ABSTRACT

DEXTER-BOONE, ABIGAIL E. Investigating Heterosis and the Genetic Control of Parthenocarpy in Tobacco. (Under the direction of Dr. Ramsey S. Lewis).

Development of high-yielding cultivars is a major objective in plant breeding programs, although it can be difficult to identify parental combinations that will produce high frequencies of improved derived lines. It has been previously suggested that measuring yield heterosis in early generations might serve as a predictive factor for the potential of a cross combination to produce transgressive segregates, or lines outperforming the superior parent. Use of heterosis measurements might inform cross selections to emphasize in early generations to increase plant breeding program efficiency. Past research has indicated $F_1$ heterosis within flue-cured tobacco to be of marginal significance, however.

In Chapter 1, fourteen diverse flue-cured tobacco inbred lines were hybridized in all possible combinations to produce ninety-one hybrid combinations which were evaluated for yield, quality, and black shank resistance characteristics. Of these ninety-one hybrids, sixty-six exhibited positive better-parent heterosis for yield, indicating potential value for utilizing heterosis in a flue-cured tobacco breeding program. Populations corresponding to three low heterotic crosses and three high heterotic crosses were advanced via single seed descent to the $F_{3:4}$ generation. Derived lines were then grown and evaluated for yield, quality, and black shank resistance characteristics. Positive correlations were observed between heterosis levels measured in the $F_1$ generation and the number of transgressive segregates observed. A negative correlation was observed between polygenic black shank resistance and cured leaf yields.

Parthenocarpy, defined as fruit production in the absence of fertilization, is of interest in a number of horticultural crop species for increasing fruit production in the absence of pollination, and for improving desirability of fruit through lack of seeds. This can occur in plants capable of
exhibiting natural parthenocarpy, or through the application of certain hormones, such as auxin or gibberellins. Natural parthenocarpy had been observed in tobacco, which is useful as a model plant to gain insight on genes controlling the parthenocarpic trait.

In Chapter 2, a set of diverse tobacco lines and hybrids were initially evaluated to determine the genetic control of the parthenocarpic trait in tobacco, which was determined to be primarily a dominant trait. A doubled-haploid mapping population was subsequently used to identify a single genomic region on linkage group 22 that is strongly associated with expression of the parthenocarpic trait in tobacco. Application of gibberellic acid to tobacco flowers was found to not influence the development of parthenocarpic fruits, indicating other hormones may play a role in parthenocarpic capsule formation in tobacco.
DEDICATION

To my family- Ken, Sharon, Leah, Sarah, Maureen, Graham, and Caroline.
BIOGRAPHY

Abigail Elizabeth Dexter-Boone was born in Prairie Village, Kansas in 1994. The oldest of six children, she spent her childhood exploring the outdoors, and fell in love with gardening in high school. Early on in her education at NC State, while studying plant biology, Abigail was introduced to plant breeding as a student technician under Dr. Tommy Carter. She continued to pursue plant breeding research by performing MS thesis work under Dr. Ramsey Lewis in tobacco breeding. After completion of her MS, she will be attending the Institute for Advanced Analytics at NC State to continue her education.
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Chapter 1: Heterosis in Flue-Cured Tobacco and Its Utility in Predicting
Transgressive Segregation in Derived Populations of Inbred Lines

Abstract

A major objective of plant breeding programs is the development of high-yielding
cultivars for commercialization through crossing of existing germplasm and discovering superior
genetic combinations among derived materials. Methods for identifying cross combinations that
might lead to superior derived lines would increase the efficiency of the cultivar development
process. F₁ hybrid heterosis may be an underappreciated mechanism of increasing yields in flue-
cured tobacco, and has also been suggested in other crops a predictor of cross combinations that
could generate higher fractions of transgressive segregants. In this study, a fourteen-parent flue-
cured tobacco diallel was created, and ninety-one resulting F₁ hybrids, along with the parental
lines, were initially evaluated for yield, chemical traits, physical cured leaf quality, and black
shank resistance. Sixty-six of the ninety-one F₁ hybrids exhibited positive better-parent heterosis
for yield, with an average better-parent yield heterosis of ~5%. The results suggested high-
parent heterosis to be of potential greater importance to yield improvement than previously
appreciated.

In order to evaluate the potential value of F₁ hybrid yield heterosis for predicting
breeding crosses with increased potential to generate transgressive segregants, populations
derived from three crosses with high heterosis and three crosses with low heterosis were
advanced to the F₃,₄ generation and derived lines were evaluated in three environments for the
same previously mentioned characteristics. Results indicated positive correlations between the
degree of F₁ heterosis for yield and the number of transgressive segregates observed amongst
resulting derived lines. A negative correlation was also observed between polygenic black shank resistance and yield within derived populations. Findings suggest that breeding for increased yield in tobacco might be assisted by a preliminary evaluation of breeding crosses at the F₁ stage, and advancing populations derived from those F₁’s with high parental means and high heterosis. In summary, this research points to several valuable roles for F₁ hybrid heterosis in a flue-cured tobacco cultivar development program.

**Introduction**

Development of higher yielding cultivars is a major objective of most breeding programs. Selection of breeding crosses with a high potential to generate progeny with superior performance relative to the best parent can be inefficient. Methods to predict crosses that might lead to increased frequencies of transgressive segregants would increase the efficiency of breeding programs. In search of methods for identifying successful cross combinations, studies have been performed to identify correlations for important traits between early and late generations of inbreeding. Positive correlations have been found between early generation bulk yields and derived line performance in spring wheat (Cregan and Busch, 1977), with similar results observed in soybean (Leffel and Hanson, 1961). Studies in soybeans have explored the use of early generation heterosis as a predictor for frequency of transgressive segregates in derived populations, but only correlations between heterosis level and genetic variance were observed (Friedrichs et al., 2016). Positive results have been observed in bread wheat for using levels of heterosis as a predictive factor, along with other considerations, as a means to determine which populations might produce more transgressive segregates (Ginkel and Ortiz, 2018).
Heterosis is frequently exploited in cultivar development programs to produce superior cultivars, such as in rice (*Oryza sativa*) (Huang et al., 2015; Huang et al., 2016), maize (*Zea mays*), sorghum (*Sorghum bicolor*), and sunflower (*Helianthus annuus*) (Duvick, 1999). Heterosis can contribute to increased crop yields, in addition to benefits such as cultivar uniformity (Janick, 1999). Heterosis refers to the improved performance of a hybrid individual over performance of the parental lines, or the mid-parent value (Fehr, 1991). First described in 1876 by Charles Darwin (Darwin, 1876), the first explanation of heterosis considered dominance to be the primary contributor to the phenomenon (Jones, 1917). By the 1930’s, however, dominance was beginning to be questioned as the only explanation of heterosis (East, 1936), and multiple theories currently exist as to the true genetic basis of observed heterosis. Heterosis levels are trait and cross specific, and hybrids will not always show an increased heterotic phenotypic. In some cases, F1 hybrids are inferior in performance to both parental inbred lines (Hua et al., 2002).

While measured heterosis levels are situation specific, some common trends are observed. In general, crosses between genetically divergent materials exhibit greater levels of heterosis, particularly in crosses between different species (East, 1936). Selecting cross combinations for the increased genetic diversity of the parents to maximize heterotic increase is commonly seen in breeding schemes of certain crops, such as the use of heterotic groupings in maize (Hallauer et al., 1988; Reif et al., 2005). Also used in breeding is the phenomenon of increased heterosis of polyploids. Polyploids tend to exhibit higher levels of heterosis than that of diploid individuals, such as the progressive heterosis observed in alfalfa double and three-way crosses (Groose et al., 1989). These observations are thought to be due to involvement of
epistatic effects (Bingham et al., 1994). Previous work in rice and potatoes has also shown progressive heterosis to lead to higher yield increases (Mok and Peloquin, 1975; Tu et al., 2007).

Three general models are used to explain heterosis in plants: those involving dominance, overdominance, and epistasis (Schnable and Springer, 2013). The dominance theory, first proposed by East and Hayes, 1912, hypothesizes that heterotic effects are due to the accumulation of favorable dominant alleles from the parental lines in the hybrid progeny, resulting in superior performance relative to the parents (Jones, 1917). Studies in maize have indicated yield heterosis to be primarily due to dominance gene action, especially incomplete dominance, while epistasis and overdominance were found to be of lesser importance (Cockerham and Zeng, 1996; Garcia et al., 2008; Yang et al., 2017). A major role for dominance in yield heterosis has been reported in rice (Xiao et al., 1995; Luo et al., 2001; Li et al., 2008; Li et al., 2010; Huang and Han, 2018).

The theory of overdominance accounting for heterosis is explained by allelic interactions resulting in a superior heterozygous individual (Shull, 1908), or through pseudo-overdominance, in which linkage between dominant loci in repulsion give the heterozygous state increased performance (Stuber et al., 1992). Overdominance has been suggested as a major contributor to heterosis in rice (Li et al., 2001; Luo et al., 2001; Li et al., 2008; Li et al., 2010) and sorghum (Li et al., 2015). Pseudo-overdominance has been described in some studies as a possible explanation and confounding factor (Falk, 1961; Muller and Falk, 1961; Stuber et al., 1992), with theoretical work attempting to distinguish between the two mechanisms and account for the influence of epistasis (Melchinger et al., 2007). It has been argued that the phenomena of inbreeding depression is also due to recessive deleterious alleles is consistent with both the dominance and overdominance explanations for heterosis (Charlesworth and Willis, 2009).
Finally, the third explanation of heterosis is that involving epistatic effects, which are due to interactions between non-allelic genes (Powers, 1944). Epistatic effects contributing to biomass and grain yield component heterosis have been seen in rice (Yu et al., 1997; Luo et al., 2001; Li et al., 2001; Li et al., 2008; Li et al., 2010; Zhou et al., 2012), and in bread wheat (Jiang et al., 2017). However, cases in tomato have shown heterotic effects for yield in absence of measurable epistasis, indicating that epistatic effects are not always a significant contributor to heterosis (Semel, 2006).

Widespread differential gene expression has been observed between parental lines and their resulting hybrids. Changes in miRNA and siRNA expression and DNA methylation levels in hybrids have also been associated with a heterotic response in some species (Chen, 2013; Groszmann et al., 2013; Zhang et al., 2014; Kawanabe et al., 2016), and QTL associated with regions containing epigenetic-related genes have been correlated with heterotic phenotypes (Venu et al., 2014). This suggests epigenetic regulation can play a role in observed heterosis levels.

Increased expression of genes involved in carbon-fixation, photosynthesis, and carbon metabolism pathways has been observed in rice hybrids (Song et al., 2010; Li et al., 2016). In Arabidopsis, increased expression of transcription factors associated with control of auxin pathways was associated with observed heterosis (Wang et al., 2017). DNA damage in yeast hybrids has led to the theory that heterosis affecting increases in biomass results from the loss of regulatory checks, suggesting that heterosis can be a manifestation of hybrid incompatibility (Herbst et al., 2017). Genes associated with circadian regulation have also been connected with hybrid vigor (Ni et al., 2009), evidence of the widespread molecular-level changes that can underlie observed heterotic phenotypes.
Multiple studies have identified loci associated with high heterosis levels, commonly referred to as Heterotic Trait Loci (HTL), in *Arabidopsis* (Redei, 1962; Yang et al., 2017), *Drosophila* (Wallace, 1957; Wallace, 1958), sorghum (Ben-Israel et al., 2012), and maize (Lariepe et al., 2012). Individual alleles have also been shown to be highly associated with increased vigor in hybrids (Dollinger, 1985; Goff, 2011; Herbst et al., 2017). In tomatoes, chromosomal introgressions from wild species were associated with heterosis for yield-related traits (Semel et al., 2006).

Previous studies have observed dosage dependent components of heterosis, with examples in tobacco (East, 1936), rice (Hua et al., 2003; Huang et al., 2016), tomato (Krieger et al., 2010), maize (Yao et al., 2012), and oil palm (Singh et al., 2013); which is suggestive of single gene overdominance contributing to heterosis. These dosage sensitivities seem to be species-specific, however, as dosage dependent genes fail to demonstrate a heterotic response in genetically engineered *Arabidopsis* (Jiang et al., 2013).

Heterosis is frequently considered to be a measurement of the degree of genetic relationship between parental materials, with the most divergent material typically exhibiting the greatest heterosis and the highest potential to produce derived progeny with significantly better performance than either parent (Burton and Brownie, 2006). Studies in *Arabidopsis* have found significant heterosis for plant biomass traits even among genetically similar parents, however (Groszmann et al., 2014). Attempts to predict heterosis levels through molecular analysis and prediction of genetic distance through the use of DNA markers have shown mixed success, with some studies showing no correlation between calculated genetic differences and heterosis for various traits, and other studies showing positive correlations (Kotzamanidis et al., 2008; Krystkowiak et al., 2009). Coefficient of parentage and restriction fragment length
polymorphisms to estimate diversity have also shown low correlations with heterosis levels or yield in later generations (Cox and Murphy, 1990; Manjarrez-Sandoval et al., 1997). Flue-cured tobacco germplasm in the U.S. is considered to have a narrow genetic base and has relatively low genetic variability, as measured through allelic counts at microsatellite loci (Moon et al., 2009).

Initial research in *Nicotiana* found extremely high levels of heterosis in crosses between *N. tabacum* and *N. rustica*, although this was not well quantified (East, 1936). Crosses between *N. tabacum* cultivars and suspected diploid progenitor species such as *N. otophora* and *N. tomentosiformis* exhibited significant mid-parent heterosis (Matzinger and Wernsman, 1967). Crosses between flue-cured tobacco and diverse tobacco germplasm accessions exhibited little to no yield heterosis, however (Vandenberg and Matzinger, 1970). In general, inter-type tobacco hybridizations are avoided in commercial breeding programs due to quality concerns (Deverna and Aycock, 1983a), but such crosses have produced yield better-parent heterosis levels of 11.8% (Deverna and Aycock, 1983b). Mid-parent yield heterosis values of 15% have been observed among Turkish tobacco crosses (Kara and Esendal, 1995), while crosses made among Oriental tobacco varieties averaged 21% mid-parent heterosis for yield (Marani and Sachs, 1966). Significant mid-parent heterosis in tobacco has been observed in hybrids resulting from crosses between Oriental and flue-cured varieties (Matzinger and Wernsman, 1968). Better-parent heterosis values of up to 51% were seen in hybrids between cigar filler tobacco lines from diverse backgrounds (Rao, 1995). Significant yield heterosis was observed from crosses between U.S. cultivars and Polish burley tobacco lines, with forty-seven out of fifty-five exhibiting yields higher than the best parent (Wilkinson and Rufty, 1990). Some crosses among burley tobacco genotypes have averaged 9.8% mid-parent heterosis for yield, with eleven of twenty-eight tested families exhibiting positive better-parent yield heterosis (Matzinger et al.,
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1971). Other hybrids between burley types have averaged 4.77% mid-parent heterosis (Legg et al., 1970).

Mid-parent heterosis values for various flue-cured tobacco traits have ranged from between 5% and 21% among lines selected for their unrelatedness (Murty, 1965). When cultivars from different tobacco classes were hybridized, significant levels of heterosis for yield (9%-10%) were observed, with the highest levels of intra-class heterosis being observed in the flue-cured tobacco crosses when cigar and oriental types were excluded (Aycock, 1980). A diallel among 8 flue-cured tobacco varieties representing 95% of the tobacco acreage at the time showed mid-parent yield heterosis levels between -7.45% - 9.50% (Matzinger et al., 1962), although other studies found negligible heterosis among crosses between flue-cured varieties (Matzinger and Mann, 1962).

Within tobacco, traits in cigar-wrapper tobacco have shown dominance effects (Dean, 1974), and within highly heterotic crosses of tobacco cultivars, additive, dominant, and epistatic effects were found to contribute to heterosis (Pooni, 1994). Studies in Turkish tobacco have suggested that the presence of significant general combining ability (GCA) and heterosis, together being indicative of a predominance of additive gene action (Kara and Esendal, 1995). Previous studies in heterosis of various traits in tobacco have shown significant environmental variation, with yield heterosis as much as doubling from one environment to another (Ahmed et al., 2016).

Recent studies on heterosis in rice have implied that a major contributing factor to observed heterosis is partial dominance effects in this crop species (Huang et al., 2015). This has led to the suggestion that heterotic effects could be fixed in inbred lines. Limited work in Arabidopsis has found that F₁ heterotic phenotypes can be fixed in later generations (Wang et al.,
2015), although this is not a phenomena commonly observed in crop species exhibiting significant heterosis levels. It has also been suggested by Burton and Brownie (2006) that heterosis levels in some crop species can be due to superior gene complementation and, as such, early generation heterosis measurements might be a predictor of the potential of favorable gene complementation of the parents. This reasoning would translate into cross combinations with highest heterosis levels also be capable of producing superior inbreds, as the complementary alleles can be fixed during inbreeding. Therefore, if observed early-generation heterosis is indicative of potential yield increases from combinations of superior alleles, then measuring heterosis in the F1 generation to screen cross combinations could provide insight on biparental combinations with the greatest potential to produce higher yielding inbred progeny and permit for a much better use of breeding program resources. By testing many crosses at the F1 stage, only the families with higher chances of superiority might be advanced through the inbreeding process, making for a more efficient breeding program. Additional methodology to improve parental selection in order to maximize the possibility of observing transgressive segregates for the trait of interest include the PopVar method of parental selection (Mohammadi et al., 2015). To utilize this program however, large amounts of genotypic and phenotypic data are required to predict mean, genetic variance, and the potential for superior progeny resulting from different parental crosses. Lacking the extensive genotypic information required, however, leaves breeding programs with few guiding resources to assist in parental selection.

Black shank is one of the most devastating diseases in tobacco production. The disease is caused by a soilborne oomycete, *Phytophthora nicotianae* (Rivera and Thiessen, 2017), and symptoms of black shank include leaf yellowing, plant wilting, and ultimate plant death (Thiessen, 2018). Using cultivars with resistance to black shank is one method of combatting
economic losses due to the pathogen. Resistance to race 0 is conferred by \textit{Php}, an introgression into \textit{N. tabacum} from \textit{N. plumbaginifolia}; and by \textit{Phl} an introgression into \textit{N. tabacum} from \textit{N. longiflora} (Valleau et al., 1960; Chaplin, 1962). Additional sources of resistance include the \textit{Wz} gene introgressed into \textit{N. tabacum} from \textit{N. rustica} that confers a high level of resistance to all currently known races of the pathogen (McCorkle et al., 2018). Polygenic disease resistance, such as that found originally in Florida 301 (Tisdale, 1931; Xiao et al., 2013), has been studied in order to develop a more durable resistance to the pathogen. Several QTL conferring black shank resistance of this polygenic nature have been identified (Vontimitta and Lewis, 2012). Breeding for increased disease resistance can be complicated by observed negative correlations between yield and cured leaf quality characteristics, however (Chaplin and Ford, 1958; Lewis, 2011).

The first objective of the following study was to determine heterosis levels within a collection of F\textsubscript{1} hybrids produced through crossing of a diverse set of U.S. flue-cured tobacco inbred lines to determine the potential for capitalizing on yield heterosis in a flue-cured tobacco breeding program. The second objective was to investigate the association, if any, between yield heterosis in the F\textsubscript{1} generation and the number of transgressive segregates among resulting derived inbred lines. A further aspect of this research was to examine the relationship between yield and polygenic black shank resistance, in order to determine if there is, in fact, a negative correlation between the two traits.

**Materials and Methods**

**Heterosis Experiment**

Fourteen Unites States flue-cured tobacco parental lines of historical and recent importance and of diverse origin (Table 1.1) were hybridized in diallel fashion during the
summer 2015 growing season at the Central Crops Research Station in Clayton, NC. Seed from reciprocal crosses were bulked.

In summer 2016, yield trials of the resulting ninety-one F₁ hybrids and fourteen corresponding parental lines were conducted at three North Carolina field locations: the Lower Coastal Plain Research Station near Kinston; the Upper Coastal Plain Research Station near Rocky Mount; and the Oxford Tobacco Research Station at Oxford. The experimental design at each location was an alpha-lattice design with four replications. Plots consisted of single 20 plant rows with 56 cm plant and 122 cm row spacing at the Upper Coastal Plain Research Station and the Oxford Tobacco Research Station, and 56 cm plant spacing and 112 cm row spacing at the Lower Coastal Plain Research Station. Tobacco plots were grown according to standard flue cured production practices for North Carolina. Plots were harvested in four primings and harvested leaves were flue-cured and weighed. Weights were translated into yield per plot (kg ha⁻¹) and adjusted for missing plants on a per plot basis using the method of Crews and Jones (1962). Cured leaf from each priming was assigned a standard USDA grade by a former USDA grader, and grade index, value 100 kg⁻¹ (USD) and value ha⁻¹ (USD) were subsequently calculated using 2017 flue-cured price index (North Carolina Cooperative Extension, 2017). Fifty gram composite samples were also collected and analyzed for percent total alkaloids and percent reducing sugars according to the methodology of Davis (1976).

The 91 hybrids and their parental lines were also evaluated for field black shank resistance in two soil-borne disease nurseries during the 2016 growing season: the Lower Coastal Plain Research Station and at the Upper Coastal Plain Research Station. Plots consisted of single 14-plant rows with 56 cm between plants and 122 cm between rows at the Upper Coastal Plain Research Station, and 56 cm between plants and 112 cm spacing between rows at the Lower
Coastal Plain Research Station. Disease data was recorded as the number of plants surviving after an initial stand count taken approximately 25 days after transplanting. Black shank incidence was recorded at the Lower Coastal Plain Research Station 22, 38, 51, 63, 69, 77, 88, 95, and 107 days after transplanting; and at the Upper Coastal Plain Research Station 27, 43, 56, 68, 74, 82, 90, 100, and 110 days after transplanting. Area Under the Disease Progress Curve (AUDPC) for each plot was calculated according to Madden et al. (2007), and a square root transformation was applied to the AUDPC values to reduce heterogeneity of error variances.

Mid-parent heterosis for measured traits was calculated as:

Mid-parent heterosis (%) = \((F_1 - MP)/MP*100\)

where, \(F_1\) = average performance of the \(F_1\) hybrid;

\(MP\) = average performance of the parental lines, \((\text{parent 1} + \text{parent 2})/2\).

Better parent heterosis for yield, quality, and black shank resistance was calculated as:

Better-parent heterosis (%) = \((F_1 - BP)/BP*100\).

where, \(F_1\) = average performance of the \(F_1\) hybrid;

\(BP\) = average performance of the superior parent (Fehr, 1991).

Data from the 2016 field experiments were analyzed using PROC MIXED of SAS 9.4 (SAS Institute, Cary, NC) for yield, value kg\(^{-1}\), value ha\(^{-1}\), grade index, percentage total alkaloids, percentage reducing sugars, and black shank AUDPC. Single degree-of-freedom CONTRAST statements were executed within PROC MIXED to compare each hybrid’s performance to the corresponding better-parent value, and also to compare each hybrid to the mid-parent value.
Development and Evaluation of Derived $F_{3:4}$ Families

All $F_1$ hybrids were self-pollinated in the greenhouse during the winter of 2015 to produce 91 $F_2$ populations. During the 2016 growing season, $F_{2:3}$ seed was collected from 100 individuals from each $F_2$ population at the Central Crops Research Station. Based upon preliminary data related to $F_1$ field performance, $F_{2:3}$ families from selected pedigrees were grown at an off-season nursery near Homestead, FL, and $F_{3:4}$ seed was collected for field testing during the 2017 growing season.

In order to investigate the relationship between $F_1$ hybrid and derived inbred line performance, the 2016 heterosis data was used to select $F_{3:4}$ families from six pedigrees for evaluation during the 2017 growing season, along with corresponding parental lines and their $F_1$ hybrids. The objective was to evaluate progeny for transgressive segregation and genetic variation that were derived from both heterotic and non-heterotic $F_1$ hybrids. Three cross combinations were selected because of their high mid-parent $F_1$ heterosis levels and high mean, while the other three cross combinations were selected because they corresponded to $F_1$ hybrid combinations exhibiting high means and low mid-parent heterosis (Figure 1.1).

During the 2017 growing season, forty-seven $F_{3:4}$ lines derived from each of the six selected families were evaluated in combination with their parental lines and corresponding $F_1$ hybrids for yield and quality determinations at the three research stations mentioned previously. The experimental design was an alpha-lattice design with two replications at each environment. Plot size and spacing were as previously described. Field resistance to black shank was also evaluated in soil-borne disease nurseries at the Kinston and Rocky Mount locations. Stand counts were taken as previously described at the Lower Coastal Plain Research Station at 15, 28, 37, 50, and 64 days after transplanting, and at the Upper Coastal Plain Research Station stand
counts were taken at 22, 37, 52, 64, and 79 days after transplanting. Area Under the Disease Progress Curve was calculated from the black shank stand counts according to Madden et al. (2007), and a square root transformation was applied to the AUDPC values to reduce heterogeneity of error variances.

Yield, quality, cured leaf chemistry, and black shank AUDPC data were analyzed as previously described using PROC MIXED of SAS 9.4 (SAS Institute, Cary, NC). Single degree-of-freedom CONTRAST statements were used to compare each hybrid to the corresponding better-parent value and also to compare each hybrid to the mid-parent value. Genetic variances for each population were also calculated using the COVTEST option of PROC MIXED.

PROC CORR of SAS 9.4 (SAS Institute, Cary, NC) was used to test for correlations between yield and black shank AUDPC values for F3:4 lines derived from the pedigrees Speight G-70 × L09-1305-1, Speight G-70 × NC61 Line D, and NC606 × NC925 that were tested during the 2017 growing season. Pedigrees that were segregating for the black shank resistance gene, \(W_z\), were excluded from this analysis because the strong effect of this gene overshadows the effects of partial effect black shank resistance QTL which we primarily interested in investigating for their relationship with yield.

PROC CORR was also used to test for correlations between 2016 and 2017 heterosis data, and the number of derived lines numerically better yielding than the higher yielding parental line (i.e. transgressive segregants) and the number of derived lines significantly \((P < 0.05)\) better yielding than the superior parent. Correlations between 2016 and 2017 mid-parent and better-parent heterosis levels and genetic variances of derived lines were also calculated.
Results

An analysis of variance on data from the 2016 field evaluation of ninety-one F1 hybrids and associated parental lines found significant differences amongst entries for yield, cash return, percent total alkaloids, percent reducing sugars, and black shank AUDPC (Table 1.2). Of the ninety-one hybrid combinations tested, sixty-six exhibited positive numerical better-parent heterosis for yield, with twelve hybrids yielding significantly greater than the better parent (Figure 1.2). Eighty-five hybrids exhibited numerically positive mid-parent heterosis for yield, with twenty-six performing significantly better than the average of the parental lines (Figure 1.3). Twenty-nine hybrids exhibited numerical better-parent heterosis for grade index, with one hybrid exhibiting a significantly higher grade index (Figure 1.4). Thirty-two hybrids exhibited numerical better-parent heterosis for value per cwt, but none were significantly higher than the better-parent (Figure 1.5). Seventy hybrids exhibited numerically better-parent heterosis for value ha\(^{-1}\), and three were significantly better than the superior parent (Figure 1.6). Twenty-three hybrids exhibited better-parent heterosis for total alkaloids, with two hybrids exhibiting significantly higher total alkaloid content than the corresponding highest parental lines (Figure 1.7). Twenty-nine hybrids exhibited better-parent heterosis for percent reducing sugars, with one hybrid exhibiting significantly higher reducing sugar content (Figure 1.8). Thirty-four hybrid combinations had negative better-parent AUDPC heterosis values, and two were significantly lower than the lowest parent (Figure 1.9).

Based upon the 2016 data, F\(_{3:4}\) families were derived from three F1 hybrids exhibiting high heterosis and high means: Speight G-70 × NC8640, Speight 168 Line A × NC8640, and Speight 168 Line A × OX2047 Wz/Wz. For comparison, F\(_{3:4}\) families were derived from three F1 hybrids exhibiting high means and low heterosis: Speight G-70 × L09-1305-1, Speight G-70 ×
NC61 Line D, and NC606 × NC925. These materials were tested during the 2017 growing season to investigate the relationship, if any, between F1 hybrid heterosis and the potential for increased transgressive segregation. An ANOVA carried out on these data indicated significant differences amongst entries for yield, grade index, value per cwt, cash return, total alkaloids, reducing sugars, and black shank AUDPC (Table 1.3). Across all six populations, a total of ninety-six derived lines yielded numerically higher than the superior parent, with ten derived lines performing significantly better than the better-parent (Figures 1.10 to 1.15). The populations derived from F1 hybrids exhibiting high heterosis had a total of sixty numerically higher yielding derived lines, with seven significantly higher, while the populations selected for low heterosis had a total of thirty-six numerically higher yielding derived lines, with three of them being significantly higher (Table 1.4). One hundred and one F3:4 lines exhibited black shank AUDPC values lower than the most resistant parent, with fifteen derived lines having significantly lower AUDPC values than the lower parent (Figures 1.16 to 1.21). Estimated genetic variances for all families were statistically significant at \( P < 0.05 \) (Table 1.5).

The correlations between 2016 measured better-parent heterosis and the number of lines per population with numerically greater yields than the better-parent, as well as the correlation between the 2016 better-parent heterosis level and number of significantly transgressive segregants were positive, at \( r = 0.59 \) and \( r = 0.23 \) respectively, but statistically insignificant with \( P \)-values of 0.22 (Figure 1.22) and 0.6648 (Figure 1.23), respectively. The correlation between 2017 better-parent heterosis levels and the number of lines numerically higher yielding than the better-parent, as well as the correlation between the 2017 better-parent heterosis levels and the number of significantly higher yielding derived lines had Pearson’s correlation coefficients of \( r = 0.88 \) and \( r = 0.86 \) with \( P \)-values of 0.02 (Figure 1.24) and 0.03 (Figure 1.25), respectively.
Correlations between 2016 mid-parent heterosis levels and the number of derived lines with yielding ability superior to the better-parent had an \( r \) of 0.64059 with a \( P \)-value of 0.1705 (Figure 1.26). The correlation between 2016 mid-parent heterosis levels and number of significant transgressive segregates had an \( r \) of 0.27749 with a \( P \)-value of 0.5944 (Figure 1.27). The correlations between 2017 mid-parent heterosis levels and the number of numerically higher-yielding derived lines, and between 2017 mid-parent heterosis levels and the number of significantly transgressive segregates had \( r \) values of 0.71610 and 0.47115 with \( P \)-values of 0.1095 (Figure 1.28) and 0.3456 (Figure 1.29), respectively.

Correlations between 2016 and 2017 better-parent heterosis and genetic variances amongst derived F\(_{3:4}\) families were found to be \( r = -0.12759 \) and \( r = -0.11811 \) with \( P \)-values of 0.8096 and 0.8237, respectively (Figures 1.30 and 1.31). The correlation between 2016 mid-parent heterosis and genetic variance amongst F\(_{3:4}\) families tested in 2017 had an \( r \) of -0.29445 with a \( P \)-value of 0.5711 (Figure 1.32). The correlation between 2017 mid-parent heterosis and genetic variance amongst F\(_{3:4}\) families tested in 2017 was \( r = -0.52912 \), with a \( P \)-value of 0.2804 (Figure 1.33).

We were also interested in testing the relationship between cured leaf yields and exhibited levels of polygenic black shank resistance as indicated by AUDPC. Results from derived lines from populations derived from Speight G-70 × L09-1305-1, Speight G-70 × NC61 Line D, and NC606 × NC925 indicated a significant, slightly positive linear relationship between yield and AUDPC value with \( r = 0.2631 \) and a \( P \)-value of 0.001 (Figure 1.34).
Discussion

The high frequency and level of heterosis observed in this study indicated the potential role that heterosis can play in future flue-cured tobacco breeding efforts for increased yield. Significant better-parent heterosis levels for yield of up to 35% were observed, with better-parent heterosis levels of up to ~ 40% for value per cwt. Among the tested cross combinations, a large percentage of hybrids exhibited phenotypes that were superior to the better-parent for yield, quality, and disease resistance. This contrasted somewhat with historical data on heterosis in flue-cured tobacco which reported a lower frequency of high-parent heterosis in smaller studies (Matzinger and Mann, 1962; Legg et al., 1970; Vandenberg and Matzinger, 1970). It is possible that previous work in flue-cured tobacco failed to properly capture the potential for heterosis among flue-cured tobacco cultivars, as many past studies likely used less diverse genetic materials (Matzinger and Mann, 1962), whereas the parental lines selected within this study were selected not only to be a diverse representation of flue-cured germplasm, but also included several parents of semi-exotic genetic backgrounds.

Hybrids are currently the predominant cultivar form for flue-cured tobacco worldwide because of the efficiency of deploying certain dominant disease resistance factors with reduced deleterious linkage drag effects (Valleau et al., 1960; Chaplin and Mann, 1978; Johnson, 1999), and because cytoplasmic male sterility provides a mechanism of intellectual property protection in the international tobacco seed market. The current research suggests that F₁ hybrids may also play a currently underappreciated role in positively affecting yield if parental lines are strategically selected to maximize heterosis.

Observed positive correlations between yield F₁ heterosis and the frequency of transgressive segregation in derived populations also indicated a potential use of heterosis
measurements in flue-cured tobacco as a predictive factor to assist in early identification of
cross combinations with increased potential to produce new cultivars with improved agronomic
performance. Previous heterosis research has suggested that heterotic yield increases due to
dominance effects could be fixed in the inbred lines (Huang et al., 2015), although debate has
occurred due to an absence of many examples of “fixing” heterosis in inbred lines. Our results
suggested that heterotic phenotype observed in the F1 generation can indeed be recovered in
inbred lines through observing transgressive segregates that are equivalent to F1 performance and
improved over the parental lines.

The analyses performed indicate that, while there were positive correlations between
heterosis in the F1 and increased transgressive segregation within the same year, this correlation
can be less significant when measuring F1 heterosis in the first year in order to inform selections
in the second year, possibly due to the heavy environmental influences on observed levels of
heterosis (Ahmed et al., 2016). As this was a sampling of only six populations, however, it is
possible that a larger sample of populations would provide increased clarity on the relationship
between heterosis in early generations and the potential for transgressive segregation. Although
the observed correlation between 2016 heterosis levels and 2017 derived line performance was
not statistically significant, a strong positive relationship was observed that could be useful when
applied as a screen when selecting for increased yield potential in a tobacco breeding program.

The parental lines tested also differed for the level of polygenic resistance to the black
shank pathogen. We were interested in evaluating the relationship, if any, between the level of
polygenic black shank resistance and cured leaf yields. Although several lines were identified
with greater levels of black shank disease resistance than the more resistant parent, there was a
general observed negative correlation between polygenic black shank resistance and yield. This
unfavorable relationship can compromise the objective of combining high yielding ability with high levels of polygenic black shank resistance.

In conclusion, we have shown here that F₁ hybrid heterosis *per se* can be used to significantly improve cured leaf yields in flue-cured tobacco cultivars. In addition, the presented data indicated a potential role for F₁ heterosis in predicting which of many cross combinations might have increased potential for yielding transgressive segregants. By making a diverse series of crosses within a breeding program and yield testing at the F₁ stage, a breeder would have information on which families to advance within a breeding program. Selecting for high parental mean and high F₁ heterosis, the selected populations would then be inbred and derived lines from these populations could be placed in yield trials for evaluation. The selected populations might have a higher percentage of transgressive segregates. This allows a breeder to maximize program resources by investing into populations most likely to result in high performing materials instead of using time and space on populations that have a low probability of producing improved lines. The data do point, however, to the negative potential influence that environmental variation can have on heterosis and on predictive ability.
References


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Chapter 2: Investigating the Genetic Control of Natural Parthenocarpy in

*Nicotiana tabacum* L.

Abstract

Parthenocarpy, or fruit development in the absence of pollination, is of interest in many horticultural crop species as a means to expand the production season, to reduce pollination requirements while maintaining yields, or to produce seedless fruits and vegetables. Natural parthenocarpy has been observed to occur in many species, although few genes involved in the control of this phenomenon have been identified. Parthenocarpy occurs in some genotypes of tobacco, a model species in plant biology research. The objective of this research was to gain insight on the genetic control of parthenocarpy in tobacco and to identify genomic regions and possible candidate genes responsible for the trait. Such information could contribute to innovation on engineering parthenocarpy in other crop species.

To examine the genetic control of natural parthenocarpy in tobacco, a diverse set of inbred lines and various F₁ hybrids were first evaluated for seed capsule development in the absence of pollination, a characteristic that was found to exhibit a high degree of dominance in these materials. Second, a doubled haploid population derived from a cross between cigar tobacco cultivar ‘Beinhart 1000’ and flue-cured tobacco cultivar ‘Hicks’ and segregating for the parthenocarpic trait was phenotyped and genotyped with SNP and microsatellite markers to identify genomic regions associated with parthenocarpy. A single region on linkage group 22 was found to be strongly associated with parthenocarpy in this population. Finally, because genes affecting phytohormone levels in floral structures have been implicated in parthenocarpy in some species, a subset of non-parthenocarpic lines were treated with gibberellic acid (GA₃) to determine if hormone application could induce parthenocarpic fruit development. Results did
not support a role for genes encoding for increased GA₃ levels inducing parthenocarpy in tobacco in our experiments.

**Introduction**

Natural parthenocarpy is defined as fruit development in the absence of fertilization, a phenomenon that can lead to the production of seedless fruit, and that has been observed to occur to different degrees in more than 98 different species (Gustafson, 1942; Selleri et al., 2010). Parthenocarpy is of great interest to growers of certain crops, as it reduces pollination requirements without sacrificing fruit production, and can also contribute fruit quality benefits in terms of increased processing efficiency and positively affecting consumer perception (Lipari and Paratore, 1987; Rotino et al., 1997; Yao et al., 2001; Liu et al., 2018). Parthenocarpy has been observed in tobacco, both as natural parthenocarpy (Gustafson, 1942) and as induced parthenocarpy through the application or injection of pollen extracts to the stigma or ovary (Gustafson, 1937). The initiation of fruit set and proper fruit development in non-parthenocarpic plants is dependent upon signals from the pollination and fertilization processes, although the nature of these signals is not clearly understood (Raghavan, 2003). In non-parthenocarpic plants, lack of pollination leads to degeneration and abscission of the ovary (Vercher et al., 1984; Vercher et al., 1989).

Parthenocarpy has been widely observed across many unrelated species from various geographical regions (Selleri et al., 2010), suggesting either an advantage to plant fitness or a lack of negative effects of seedless fruit production. Several studies have examined the benefits of parthenocarpic fruit in pest avoidance and damage. In yucca, for example, yucca moths were found to prefer seeded fruits. Encountering parthenocarpic fruits led to frequent plant
abandonment, feeding more on plants with high rates of seeded fruits, and leaving plants with high levels of parthenocarpy unscathed (Ziv and Bronstein, 1996). Preferential predation on plants with higher percentages of fertile fruit was also observed in studies on Juniperus osteosperma and bird predation on the fruits (Fuentes and Schupp, 1998). Conversely, in wild parsnip, preferential feeding by the parsnip webworm was observed on parthenocarpic fruit, where thirteen times as many parthenocarpic fruit were consumed as compared to seeded fruits, perhaps due to lower furanocoumarins levels (Zangerl et al., 1991). In other systems, such as predation by chalcidoid wasps in Pistacia terebinthus, pests are unable to distinguish parthenocarpic from fertile fruit (Traveset, 1993), but a higher percentage of parthenocarpic fruits translates into a reduced chance that a fertile fruit will be damaged.

It is believed that ovary development is a process that is partially dependent upon phytohormones, primarily auxins and gibberellins (GAs), that may be naturally emitted by pollen or the pollen tube on the stigma during the fertilization process (Gustafson, 1936; Muir, 1942; Gillaspy, 1993; Srivastava and Handa, 2005). Of the various auxin compounds, indole-3-acetic acid (IAA) is of primary importance to plants (Zhao, 2008). As observed in tomato, IAA causes initiation of ovary swelling and fruit enlargement, first through rapid cell division, and subsequently through cell expansion (Chevalier et al., 2014).

Phytohormone levels in naturally parthenocarpic fruits have been studied, and differential levels of gibberellic acids (GAs) through varying fruit development stages has been noted (Mapelli et al., 1978), with the primary difference being greater hormone levels in parthenocarpic fruits (Gustafson, 1939a; Talon et al., 1990; Talon et al., 1992). Differences in auxin content between non-parthenocarpic and parthenocarpic fruit has also been noted, with naturally parthenocarpic plants having higher levels of measured auxin within the fruit.
(Gustafson, 1939b). This observed increase in auxin content within the ovary is thought to be GA mediated (Serrani et al., 2008), although auxin has also been found in pollen extracts (Larsen and Tung, 1950). Increased levels of polyamines have also been observed in parthenocarpic fruits (Fos et al., 2001; Fos et al., 2003).

Differential hormone levels have been observed in parthenocarpic mutants prior to the stage in which fertilization would occur (Mapelli et al., 1978; Fos and Nuez, 1996; Fos and Nuez, 1997). Additionally, it has been noted that parthenocarpic fruits are typically smaller than pollinated fruits (Mapelli et al., 1978), possibly due to GAs associated with seed development, as seed GA-deficient mutants produce smaller fruits than those with GA-sufficient seeds (Groot et al., 1987). Cytokinin levels are also decreased in parthenocarpic mutants as compared to normal fruits (Mapelli, 1981), and differential protein expression has been observed between parthenocarpic and pollinated fruits (Barg et al., 1990), some of which have been hypothesized to be part of novel, hormone-independent parthenocarpic pathways within cucumber (Li et al., 2017).

An array of plant species have been treated with phytohormones such as GAs and auxins to successfully induce parthenocarpic fruit set (Gustafson, 1936; Gardner and Marth, 1937; Serrani et al., 2007), and early studies found application or injection of pollen extracts to cause variable parthenocarpic fruit development (Gustafson, 1937; Gustafson, 1938). Timing of hormone application is thought to be critical to the effective induction of parthenocarpic fruits (Sawhney, 1984). Applied GA3 has been observed to increase parthenocarpy levels in non-parthenocarpic citrus (Talon et al., 1992), as well as in Arabidopsis (Jacobsen and Olszewski, 1993; Chaudhury et al., 1994; Vivian-Smith and Koltunow, 1999) and tomato (Sawhney, 1984). In pear, the application of a specific combination of GAs to the ovary led to an increase in IAA
transporters and parthenocarpic fruit development, but no detectable increase in auxin biosynthesis (Liu, et al., 2018).

Several loci associated with the ability to produce parthenocarpic fruit have been identified in a number of crop species. Differential control of parthenocarpy among species has previously hypothesized to be due to differences in which floral tissues contribute to fruit development (Yao et al., 2001). Some of the first identified loci associated with parthenocarpy were reported in tomato: *pat, pat-2, pat-3*, and *pat-4* (Soressi and Salamini, 1975; Philouze and Maisonneuve, 1978a; Philouze and Maisonneuve, 1978b; Pecaut and Philouze, 1978; Lin et al., 1984). These loci are thought to influence GA levels in tomato ovaries (Mazzucato et al., 1998; Fos et al., 2000; Antognoni et al., 2002), although precise mechanisms are not understood. High *GA_{1}* and *GA_{20}* levels have been observed in *pat-2* mutants (Fos et al., 2003), and high *GA_{1}* and *GA_{3}* are found in *pat-3* and *pat-4* mutants (Fos et al., 2001).

Investigations of the inheritance of parthenocarpy in plants have been few. Recessive alleles are thought to control the parthenocarpic trait at the *pat, pat-2, pat-3*, and *pat-4* loci in tomato, while epistatic effects may be involved with parthenocarpy controlled by the *pat-3* and *pat-4* loci (Soressi and Salamini, 1975; Philouze and Maisonneuve, 1978a; Philouze and Maisonneuve, 1978b; Pecaut and Philouze, 1978; Lin et al., 1984). Parthenocarpy due to *MdPI* in apple (Yao et al., 2001), and parthenocarpy due to mutations in *procer* (Vardy et al., 1989a), *SILAGL6* (Klap et al., 2016) and *IAA9* (Wang et al., 2005) in tomato, are recessive traits. In contrast, parthenocarpy mediated by *SmARF8* in eggplant, is dominantly expressed (Du et al., 2016).

Silencing of genes encoding for GA2ox enzymes, which leads to an increased level of active GAs, has led to parthenocarpic fruit formation in tomato (Martinez-Bello et al., 2015).
Also in tomato, *procera*, a mutant with a mutation in a *DELLA* protein that confers seedless fruits, and also the *mp* gene of unknown function that interacts with *pat-2* (Vardy et al., 1989a,b) have been identified. Many of these previously studied parthenocarpic mutations have also been associated with pleiotropic negative phenotypic changes in leaf structure and growth habit. A mutation in the MADS-box gene *SlAGL6* in tomato however, allowed for the production of parthenocarpic fruit through an unknown mechanism with negligible phenotypic impacts (Klap et al., 2016).

Auxin response factor (ARF) or indole-3-acetic acid (IAA)-related genes have been widely studied for their connection to parthenocarpic fruit production. *Auxin Response Factor 8* (*ARF8*) appears to play a role in negative regulation of parthenocarpy, with *ARF8* mutants in *Arabidopsis*, tomato, and eggplant exhibiting parthenocarpic fruit development (Goetz et al., 2007; Du et al., 2016). Similar observations were made for *Auxin Response Factor 7* (*ARF7*) (De Jong et al., 2010), *IAA9* (Wang et al., 2005; Ueta et al., 2017), *IAAM* and *ROLB* (auxin synthesis and response genes) mutants in tomato (Martinelli et al., 2009). Silencing of *Aucsia* genes in tomato also causes parthenocarpic fruit development phenotype due to auxin accumulation (Molesini et al., 2009). Parthenocarpy has been observed in apple due to a mutation to the MADS-box transcription factor gene, *MdPI*, thought to be MADS-box gene that encodes for a transcription factor important to proper floral development (Yao et al., 2001). Downregulation of the MADS box gene *TM29* in tomato also induces seedless fruit set (Ampomah-Dwamena et al., 2002).

Parthenocarpy is of commercial interest in many horticultural crops and has been studied extensively in plants with environmentally sensitive pollination processes, such as tomatoes which exhibit poor fruit set under cold greenhouse conditions (Lipari and Paratore, 1987). It is
also of interest in many fruit tree crops such as apple or pear in which self-incompatibility is common, and in which orchard space must be devoted to pollinator varieties for proper fruit production (Yao et al., 2001; Liu et al., 2018). Seedless fruits can also be beneficial from processing and consumer desirability standpoints (Carmi et al., 2003). Seedless cultivars of eggplant, for example, have improved consumer perception because of reduced bitterness (Rotino et al., 1997). Rate of increased parthenocarpy has been a trait under conventional selection within some crop breeding programs (Sykes and Lewis, 1996), but most genetic approaches have focused primarily on the development of transgenic plants engineered to have reduced or increased expression to produce parthenocarpic fruits (Rotino et al., 1997; Carmi et al., 2003; Mezzetti et al., 2004; Yin et al., 2006; Ueta et al., 2017). This includes the engineering of parthenocarpic tobacco capsules through the use of the \( iaaM \) gene from \textit{Pseudomonas syringae} (Rotino et al., 1997).

Tobacco is a model species in plant biology, and increased insight on the genetic control of parthenocarpy in this species could prove to be useful in the innovation of techniques to confer parthenocarpy to other crop species. The purpose of this research was therefore to examine the genetic control of natural parthenocarpy in tobacco through evaluation of a set of diverse materials and selected F\(_1\) hybrids. Second, a major objective was to use mapping to identify the primary genomic region(s) controlling parthenocarpy in Beinhart 1000, a cigar tobacco cultivar, with the ultimate goal of identifying causal DNA sequence variation. Finally, given the known role of phytohormones on the control of parthenocarpy in other crop species, we investigated whether hormone application to tobacco flowers could induce the formation of parthenocarpic capsules.
Materials and Methods

To investigate the expression and additivity of genes affecting parthenocarpy in a diverse set of tobacco materials, fifteen lines and seven F1 hybrids (Table 2.1) were evaluated in a field environment during the summer of 2017. To determine the degree of parthenocarpy amongst these materials, flowers were emasculated prior to flower opening and anther dehiscence. After emasculation, stigmas were covered by a paper straw, folded at one end (Figure 2.1), to prevent inadvertent pollen transfer. Two flowers per genotype were emasculated per day, for five days, for a total of 10 emasculations per genotype. Tobacco seed capsules fully mature 25 to 30 days after pollination, and the degree of parthenocarpy was thus determined at least 40 days post-emasculuation.

Based upon preliminary evaluations, it was determined that cigar tobacco cultivar ‘Beinhart 1000’ was highly parthenocarpic, while old-line flue-cured tobacco cultivar ‘Hicks’ was found to be non-parthenocarpic. To investigate the genetic control of parthenocarpy in Beinhart 1000, a doubled haploid mapping population derived from a cross between Beinhart 1000 and Hicks was generated (Vontimitta and Lewis, 2012) and evaluated for parthenocarpy. Single plants from each of 118 doubled haploid lines, along with parental plants, were grown and evaluated in a greenhouse setting for parthenocarpy during the spring of 2017. Ten flowers per plant were emasculated (two flowers per day for five days) and stigmas of emasculated flowers were covered by paper straws folded on one end and secured in place using thin wire. For comparison, seven self-pollinations were also made by hand per plant. Capsule formation was monitored after pollination, and flower abscission without capsule formation was recorded. Presumed parthenocarpic capsules remaining on the plant were harvested at least 40 days post-emasculuation.
After harvest, the length of all parthenocarpic and self-pollinated capsules was measured using a caliper. PROC TTEST of SAS 9.4 (SAS Institute, Cary, NC) was used to analyze differences between parthenocarpic and self-pollinated capsule sizes. Presumed parthenocarpic capsules were then shelled and the contents seeded to test for the presence of viable seedlings. Because haploid seedlings can result from parthenogenesis at low frequencies in tobacco (Dunwell, 2010), we also grew any viable seedlings to maturity to test for haploidy (suggested by small, infertile flowers). For comparison, we also determined the frequency of haploidy due to parthenogenesis on parthenocarpic genotypes by pollinating them with a tobacco genetic stock homozygous for a 35S:PAPI transgene insertion that confers a purple seedling color and that permits for identification of maternal haploids at the seedling stage (Lewis and Rose, 2011).

To associate phenotypic variability for parthenocarpy with genetic variability, we genotyped the doubled haploid population with a set of 1898 polymorphic SNP markers differentiating Beinhart 1000 and Hicks using a custom 30K Infinium iSelect HD BeadChip SNP chip (Illumina Inc., San Diego, CA). Polymorphic SNPs, along with previously reported microsatellite markers (Vontimitta and Lewis, 2012) were used to map genomic regions affecting parthenocarpy using multiple approaches.

First, parthenocarpy was treated as a binary trait (zero parthenocarpic capsules observed versus at least one parthenocarpic capsule observed), and an assumed single controlling gene was mapped using R/QTL software (Broman et al., 2003) and the Kosambi mapping function with an error rate of 0.0001 and genome-wide thresholds for significant marker association being based upon 1000 permutations. Second, parthenocarpy was treated as a quantitative trait, with the phenotypic data being the number of observed parthenocarpic capsules out of ten. QTL analysis for parthenocarpy was carried out using R/QTL software (Broman et
al., 2003) and the Kosambi mapping function with an error rate of 0.0001, and genome-wide thresholds for QTL identification being based upon 1000 permutations. Finally, a third method involved a summation of the two separate models according to Holland and Coles (2011).

Because of the observed role of phytohormone accumulation, particularly gibberellic acid, in parthenocarpy in other plant species, we also carried out a small experiment to determine if gibberellic acid (GA3) application could cause parthenocarpy in ordinarily non-parthenocarpic doubled haploid lines. In a greenhouse in Raleigh, NC during the spring 2018, six non-parthenocarpic doubled haploids from the previously mentioned Beinhart 1000 × Hicks population were grown and treated with GA3 to test whether the application of GA3 could produce the parthenocarpic phenotype in non-parthenocarpic lines. After emasculation, a 5 µL drop of 1.0 µM GA3 in 5% (v/v) ethanol and 0.1% Triton X-100 was applied to the pistil of each flower and covered with a paper straw to protect from pollen contamination. A control of 5% (v/v) ethanol and 0.1% Triton X-100 was applied to emasculated pistils in the same manner. Two flowers a day over five days were treated with each application. Treated flowers were then monitored to determine whether the ovary dehisced without swelling, whether minor swelling occurred before the ovary dehisced and prior to full ovary maturity, or if mature capsules formed. Ovaries from treated flowers were then collected at least 40 day post-treatment and measured using a caliper. PROC TTEST of SAS 9.4 (SAS Institute, Cary, NC) was used to analyze differences in size between GA3 treated ovaries and the control.

Results

Field evaluation of a diverse series of 15 tobacco inbred lines for parthenocarpic potential found White Stem Orinoco, K326, K326 Php/Php, K326 Wz/Wz, TI 1068, Beinhart 1000, and
Florida 301 to be parthenocarpic; while synthetic tobacco [4x(N. sylvestris x N. tomentosiformis)], 400, Virginia Bright Leaf, Yellow Special A, Hicks, K346, Red Russian, and TN90 were non-parthenocarpic (Figure 2.2). Evaluation of selected F₁ hybrids between parthenocarpic and non-parthenocarpic lines suggested parthenocarpy to be a dominant, or partially dominant trait, as F₁ hybrids between parthenocarpic and non-parthenocarpic lines exhibited a tendency to produce slightly lower frequencies of parthenocarpic capsules from emasculated flowers (Figure 2.3).

Cigar tobacco cultivar Beinhart 1000 and flue-cured tobacco cultivar Hicks were found to be highly parthenocarpic (with eight of ten emasculated flowers producing parthenocarpic capsules) and non-parthenocarpic, respectively. In order to further investigate the genetic control of parthenocarpy in Beinhart 1000, a doubled haploid mapping population derived from a cross between the two lines was evaluated. High variability for parthenocarpy was observed among the 118 doubled haploid lines that were tested. Fifty-six doubled-haploid lines produced at least one parthenocarpic capsule (with a range of 1 to 10 parthenocarpic capsules) and sixty-two doubled-haploid lines failed to produce parthenocarpic capsules (Figure 2.4). Differences in size were observed between self-pollinated and parthenocarpic capsules (Figure 2.5). Parthenocarpic capsules were noticeably smaller when compared to self-pollinated capsules (Figure 2.6, Figure 2.7), with parthenocarpic capsules being, on average, ~17% smaller in length than the self-pollinated capsules. The mean length of parthenocarpic capsules was 15.11 mm, while the mean of self-pollinated capsules was 18.18 mm (Table 2.2).

Marker-trait analyses for data collected for the doubled haploid population using R/QTL with a 5% LOD threshold of 5.46 identified a single region on N. tabacum linkage group 22 of Bindler et al. (2007) to be strongly associated with parthenocarpy in the Beinhart 1000 × Hicks
doubled haploid mapping population (Figure 2.8). This identified region, with a peak at 70.3 cM, 6 cM to the right of marker Nt1AF1252, and 0.3 cM to the left of marker Nt1AD2392 (Figure 2.9) was most closely associated with parthenocarpy. The region was only statistically significant when parthenocarpy was analyzed as a binary trait and in the combined analysis.

To preliminarily investigate the potential that genetic differences for phytohormone accumulation might be responsible for parthenocarpy in Beinhart 1000, we evaluated the potential of exogenous gibberellic acid application at to the stigma to determine if parthenocarpic potential might be conferred in non-parthenocarpic genotypes. Application of GA₃ to the pistil resulted in slight swelling of the ovary in some cases, but not complete parthenocarpic fruit development. The average ovary size of GA₃ treated capsules was 10.03 mm, while the control ovaries averaged 9.4 mm (Table 2.3). While GA₃ ovaries were slightly larger in size than the controls, size distribution for both GA₃ treated ovaries and the control overlapped (Figure 2.9).

Discussion

Based on the field evaluation of a diverse set of lines and hybrids, it appears that the parthenocarpic trait in the studied tobacco genotypes is dominant or partially dominant. Hybrids with parthenocarpic lines, in all cases, displayed parthenocarpic capsule formation, although this was expressed to a slightly lesser degree (in terms of observed frequency) than in the parthenocarpic parental lines. This is in contrast to other identified loci or genes in tomato and apple which appear to act in a recessive manner or are involved in epistatic interactions to confer parthenocarpy (Soressi and Salamini, 1975; Philouze and Maisonneuve, 1978a; Philouze and Maisonneuve, 1978b; Pecaut and Philouze, 1978; Lin et al., 1984; Yao et al., 2001). This
finding suggests that the gene controlling parthenocarpy observed within tobacco germplasm may be associated with an alternative function.

Based on genetic analyses of the Beinhart 1000 × Hicks doubled haploid population, a single genomic region on linkage group 22 was found to be associated with the parthenocarpic phenotype in Beinhart 1000. Now that this region has been identified, it would be of interest to examine this region of the tobacco chromosome to determine what genes are in the vicinity of the strongly associated markers, and to begin to work towards identifying the causal gene for parthenocarpy in order to gain insight into the mechanism and evaluate whether the gene could be used as a novel source of parthenocarpy in other crop species.

Although the performed GA₃ treatment to tobacco flowers was inconclusive in the inducement of parthenocarpy, it has been observed in some plants that the type of GA applied is crucial to successful GA induction of parthenocarpic fruit, sometimes requiring a certain combination of GAs applied to the ovary to induce parthenocarpic fruit set (Liu et al., 2018). As such, applying different GAs at various concentrations could allow for inducible parthenocarpy to be observed in tobacco. Additionally, previous work in tobacco successfully induced parthenocarpic fruit production through the application of pollen extracts from different species. Pollen extracts have been found to contain auxin (Larsen and Tung, 1950), so it is possible that in the case of tobacco, auxin is of greater importance than GA in fruit development, and the identified genomic region may contain a gene playing a role in auxin biosynthesis, accumulation, or sensitivity.

The results from this research will be useful for future work to gain insight into the expression and genetic control of natural parthenocarpy in tobacco, with the hope that a novel genetic mechanism might be identified and potentially utilized in other crops. Parthenocarpic
fruit production is of interest in many horticultural crops, primarily to maintain yields in
inclement environmental conditions by reducing pollination requirements as well as to providing
a more appealing product to the consumer. Identifying the gene responsible for the
parthenocarpic phenotype in the model plant tobacco could assist in the use of the identified gene
in other species.
References


Tables and Figures
Table 1.1. List of fourteen parental genotypes used in the diallel crossing scheme.

<table>
<thead>
<tr>
<th>Parents</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Speight G-28</td>
<td></td>
</tr>
<tr>
<td>McNair 944</td>
<td></td>
</tr>
<tr>
<td>Speight G-70</td>
<td></td>
</tr>
<tr>
<td>NC82</td>
<td></td>
</tr>
<tr>
<td>K149</td>
<td></td>
</tr>
<tr>
<td>NC606</td>
<td></td>
</tr>
<tr>
<td>Speight 220</td>
<td></td>
</tr>
<tr>
<td>NC925</td>
<td></td>
</tr>
<tr>
<td>L09-1305-1</td>
<td></td>
</tr>
<tr>
<td>NC61 Line D</td>
<td></td>
</tr>
<tr>
<td>Speight 168 Line A</td>
<td></td>
</tr>
<tr>
<td>NC8640</td>
<td></td>
</tr>
<tr>
<td>Ox2047 Wz/Wz</td>
<td></td>
</tr>
<tr>
<td>Family #17</td>
<td></td>
</tr>
</tbody>
</table>
Table 1.2. Analysis of Variance for 2016 evaluation of hybrid and parental lines for yield, quality characteristics, and black shank AUDPC.

<table>
<thead>
<tr>
<th>Source</th>
<th>df*</th>
<th>Yield (kg ha⁻¹)</th>
<th>Grade index</th>
<th>Value ($ cwt⁻¹)</th>
<th>Cash return ($ ha⁻¹)</th>
<th>Total alkaloids %</th>
<th>Reducing sugars %</th>
<th>sqrt AUDPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment</td>
<td>2 (1)</td>
<td>11710690</td>
<td>* 9963</td>
<td>259708 ***</td>
<td>130788217 ***</td>
<td>168.33 ***</td>
<td>634.43 ***</td>
<td>158.39</td>
</tr>
<tr>
<td>Replication(Environment)</td>
<td>9 (2)</td>
<td>2494704 ***</td>
<td>186 **</td>
<td>4729 ***</td>
<td>40352036 ***</td>
<td>2.88 **</td>
<td>116.89 **</td>
<td>13.25 *</td>
</tr>
<tr>
<td>Block(Environment*Replication)</td>
<td>72 (24)</td>
<td>469735 ***</td>
<td>54 ***</td>
<td>1241 **</td>
<td>761204 ***</td>
<td>0.81 ***</td>
<td>34.48 ***</td>
<td>1.91 ***</td>
</tr>
<tr>
<td>Entry</td>
<td>104 (104)</td>
<td>480899 ***</td>
<td>56</td>
<td>1418</td>
<td>8802365 ***</td>
<td>0.62 ***</td>
<td>13.40 ***</td>
<td>3.53 ***</td>
</tr>
<tr>
<td>Entry x Environment</td>
<td>207 (104)</td>
<td>203073 **</td>
<td>49 ***</td>
<td>1293 ***</td>
<td>4470338 ***</td>
<td>0.12</td>
<td>5.38</td>
<td>0.92</td>
</tr>
<tr>
<td>Pooled error</td>
<td>980 (184)</td>
<td>149619</td>
<td>30</td>
<td>801</td>
<td>2774069</td>
<td>0.11</td>
<td>5.12</td>
<td>0.78</td>
</tr>
</tbody>
</table>

a: df for sqrtAUDPC listed in parentheses. *Significant at \( P < 0.05 \); **Significant at \( P < 0.01 \); ***Significant at \( P < 0.001 \)

Table 1.3. Analysis of Variance for 2017 evaluation of parental lines, F₁ hybrids, and derived F₃:₄ lines.

<table>
<thead>
<tr>
<th>Source</th>
<th>df*</th>
<th>Yield (kg ha⁻¹)</th>
<th>Grade index</th>
<th>Value ($ cwt⁻¹)</th>
<th>Cash return ($ ha⁻¹)</th>
<th>Total alkaloids %</th>
<th>Reducing sugars %</th>
<th>sqrt AUDPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment</td>
<td>2 (1)</td>
<td>65812576</td>
<td>66673.00 **</td>
<td>2035739.00 **</td>
<td>436285598 *</td>
<td>16.12</td>
<td>3447.74</td>
<td>11.20</td>
</tr>
<tr>
<td>Replication(Environment)</td>
<td>3 (2)</td>
<td>22516151 ***</td>
<td>677.62</td>
<td>27934.00 *</td>
<td>155950840 **</td>
<td>5.32</td>
<td>371.23</td>
<td>1.79</td>
</tr>
<tr>
<td>Block(Environment*Replication)</td>
<td>66 (44)</td>
<td>801404 ***</td>
<td>178.70 ***</td>
<td>5053.23 ***</td>
<td>14274210 ***</td>
<td>0.55</td>
<td>14.48</td>
<td>2.69 ***</td>
</tr>
<tr>
<td>Entry</td>
<td>299 (299)</td>
<td>559801 ***</td>
<td>119.55 ***</td>
<td>2606.73 ***</td>
<td>7760769 ***</td>
<td>0.48</td>
<td>8.82</td>
<td>4.99 ***</td>
</tr>
<tr>
<td>Entry*Environment</td>
<td>598 (299)</td>
<td>138312 *</td>
<td>60.25 ***</td>
<td>1484.40 ***</td>
<td>2918756 ***</td>
<td>0.16</td>
<td>5.50</td>
<td>0.80 *</td>
</tr>
<tr>
<td>Pooled error</td>
<td>831 (552)</td>
<td>116223</td>
<td>43.45</td>
<td>1065.21</td>
<td>2035027</td>
<td>0.15</td>
<td>5.22</td>
<td>0.67</td>
</tr>
</tbody>
</table>

a: df for sqrtAUDPC listed in parentheses. *Significant at \( P < 0.05 \); **Significant at \( P < 0.01 \); ***Significant at \( P < 0.001 \)
Table 1.4. Summary of population statistics from 2017 yield testing along with the 2016 F₁ heterosis level group selected for.

<table>
<thead>
<tr>
<th>Pedigree</th>
<th># lines numerically better than better parent</th>
<th># lines significantly better than better parent</th>
<th>Heterosis class (high/low)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speight G-70 x L09-1305-1</td>
<td>15</td>
<td>2</td>
<td>low</td>
</tr>
<tr>
<td>Speight G-70 x NC61 Line D</td>
<td>14</td>
<td>1</td>
<td>low</td>
</tr>
<tr>
<td>NC606 x NC925</td>
<td>7</td>
<td>0</td>
<td>low</td>
</tr>
<tr>
<td>Speight 168 Line A x NC8640</td>
<td>27</td>
<td>4</td>
<td>high</td>
</tr>
<tr>
<td>Speight 168 Line A x OX2047 Wz/Wz</td>
<td>15</td>
<td>0</td>
<td>high</td>
</tr>
<tr>
<td>Speight G-70 x NC8640</td>
<td>18</td>
<td>3</td>
<td>high</td>
</tr>
</tbody>
</table>
Table 1.5. Estimated genetic variance for yield for each derived population.

<table>
<thead>
<tr>
<th>Family</th>
<th>Heterosis Grouping</th>
<th>Estimate of Variance</th>
<th>Standard Error</th>
<th>Z-value</th>
<th>Pr &gt; Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC606 × NC925</td>
<td>Low</td>
<td>28469</td>
<td>11220</td>
<td>2.54</td>
<td>0.0056</td>
</tr>
<tr>
<td>Speight G-70 × L09- 1305-1</td>
<td>Low</td>
<td>101660</td>
<td>25270</td>
<td>4.02</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Speight G-70 × NC61 Line D</td>
<td>Low</td>
<td>60184</td>
<td>16751</td>
<td>3.59</td>
<td>0.0002</td>
</tr>
<tr>
<td>Speight 168 Line A × NC8640</td>
<td>High</td>
<td>42779</td>
<td>13285</td>
<td>3.22</td>
<td>0.0006</td>
</tr>
<tr>
<td>Speight 168 Line A × OX2047 Wz/Wz</td>
<td>High</td>
<td>48340</td>
<td>15226</td>
<td>3.17</td>
<td>0.0007</td>
</tr>
<tr>
<td>Speight G-70 × NC8640</td>
<td>High</td>
<td>73931</td>
<td>19327</td>
<td>3.83</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
Table 2.1 List of lines and hybrids tested in 2017 for parthenocarpy.

<table>
<thead>
<tr>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. sylvestris × N. tomentosiformis</em> (4N)</td>
</tr>
<tr>
<td>400</td>
</tr>
<tr>
<td>Virginia Bright Leaf</td>
</tr>
<tr>
<td>White Stem Orinoco</td>
</tr>
<tr>
<td>Yellow Special A</td>
</tr>
<tr>
<td>Hicks</td>
</tr>
<tr>
<td>K326</td>
</tr>
<tr>
<td>K326 <em>Php</em>/<em>Php</em></td>
</tr>
<tr>
<td>K326 <em>Wz</em>/<em>Wz</em></td>
</tr>
<tr>
<td>K346</td>
</tr>
<tr>
<td>TI 1068</td>
</tr>
<tr>
<td>Red Russian</td>
</tr>
<tr>
<td>Beinhart-1000</td>
</tr>
<tr>
<td>Florida 301</td>
</tr>
<tr>
<td>TN90</td>
</tr>
<tr>
<td>K326 × Beinhart-1000</td>
</tr>
<tr>
<td>Hicks × Beinhart-1000</td>
</tr>
<tr>
<td>K326 × Hoja Parado</td>
</tr>
<tr>
<td>Hicks × Hoja Parado</td>
</tr>
<tr>
<td>K326 × Hicks</td>
</tr>
<tr>
<td>Kicks × Florida 301</td>
</tr>
<tr>
<td>K326 × Florida 301</td>
</tr>
</tbody>
</table>
Table 2.2 Summary of capsule size measurements between parthenocarpic and self-pollinated capsules produced from PROC TTEST in SAS 9.4.  CL= Confidence Level; STD= Standard Deviation.

<table>
<thead>
<tr>
<th>Capsule type</th>
<th>Mean  mm</th>
<th>95% CL Mean</th>
<th>STD Dev</th>
<th>95% CL Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parthenocarpic</td>
<td>15.1176</td>
<td>14.971</td>
<td>1.4953</td>
<td>1.3986</td>
</tr>
<tr>
<td>Self-pollinated</td>
<td>18.1805</td>
<td>18.0856</td>
<td>1.3969</td>
<td>1.333</td>
</tr>
</tbody>
</table>

Table 2.3 Summary of capsule size measurements between capsules treated with GA₃ and the control from PROC TTEST in SAS 9.4.  CL= Confidence Level; STD= Standard Deviation.

<table>
<thead>
<tr>
<th>Capsule treatment</th>
<th>Mean  mm</th>
<th>95% CL Mean</th>
<th>STD Dev</th>
<th>95% CL Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA₃</td>
<td>10.0380</td>
<td>9.5611</td>
<td>1.8298</td>
<td>1.5489</td>
</tr>
<tr>
<td>control</td>
<td>9.4835</td>
<td>8.6948</td>
<td>2.6557</td>
<td>2.2028</td>
</tr>
</tbody>
</table>
Figure 1.1. Graph of percent mid-parent yield heterosis and average parental yield for ninety-one hybrids tested in 2016. Red dots = families selected for further testing. Red dots within black circle = populations selected for high parental mean and low mid-parent heterosis. Red dots within red circle = populations selected for high parental mean and high mid-parent heterosis.
Figure 1.2. Better-parent heterosis levels in percent for yield in F₁ hybrids tested in 2016. Blue bars indicate hybrids that performed significantly better than the better-parent at $P < 0.05$. 
Figure 1.3. Percent mid-parent heterosis levels for yield in F₁ hybrids tested in 2016. Blue bars indicate hybrids that performed significantly better than the mid-parent at $P < 0.05$. 
Figure 1.4. Percent better-parent heterosis levels for grade index in F₁ hybrids tested in 2016. Blue bars indicate hybrids that performed significantly better than the better-parent at $P < 0.05$. 
Figure 1.5. Percent better-parent heterosis levels for value per cwt in F₁ hybrids tested in 2016.
Figure 1.6. Percent better-parent heterosis levels for cash return in $ ha\(^{-1}\) in F\(_1\) hybrids tested in 2016. Blue bars indicate hybrids that performed significantly better than the better-parent at \(P < 0.05\).
Figure 1.7. Percent better-parent heterosis levels for percent total alkaloids in F1 hybrids tested in 2016. Blue bars indicate hybrids that performed significantly better than the better-parent at $p = 0.05$. 
Figure 1.8. Percent better-parent heterosis levels for percent reducing sugars in F₁ hybrids tested in 2016. Blue bars indicate hybrids that performed significantly better than the better-parent at $P < 0.05$. 
Figure 1.9. Percent better-parent black shank AUDPC heterosis values for F1 hybrids tested in 2016. Blue bars indicate hybrids that performed significantly better than the better-parent at $P < 0.05$. 
Figure 1.10. Least square means for yield in kg ha\(^{-1}\) for Speight G-70 × L09-1305-1 F3:4 derived lines and associated parental lines and the corresponding F\(_1\) hybrid. Red bars = parental lines; Black bars = hybrids; Blue bars = F\(_3:4\) derived lines; § = significantly different from high parent at \(P < 0.05\); h = positive better-parent heterosis.
Figure 1.11. Least square means for yield in kg ha$^{-1}$ for Speight G-70 × NC61 Line D F$_{3:4}$ derived lines and associated parental lines and the corresponding F$_1$ hybrid. Red bars = parental lines; Black bars = hybrids; Blue bars = F$_{3:4}$ derived lines; § = significantly different from high parent at $P < 0.05$; h = positive better-parent heterosis.
Figure 1.12. Least square means for yield in kg ha$^{-1}$ for Speight G-70 × NC8640 F$_{3.4}$ derived lines and associated parental lines and the corresponding F$_1$ hybrid. Red bars = parental lines; Black bars = hybrids; Blue bars = F$_{3.4}$ derived lines; § = significantly different from high parent at $P < 0.05$; h = positive better-parent heterosis.
Figure 1.13. Least square means for yield in kg ha\(^{-1}\) for NC606 × NC925 F\(_{3:4}\) derived lines and associated parental lines and the corresponding F\(_1\) hybrid. Red bars = parental lines; Black bars = hybrids; Blue bars = F\(_{3:4}\) derived lines; § = significantly different from high parent at \(P < 0.05\); h = positive better-parent heterosis.
Figure 1.14. Least square means for yield in kg ha$^{-1}$ for Speight 168 Line A × NC8640 F$_{3:4}$ derived lines and associated parental lines and the corresponding F$_1$ hybrid. Red bars = parental lines; Black bars = hybrids; Blue bars = F$_{3:4}$ derived lines; § = significantly different from high parent at $P < 0.05$; h = positive better-parent heterosis.
Figure 1.15. Least square means for yield in kg ha\(^{-1}\) for Speight 168 Line A × OX2047 Wz/Wz F\(_{3:4}\) derived lines and associated parental lines and the corresponding F\(_1\) hybrid. Red bars = parental lines; Black bars = hybrids; Blue bars = F\(_{3:4}\) derived lines; § = significantly different from high parent at \(P < 0.05\); h = positive better-parent heterosis.
Figure 1.16. AUDPC values for Speight G-70 × L09-1305-1 F$_{3:4}$ derived lines and associated parental lines and the corresponding F$_1$ hybrid. Red bars = parental lines; Black bars = hybrid lines; Blue bars = F$_{3:4}$ derived lines; § = significant at $P < 0.05$; l = lower than lowest parent.
Figure 1.17. AUDPC values for Speight G-70 × NC61 Line D F3:4 derived lines and associated parental lines and the corresponding F1 hybrid. Red bars = parental lines; Black bars = hybrid lines; Blue bars = F3:4 derived lines; § = significant at $P < 0.05$; 1 = lower than lowest parent.
Figure 1.18. AUDPC values for Speight G-70 × NC8640 F$_{3:4}$ derived lines and associated parental lines and the corresponding F$_1$ hybrid. Red bars = parental lines; Black bars = hybrid lines; Blue bars = F$_{3:4}$ derived lines; § = significant at $P < 0.05$; l = lower than lowest parent.
Figure 1.19. AUDPC values for NC606 × NC925 F₃:₄ derived lines and associated parental lines and the corresponding F₁ hybrid. Red bars = parental lines; Black bars = hybrid lines; Blue bars = F₃:₄ derived lines; § = significant at $P < 0.05$; l = lower than lowest parent.
Figure 1.20. AUDPC values for Speight 168 Line A × NC8640 F3:4 derived lines and associated parental lines and the corresponding F1 hybrid. Red bars = parental lines; Black bars = hybrid lines; Blue bars = F3:4 derived lines; § = significant at $P < 0.05$; l = lower than lowest parent.
Figure 1.21. AUDPC values for Speight 168 Line A × OX2047 Wz/Wz F3:4 derived lines and associated parental lines and the corresponding F1 hybrid. Red bars = parental lines; Black bars = hybrid lines; Blue bars = F3:4 derived lines; § = significant at $P < 0.05$; l = lower than lowest parent.
Figure 1.22. Correlation between observed 2016 percent better parent heterosis and number of derived lines numerically outyielding the better-parent.
Figure 1.23. Correlation between observed 2016 percent better-parent heterosis and number of derived lines significantly outyielding the better-parent.

$r = 0.23$

$P = 0.66$
Figure 1.24. Correlation between observed 2017 percent better-parent heterosis and number of derived lines numerically outyielding the better-parent.
Figure 1.25. Correlation between observed 2017 percent better-parent heterosis and number of derived lines significantly outyielding the better-parent.
Figure 1.26. Correlation between observed 2016 percent mid-parent heterosis and number of derived lines numerically outyielding the better-parent.

$r = 0.64$

$P = 0.17$
Figure 1.27. Correlation between observed 2016 percent mid-parent heterosis and number of derived lines significantly outyielding the better-parent.
Figure 1.28. Correlation between observed 2017 percent mid-parent heterosis and number of derived lines numerically outyielding the better-parent.
Figure 1.29. Correlation between observed 2017 percent mid-parent heterosis and number of derived lines significantly outyielding the better-parent.
Figure 1.30. Correlation between 2016 percent better-parent heterosis and genetic variance among derived F3:4 families.
Figure 1.31. Correlation between 2017 percent better-parent heterosis and genetic variance among derived F3:4 families.
Figure 1.32. Correlation between 2016 percent mid-parent heterosis and genetic variance among derived F3:4 families.
Figure 1.33. Correlation between 2017 percent mid-parent heterosis and genetic variance among derived F3:4 families.
Figure 1.34 Correlation between yield and AUDPC value of parentals, hybrids, and derived lines from populations Speight G-70 x L09-1305-1, Speight G-70 x NC61 Line D, and NC606 x NC925 in 2017.
Figure 2.1 Images of flowers showing technique used for making emasculations to test for natural parthenocarpy in tobacco. (A) Image of flower at the appropriate stage for emasculation. (B) Opened flower shown with anthers prior to pollen dehiscence. (C) Opened flower emasculated through removal of anthers. (D) Flower with stigma protected by paper straw and fastened with a wire tie.
Figure 2.2 Number of parthenocarpic capsules harvested from ten emasculated flowers per genotype in 2017 field study.
Figure 2.3 Number of parthenocarpic capsules harvested per genotype from 2017 field evaluation of hybrids and parental lines. Black bars = parental lines; Red bars = hybrids.
Figure 2.4 Number of parthenocarpic capsules (from 10 emasculated flowers) harvested per genotype from the Beinhart 1000 × Hicks doubled haploid mapping population. Black dots = double haploid lines; Red dots = parental lines.
Figure 2.5 Length of measured capsules in mm. Black dots = parthenocarpic capsules; Red dots = self-pollinated capsules.
Figure 2.6 Image of parthenocarpic and self-pollinated capsules harvested from a representative single doubled haploid line. Top row: parthenocarpic; Bottom row: self-pollinated.
**Figure 2.7** Distribution of capsule size in mm showing differences between parthenocarpic and self-pollinated capsules with overlaid normal and kernel density estimation; boxplots of parthenocarpic and self-pollinated capsules.
Figure 2.8 Top: LOD scores for region identified to be associated with parthenocarpy in the Beinhart 1000 × Hicks population. Black line = total LOD score; Blue line = LOD score for binary trait; Red line = LOD score for quantitative trait. Dotted lines = α of 0.1; Solid lines = α of 0.05. Bottom: Detailed map showing markers present within the region of significant LOD scores.
Figure 2.9 Distribution of capsule size in mm showing differences between capsules treated with GA$_3$ and the control, with overlaid normal and kernel density estimation; boxplots of parthenocarpic and self-pollinated capsules.