20 (NC413 x L99-35)
20
16
12
8
4
0
P M
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Clinton
Kinston

# Chapter 4: Summary

Blake Bowen

### 4.1 Previous research:

Previous research showed that cutting increased sprouting per root (Allen and Phillis, 1979; Bouwkamp, 1982; Edmond and Ammerman, 1971; Kobayashi, 1968; Phillis and Allen, 1979). and also increased sprouting in distal sections compared to uncut treatments. Phillis and Allen showed that middle pieces when planted had the least seed root enlargment. However, they also had the least yield and it is speculated because they rotted in the field post planting. Multiple researchers from Japan and the US both found planting using cut root pieces decreased plant yields, increased shape variability, and decreased root appearance compared to conventional planting with slips. Lastly, Kusuhara et al. (1972) found that indirect daughter roots and seed root enlarging type lines were highly heritabile, while daughter root types were moderately heritable.

## 4.2 My Research:

While past research covered a wide range of information, this research also added valuable information for root piece planting of current varieties and lines used in conventional planting in North Carolina. We found that the cutting promoted sprouting in the middle and distal root sections while keeping the total amount of sprouts the same as the uncut treatment in our greenhouse experiment. Our research showed that the proximal root piece held the lowest enlarged seed root (ESR) yield and maximized the partitioning index in the root piece section study. There were two lines in the root piece section study that held the same daughter root and total yield regardless of treatment (root piece section) used (NC03-395 and NCDM04-226). In the planting type study (conventional vs root piece) significant differences were seen for most clones in all traits tested except for a few. The exceptions were NC03-417 and NC03-395 that across all three years were not significantly different for partitioning index or daughter root yield respectively. Few other clones, such as NCDM02-105, NC04-097, and NC03-089 showed values in one or both traits at least one year. In the heritability study important correlations showed strong positive correlation between daughter root yield and partitioning index and a strong negative correlation between enlarged seed root yield and partitioning index. Lastly, the heritability study showed high heritability for partitioning index and dry matter, moderate heritability for seed root survival, and low heritability for daughter root and total yield.

### 4.3 Issues/Mistakes:

Unfortunately, no experiment is perfect and as time went on we learned and amended how experiments were planned out and executed. Rot during pre-sprouting was an issue that caused us to lose clones in our research and substitutions had to be made. This was unfortunate because it did not enable us to have a uniform group of clones to test each year to draw yearly comparisons based off of. Also, as we became more experienced we learned of more traits that we could assess and added them in for later years.

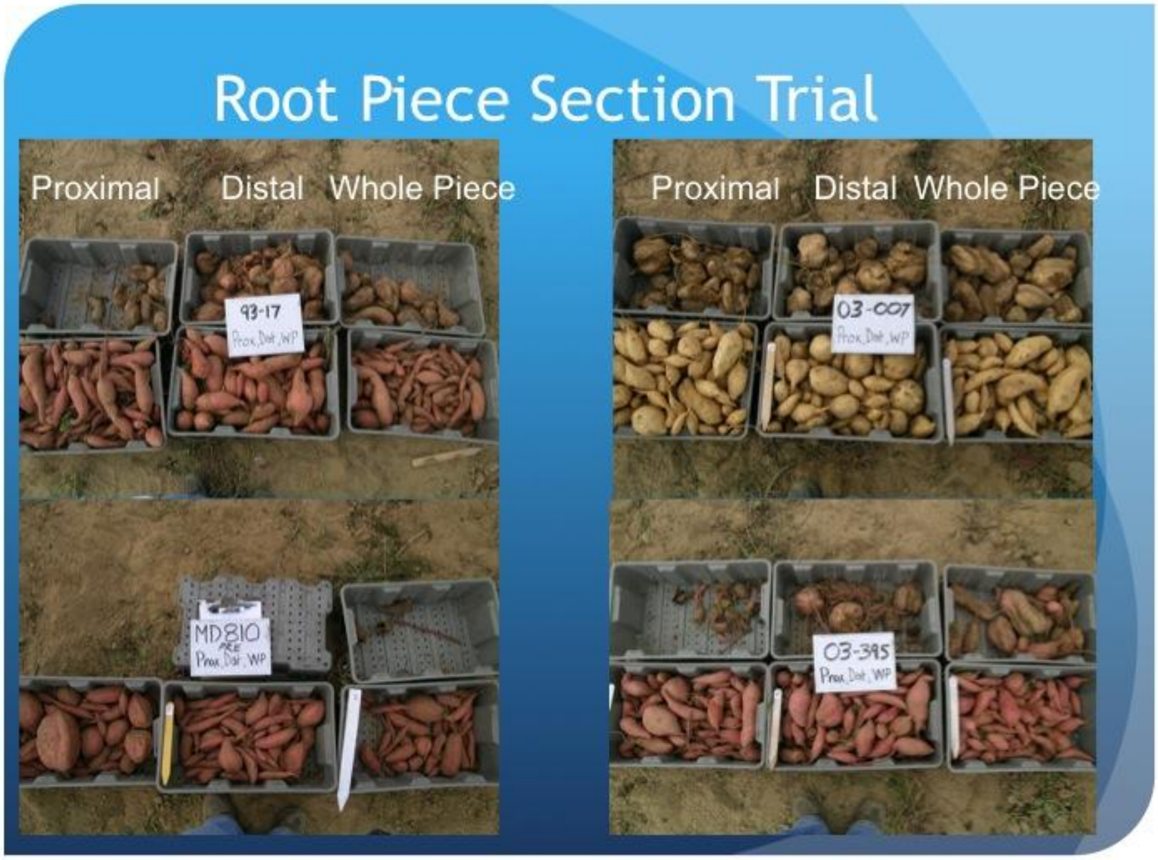
### 4.4 Future experiments:

In the future more work needs to be done in order to fine tune this planting system to a specific variety(s). Ways this can be done include. 1) focus on alternate cultural methods to develop systems for cut root piece seed production, 2) cultural methods to control for root piece enlargement, 3) screen germplasm for high PI lines with sufficient total yield, 4) Develop efficient systems for mechanical harvest, 4) study storage effects for mechanical harvesting prior to industrial processing.

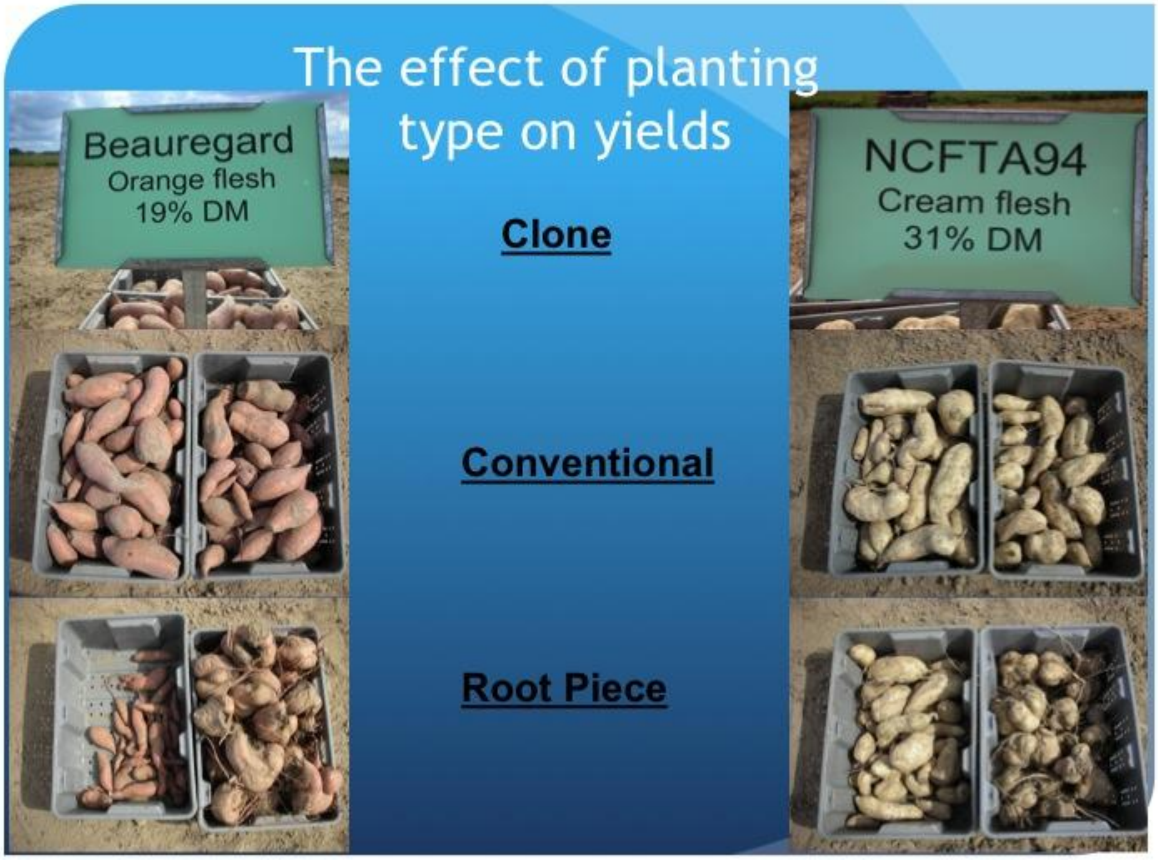
Appendix: Pictures from my studies


Cut Root Piece planting may
alleviate planting costs


Challenges
MD 810
Clinton Rd
5/15/08
AVERY


Root Piece Section Trial
Proximal
Distal
Whole Piece
93-17
Prox, Dist, WP
MD810
PRE
Prox, Dist, WP
Proximal
Distal
Whole Piece
03-007
Prox, Dist, WP
03-395
Prox, Dist, WP


What does NS between treatments look like?
03-395
Prox, Dist, WP
DM04-226
Prox, Dist, WP


The effect of planting type on yields
Beauregard
Orange flesh
19% DM
NCFTA94
Cream flesh
31% DM
Clone
Conventional
Root Piece


A closer look between lines in the root piece vs plants study
NCDM02-180
Cream flesh
34% DM
NCDM02-105
Cream flesh
28% DM
NC03-417
Orange flesh
19% DM