ABSTRACT

DRAKE, KATHERINE. Genetic Approaches to Reduce Production Costs and Chemical Applications to Flue-Cured Tobacco. (Under the direction of Dr. Ramsey Lewis).

Tobacco is an economically significant crop throughout the world. In 2010, 5.7 billion kg of tobacco were produced worldwide, a 2.8 percent increase from 2009. The United States produced 316 million kg of tobacco in 2009 with a value of value of $1.5 billion. About 44% of total production, 138 million kg, was exported. As the overall demand for tobacco remains stable or increases, tobacco production will likely continue to be a sustainable source of income for many farmers in the Southeastern United States if production practices can keep practices competitive with other countries.

Strategies to reduce production costs and to comply with stringent chemical residue regulations are needed in all tobacco-producing regions. One of the most attractive and effective ways to do this is through genetic approaches. Disease management and sucker control are two significant inputs in tobacco production, both of which rely on multiple chemical applications during the growing season. This research had two major objectives. The first involved investigation of a potentially novel source of genetic variation affecting black shank resistance and the second involved investigation of a transgenic method for eliminating sucker production in tobacco.

Black shank, caused by *Phytophthora nicotianae*, is typically one of the most important pathogen affecting tobacco production in the US. Several recently commercialized flue-cured tobacco hybrids possess a genomic region designated as *Wz* that was derived from a Zimbabwean breeding line called ‘WZ.’ This region was reportedly introgressed from *N. rustica*. The effect of this genomic region against multiple races of the black shank pathogen

has not previously been investigated in a systematic way. A doubled haploid (DH) mapping population of 71 lines was generated from a cross between WZ and the black-shank susceptible cultivar, 'NC 55.' A BC_1F_1 population also was generated using the cross WZ/Hicks//Hicks, where 'Hicks' is a highly susceptible flue-cured tobacco cultivar. The DH population was evaluated for resistance to *P. nicotianae* in six field environments and by using race-specific inoculations in growth chambers. The BC_1F_1 population was tested for segregation of resistance to race 0 of *P. nicotianae* using growth chamber inoculations. Both populations also were genotyped using AFLP markers found to be polymorphic between NC55 and WZ. Results suggested the introgressed *N. rustica* region has a large effect on resistance to race 0 and race 1 of the black shank pathogen. Seven AFLP markers of *N. rustica* origin were found to cosegregate and were linked with the introgressed black shank resistance factor. This genetic variation and associated markers may be of value for breeding for black shank resistance.

Tobacco exhibits exceptionally strong apical dominance. Molecular signals from the shoot apical meristem mediate a hormonal environment that effectively inhibits outgrowth of lateral buds. Upon decapitation (topping), this signal is lost, enabling the formation of new shoots at one or more leaf axils. Because of negative relationships between development of axillary meristems and tobacco leaf quality, chemicals such as maleic hydrazide (MH) and flumetralin are administered to topped tobacco plants to prevent outgrowth of axillary meristems, or suckers. In *A. thaliana*, the gene *BRANCHED1* was previously shown to be involved in the suppression of lateral branching. A similar gene has the same effect in tomato. A transgenic approach involving over-expression of the *A. thaliana* TCP transcription factor, *BRANCHED1*, was investigated for its potential to suppress sucker

growth in tobacco. Plants of cultivar ‘K326’ were transformed with the *BRANCHED1* gene under the control of the CaMV 35S constitutive promoter. Field testing of R_1 plants did not reveal the desired phenotype of reduced suckering after topping.

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Genetic Approaches to Reduce Production Costs and Chemical Applications to Flue-Cured Tobacco

by
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DEDICATION

To my papa, AJ Drake, II for giving me an appreciation and respect for North Carolina agriculture, especially tobacco farming.

BIOGRAPHY

Katherine Elizabeth Drake, daughter of Paul and Melissa Drake was born in 1987 in Pinetops, NC, a small, rural town in Edgecombe County. She was raised on a family farm that grows tobacco, cotton, and American beachgrass, and also raises hogs and cattle. Katherine spent her summers “putting in” tobacco and working with livestock. After graduation from SouthWest Edgecombe High School in 2005 she went on to North Carolina State University. She graduated in 2009 with a Bachelor’s degree in Polymer and Color Chemistry and a minor in Crop Science. While still an undergraduate, she met Dr. Ramsey Lewis and began working in the tobacco breeding lab where an interest in plant breeding was discovered. In 2009 she was admitted as a graduate student in the NCSU Crop Science department and began a Master’s of Science under the direction of Dr. Lewis. Katherine plans to continue to work in the Lewis lab after graduation.

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Chapter 1: Literature Review

INTRODUCTION

Tobacco Classification

The genus *Nicotiana* includes 76 naturally occurring species and is the sixth largest genus in the family Solanaceae. The majority of the species are native to the Americas and Australia, with only one species inative to Africa. While there are many different *Nicotiana* species, *N. tabacum* is the only species of major commercial value in the United States (Knapp et al., 2004).

Commercial tobacco, *N. tabacum* L., is a 48-chromosome (n=24) allotetraploid species that likely resulted from hybridization of two *Nicotiana* species, *N. sylvestris* and *N. tomentosiformis*, followed by chromosome doubling. Both progenitor species have 24 chromosomes and each contributed 12 pairs to *N. tabacum* (Wernsman and Matzinger, 1980). Chromosome homology, isoenzyme patterns, isoelectric analysis of Fraction 1 protein, and plastid and mitochondrial genome sequencing demonstrate that *N. sylvestris* was the maternal parent and contributor of the cytoplasm (Gray et al., 1974; Chase et al., 2003), while a species closely related to modern-day *N. tomentosiformis* was the paternal parent (Lim et al., 2000; Murad et al., 2002).

The progenitor species of *N. tabacum* are widely diverged and a number of intergenomic translocations have occurred since the initial speciation event (Lim et al., 2004; Bindler et al., 2011). Intergenomic translocations may arise to restore fertility of a polyploid (Leitch and Bennett, 1997). Despite the two genomes of *N. tabacum* being widely diverged, there are probably still many genes that are highly conserved, resulting in a great deal of genetic redundancy. Over time, these two progenitor genomes have become interdependent

as indicated by the non-viability of most nullisomics (plants missing both copies of a specific chromosome) (Wernsman, 1999).

Market Classes of Tobacco

Commercial tobacco is sub-divided into classes based on production and curing methodologies. The major classes include flue-cured, burley, Maryland, dark air-cured, cigar filler and wrapper, fire-cured, sun-cured, and Oriental. In the United States, the majority of the acreage is occupied by flue-cured and burley types. Flue-cured tobacco is harvested over time and is cured in closed barns where the heat, temperature, and moisture are precisely controlled. The cured leaf is yellow to orange in color and has high concentrations of reducing sugars and moderate concentrations of nicotine (Wernsman and Rufty, 1987). Burley tobacco appears lighter green to yellow in color due to the presence of a set of double recessive genes affecting chlorophyll content and plant color (Clausen and Cameron, 1944). Burley tobacco is harvested by cutting the plant at the ground level and hanging the entire plant for air-curing in open barns. This results in reddish-brown leaves that have low concentrations of reducing sugars and higher concentrations of nicotine (Wernsman and Rufty, 1987).

Botanical Characteristics

Tobacco is naturally a perennial plant in warm, tropical habitats, but is cultivated as an annual crop in the United States. It is composed of a single erect stem with a terminal inflorescence. Leaves originate from this stem in a spiral pattern. They are typically oblong and ovate in shape but there are some small differences between classes and even cultivars. Flue-cured cultivars have leaves that range from 18 decimeters^2 at the lower nodes to 12

decimeters^2 at the upper most nodes. Both leaf and stem surfaces are typically covered with trichomes that produce exudates. These exudates give the plant a sticky feel and are composed of precursors to compounds that give rise to the distinctive aroma and flavor of the cured leaf. There is also evidence of exudates providing resistance to certain insects (Tso, 1999).

Each leaf axil of the stalk contains three axillary buds. Because tobacco exhibits apical dominance, these axillary buds typically remain dormant as long as the apical inflorescence remains intact. In conventional flue-cured tobacco production, the apical inflorescence is removed in the button stage (referred to as ‘topping’) to prevent formation of a seed head. This allows resources to be allocated to growth in leaf tissues as opposed to reproductive development, thus increasing yield and providing more desirable leaf chemistry. Removal of the inflorescence alleviates apical dominance and leads to development of the lateral meristems known as ‘suckers’. If allowed to grow, these suckers develop into large branches, each with an inflorescence (Tso, 1999).

The top inflorescence is capable of producing hundreds of flowers. *N. tabacum* flowers are typically pink in color, although they can range from white to red. The flowers are perfect and easily manipulated by hand, making both self and cross-pollinations convenient. Naturally occurring cross-pollination frequencies are typically less than five percent, but this may vary with the environment and cultivar. Cross-pollination can occur due to insects, but pollen is not wind borne. Pollination of an individual flower can yield up to 3000 seed (Wernsman and Rufty, 1987).

Economic Importance

Tobacco is an economically significant crop throughout the world. In over 100 different countries, farmers depend on tobacco as a source of income and there are many others who depend on the processing, manufacture, and distribution of tobacco for employment (Davis and Nielsen, 1999). In 2010, 5.7 billion kg of tobacco were produced worldwide, a 2.8 percent increase from 2009 (Universal Leaf Tobaco Company, Inc, 2011). The United States produced 316 million kg in 2009, with a production value of $1.5 billion. Roughly 50 percent, 138 million kg, was exported. While the domestic demand for tobacco is declining, global demand is increasing (Brown, 2011). As the overall demand for tobacco increases, tobacco production will likely continue to be a sustainable source of income to many farmers worldwide.

TOBACCO BREEDING

History

The goal of any plant breeding program is to develop cultivars desired by growers and manufacturers. Tobacco breeding programs seek to develop cultivars that produce high yields of cured leaf with acceptable quality and resistance to multiple diseases. Additionally, over the last five years, there has been an interest in the use of plant breeding as a component of harm-reduction strategies. All new cultivars must meet a set of standards determined by the industry that takes into consideration cured leaf chemical composition, physical quality, and smoke flavor. Moreover, in order to be popular with growers, a new cultivar should minimize potential for loss due to diseases, provide acceptable yields of high quality cured leaf, and be easy to handle and cure (Legg and Smeeton, 1999).

The major tobacco classes are partially the result of farmer selection within broad germplasm pools. In the 1930's, the United States Department of Agriculture (USDA) initiated plant breeding programs for flue-cured and burley types in order to increase levels of disease resistance in available cultivars (Valleau, 1947). Experimental stations and public universities in tobacco growing states followed the USDA's lead and began their own breeding programs around the same time. By the 1940's, private tobacco breeding programs were initiated and quickly released a number of varieties that became very popular (Wernsman and Rufty, 1987). Today, the majority of tobacco breeding work is being conducted at a few public universities and within the private sector. Over the last several years, cigarette manufacturing companies have begun to allocate resources to their own breeding programs.

Breeding efforts have contributed to significant improvements in disease and pest resistance. There are sources of resistance available for some of the most common problems in tobacco, such as black shank, Granville wilt, tobacco mosaic virus (TMV), and root-knot nematodes (Valleau, 1947). Very significant increases in yield also were realized until the 1980's, when genetic gain for this characteristic slowed. This may be related to the strong negative correlation seen between yield and nicotine concentrations. If nicotine levels fall below the minimum standards set by the industry, high-yielding experimental cultivars cannot be released commercially (Wernsman, 1999).

Sources of Genetic Resistance

Resistance to many diseases affecting tobacco cannot be found within the flue-cured tobacco class, so breeders have pursued incorporation of resistance from other market classes

or introgression from other *Nicotiana* species. Resistance to black shank, Granville wilt, and root knot nematodes has been obtained from wide intraspecific crosses, while interspecific crosses were used to enhance resistance to black shank, blue mold, TMV, and tomato spotted wilt virus (TSWV) (Wernsman, 1999). The intraspecific type of black shank resistance was transferred from the cigar cultivar Florida 301 (Tisdale, 1931), while the interspecific type of resistance was transferred from *N. longiflora* and *N. plumbaginifolia* (Valleau et al., 1960; Apple , 1962; Chaplin, 1960). Granville wilt resistance was largely derived from the *N. tabacum* accession TI 448A (Sisson and Wernsman, 1992), and root knot nematode resistance from the *N. tabacum* accession TI 706 (Slana et al., 1981). Other interspecific sources of resistance include blue mold resistance from *N. debneyi* (Clayton et al., 1967), TMV resistance from *N. glutinosa* (Holmes, 1936), and TSWV resistance from *N. alata* (Laskowska and Berbec, 2005).

While resistance from wild relatives has been introgressed into *N. tabacum*, most gene transfers were not easy. In flue-cured tobacco, there have been many problems associated with the wide intraspecific and interspecific crosses. Germplasm derived from intraspecific crosses often exhibited leaf quality and smoke flavor problems and many generations of backcrossing were required to eliminate these problems. Interspecific crosses are problematic because adequate homology often does not exist between chromosomes from *N. tabacum* and those from many of the other *Nicotiana* species. Since the chromosomes often do not pair correctly, it is often even difficult to obtain viable progeny from such crosses. Moreover, if the desired gene is transferred, an entire block of genes is commonly transferred from the donor species instead of a single gene. Reducing the size of alien

chromatin blocks often proves to be very difficult. Undesired linked genes often have substantial negative effects on quality, yield, and plant type and many times these negative agronomic effects are so great that the resistance is not commonly used in tobacco breeding (Wernsman, 1999).

Inheritance

Early studies on quantitative traits in tobacco indicated that most quantitatively inherited traits are controlled in an additive genetic fashion (Robinson et al., 1954). Additive variance is generally high in segregating tobacco populations while dominance and epistatic variances are generally low, but not zero (Wernsman and Rufty, 1987). In an investigation of genetic variability in a Hicks Broadleaf x Coker 139 population, Matzinger et. al. (1960) found additive variance to comprise the majority of the genetic variance for yield, leaf width, percent nicotine, and percent total alkaloids. In contrast, dominance variance comprised the major portion of genetic variance for days to flower and number of suckers. Furthermore, it was reported that the major component of genetic variation affecting leaf length was found to be additive x additive epistatic variance.

Little heterosis has been exhibited in tobacco and the performance of F_1 hybrids is typically near the midparent value for most important traits. Tobacco also exhibits little inbreeding depression. Consequently, breeders historically produced pure-line cultivars rather than hybrids (Wernsman and Rufty, 1987). Recently, however, hybrids have become very common in the marketplace. The production of hybrid cultivars allows for varietal protection and the efficient deployment of certain disease resistance genes. Cytoplasmic male sterile F_1 hybrids prohibit persons from increasing seed of the variety, and hybrids also

facilitate deployment of dominant disease resistance genes from wild relatives. The full level of disease resistance can often be achieved in heterozygous F_1 hybrids, yet the deleterious linkage drag effects on yield and quality can be dramatically reduced (Wernsman, 1999).

Breeding Methods

Given the ability of tobacco to be easily self- or cross-pollinated, a number of breeding methods have been used successfully in improving the crop. Early work on domestication and improvement of *N. tabacum* was the result of mass and pure-line selection. Genetic variants were common in these populations that could be visually selected, eventually giving rise to the different market classes. Almost all of the earliest cultivars in these classes were probably the result of farmer selection (e.g. Hicks Broadleaf, Barnett Special, Adkins Madole). Over time, a better understanding of heredity led to the development of science-based breeding methods that enhanced tobacco improvement. Occasionally, however, mass selection is still used in some population development research (Wernsman, 1999).

Like other self-pollinated crops, pedigree selection is one of the most common methods used for variety development. Pedigree selection is useful for combining desirable traits found in two or more parents into new lines. The method involves initial development of a variable population (from the cross of two lines or from a random-mated population) and then selecting the best plants to be self-pollinated. The seeds resulting from each of the selected plants are typically kept together to maintain a family structure. In the next generation, the best families can be selected, followed by selection of the best individual plants in each of the best families. This is repeated for several generations until a near

homozygous state is reached (Fehr, 1993). The pedigree method partitions the genetic variability of the parents into differences among recombinant inbred families. As homozygosity is approached, the variability within families decreases while that between families increases allowing the breeder to effectively select the best families (Wernsman and Rufty, 1987).

Backcrossing

The backcross method is commonly used to transfer a simply inherited character to an already proven cultivar that may be deficient in that specific area. The proven cultivar or advanced breeding line (known as the recurrent parent) is crossed to a line carrying the trait of interest. The progeny, with the trait of interest, are then crossed back to the recurrent parent to produce the next generation. With each generation and cross back to the recurrent parent, the progeny become more similar to the recurrent parent but still maintain the desired trait that the recurrent parent was originally lacking (Fehr, 1993). This method has proved useful in transferring disease resistance from wild relatives as well as transferring simply inherited resistance traits between proven lines.

Recently, a modified backcrossing program has been introduced in tobacco breeding. The *Arabidopsis thaliana* gene *FT* (*FLOWERING LOCUS T*), which regulates the induction of early-flowering in plants, was over-expressed in tobacco via transgenic methods. The over-expression of *FT* reduces the time from germination to flowering from roughly 120 days to about 39 days. These plants, termed “early flowering” produce normal flowers which can be crossed with pollen from the donor parents, shortening the amount of time needed to achieve five generations of backcrossing from 1150 days to 604 days, and thus allowing

breeders to develop cultivars more quickly and efficiently. In the final generation of backcrossing, the *FT* gene is selected against, resulting in normal flowering plants with the desired gene of interest (Lewis and Kernodle, 2009).

Doubled Haploids

The production of haploid plants followed by chromosome doubling to produce "doubled haploids" provides a rapid method for achieving completely inbred lines. Tobacco is an ideal crop for production of doubled haploids because it is easy to identify haploid plants and then subsequently double them (Legg and Smeeton, 1999). Haploid plants only have one set of chromosomes (n=24) and are therefore sterile. Their chromosomes must be doubled (to $2n$=48) in order to generate a plant that produces viable seed. This doubling results in a plant that is theoretically homozygous at every locus and thus the completely inbred state is reached in a single generation.

Haploid plants can be produced from microspores via *in vitro* anther culture or from egg cells by a technique involving hybridization of tobacco with pollen from *N. africana*. Gynogenic tobacco haploids are more commonly used in breeding programs because of reduced yields associated with anther culture-derived haploids (Wernsman et al., 1989). The *N. africana* method is used to identify parthenogenic (reproduction without fertilization) haploids. Parthenogenic haploids occur in many plant species with the frequency of about one in a thousand. The *N. tabacum* x *N. africana* cross produces viable seeds that germinate, but the true F_1 hybrid seedlings die quickly after germination. The parthenogenic haploids remain as they do not possess the *N. africana* background allowing for easy identification of haploid plants (Wernsman, 1999).

Haploid plants can be doubled via *in vitro* tissue culture methods or with colchicine treatment. While colchicine is quite popular in other crops, its use in tobacco is limited due to low probability of success. The midvein tissue culture method of aged leaves described by Kasperbauer and Collins (1972) is more common as it provides a higher rate of success. This doubling is induced by the tissue culture process without the need for colchicine.

There are a number of advantages to using doubled haploids in a tobacco breeding program. The methods described above allow for the quick production and identification of haploid plants and subsequent doubling. Completely homozygous progeny can be obtained from a heterozygous population or F_1 plant in less than a year. This greatly reduces the time it takes to develop a cultivar by three or four years (Wernsman and Rufty, 1987), which is an obvious advantage. Doubled haploids are also advantageous in terms of recurrent selection programs. Use of doubled haploids can increase the efficiency of selection because the amount of additive variance in a doubled haploid population is twice that of a random mated population and there is no dominance variance in doubled haploid populations. Recurrent selection methods that use doubled haploids have an increased efficiency, as additive variance is the variance upon which selection is dependent (Griffing, 1975). In a simulation study by Choo and Kannenberg (1978), mass selection with doubled haploids was about 1.4 times more efficient than mass selection using traditional methods. In addition, doubled haploids are invaluable to genetic mapping and genomics. They are ideal for the production of mapping populations because they contain no residual heterozygosity. In tobacco, doubled haploid populations have been used to identify markers associated with both black shank resistance provided by the *Ph* gene (Johnson et al., 2002) and blue mold resistance (Milla et

al., 2005). They were also used to map quantitative trait loci (QTL) associated with bacterial wilt resistance (Nishi et al., 2003) and black shank resistance (Vontimitta and Lewis, 2012).

One disadvantage of using doubled haploids to develop cultivars is the amount of undesirable genotypes carried along before one is able to evaluate genotypes for plant type and other important quantitative traits. Detached leaf tests can be used to screen haploid plants for resistance to TMV, potato virus Y (PVY), and *Meloidogyne incognita* (root knot nematode) prior to the chromosome doubling process (Rufty et al., 1987). In addition, the efficiency of the doubled haploid process can be improved by using molecular markers to screen haploids for certain resistance genes before doubling. Molecular markers have been identified that are closely linked to genes conferring resistance to race 0 of the black shank pathogen (Johnson et al., 2002), TMV (Whitham et al., 1994), blue mold (Milla et al., 2005), root knot nematodes (Yi and Rufty, 1998), wildfire (Yi et al., 1998), and black root rot (Bai et al., 1995). Using molecular markers and detached leaf tests for screening prior to chromosome doubling helps decrease the number of undesirable plants that are doubled, thus saving time and money as the doubling process requires a significant amount of resources. While haploids can be screened for simply inherited traits, there are currently no tools for screening for more complex traits like yield or plant type due to morphological differences between haploid and diploid plants and because traits with low heritability are difficult to evaluate on a single-plant basis.

Transgenics

Novel genes from other species can be incorporated into *N. tabacum* via transgenic approaches. This is an attractive breeding procedure because the gene pool from which one

can transfer desired traits becomes essentially limitless. Given the amenability of tobacco to *in vitro* tissue culture methods, it quickly became a model organism for plant transformation (Horsch et al., 1985) and hundreds of genes have been transferred to tobacco (Wernsman, 1999).

While *N. tabacum* can be transformed using a number of different methods, the *Agrobacterium tumefaciens* vector system is the most widely used method. *A. tumefaciens* contains a large tumor-inducing (Ti) plasmid in addition to the primary bacterial chromosome. In normal *A. tumefaciens* infection, after attachment to the cell walls of wounded cells, the *Agrobacterium* bacterium transfers part of its Ti-plasmid, known as the T-region, into the host plant where it becomes integrated into the genome as T-DNA. Several virulence genes are also located on the Ti-plasmid that facilitate plant cell recognition and T-DNA transfer. In addition, the T-DNA carries oncogenes that induce the abnormal growth of cells at the infection site which results in the formation of a crown gall tumor (Weising et al., 1988).

The natural *A. tumefaciens* system has been modified in order to transfer foreign genes of interest into plant cells. A binary vector system was developed in which there are two plasmids in *Agrobacterium*. A large plasmid carries the set of virulence genes required for infection, while a smaller plasmid carries the T-DNA and border sequences, an origin of replication, selectable marker genes, and a multiple cloning site (An et al., 1986). The smaller plasmid can be easily propagated and manipulated in *Escherichia coli*, allowing one to insert a gene of interest as well as the desired selectable marker genes (Brandle and Bai, 1999). The Ti-plasmid was disarmed by removing the oncogenes responsible for tumor-

induction and opine synthesis, so that plant cells transformed by the plasmid could be regenerated into healthy, fertile plants (Weising et al., 1988).

To generate transformed plants, leaf disks are cut from surface sterilized leaves and soaked in a culture of *A. tumefaciens* containing both the Ti-plasmid and the plasmid carrying the novel gene. Soaking the disks allows the *A. tumefaciens* to infect the cells along the cut edges and to insert its T-DNA (containing the gene of interest) into the plant cells. The disks are placed on plates containing regeneration media with antibiotics corresponding to the selective resistance genes (Horsch et al., 1985). These selective resistance genes code for resistance to specific antibiotics. Regeneration media containing the antibiotic ensures that only plant cells carrying the resistance gene survive. The selective resistance gene is typically placed adjacent to the inserted gene of interest in the plasmid. Therefore, when the T-DNA is transferred into the plant cell, it should theoretically have both the antibiotic resistance genes and the desired insert gene (Weising et al., 1988). Calli on the surviving disks will begin to grow, eventually followed by shoots. The shoots are then transferred to flasks with rooting media where they grow roots and are then transferred to soil where they develop into whole plants (Horsch et al., 1985).

Although *N. tabacum* is an ideal crop to transform and many genes have been transferred into tobacco, there are currently no commercial transgenic cultivars available. While the use of transgenic tobacco could have an immediate impact on tobacco production by providing resistance to important pathogens such as TSWV or TMV, the industry currently does not accept transgenic tobacco (Fortnum et al., 2010). The negative perception

associated with genetically modified crops both internationally and in the US is the primary reason for the industry's current reluctance to use transgenic tobacco cultivars.

DISEASES AFFECTING TOBACCO

Diseases of tobacco are one of the major causes of yield loss. Diseases, caused by fungi, bacteria, viruses, and nematodes, affect tobacco at all stages of development, and can occur on all plant parts (Shew and Lucas, 1991). While there are approximately 50 different organisms that are known to attack tobacco, only a few of these pathogens are responsible for major crop losses in North Carolina. Over the last five years, the five most economically important diseases in North Carolina were black shank, Granville wilt, TSWV, target spot, and Pythium rot (Mila and Radcliff, 2010). In addition, losses caused by TMV, PVY, blue mold, and root-knot nematodes are important problems internationally.

In North Carolina in 2010, the total tobacco crop loss due to disease was 13.3% and 82.3% of this loss could be attributed to the five major diseases listed above. Yield loss from diseases cost North Carolina tobacco farmers $401.5 million over the last five years. On average, black shank caused the greatest yield loss with an average annual loss of three percent and a total cost of $103.8 million to farmers from 2006 to 2010 (Mila and Radcliff, 2010).

Black Shank

Phytophthora nicotianae Breda de Haan is an oomycete that causes black shank, a root and stem rot disease, on all major types of tobacco (Shew and Lucas, 1991). It was first described by Van Breda de Haan in Indonesia in 1896 and has since spread to tobacco producing areas throughout the world (Shoemaker et al., 1999). It was first observed in the

United States in Georgia in 1915, and by 1931 it had moved into North Carolina (Shew and Lucas, 1991). As tobacco is the only known host for the causal agent of black shank, it is speculated that the oomycete was spread on tobacco and tobacco products as they were moved between countries (Shoemaker et al., 1999).

P. nicotianae is a polycyclic pathogen. Infection is caused by chlamydospores in the soil. In warm, moist soil, the chlamydospores germinate to produce germ tubes. These germ tubes infect tobacco roots directly or produce a sporangium. The sporangia then germinate indirectly to produce zoospores or directly to produce a germ tube. If zoospores are produced, they are released into the soil upon saturation and are attracted by chemotaxis to the roots where they encyst and germinate within an hour. The pathogen then reproduces within the host to produce more chlamydospores or sporangia and the cycle repeats itself (Shew and Lucas, 1991). The cycle occurs quickly during favorable conditions such that a new generation of motile spores can be produced as fast as every 72 hours (Melton and Shew, 2000).

Temperature, precipitation, and number of drought days have been consistently correlated to black shank disease incidence (Jacobi et al., 1983). Black shank is a warm-weather disease and soil temperatures above 20°C are required for significant levels of infection to occur. Infection can occur at temperatures as low as 16°C, but expansion is greatest at temperatures of 22-28°C (Shew and Lucas, 1991). In addition, high soil moisture is required for infection because soil saturation is required for zoospore production and dispersal. The pathogen causes little infection at soil matrix potentials lower than those favorable for zoospore production and dispersal, indicating that zoospores are the major

infective propagules (Shew, 1983). While black shank can occur in both acid and alkaline soils, the optimum pH for the disease is between six and seven. Also, increased levels of calcium and magnesium and decreased levels of aluminum are correlated with increased disease incidence (Shew and Lucas, 1991).

Black shank is characterized by a darkening of the lower stem near the soil line. The oomycete primarily affects the roots and lower stem region but symptoms can be seen on all parts of the plant. In young seedlings, which are very susceptible, a damping off is observed. In the field, the first above ground symptom includes wilting and yellowing of the leaves. If the plant is pulled up, the large lateral roots are blackened and the fine adventitious roots are decayed. The infection then spreads into the stem and the entire root system as the disease progresses. The characteristic blackening of the lower stem occurs in the final stages of the disease. If the stem is split vertically, the pith will appear dry and brown to black in color. Additionally, the pith may be separated into disks. At this point the leaves turn brown, shrivel up, and are no longer harvestable (Shew and Lucas, 1991). Plants are typically most susceptible to black shank six to eight weeks after transplanting. In North Carolina, there is a significant rise in average temperature and a decrease in rainfall during this period. In addition, this is the period of time when the plants are transitioning from very aggressive vegetative growth to reproductive growth. The combination of these environmental and physiological changes may reduce the plant's ability to resist infection (Jacobi et al., 1983).

Races

Different races of the black shank pathogen are defined by their ability to overcome different resistance genes. Four physiological races of black shank have been reported (0, 1,

2, 3). The first race differentiation was described by Apple (1962a) who classified race 0 as the strain that was nonpathogenic to *N. plumbaginifolia*, and race 1 as the strain which could infect *N. plumbaginifolia*. Since then, it has been observed that race 1 isolates can also infect *N. longiflora.* Race 2 was reported in South Africa (Sullivan et al., 2005) but has not been described elsewhere and is considered epidemiologically insignificant (Gallup and Shew, 2010). Race 3 was first reported on cigar wrapper tobacco in Connecticut in 1978 (McIntyre and Taylor, 1978), but was recently observed in fields in North Carolina. Race 3 has been defined as a strain which can overcome resistance in L8 (carrying resistance) from *N. longiflora*) but not resistance from NC1071 possessing the *Php* gene from *N. plumbaginifolia* (Gallup and Shew, 2010).

Race 0 and race 1 are the two most prevalent races of *P. nicotianae* in the United States. In North Carolina, race 0 had been the predominant race in flue-cured areas since the pathogen was first observed. Since the introduction of cultivars containing either the *Php* or *Phl* genes, however, there has been an increase in race 1 infestation (Greene et al., 2007). Race 0 has been described as the “more fit” race for many years (Apple, 1957; Greene et al., 2007). A thorough investigation by Sullivan et. al (2005) revealed that race 0 isolates were more aggressive than race 1 isolates, but that race 1 isolates caused a greater stunting on plants with moderate to high levels of quantitative resistance. The decrease in race 1 relative to race 0 in fields where cultivars with partial resistance are planted is most likely due to the differences in aggressiveness in the pathogen races along with race 0’s enhanced ability to overwinter (Sullivan et al., 2005).

Genetic Resistance

While cultural practices and chemical treatments can be used to combat black shank, the least expensive and most practical approach to this disease problem is incorporating genetic resistance into high yielding cultivars (Shew and Lucas, 1991). There are currently two major types of black shank resistance used in commercial cultivars. The first is a quantitative or polygenic resistance identified by Tisdale in the mid 1920's that confers partial resistance to both race 0 and race 1 (Tisdale, 1931). The second is a dominant monogenic factor from *N. plumbaginifolia* (Apple, 1962b; Chaplin, 1962) or *N. longiflora* (Valleau et al., 1960) that confers high resistance to race 0.

Tisdale selected plants from cigar wrapper cultivars that exhibited resistance in infested fields. By crossing a plant selection from 'Big Cuba' with a selection from 'Little Cuba' followed by several years of continued selection and inbreeding, Tisdale developed a resistant cigar wrapper line designated as Florida 301 (Tisdale, 1931). Florida 301 is in the pedigrees of most modern cultivars having some degree of partial resistance to black shank (K346, for example). This partial resistance does not totally prevent the growth and reproduction of the pathogen, but reduces lesion number, slows the rate of root colonization, and reduces inoculum production. This, in turn, results in a reduction of epidemic development (Shew and Shew, 1994).

In 1962, the *Php* resistance locus was successfully transferred from *N. plumbaginifolia* into flue-cured varieties (Apple, 1962b; Chaplin, 1962). The introgressed locus was incorporated into NC 1071 (Apple, 1962b), a breeding line now used as an indicator to check for the presence of race 1 (Csinos et al., 1984). Investigations by Goins

and Apple (1970) confirmed that the resistance from *N. plumbaginifolia* is controlled by a single dominant gene. In 1984, the highly resistant variety Coker 371-Gold was released. The high level of resistance exhibited by this cultivar was a result of pyramiding Florida 301 resistance genes with the *Php* gene (Johnson. et al., 2002).

In 1960, *Phl* was successfully transferred from *N. longiflora* into the burley line L8 (Valleau et al, 1960). While *Php* has been deployed in many flue-cured cultivars, *Phl* has been used primarily in burley tobacco. Like *Php*, it has also been confirmed that *Phl* associated resistance is due to a single dominant gene (Collins et al., 1971).

While these three sources of resistance are commonly used in current commercial cultivars, there is still room for improvement. *Php* and *Phl* provide immunity to race 0, but no resistance to race 1. Over the last 20 years, wide scale planting of cultivars containing *Php* has lead to race shifts and an increased prevalence of race 1 (Sullivan et al., 2005). There is currently no complete resistance available for race 1. Although the resistance from Florida 301 is partial and race non-specific, the resistance may be negatively correlated with yield and/or quality (Ramsey Lewis, personal communication, 2011).

Previous studies have reported resistance to both race 0 and race 1 in several other *Nicotiana* species including *N. rustica*, *N. debneyi*, *N. repanda*, *N. megalosiphon* and, *N. suaveolens* (Lautz, 1957; Litton et al., 1970; Li et al., 2006). The transfer of the resistance to *N. tabacum* from any of these species could provide a new source of resistance, but interspecific gene transfer is difficult. In addition, there is resistance from within *N. tabacum* in the cigar cultivar Beinhart-1000 that has not yet been used successfully in flue-cured tobacco breeding. A recent mapping study identified 6 QTL which explained 73.1% of the

genetic variation in a doubled haploid mapping population derived from a cross between Beinhart-1000 and Hicks, a susceptible cultivar (Vontimitta and Lewis, 2010). With this information, resistance from Beinhart-1000 may become a useful source of resistance in the near future.

MOLECULAR MARKERS IN TOBACCO

With the development of new molecular techniques over the last 30 years, there has been an increase in the use of molecular markers in plant breeding. Molecular markers are advantageous as they allow breeders to select germplasm based on the presence or absence of molecular markers closely linked to a gene or genes controlling a trait of interest. This early screening, may improve selection efficiency. Unlike phenotypic markers, plants can be tested for the presence or absence of a marker at a young stage, saving time and money. Furthermore, molecular markers are unaffected by environmental conditions. Marker assisted selection (MAS) has been useful in other crop species in selecting for traits such as resistance to pathogens, tolerance to abiotic stresses, quality parameters, and other quantitative traits (Mohan et al., 1997).

Restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP) marker systems have yielded few usable marker polymorphisms among lines of *N. tabacum*. Therefore, the majority of marker-trait association studies in tobacco were initially conducted on traits introgressed from other *Nicotiana* species (Bai et al., 1995; Ren and Timko, 2001). Markers have been identified that are linked to a number of disease resistance traits including resistance to black root rot (Bai et al., 1995), black shank (Johnson et al., 2002), blue mold

(Milla et al., 2005), PVY (Lewis, 2005), root knot nematodes (Yi and Rufty, 1998), TMV (Lewis et al., 2005), and wildfire (Yi et al., 1998). The majority of these traits are thought to be controlled by single genes. The use of molecular markers for identifying quantitative trait loci (QTL) and other genome-wide mapping experiments has been limited in tobacco, but with the development of microsatellite markers for tobacco, the opportunity for this approach has been improved greatly. Microsatellite markers exhibit a greater degree of polymorphism than the other classes of markers and are more reproducible (Bindler et al., 2007).

Before microsatellite markers were available, there were only a few QTL mapping experiments published for tobacco. One study identified QTLs affecting bacterial wilt resistance using AFLP markers (Nishi et al., 2003), while another used a combination of AFLP, sequence characterized amplified regions (SCAR), inter simple sequence repeat (ISSR), and sequence specific amplified polymorphism (SSAP) markers to identify a number of QTLs linked to leaf and smoke properties and to develop a partial linkage map (Julio et al., 2006).

Microsatellites

Microsatellites were not used in tobacco until genomic sequences for tobacco were available from the Tobacco Genome Initiative. They are useful for mapping in allopolyploids because a large number of markers amplify only single fragments. In tobacco, nearly 60% of all markers evaluated amplified only a single fragment from one of the two genomes (Bindler et al., 2007). This specificity ensures few perturbations in data analysis due to loci amplified from other genomes. In addition, microsatellites are useful in mapping because, unlike RAPD's or AFLP's that can only be analyzed as dominant markers, they are one of the only

marker systems that can be analyzed in a co-dominant fashion in segregating populations (Bindler et al., 2007). Since microsatellite markers have become available for tobacco, they have been used to identify QTLs associated with black shank resistance (Vontimitta and Lewis, 2010), for creating a linkage map (Bindler et al., 2007; Bindler et al., 2011), and to characterize diversity and genetic variation in tobacco germplasm (Moon et al., 2009a; Moon et al., 2009b).

AFLP's

Amplified fragment length polymorphism (AFLP) is a favorable method for identifying polymorphisms between tobacco lines because of its ability to amplify fragments between closely related specimens. In addition, AFLP's provide maximum coverage of the entire genome in a short amount of time, allowing one to identify markers linked to a gene of interest very quickly (Savelkoul et al., 1999). The AFLP protocol is based on the amplification of subsets of genomic restriction fragments using PCR. The technique involves a three step process which includes restriction/ligation, amplification, and gel analysis (Vos et al., 1995).

The restriction/ligation step involves cutting the DNA with restriction enzymes and ligating double-stranded adapters to the ends of the DNA fragments to generate template DNA for amplification. Two restriction enzymes are used to cut the DNA, a rare cutter (typically *EcoRI*) and a frequent cutter (typically *MseI*). Only DNA fragments with the rare cutter sequence at one end and the frequent cutter sequence at the other are amplified. Using the frequent cutter ensures that small DNA fragments are generated which are optimum for separation on a gel. Use of the rare cutter reduces the number of fragments amplified which

limits the number of nucleotides needed for selective amplification (Vos et al., 1995).

The amplification step requires two polymerase chain reactions (PCR). The first, a pre-amplification reaction, uses two oligonucleotide primes, one corresponding to the *EcoRI*-ends and the other to the *MseI*-ends. The primers consist of two sections, a core sequence and an enzyme-specific sequence. The diluted restriction/ligation product is used as a template and these primers anneal to the adapters ligated to the cut sites and amplify the DNA sequence between the two cut sites. This pre-amplification reaction is then diluted tenfold and used as the template for the selective amplification. Like the pre-amplification, the selective amplification uses two oligonucleotide primers corresponding to the two ends of the fragment. In addition to the core sequence and enzyme specific sequence, they also contain a selective extension sequence. The selective extension sequence consists of three nucleotides. Primers are designed so that only the three selective nucleotides vary which allows amplification of different bands in each reaction (Vos et al., 1995).

The final step, gel analysis, involves running the selective amplifications on an eight percent polyacrylamide gel. The gel separates DNA fragments by size and then these fragments can be visualized because they have been labeled. Pictures can be taken of the gels so they can be visualized digitally (Vos et al., 1995).

TOPPING AND SUCKER GROWTH

The act of removing the central, terminal meristem from tobacco plants in conventional production, in a process known as topping, diverts resources into the leaf rather than seed production. Topping stimulates root growth and slows the rate of decline in net photosynthesis in the remaining leaves, which relieves stress on the plant and results in plants

having a greater leaf area and leaf mass (Papenfus, 1987). In addition to contributing to higher yields, topping increases the concentration of compounds in the cured leaf that are desirable to the industry, such as nicotine (Elliot, 1975). It also increases levels of most of the volatile constituents associated with a desirable smoke character (Weeks and Seltmann, 1986).

While topping has many positive effects on leaf quality and chemistry, it also stimulates the growth of suckers. Suckers, or lateral meristems that grow in the leaf axils after topping, serve as a substitute for the lost terminal inflorescence of the plant (Papenfus, 1987). They act as a sink for water, light, and nutrients, and if not removed, they can have a negative impact on leaf quality and chemistry. Sucker removal contributes to yield increases and the accumulation of desirable cured leaf chemistry (Weeks and Seltmann, 1986).

Sucker Control

To maintain the quality of cured leaf desired by the industry, it is essential that growers remove suckers by hand or inhibit their growth with chemical inputs. If not controlled by chemicals, a grower may have to remove suckers by hand four or five times a season, a process which greatly increases cost of production (Steffens, 1970). There are currently three types of sucker controls available: fatty alcohol contacts, contact-local systemics, and systemics.

Fatty alcohol contacts are composed of chains of *n*-octanol and *n*-deconal mixtures. In order to be effective, the fatty alcohols must wet the bud directly and are therefore applied directly over the top of upright plants to permit the chemical to run down the stalk and into the leaf axils (Peedin, 1999). Fatty alcohols work by disrupting the semi-permeable cell

membranes in the young, tender tissue of the buds which causes the cell contents to dry out and thus die (Wheeler et al., 1991). Fatty alcohols are rarely used as the sole sucker control chemical because they require each sucker bud to be wetted by the chemical. If one is missed, it will be too big at the next application for the fatty alcohol to be effective (Collins et al., 1970).

Contact-local systemics are dinitroanalines, with the most common being flumetralin. Like fatty alcohols, their effectiveness is dependent upon being directly absorbed by meristemic tissues. They are therefore applied over the top of upright plants to permit the chemical to run down the stalk and into the leaf axils (Peedin, 1999). Flumetralin functions by interfering with cell division. Unlike a fatty alcohol, flumetralin has no true contact activity, so the buds will not look brown or burned but rather look yellow and deformed, and will stop growing. Contact-local systemics should only be applied at the elongated button to the early flower stage as they can stunt growth of top leaves if the leaves are too young when the chemical is applied (Fisher et al., 2011).

The only true systemic used for sucker control in tobacco is maleic hydrazide (MH). There is no requirement for MH to wet the bud, because unlike the other two categories of sucker control, MH is directly absorbed by the plant and then translocated (Peedin, 1999). It is transported via the xylem and phloem to meristematic regions where it inhibits cell division but not cell elongation, thus preventing growth of new suckers without retarding growth of mature leaves (Meyer et al., 1987). Maleic hydrazide is only applied late in the season after the upper leaves are at least sixteen inches long to reduce the chances of stunting

and discoloration. In addition, MH should be applied after a rain or when the plants are still wet with dew as it is absorbed best under these conditions (Fisher et al., 2011).

Many sucker control programs include a combination of the three types of chemicals. A common sucker control program begins with an application of a fatty alcohol when plants are in the button stage (approximately two days prior to topping). Plants are then topped and a second application of contact fatty alcohol is applied three to five days after the first application. Several days after the second application, when leaves are ten to twelve inches long, an application of flumetralin may be made followed by a final application of MH approximately a week later. If late sucker growth is anticipated, a final fatty alcohol or flumetralin application can be made about three weeks after the MH application. In addition to the chemical application, growers often walk over their fields at least once a season to manually remove missed suckers (Fisher et al., 2011).

Problems Associated with Chemical Sucker Control

A sucker control program is one of the most significant economic inputs in flue-cured tobacco production, with the cost of the chemicals described above being approximately $186 per acre. This doesn't include the fuel costs or labor costs associated with hand removal. Sucker control can ultimately cost growers up to $300 per acre, which is approximately ten percent of the total expected costs associated with production (Fisher et al., 2011).

In addition to contributing to high production costs, chemical sucker control is problematic because of concerns associated with MH residues. Maleic hydrazide is metabolically stable within the plant and is not degraded by ultraviolet radiation or extreme

temperatures associated with curing. Treated leaves usually therefore contain MH residues (Collins and Hawks, 1993). Since MH was first introduced, there has been debate on whether MH is a carcinogen or mutagen, and the degree of risk associated with its use. There have been conflicting reports over the last fifty years concerning the possibility of metabolism of MH in animals and any resulting harm. A number of studies in the 1960s and 1970s found MH to have no negative effects on animals (Barnes et al., 1957; Akin, 1976; Cabral and Ponomarkov, 1982), while a number of other studies reported negative effects from exposure to MH (Epstein et al., 1967; Hunter et al., 1973; Dickens and Jones, 1975). A possible explanation for the contradictory results is that commercial samples of MH from the 1960s to the 1980s contained hydrazine concentrations ranging from 0.14 mg/kg to 870 mg/kg (Lui et al., 1974; Bakker et al., 1983). Hydrazine is a known liver and lung carcinogen, so high levels of hydrazine in some samples may have been contributing to the negative effects reported (Meyer et al., 1987). The United States Environmental Protection Agency (US-EPA) conducted their own investigations, and after several studies, concluded that MH is not oncogenic in mice or rats and that no further oncogenicity testing was required (Taylor, 1985).

As of 2006, the US-EPA still classified MH as a Group E (Evidence of Non-Carcinogenicity for Humans) chemical (USEPA, 2006). Despite being classified as a non-carcinogen in the United States, MH is still scrutinized internationally. In 1978, the Federal Republic of Germany enacted a new food law which required that all pesticides approved for use meet tolerance levels established for foods. Under this law, tobacco was considered a food product (Hunt et al., 1977) and cured leaf MH residues were not to exceed 80 ppm

(Wittekindt, 1978). Since this law was enacted, several other countries in the European Union have also established a MH residue tolerance of 80 ppm. Consequently, growers in major flue-cured tobacco-producing countries such as Brazil, Canada, and Zimbabwe avoid the use of MH (Peedin, 1999). United States growers must be willing to ensure they are producing a product that has no more than 80 ppm MH residue in order to continue to be one of the major exporters of tobacco. Tobacco manufacturers are beginning to take pesticide residues very seriously and, in 2011, one manufacturer purchased only MH free tobacco (Fisher et al., 2011). It is therefore essential that the tobacco industry continues to find ways to reduce chemical residues on cured leaf.

Axillary Shoot Growth

One potential way to reduce pesticide residues is to eliminate the need for chemical treatment for sucker control. Genetic strategies to reduce or eliminate cell division or cell elongation in leaf axils may provide an attractive way to generate non-suckering tobacco varieties. Axillary shoot growth is a complex process within the plant and is a subject to which much study has been dedicated. While some regulatory aspects of axillary shoot growth have been described, there are still many aspects that remain unknown.

The shoot apical meristem (SAM) is the source of all above-ground organs. After germination, the SAM generates the main shoot, leaf primordia, and new meristems. Axillary meristems (AMs) are formed in the axils of leaves and, after initiation, develop into axillary buds (Aguilar-Martinez et al., 2007). Whether a plant branches (suckers) depends on whether these axillary buds grow out or whether they remain small and dormant in the leaf axils (Gerb et al., 2003). When the main shoot exerts control over the outgrowth of the lateral

buds, the plant is under apical dominance. Topping of the plant leads to a loss of apical dominance and the lateral buds are permitted to grow. The apical dominance phenomenon is mediated by plant hormones. Auxin inhibits lateral bud outgrowth while cytokinins promote it (Cline, 1997).

Much of the work conducted to understand lateral shoot branching has used the model plant *A. thaliana*. A number of genes involved in shoot branching have been identified and described. Genes involved in AM initiation include the following: *LATERAL SUPPRESSOR* (in *Arabidopsis* (Gerb et al., 2003) and tomato (Schumacher et al., 1999)), *MONCULM1* (in rice (Li et al., 2003)), *Blind* (in tomato (Schmits et al., 2002)), *REGULATOR OF AXILLARY MERISTEMS1* (in *Arabidopsis* (Keller et al., 2006)), and *LAX PANICLE* (in rice (Komatsu et al., 2003)). Once the AM is initiated, auxin (synthesized in the shoot apex) and strigolactone (synthesized in the roots) act as long distance signals to suppress branching (Martin-Trillo et al., 2011). Strigolactone is a carotenoid-derived hormone produced by *MORE AXILLARY GROWTH* (*MAX*) genes in *A. thaliana* (Dun et al., 2009). Both of these hormones act outside the axillary buds, but they are integrated by pathways that respond to the hormones that act inside the bud to suppress outgrowth. In monocots, *teosinte branched1* (*tb1*) genes act to prevent bud outgrowth, while in dicots there are several *tb1*-like genes that do the same (Martin-Trillo et al., 2011).

Branched1

Teosinte branched1 is classified as part of a major family of transcription factors that affect plant growth and development and that includes the TB1, CYCLOIDEA, or PCF (TCP) genes (Cubas et al., 1999). In dicots, there have been duplications of ancestral *tb1*-like

genes leading to a clade of genes that affect plant growth and development. The *BRANCHED1* (*BRC1*) gene is a part of this clade and acts to integrate hormone signals in the bud to suppress branching in *A. thaliana* (Aguilar-Martinez, 2007). In addition, homologs of *BRANCHED1* have been identified in tomato where they also suppress branching (Martin-Trillo et al., 2011).

Aguilar-Martínez et. al. (2007) described the function of *BRC1* in *A. thaliana*. They reported that *BRC1* plays a central role in axillary bud development. Before flowering when AMs were not yet initiated, *BRC1* transcripts were not detectable. After flowering (when the AMs became visible), *BRC1* transcripts were reported in all cell layers of the meristems. At the time of bud outgrowth, *BRC1* was down-regulated. *BRANCHED1* mutants had a significantly higher number of branches than the wild type, demonstrating that expression of *BRC1* is inversely correlated with branching. The team also reported that auxin and the *MAX* genes act though *BRC1* to promote bud arrest and that *BRC1* also responds to environmental and endogenous signals which influence bud outgrowth. This suggests that *BRC1* acts as a local integrator of genetic pathways controlling bud outgrowth.

Martín-Trillo et. al. (2001) investigated the role of *BRC1*-like genes in tomato. They identified two *BRC1*-like paralogs in tomato: *SlBRC1a* and *SlBRC1b*. Like the *Arabidopsis BRC1*, both tomato paralogs were expressed in arrested axillary buds and down-regulated upon bud activation. *SlBRC1b* transcript levels were much higher than the *SlBRC1a* levels, however. The identification of two *BRC1*-like genes in tomato suggests that a duplication of *BRC1* occurred after the separation of the Brassicaceae and Solanaceae, probably in a whole-genome duplication event. Since the second copy of the gene was not lost during evolution,

the *SlBRC1a* gene may have lost function or diverged in function. The authors reported that *SlBRC1b* retained the ancestral *BRC1*-like gene function of suppressing shoot branching.

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Chapter 2: Investigation of Black Shank Resistance Conferred by an Introgressed *Nicotiana rustica* Genomic Region in Flue-Cured Tobacco

ABSTRACT

Black shank, caused by *Phytophthora nicotianae*, is one of the most important pathogens affecting tobacco production in the United States. Host resistance offers the most effective means of reducing economic loss due to this pathogen. Several recently commercialized flue-cured tobacco hybrids possess a genomic region designated as *W*z that was derived from a Zimbabwean breeding line called 'WZ.'. This region was reportedly introgressed from *Nicotiana rustica*. The effect of this genomic region against multiple races of the black shank pathogen has not previously been investigated in an systematic way. A doubled-haploid (DH) mapping population of 71 lines was generated from a cross between WZ and black-shank susceptible cultivar, 'NC 55.' A BC_1F_1 population also was generated using the cross WZ/Hicks//Hicks, where 'Hicks' is a highly susceptible flue-cured tobacco cultivar. The DH population was evaluated for resistance in six field environments and also using race-specific inoculations in growth chambers. The BC_1F_1 population was tested for segregation of resistance to race 0 of *P. nicotianae* using growth chamber inoculations. Both populations also were genotyped using AFLP markers that were polymorphic between NC55 and WZ. Results suggest that the introgressed *N. rustica* region has a large effect on resistance to race 0 and race 1 of *P. nicotianae*. Seven AFLP markers of *N. rustica* origin were found to cosegregate with each other and were found to be linked with the introgressed black shank resistance factor. This genetic variation and associated markers may be of value for breeding for black shank resistance in U.S. tobacco.

INTRODUCTION

Black shank, caused by the soil borne pathogen *Phytophthora nicotianae* Breda de Haan, is one of the most destructive diseases of tobacco (*Nicotiana tabacum*) in the southeastern United States and also in other tobacco producing areas worldwide (Csinos et al., 1984; Shew and Lucas, 1991). The disease causes approximately 2 to 3% crop loss in North Carolina annually (Mila and Radcliff, 2010). The hemibiotrophic oomycete can infect tobacco roots, stems, and leaves at any stage of plant development resulting in symptoms that include root and stem necrosis, wilting, chlorosis, stem lesions, and stunting of plants (Csinos and Minton, 1983). Genetic resistance is an attractive means of combating black shank in tobacco and plays a major role in overall strategies to reduce the economic impact of this disease.

Both monogenic and oligogenic types of resistance to *P. nicotianae* have been used in the development of tobacco cultivars resistant to black shank. In 1931, Tisdale developed *N. tabacum* cigar cultivar 'Florida 301' (Tisdale, 1931). This cultivar possesses resistance of the classic polygenic type, where resistance is partial, non-race specific, and controlled by multiple genes (Smith and Clayton, 1948; Crews et al., 1964; Chaplin, 1966). Initially, Florida 301 was the only source of black shank resistance available to breeders and thus was the parent from which most resistance in burley and flue-cured tobacco was derived until 1964 (Lucas, 1975). Continuous exposure to this resistance may result in increased aggressiveness in pathogen populations (Dukes and Apple, 1961; Sullivan et al., 2005a). In addition, varieties that possess this type of resistance have historically been considered to produce cured leaf of a lower quality (Chaplin and Ford, 1958).

Monogenic resistance, controlled by single genes designated as *Php* and *Phl*,has been introgressed into *N. tabacum* from *N. plumbaginifolia* Viv. (Chaplin, 1962) and *N. longiflora* Cav. (Valleau et al., 1960), respectively. Both genes confer immunity to race 0, but confer no resistance to race 1 (Sulliva et al., 2005a). Historically, the *Php* locus has been used in flue-cured varieties, while the *Phl* introgression was used solely in burley varieties. Recently, *Php* has been incorporated into burley varieties, as well (Mila and Radcliff, 2011).

Apple (1962b) successfully transferred *Php* from *N. plumbaginifolia* to cultivated tobacco in the development of the breeding line 'NC 1071'. As NC 1071 has very little resistance to infection by race 1 of *P. nicotianae*, it is often used as an indicator of race 1 presence in field and growth chamber trials. The first commercial cultivar of any significance with the *Php* gene, 'Coker 371-Gold' (C 371-G), was not released until 1986 (Johnson et al., 2002). Resistance in C 371-G is provided by a combination of *Php* with genes derived from Florida 301 (Carlson et al., 1997). Although C 371-G was not widely popular with growers due to its poor agronomic traits, it was used in the pedigrees of more popular recent flue-cured tobacco hybrids such as NC71 and NC196.

In 1953, *Phl* was transferred from *N. longiflora* to the burley breeding line 'L8'. L8 is homozygous for *Phl* resistance and is not acceptable for commercial production (Valleau et al., 1960). To overcome this detrimental characteristic, *Phl* is only used commercially in hybrids which carry *Phl* in the heterozygous condition. As was observed with the wide-scale deployment of varieties carrying *Php*, planting of burley varieties with *Phl* has led to an increase in prevalence of race 1 isolates (Litton et al, 1966; Sullivan et al., 2005b).

Four physiological races (0, 1, 2, and 3) of *P. nicotianae* have been reported worldwide. Historically, race 0 was the predominant race found in flue-cured areas in North Carolina and worldwide (Lucas, 1975). However, with the extensive planting of cultivars with the *Php* gene in the 1990's, the prevalence of race 1 increased rapidly (Sullivan et al., 2005a). Race 2 has been reported in South Africa (Jaarsveld et al., 2002), but given that it has not been described elsewhere, it has been considered epidemiologically insignificant. In 1978, race 3 was reported in cigar-wrapper growing areas of Connecticut (McIntyre and Taylor, 1978). This race is also present in flue-cured production areas in North Carolina (Gallup and Shew, 2010).

Because complete resistance to races 0 and race 1 does not currently exist among tobacco cultivars, identification of additional genetic factors that could extend the range and level of resistance to *P. nicotianae* would be of great value to growers. Burk and Heggestad (1966) reported that, in addition to *N. longiflora* and *N. plumbaginifolia*, *N. rustica* L. also exhibited high levels black shank resistance that might be of value if transferred to cultivated tobacco. A recent evaluation of 86 *N. rustica* accessions revealed that almost all of the accessions had very high resistance to *P. nicotianae* race 0, and that 20 accessions had resistance to race 1 that was greater than the resistant flue-cured check, 'K 346' (Nifong et al., 2011).

Dominant resistance to *Pseudomonas syringae* pv. *tabaci* (the causal agent of the wildfire disease) and *P. syringae* pv. *angulata* (the causal agent of angular leaf spot [ALS]) was transferred from *N. rustica* var. Brasilia to *N. tabacum* (Woodend and Mudzengerere, 1992). A breeding line designated as 'WZ' with an introgressed chromosome segment from

N. rustica was derived from this research. Preliminary observations based on field testing of the WZ line and flue-cured tobacco hybrids with WZ in their pedigree, suggested that the introgressed *N. rustica* region might also be associated with resistance to multiple races of the black shank pathogen (Ramsey Lewis, unpublished data, 2011). If so, this novel resistance could be of great economic importance to tobacco growers. In addition, molecular markers linked to the introgressed *N. rustica* region could increase the efficiency of developing future tobacco cultivars carrying this resistance. The objectives of this study were (1) to investigate the nature of black shank resistance observed in WZ with special attention to race specificity, and (2) to identify molecular markers linked to the genomic region controlling this resistance.

MATERIALS AND METHODS

Production of Mapping Populations

WZ x NC 55 Doubled Haploid Population

The Zimbabwean flue-cured breeding line, WZ, which was preliminarily observed to exhibit resistance to race 0 and race 1 *P. nicotianae* (Ramsey Lewis, unpublished data), was hybridized with the fertile version of flue-cured cultivar 'NC 55', which possesses a low level of black shank resistance probably derived from Florida 301. F_1 individuals from this cross were then crossed as females with *N. africana* to generate maternally derived haploids according to the procedure described by Burk et al. (1979). The chromosome number of these haploid plants was then doubled using the midvein tissue culture procedure of Kasperbauer and Collins (1972). A total of 71 doubled haploid (DH) plants were generated and self-pollinated to produce 71 DH lines.

(WZ x Hicks) x Hicks BC_1F_1 Population

The breeding line WZ also was crossed to the black shank susceptible flue-cured cultivar, 'Hicks'. A single F_1 individual was subsequently backcrossed to Hicks to generate a BC_1F_1 population. This population was expected to segregate 1:1 for the introgressed *N. rustica* region.

Field Evaluation

The DH population, the parental lines (NC 55 and WZ), and the race 0 resistant but race 1 susceptible check, NC 1071, were evaluated in black shank nurseries at three locations (Cunningham Research Station, Kinston, NC; Upper Coastal Plain Research Station, Rocky Mount, NC; Oxford Tobacco Research Station, Oxford, NC) in eastern North Carolina during 2010 and 2011. In addition, the Zimbabwean flue cured tobacco cultivar 'Kutsaga51' (K51) which has very low levels of black shank resistance was included in 2011 as a check. The experimental design was a randomized complete block (RCBD) with three replications in each environment. Experimental units consisted of single 12-plant plots at both the Cunningham Research Station and the Upper Coastal Plain Research Station, and 22-plant plots at the Oxford Tobacco Research Station. Plants were transplanted and managed in accordance with common cultural practices for flue-cured tobacco production in North Carolina (North Carolina Cooperative Extension, 2011).

Approximately 35 days after transplanting, the number of plants killed by black shank in each plot was recorded. Disease counts were repeated approximately every 21 days in 2010 and every 28 days in 2011. At the end of the season (approximately 110 days after transplanting), end percent survival (EPS) and average percent survival (APS) were

calculated for each plot, along with the area under disease progress curve (AUDPC) and a disease index (DI). The AUDPC was calculated using the trapezoidal method described by Madden et al. (2007), which calculates the average disease intensity between each pair of adjacent time points. The following formula was used:

$$A_k = \sum_{i=1}^{N_i-1} \frac{(y_i+y_{i+1})}{2}(t_{i+1} - t_i),$$

where $\{t_i\}$ represents the time points in a sequence (the time interval between two time points can be consistent or vary), $\{y_i\}$ is the sequence of associated measures of disease, and $y(0) = y_o$ is defined as the initial disease level at $t = 0$. Percent survival was used as the measure of disease. The DI was calculated using the formula described by Csinos et al. (1984):

$$DI = \frac{\sum_{i=i}^{n} X_i\left[100-\left\{(i-1)\frac{100}{n}\right\}\right]}{I},$$

where i is the ordinal evaluation number, n is the number of evaluations (excluding initial stand count), X is the number of dead plants since last count, and I is the initial number of plants in the plot.

Growth Chamber Evaluation

Inoculum Preparation

Cultures of *P. nicotianae* were started on carrot agar one month prior to transplanting plants. Carrot agar medium was prepared by mixing 50 mL of organic carrot juice (Bolthouse Juice Products LLC, Bakersfield, CA) and 20 g of agar (Sigma Chemical Co. St Louis, MO) in 950 mL distilled water. Autoclaved medium was poured into 100 x 20 mm Petri dishes. An agar plug containing either a race 0 or race 1 *P. nicotiane* isolate of *P. nicotianae* (provided by Dr. David Shew, NCSU, Raleigh, NC) was placed on the medium and allowed

to grow until hyphae were spread across most of the plate (about 7 days at room temperature without light). Sterilized oat grains were then spread over the plate in a single layer. Cultures were considered ready to use when hyphae could be seen covering the majority of the oat grains (approximately 15 days at room temperature without light).

WZ x NC55 DH Population

The DH population, the parental lines (NC 55 and WZ), and the race 0 resistant, race 1 susceptible check NC 1071 were evaluated for race-specific resistance in growth chambers in the North Carolina State University Phytotron (Raleigh, NC). The experimental design was a split-plot with three replications conducted over time. The main-plot factor was pathogen race and the sub-plot factor was entry. The entries were randomized within main-plots. There were six plants per replication for each subplot. Plastic trays (Landmark Plastic Corporation, Akron, OH) containing eight sectors of six cells (6 cm x 4 cm x 5 cm) each were filled with a 2:1 peat:sand mixture. Three-week-old seedlings were transplanted to individual cells. Two weeks after transplanting, each plant was inoculated with black shank by placing three oat grains into the soil in the corners of each cell as described above. The growth chamber was maintained at a temperature of 30°C for 16 hours of daylight and decreased to 25°C at night. Soil moisture was maintained through sub-irrigation.

Disease incidence was recorded seven days post-inoculation and every seven days over 35 days. Disease measures including EPS, APS, AUDPC, and DI were calculated as described above for field evaluation.

(WZ x Hicks) x Hicks BC_1F_1 Population

Plants from the (WZ x Hicks) x Hicks BC_1F_1 population, along with the original parental lines (WZ and Hicks), were evaluated for race 0 resistance in a growth chambers as described above. One-hundred and forty BC_1F_1 plants along with nine of each of the parental lines were transplanted into individual cells (8 cm x 8 cm x 5 cm) of 18 cell plastic trays (Landmark Plastic Corporation, Akron, OH). The parental lines were randomly interspersed among the 140 plants. Two weeks after transplanting, each plant was inoculated by inserting five oat grains into the soil of each pot at a depth of approximately 1 cm. The growth chamber was maintained at a temperature of 30°C for 16 hours of daylight and decreased to 25°C at night. Soil moisture was maintained through sub-irrigation. Plants were observed every day for 31 days post-inoculaton and the number of days of survival was recorded for each entry.

DNA Extraction and Quantification

Leaf tissue samples (two to three small leaves) were collected from each of the doubled haploid lines, each of the BC_1F_1 individuals, the parental lines, and *N.rustica* (TW 117) approximately three weeks after seeding. DNA was isolated using a modified cetyltrimethylammonium bromide (cTAB) procedure (Afanador, Haley and Kelly, 1993; Johnson et al., 1995), with the exception that a BIO 101 FastPrep machine (BIO 101, Holbrook, N.Y.) was used to grind leaf samples. DNA was quantified using a Hoefer fluorometer (Hoefer Scientific Instruments, San Francisco, CA). After quantification, DNA was diluted to 25 ng μL^{-1} and stored at -20°C.

AFLP Genotyping

All amplified fragment length polymorphism (AFLP) reactions were performed according to the protocol of Milla et al. (2005) which is based on the original protocol described by Vos et al. (1995). Labeled *EcoRI* + 3 primers were purchased from LI-COR Inc. (Lincoln, NE). All other AFLP primers and adapters were purchased from Sigma Genosys (The Woodlands, TX). After amplification, AFLP fragments were denatured at 94°C and separated by polyacrylamide gel electrophoresis (PAGE) on a LI-COR 4300 DNA analyzer sequencer using 8% v/v denaturing polyacrylamide gels. AFLP bands were scored as binary data (band presence indicated by 1 and absence indicated by 0) using the software package AFLP-Quantar 1.0 (Keygene Products, 2000). To determine the size of bands of interest, IRDye 700- or 800-labeled molecular weight standards (50 – 700 bp) also were loaded on each gel. A total of 224 AFLP primer combinations were initially screened to identify those primer pairs that amplified polymorphic bands between WZ and NC55. Genotyping was also conducted for *N. rustica* in order to deduce the likely origin of polymorphic bands. Candidate coupling phase markers (those that were amplified for WZ and *N. rustica*, but not for NC55) were then used to genotype the entire population of DH lines (Table 2.1). Candidate repulsion phase markers (those that were amplified for NC55, but not WZ or *N. rustica*) were also identified and used to genotype the DH population (Table 2.1). The (WZ x Hicks) x Hicks BC_1F_1 population consisting of 140 individuals was subsequently genotyped using a subset of markers found to be significantly associated with black shank resistance in the DH population (Table 2.1).

Statistical Analysis

WZ x NC 55 DH Population

A total of five different disease measures were analyzed which included EPS, APS, AUDPC, DI, and a normalized AUDPC (arAUDPC). Because field data was collected across different time intervals, a normalized AUDPC was analyzed in addition to the original AUDPC. The normalized AUDPC is referred to as the relative AUDPC and is calculated as the AUDPC divided by the total area of the graph, i.e., the number of days from inoculation to the end of observations x 100 (Vidhyasekaran, 2004). An arcsin transformation was performed on the relative AUDPC to better approximate a normal distribution prior to data analysis (arAUDPC).

The PROC GLM procedure of SAS 9.1 (SAS Institute, Cary, NC) was used to conduct an analysis of variance (ANOVA) and to calculate means for each of the five disease measures for the field and race-specific growth chamber experiments. The PROC CORR procedure was used to calculate Pearson correlation coefficients between the five disease measures. PROC GLM was also used to compute simple F-tests for each marker to determine if significant differences between marker groups existed for each disease measure.

WZ x Hicks BC_1F_1 Population

Simple t-tests were performed using the PROC TTEST procedure of SAS 9.1 to determine the significance of differences in the number of days of survival after inoculation between the two marker groups (with and without marker present) for each of seven selected markers.

Investigation of the Relationship Between *Php* and the Introgressed Wz Region

To determine if *Php* and *Wz* were introgressed on the same chromosome, WZ was first hybridized with NCTG-61, an N.C. State University breeding line homozygous for *Php* derived from *N. plumbaginifolia*. The F_1 was then crossed with Hicks which possesses neither *Wz* or *Php*. Thirty-six progeny from the (WZ x NC 61) x Hicks cross were genotyped with the selected subset of AFLP markers identified to be linked to the introgressed *N. rustica* region (Table 2.1) and also with an unpublished *Php* sequence characterized amplified region (SCAR) marker derived from RAPD marker SOPZ-5 published by Johnson et al. (2002). A Chi-square test for independence using a 2 x 2 contigency table was performed on the genotypic data to test for independent segregation of *Wz* and *Php*.

RESULTS

WZ x NC 55 DH Population Field Data

Phenotypic Variation

The means for the DH lines and the parents averaged over all six field environments for each of the five disease measures are presented in Table 2.2. The five disease measures – (EPS, APS, arAUDPC, AUDPC, and DI) were highly correlated with each other with Pearson correlation coefficients ranging from $r_{\text{Pearson}} = 0.96$ to 0.99 ($P < 0.0001$ for all comparisons). The DH population of 71 lines exhibited wide variation for disease resistance. End percent survival ranged from 7.5% to 77.4% (Figure 2.1), APS ranged from 37.2% to 88.1% (Figure 2.2), arAUDPC (higher the number, greater the survival rate) ranged from 0.83 to 1.35 Figure 2.3) AUDPC (higher the number, greater the survival rate) ranged from 5252.6 to 9064.7 (Figure 2.4), and DI (lower the number, greater the survival rate) ranged

from 61.8 to 11.6 (Figure 2.5). The distribution of each of the disease measures was skewed slightly towards high survival rates. The resistant parent, WZ, was superior to the partially resistant parent NC 55 for each of the disease measures. The means for WZ were 68.91%, 83.87%, 1.26, 8788.4, and 16.0, while the means for NC55 were 37.1%, 63.1%, 1.07, 7218.1, and 36.7 for EPS, APS, arAUDPC, AUDPC, and DI, respectively. The means for the check NC 1071 were similar to those for NC55 with values of 41.68%, 61.71%, 1.0825, 6866.49, and 37.13 for EPS, APS, arAUDPC, AUDPC, and DI, respectively.

Each of the five disease measures were subjected to an ANOVA (Tables 2.3-2.7) which revealed not only significant genotypic differences, but also significant environmental effects, and significant genotype × environment interaction (GEI). Despite significant GEI, environment was considered a random variable and further analysis was based upon averages over environments.

Marker Analysis

A total of 46 polymorphic bands (Table 2.1) were identified from the initial screening of 224 AFLP primer combinations on DNA isolated from *N. rustica*, WZ, NC 55, and K51. Thirty of these were putative coupling phase markers (amplification products present for WZ and *N. rustica*, but absent for NC55), while 16 were putative repulsion phase markers (present for NC55, but absent for WZ and *N. rustica*). All 46 of the identified markers were used to genotype the population of 71 DH lines (Table 2.1). Thirty-one lines tested positive for the markers present for *N. rustica*, while 40 did not test positive for the 46 AFLP markers. A Chi-square test for segregation distortion indicated that there was not significant difference between the observed and the expected (1:1) segregation ratios (P = 0.33).

F-tests revealed significant differences ($P < 0.0001$) between the means of the two marker groups (with the marker versus not having the marker) for each of the 46 markers tested for each of the five disease measures (Table 2.8). The means for the two marker groups are presented for each marker in Table 2.9. For each disease measure, the group carrying the *N. rustica* marker had significantly higher levels of resistance than the group that did not. An example gel image for both a coupling phase (M14) and repulsion phase (M33) marker in a sample of the WZ x NC 55 DH lines is shown in Figure 2.6.

WZ x NC55 Growth Chamber Data

Phenotypic Variation

The means for each of the five disease measures are reported in Table 2.10 for race 0 inoculation of the DH lines, and in Table 2.11 for race 1 inoculation. The five measure (EPS, APS, arAUDPC, AUDPC, and DI) were highly correlated with each other, with $r_{\text{Pearson}} =$ 0.93 to 0.97 ($P < 0.0001$ for all pairwise combinations) for the race 0 inoculation. Correlation coefficients ranged from $r_{\text{Pearson}} = 0.98$ to 0.99 ($P < 0.0001$) for all pairwise combinations for race 1 inoculations. The 71 DH lines exhibited wide variation for disease resistance. For race 0, EPS ranged from 0% to 100% (Fig 2.7), APS ranged from 34.5% to 100% (Fig 2.8), arAUDPC ranged from 0.729 to 1.5708 (Fig 2.9), AUDPC ranged from 1556 to 3500 (Fig 2.10), and DI ranged from 0 to 95.6 (Fig 2.11). Distribution of EPS data was bimodal, while that of the other disease measures was more varied. For race 1, EPS ranged from 0% to 100% (Fig 2.12), APS ranged from 25.6% to 100% (Fig 2.13), arAUDPC ranged from 0.615 to 1.571 (Fig 2.14), AUDPC ranged from 1206 to 3500 (Fig 2.15), and DI ranged from 0 to 74.44 (Fig 2.16). The distributions for all disease measures after race 1 inoculation

were skewed towards a high survival rate. The WZ parent was superior to the partially resistant parent, NC 55, after inoculation with both races. There was a significant difference between the two cultivars for race 0 inoculations, but the differences were not significant race 1. NC 1071 exhibited 100% EPS after inoculation with race 0 and an EPS of 22.2% after inoculation with race 1. Significant differences between WZ and NC 1071 were present for each of the five disease measures after inoculation with race 1.

Data for each of the five disease measures was subjected to an ANOVA (Tables 2.12-2.16) which confirmed significant genotypic differences. A significant difference was observed between races for all five disease measures.

Marker Analysis

F-tests revealed significant differences (P <0.0001) between the means of the two marker groups (with and without the marker) for each of the 46 markers tested for each of the five disease measures after both race 0 and race 1 inoculation (Table 2.17 and 2.18). Means for the two marker groups are presented for each marker in Table 2.19 for race 0 and Table 2.20 for race 1. For each disease measure, the group carrying the *N. rustica*-derived marker exhibited significantly higher levels of resistance than the group that did not.

(WZ x Hicks) x Hicks BC_1F_1 Population Analysis

Phenotypic Variation

The number of days survived postinoculation (DAI) ranged from five days to still living at day 31 when the test was terminated (reported as 31 DAI). The distribution was bimodal with 86 plants dying within nine days of inoculation and 43 plants surviving the entire observation period (Fig 2.17). WZ was highly superior to Hicks as all nine WZ plants

survived the entire observation period, while all nine Hicks plants died between six and seven days after inoculation (Fig 2.18).

Marker Analysis

A subset of seven putative coupling phase markers from the original set of 46 was used to genotype the population of 140 BC_1F_1 individuals (Table 2.1). Fifty-four plants were positive for each of the seven markers while 85 were negative. A Chi-square test for segregation distortion revealed significant differences between the observed and the expected (1:1) segregation ratios ($P = 0.01$). Significant differences ($P < 0.0001$) were found for DAI between plants with each of the seven markers versus those without (Table 2.21). The group of plants with the seven associated markers had a mean DAI of 28.5, while the group without the markers had a mean DAI of 7.5. The majority of plants with the *Wz* markers had a very high DAI and those without had a low DAI. There were a few exceptions, however. Eight plants with a low DAI tested positive for the seven coupling markers, and one plant with a DAI of 31 tested negative for the *Wz* markers (Fig 2.19). An example gel image for M14 in a sample of the (WZ x Hicks) x Hicks BC_1F_1 plants is shown in Figure 2.20. No recombination was observed among these seven markers in this population of 140 plants.

Investigation of independent segregation between Wz and Php

Of the 36 (WZ x NC 61) x Hicks individuals genotyped, 17 tested positive for the SOPZ-5 SCAR marker linked to *Php* and 23 tested positive for the three AFLP markers (M14, M19, M20) associated with *Wz*. Thirteen plants tested positive for both the *Php* gene and *Wz-associated* markers, four plants possessed only the *Php* marker, 10 possessed only the *Wz* markers, and nine plants had neither (Table 2.22). The Chi-square test for

independence failed to reject the null hypothesis that *Wz* and *Php* segregate independently of each other ($P = 0.14$).

DISCUSSION

Phenotypic analysis of two populations derived from crosses involving WZ suggested that the breeding line possesses genetic variability affecting resistance to black shank. WZ had a high survival rate in black shank infested fields and in growth chamber inoculation experiments with race 0 and race 1 isolates of *P. nicotianae*. Identification of AFLP markers associated with black shank resistance that were present in *N. rustica* indicates that a major *P. nicotianae* resistance gene was introgressed from this species along with the transfer of resistance to angular leaf spot by Woodend and Mudzengerere (1992). The significant differences in disease measures in lines or plants with this marker compared to those without the marker, suggest that this introgressed *N. rustica* segment has an effect on isolates of race 0 and race 1. The *N. rustica* segment appears to be inherited as a complete block as no recombination was observed between the seven markers genotyped on 140 BC_1F_1 plants.

NC 1071 was included as a control in the field and growth chamber experiments because it possesses the *Php* gene, meaning it has immunity to race 0, but very little resistance to race 1 of *P. nicotianae*. As expected, NC 1071 had 100% survival when inoculated with race 0 and a very low rate of survival when inoculated with race 1. A greater number of lines had a higher degree of survival when inoculated with race 1 as compared to race 0, suggesting that the race 0 isolate used in our research was more aggressive than the race 1 isolate. This might be expected as race 1 isolates have been documented to be less aggressive than race 0 isolates (Apple, 1957; Lucas, 1975; Sullivan et al., 2005a).

Others have speculated an introgressed *N. rustica* gene in WZ acts in a manner similar to the *Ph* genes and provides single-gene immunity to race 0 (Bukuta, 2002). Results from the current work, however, indicate that this may not be the case. In the (WZ x Hicks) x Hicks BC_1F_1 population, a number of plants were positive for the markers yet still had low rates of survival. Also, in growth chamber inoculations with race 0, the survival of WZ was not 100%. Futhermore, the introgressed *N. rustica* region in WZ provides at least some resistance to race 1, which is not the case for *Php*. Genotyping of the (WZ x NC 61) x Hicks progeny revealed that *Wz* and *Php* segregate independently of each other, a finding that would allow tobacco breeders to combine both genes into single genotypes in homozygous condition.

Given the dynamic nature of *P. nicotianae* and its ability to adapt relatively quickly in response to the deployment of complete resistance, there has been an increase in race 1 in fields where cultivars with the *Php* gene have been consistently grown (Sullivan et al., 2005b; Sullivan et al., 2010). The resistance provided by the introgressed *N. rustica* region in WZ may be an additional source of genetic variability useful for increasing the range and level of black shank resistance in tobacco varieties. The research described here involved only single isolates of both race 0 and race 1. The race 1 isolate used here was less aggressive than the race 0 isolate. Further research will be needed to verify the effect of the introgressed *N. rustica* region on diverse isolates of *P. nicotianae*. The level of resistance to diverse race 1 isolates is of particular interest, especially those with a high level of aggressiveness.

One would expect the *N. rustica* marker to segregate in a 1:1 fashion in both the WZ x NC 55 DH and (WZ x Hicks) x Hicks BC_1F_1 populations. The ratios were significantly

distorted in the (WZ x Hicks) x Hicks BC_1F_1 population with a dearth of segregants carrying the *N. rustica* markers. These distorted segregation ratios might be attributed to lack of homology between the introgressed *N. rustica* segment and the *N. tabacum* chromosomes (Knott and Dvorak, 1976), or gametophytic competition during the fertilization process. Woodend and Mudzengere (1992) also observed segregation anomalies in terms of resistance to wildfire and ALS.

CONCLUSIONS

This investigation determined that the introgressed *N. rustica* region in the line WZ may provides a novel source of genetic variability affecting resistance to black shank, the most important pathogen affecting tobacco in North Carolina. Unlike resistance provided by the *Ph* genes, *Wz* provides resistance to both race 0 and race 1, making it an attractive potential source of resistance for tobacco varieties in the future.

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Table 2.1. Primer combinations used for AFLP analysis of WZ-derived populations

Marker	Primer Combination	Phase	Marker	Primer Combination	Phase
M1	E-ATT/M-CCA-482[1]	Coupling	M24	E-ATT/M-CGT-223	Coupling
M2	E-ATT/M-CCA-302	Coupling	M25	E-ATT/M-CGT-206	Coupling
M3	E-ATC/M-CCC-323	Coupling	M26	E-AGA/M-CTT-288	Coupling
M4	E-ATC/M-CCC-175	Coupling	M27	E-ATT/M-CCA-324	Repulsion
M5	E-ATG/M-CCG-256	Coupling	M28	E-AGC/M-CGG-102	Repulsion
M6	E-ATG/M-CCG-88	Coupling	M29	E-ATG/M-CGA-577	Repulsion
M7	E-AGC/M-CGG-299	Coupling	M30	E-ATG/M-CGA-412	Repulsion
M8	E-ATG/M-CGA-157	Coupling	M31	E-ATT/M-CGG-107	Repulsion
M9	E-AGC/M-CGA-113	Coupling	M32	E-AAT/C-CGA-162	Repulsion
M10	E-ATT/M-CGG-167	Coupling	M33	E-AGA/M-CCG-169	Repulsion
M11	E-AGC/M-CGT-657	Coupling	M34	E-ATT/M-CCT-587	Repulsion
M12	E-AGC/M-CGT-289	Coupling	M35	E-ATT/M-CCT-407	Repulsion
M13	E-AAT/C-CGA-332	Coupling	M36	E-AGG/M-CCG-295	Repulsion
M14[ab]	E-AGA/M-CCG-150	Coupling	M37	E-AAA/M-CGC -679	Repulsion
M15	E-ATT/M-CCT-542	Coupling	M38	E-AAA/M-CGC-487	Repulsion
M16	E-ATT/M-CCT-413	Coupling	M39	E-AAA/M-CGC-208	Repulsion
M17	E-ATT/M-CCT-223	Coupling	M40	E-ATT/M-CGT-590	Repulsion
M18	E-ATT/M-CCT-206	Coupling	M41	E-ATT/M-CGT-408	Repulsion
M19[ab]	E-AGG/M-CCG -675	Coupling	M42	E-AGA/M-CTT-247	Repulsion
M20[ab]	E-AGG/M-CCG -344	Coupling	M43[a]	E-ATC/M-CCC-232	Coupling
M21[a]	E-AAA/M-CGC -96	Coupling	M44	E-ATC/M-CCC-223	Repulsion
M22	E-ATT/M-CGT-544	Coupling	M45[a]	E-ATG/M-CCG-359	Coupling
M23	E-ATT/M-CGT-413	Coupling	M46[a]	E-ATG/M-CCG-361	Coupling

[1] Number after the primer pair indicates the size of the polymorphic band
[a]Markers used to screen the BC_1F_1 population
[b] Markers used to screen (WZ x NC 61) x Hicks progeny

Table 2.2. Means for field disease measures of the WZ x NC 55 DH population, parental lines, and selected checks. Means are averages over six North Carolina environments.

Genotype	Entry	EPS[a]	APS[b]	arAUDPC[c]	AUDPC[d]	DI[e]
DH09-339-1	1	9.31	37.97	0.85	5381.17	61.76
DH09-339-3	2	17.84	48.14	0.94	6149.80	51.61
DH09-339-4	3	74.96	86.64	1.32	8979.51	13.07
DH09-339-7	4	73.78	84.05	1.28	8706.05	15.58
DH09-339-10	5	76.57	88.05	1.35	9064.33	11.84
DH09-339-17	6	64.19	76.51	1.20	8119.68	22.78
DH09-339-20	7	34.27	60.82	1.05	7053.67	38.21
DH09-339-22	8	55.40	73.16	1.17	8010.30	26.04
DH09-339-24	9	33.10	62.99	1.07	7298.28	36.55
DH09-339-26	10	62.73	78.16	1.22	8295.86	20.32
DH09-339-27	11	19.59	52.13	0.98	6515.63	46.93
DH09-339-30	12	34.98	65.17	1.09	7520.34	33.45
DH09-339-32	14	55.74	74.91	1.19	8163.13	24.81
DH09-339-36	15	75.97	84.48	1.30	8679.72	15.27
DH09-339-38	16	52.40	70.00	1.13	7756.22	29.68
DH09-339-39	17	40.09	63.73	1.07	7223.34	35.45
DH09-339-43	18	24.22	59.39	1.05	7123.91	40.11
DH09-339-45	19	61.59	79.63	1.25	8509.62	20.32
DH09-339-48	20	54.66	75.14	1.20	8296.93	23.06
DH09-339-52	21	13.37	38.65	0.84	5303.04	60.27
DH09-339-56	22	15.94	41.52	0.87	5522.53	56.01
DH09-339-57	23	32.99	65.31	1.11	7612.72	34.16
DH09-339-58	24	74.29	85.77	1.29	8840.71	13.92
DH09-339-59	25	59.06	70.89	1.13	7604.91	28.15
DH09-339-62	26	68.56	82.56	1.25	8663.65	17.27
DH09-339-63	27	34.73	62.41	1.07	7222.63	36.99
DH09-339-65	28	39.81	62.60	1.06	7126.57	37.16
DH09-339-66	29	31.78	65.07	1.11	7595.53	32.30
DH09-339-67	30	77.45	87.15	1.31	8904.64	12.65
DH09-339-68	31	27.22	54.93	0.99	6751.87	44.19

Table 2.2. Continued.

Genotype	Entry	EPS[a]	APS[b]	arAUDPC[c]	AUDPC[d]	DI[e]
DH09-339-70	32	27.92	61.53	1.07	7330.02	37.42
DH09-339-71	33	26.34	55.75	1.01	6760.54	42.70
DH09-339-74	34	14.89	42.90	0.89	5713.89	56.13
DH09-339-75	35	60.11	74.58	1.19	7980.03	25.14
DH09-339-79	36	21.84	55.40	1.01	6745.17	44.13
DH09-339-81	37	64.43	80.86	1.25	8594.95	18.66
DH09-339-89	38	26.89	59.17	1.05	7038.31	39.70
DH09-339-90	39	70.12	81.79	1.26	8543.40	18.09
DH09-339-92	40	41.54	64.53	1.09	7382.37	35.00
DH09-339-94	41	15.36	47.17	0.94	6144.07	52.03
DH09-339-96	42	66.06	78.35	1.23	8284.29	21.36
DH09-339-97	43	62.32	76.79	1.22	8276.51	21.70
DH09-339-99	44	73.38	84.65	1.31	8731.41	15.31
DH09-339-100	45	52.63	70.40	1.13	7736.11	29.15
DH09-339-103	46	13.88	41.43	0.88	5566.74	58.06
DH09-339-107	47	17.99	45.90	0.92	5952.08	52.14
DH09-339-108	48	56.44	71.98	1.17	7891.79	28.01
DH09-339-111	49	68.68	79.48	1.24	8375.99	19.98
DH09-339-122	50	7.48	37.56	0.85	5396.05	60.47
DH09-339-124	51	20.38	45.56	0.91	5840.53	53.92
DH09-339-126	52	39.60	62.60	1.07	7075.29	36.97
DH09-339-128	53	61.01	76.06	1.21	8121.80	23.66
DH09-339-135	54	12.78	37.23	0.84	5252.61	60.96
DH09-339-139	55	8.46	37.64	0.85	5308.94	60.67
DH09-339-140	56	25.89	57.01	1.02	6832.29	41.52
DH09-339-142	57	45.76	68.92	1.13	7688.06	30.97
DH09-339-145	58	62.61	76.19	1.20	8138.03	23.24
DH09-339-146	59	23.48	51.89	0.96	6395.54	47.72
DH09-339-149	60	60.38	78.06	1.22	8269.60	21.51
DH09-339-150	61	11.82	38.71	0.85	5383.57	60.85
DH09-339-151	62	26.59	54.18	0.99	6565.08	45.30
DH09-339-155	63	66.75	78.55	1.23	8298.81	21.17
DH09-339-34	64	68.56	84.10	1.30	8821.60	15.56

Table 2.2. Continued.

Genotype	Entry	EPS[a]	APS[b]	arAUDPC[c]	AUDPC[d]	DI[e]
DH05B-114-26	65	17.70	52.90	0.99	6628.34	46.49
DH05B-114-31	66	35.84	61.18	1.05	7100.96	38.31
DH05B-114-34	67	63.02	74.75	1.19	7931.16	25.25
DH05B-114-8	68	74.86	88.13	1.32	9064.70	11.58
DH05B-114-28	69	37.29	66.06	1.11	7453.78	33.41
DH05B-114-15	70	21.05	52.37	0.98	6561.55	46.55
DH05B-114-24	71	76.45	86.84	1.31	8953.98	12.98
DH05B-114-13	72	73.70	84.98	1.28	8847.87	14.77
WZ	73	68.91	83.87	1.26	8788.35	16.03
NC55	74	37.10	63.05	1.07	7218.09	36.72
NC 1071	75	41.68	61.71	1.08	6866.49	37.13
LSD[f]		29.05	19.48	0.20	1487.09	19.36

[a]End Percent Survival; [b]Average Percent Survival;
[c]arcsin relative Area Under Disease Progress Curve; [d]Area Under Disease Progress Curve;
[e]Disease Index; [f]Least Significant Difference

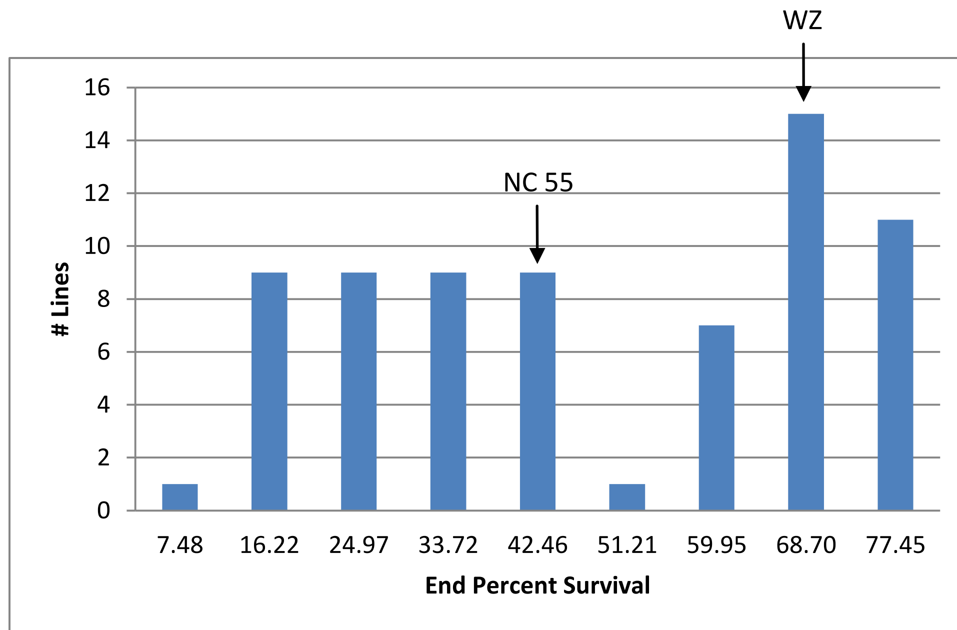

Figure 2.1. Frequency histogram for end percent survival averaged over six North Carolina environments in the field for the WZ x NC 55 DH population.

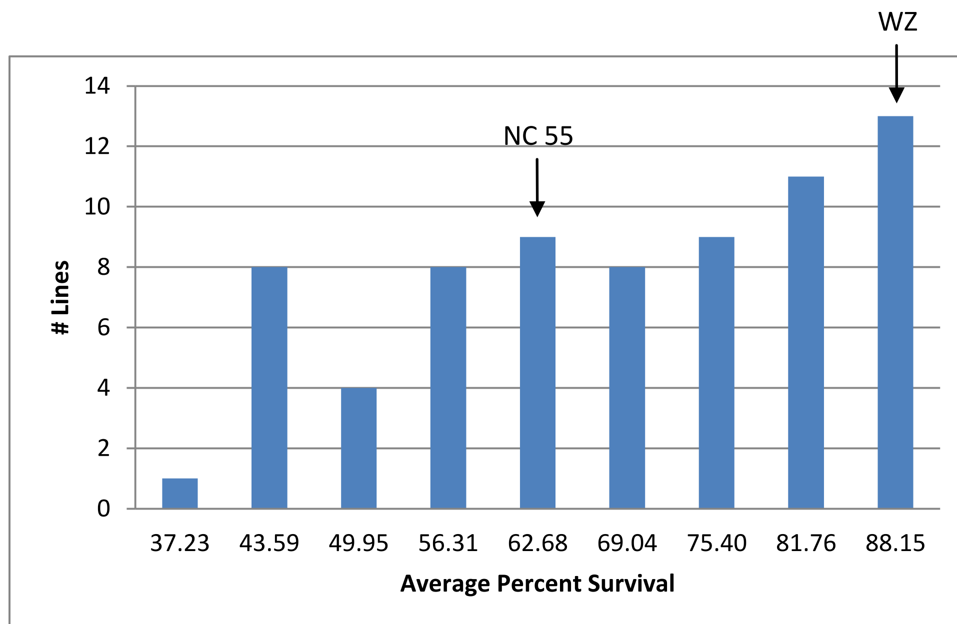

Figure 2.2. Frequency histogram for average percent survival averaged over six North Carolina environments in the field for the WZ x NC 55 DH population.

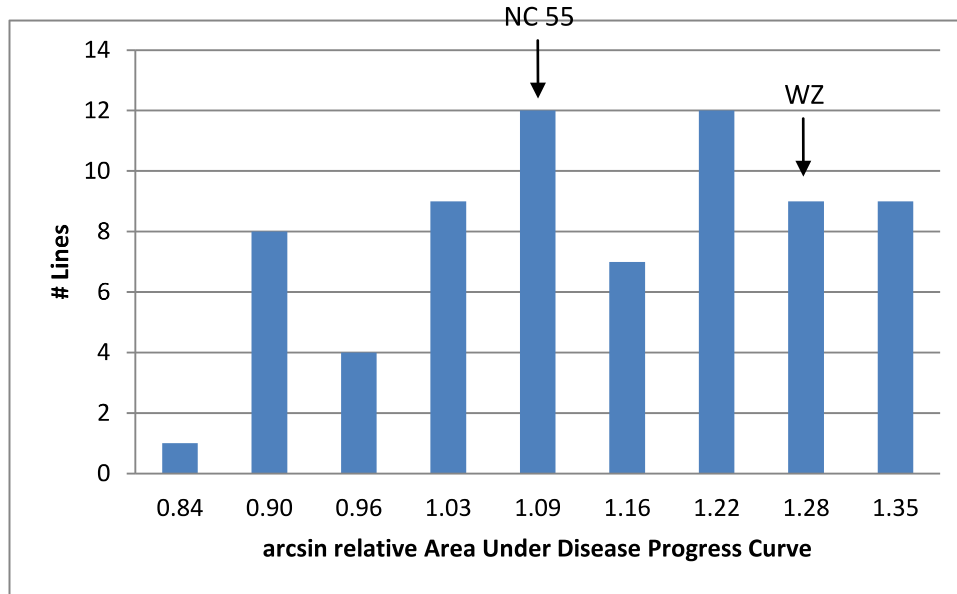

Figure 2.3. Frequency histogram for arcsin transformed relative area under disease progress curve averaged over six North Carolina environments in the field for the WZ x NC 55 DH population.

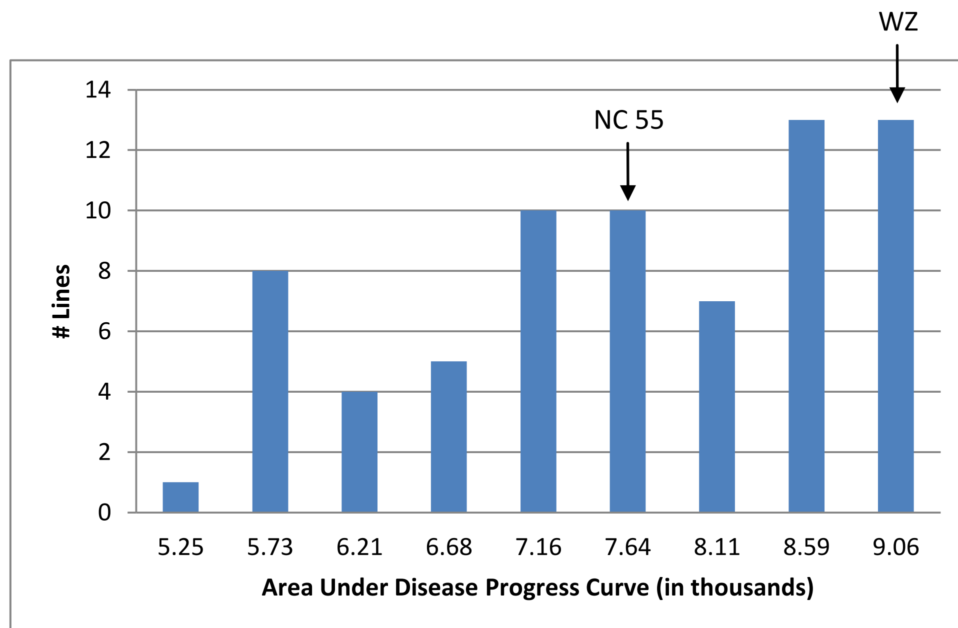

Figure 2.4. Frequency histogram for area under disease progress curve averaged over six North Carolina environments in the field for the WZ x NC 55 DH population.

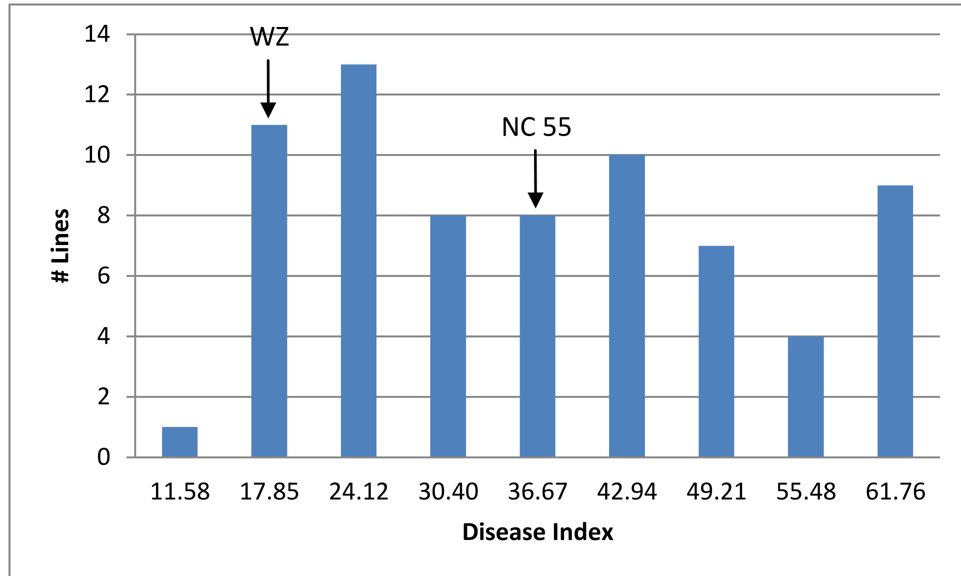

Figure 2.5. Frequency histogram for disease index averaged over six North Carolina environments in the field for the WZ x NC 55 DH population.

Table 2.3. ANOVA for end percent survival for field evaluation of a WZ x NC 55 DH population, parents, and controls.

Source	DF[a]	SS[b]	MS[c]	F-Value	P-Value
Environment	5	158238.30	31647.66	10.3	0.0005
Rep(Environment)	12	36864.69	3072.06	9.35	<.0001
Entry	74	646851.01	8741.23	10.22	<.0001
Entry*Environment	370	316459.13	855.29	2.6	<.0001
Error	888	291914.72	328.73	_	_

R^2 = 0.7987, C.V. = 41.15

[a]DF, Degrees of Freedom; [b]SS, Sum of Squares; [c]MS, Mean Square

Table 2.4. ANOVA for average percent survival for field evaluation of a WZ x NC 55 DH population, parents, and controls.

Source	DF[a]	SS[b]	MS[c]	F-Value	P-Value
Environment	5	148748.10	29749.62	33.48	<.0001
Rep(Environment)	12	10664.32	888.69	6.01	<.0001
Entry	74	303615.52	4102.91	7.99	<.0001
Entry*Environment	370	190110.80	513.81	3.48	<.0001
Error	888	131216.90	147.77	_	_

R^2 = 0.8327, C.V. = 18.49

[a]DF, Degrees of Freedom; [b]SS, Sum of Squares; [c]MS, Mean Square

Table 2.5. ANOVA for arcsin relative area under disease progress curve field evaluation of a WZ x NC 55 DH population, parents, and controls.

Source	DF[a]	SS[b]	MS[c]	F-Value	P-Value
Environment	5	17.09	3.42	36.79	<.0001
Rep(Environment)	12	1.11	0.09	5.93	<.0001
Entry	74	27.86	0.38	7.65	<.0001
Entry*Environment	370	18.21	0.05	3.14	<.0001
Error	888	0.02	_	_	_

R^2 = 0.8577, C.V. = 12.50
[a]DF, Degrees of Freedom; [b]SS, Sum of Squares; [c]MS, Mean Square

Table 2.6. ANOVA for area under disease progress curve field evaluation of a WZ x NC 55 DH population, parents, and controls.

Source	DF[a]	SS[b]	MS[c]	F-Value	P-Value
Environment	5	1531919990.50	306383998.09	115.78	<.0001
Rep(Environment)	12	31755124.10	2646260.34	3.07	0.0003
Entry	74	1634831497.40	22092317.53	5.79	<.0001
Entry*Environment	370	1411439747.30	3814702.02	4.43	<.0001
Error	888	764708995.40	861158.78	_	_

R^2 = 0.8577, C.V. = 12.50
[a]DF, Degrees of Freedom; [b]SS, Sum of Squares; [c]MS, Mean Square

Table 2.7. ANOVA for disease index field evaluation of a WZ x NC 55 DH population, parents, and controls.

Source	DF[a]	SS[b]	MS[c]	FValue	P-Value
Environment	5	143266.56	28653.31	34.15	<.0001
Rep(Environment)	12	10068.68	839.06	5.75	<.0001
Entry	74	292487.62	3952.54	7.83	<.0001
Entry*Environment	370	186681.73	504.55	3.46	<.0001
Error	888	129548.42	145.89	_	_

R^2 = 0.8300, C.V. = 35.96

[a]DF, Degrees of Freedom; [b]SS, Sum of Squares; [c]MS, Mean Square

Table 2.8. Marker F-tests for field evaluation at 6 North Carolina environments of a WZ x NC 55 DH population.

Dependent Variable	Marker	F-Value	P-Value	R^2
EPS[a]	M1	180.1	<.0001	0.7378
EPS	M2	231.4	<.0001	0.7833
EPS	M3	221.9	<.0001	0.7788
EPS	M4	221.9	<.0001	0.7788
EPS	M5	264.1	<.0001	0.8001
EPS	M6	264.1	<.0001	0.8001
EPS	M7	211.3	<.0001	0.7593
EPS	M8	240.7	<.0001	0.7874
EPS	M9	173.7	<.0001	0.7247
EPS	M10	213.0	<.0001	0.7580
EPS	M11	265.7	<.0001	0.7962
EPS	M12	265.7	<.0001	0.7962
EPS	M13	265.7	<.0001	0.7962
EPS	M14	265.7	<.0001	0.7962
EPS	M15	255.9	<.0001	0.7925
EPS	M16	255.9	<.0001	0.7925
EPS	M17	255.9	<.0001	0.7925
EPS	M18	255.9	<.0001	0.7925
EPS	M19	265.7	<.0001	0.7962
EPS	M20	252.5	<.0001	0.7953
EPS	M21	263.1	<.0001	0.7970
EPS	M22	265.7	<.0001	0.7962
EPS	M23	265.7	<.0001	0.7962
EPS	M24	265.7	<.0001	0.7962
EPS	M25	265.7	<.0001	0.7962
EPS	M26	199.2	<.0001	0.7456
EPS	M27	207.4	<.0001	0.7670
EPS	M28	206.7	<.0001	0.7552
EPS	M29	219.9	<.0001	0.7718
EPS	M30	219.9	<.0001	0.7718
EPS	M31	199.0	<.0001	0.7453
EPS	M32	244.0	<.0001	0.7821
EPS	M33	244.0	<.0001	0.7821
EPS	M34	255.9	<.0001	0.7925

Table 2.8. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
EPS	M35	255.9	<.0001	0.7925
EPS	M36	232.3	<.0001	0.7814
EPS	M37	241.2	<.0001	0.7826
EPS	M38	241.2	<.0001	0.7826
EPS	M39	241.2	<.0001	0.7826
EPS	M40	155.9	<.0001	0.6963
EPS	M41	244.0	<.0001	0.7821
EPS	M42	244.0	<.0001	0.7821
EPS	M43	265.7	<.0001	0.7962
EPS	M44	257.0	<.0001	0.7932
EPS	M45	250.1	<.0001	0.7962
EPS	M46	250.1	<.0001	0.7962
APS[b]	M1	107.8	<.0001	0.6276
APS	M2	123.9	<.0001	0.6594
APS	M3	119.6	<.0001	0.6549
APS	M4	119.6	<.0001	0.6549
APS	M5	141.6	<.0001	0.6821
APS	M6	141.6	<.0001	0.6821
APS	M7	128.2	<.0001	0.6567
APS	M8	129.9	<.0001	0.6665
APS	M9	100.2	<.0001	0.6030
APS	M10	130.3	<.0001	0.6570
APS	M11	144.9	<.0001	0.6806
APS	M12	144.9	<.0001	0.6806
APS	M13	144.9	<.0001	0.6806
APS	M14	144.9	<.0001	0.6806
APS	M15	139.0	<.0001	0.6748
APS	M16	139.0	<.0001	0.6748
APS	M17	139.0	<.0001	0.6748
APS	M18	139.0	<.0001	0.6748
APS	M19	144.9	<.0001	0.6806
APS	M20	140.9	<.0001	0.6843
APS	M21	147.9	<.0001	0.6882
APS	M22	144.9	<.0001	0.6806
APS	M23	144.9	<.0001	0.6806
APS	M24	144.9	<.0001	0.6806

Table 2.8. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
APS	M25	144.9	<.0001	0.6806
APS	M26	117.5	<.0001	0.6334
APS	M27	120.4	<.0001	0.6564
APS	M28	123.9	<.0001	0.6491
APS	M29	124.4	<.0001	0.6569
APS	M30	124.4	<.0001	0.6569
APS	M31	125.9	<.0001	0.6493
APS	M32	139.1	<.0001	0.6717
APS	M33	139.1	<.0001	0.6717
APS	M34	139.0	<.0001	0.6748
APS	M35	139.0	<.0001	0.6748
APS	M36	135.7	<.0001	0.6762
APS	M37	141.6	<.0001	0.6788
APS	M38	141.6	<.0001	0.6788
APS	M39	141.6	<.0001	0.6788
APS	M40	95.3	<.0001	0.5835
APS	M41	139.1	<.0001	0.6717
APS	M42	139.1	<.0001	0.6717
APS	M43	144.9	<.0001	0.6806
APS	M44	139.0	<.0001	0.6747
APS	M45	132.2	<.0001	0.6738
APS	M46	132.2	<.0001	0.6738
arAUDPC[c]	M1	110.4	<.0001	0.6330
arAUDPC	M2	125.5	<.0001	0.6623
arAUDPC	M3	121.0	<.0001	0.6576
arAUDPC	M4	121.0	<.0001	0.6576
arAUDPC	M5	144.8	<.0001	0.6870
arAUDPC	M6	144.8	<.0001	0.6870
arAUDPC	M7	130.1	<.0001	0.6600
arAUDPC	M8	131.6	<.0001	0.6694
arAUDPC	M9	102.9	<.0001	0.6093
arAUDPC	M10	133.2	<.0001	0.6621
arAUDPC	M11	147.0	<.0001	0.6837
arAUDPC	M12	147.0	<.0001	0.6837
arAUDPC	M13	147.0	<.0001	0.6837
arAUDPC	M14	147.0	<.0001	0.6837

Table 2.8. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
arAUDPC	M15	141.0	<.0001	0.6779
arAUDPC	M16	141.0	<.0001	0.6779
arAUDPC	M17	141.0	<.0001	0.6779
arAUDPC	M18	141.0	<.0001	0.6779
arAUDPC	M19	147.0	<.0001	0.6837
arAUDPC	M20	142.0	<.0001	0.6860
arAUDPC	M21	150.6	<.0001	0.6920
arAUDPC	M22	147.0	<.0001	0.6837
arAUDPC	M23	147.0	<.0001	0.6837
arAUDPC	M24	147.0	<.0001	0.6837
arAUDPC	M25	147.0	<.0001	0.6837
arAUDPC	M26	121.1	<.0001	0.6404
arAUDPC	M27	125.9	<.0001	0.6664
arAUDPC	M28	123.9	<.0001	0.6491
arAUDPC	M29	128.9	<.0001	0.6648
arAUDPC	M30	128.9	<.0001	0.6648
arAUDPC	M31	131.5	<.0001	0.6591
arAUDPC	M32	144.3	<.0001	0.6797
arAUDPC	M33	144.3	<.0001	0.6797
arAUDPC	M34	141.0	<.0001	0.6779
arAUDPC	M35	141.0	<.0001	0.6779
arAUDPC	M36	140.1	<.0001	0.6831
arAUDPC	M37	147.4	<.0001	0.6875
arAUDPC	M38	147.4	<.0001	0.6875
arAUDPC	M39	147.4	<.0001	0.6875
arAUDPC	M40	99.9	<.0001	0.5951
arAUDPC	M41	144.3	<.0001	0.6797
arAUDPC	M42	144.3	<.0001	0.6797
arAUDPC	M43	147.0	<.0001	0.6837
arAUDPC	M44	141.0	<.0001	0.6778
arAUDPC	M45	136.2	<.0001	0.6804
arAUDPC	M46	136.2	<.0001	0.6804
AUDPC[d]	M1	94.6	<.0001	0.5964
AUDPC	M2	105.8	<.0001	0.6231
AUDPC	M3	102.4	<.0001	0.6192
AUDPC	M4	102.4	<.0001	0.6192

Table 2.8. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
AUDPC	M5	120.0	<.0001	0.6452
AUDPC	M6	120.0	<.0001	0.6452
AUDPC	M7	111.8	<.0001	0.6252
AUDPC	M8	111.2	<.0001	0.6311
AUDPC	M9	87.3	<.0001	0.5694
AUDPC	M10	115.2	<.0001	0.6288
AUDPC	M11	123.8	<.0001	0.6455
AUDPC	M12	123.8	<.0001	0.6455
AUDPC	M13	123.8	<.0001	0.6455
AUDPC	M14	123.8	<.0001	0.6455
AUDPC	M15	118.8	<.0001	0.6393
AUDPC	M16	118.8	<.0001	0.6393
AUDPC	M17	118.8	<.0001	0.6393
AUDPC	M18	118.8	<.0001	0.6393
AUDPC	M19	123.8	<.0001	0.6455
AUDPC	M20	120.3	<.0001	0.6493
AUDPC	M21	128.4	<.0001	0.6571
AUDPC	M22	123.8	<.0001	0.6455
AUDPC	M23	123.8	<.0001	0.6455
AUDPC	M24	123.8	<.0001	0.6455
AUDPC	M25	123.8	<.0001	0.6455
AUDPC	M26	101.6	<.0001	0.5991
AUDPC	M27	104.4	<.0001	0.6236
AUDPC	M28	109.6	<.0001	0.6206
AUDPC	M29	106.4	<.0001	0.6208
AUDPC	M30	106.4	<.0001	0.6208
AUDPC	M31	111.1	<.0001	0.6204
AUDPC	M32	118.8	<.0001	0.6360
AUDPC	M33	118.8	<.0001	0.6360
AUDPC	M34	118.8	<.0001	0.6393
AUDPC	M35	118.8	<.0001	0.6393
AUDPC	M36	115.8	<.0001	0.6405
AUDPC	M37	122.8	<.0001	0.6470
AUDPC	M38	122.8	<.0001	0.6470
AUDPC	M39	122.8	<.0001	0.6470
AUDPC	M40	83.6	<.0001	0.5515

Table 2.8. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
AUDPC	M41	118.8	<.0001	0.6360
AUDPC	M42	118.8	<.0001	0.6360
AUDPC	M43	123.8	<.0001	0.6455
AUDPC	M44	118.6	<.0001	0.6389
AUDPC	M45	111.7	<.0001	0.6357
AUDPC	M46	111.7	<.0001	0.6357
DI[e]	M1	101.4	<.0001	0.6167
DI	M2	123.3	<.0001	0.6582
DI	M3	119.0	<.0001	0.6539
DI	M4	119.0	<.0001	0.6539
DI	M5	141.2	<.0001	0.6814
DI	M6	141.2	<.0001	0.6814
DI	M7	127.1	<.0001	0.6548
DI	M8	129.3	<.0001	0.6654
DI	M9	98.37	<.0001	0.5984
DI	M10	130.5	<.0001	0.6573
DI	M11	144.1	<.0001	0.6793
DI	M12	144.1	<.0001	0.6793
DI	M13	144.1	<.0001	0.6793
DI	M14	144.1	<.0001	0.6793
DI	M15	138.2	<.0001	0.6735
DI	M16	138.2	<.0001	0.6735
DI	M17	138.2	<.0001	0.6735
DI	M18	138.2	<.0001	0.6735
DI	M19	144.1	<.0001	0.6793
DI	M20	139.5	<.0001	0.6821
DI	M21	146.9	<.0001	0.6867
DI	M22	144.1	<.0001	0.6793
DI	M23	144.1	<.0001	0.6793
DI	M24	144.1	<.0001	0.6793
DI	M25	144.1	<.0001	0.6793
DI	M26	117.1	<.0001	0.6327
DI	M27	121.9	<.0001	0.6591
DI	M28	123.1	<.0001	0.6474
DI	M29	124.3	<.0001	0.6566
DI	M30	124.3	<.0001	0.6566

Table 2.8. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
DI	M31	126.6	<.0001	0.6505
DI	M32	138.9	<.0001	0.6713
DI	M33	138.9	<.0001	0.6713
DI	M34	138.2	<.0001	0.6735
DI	M35	138.2	<.0001	0.6735
DI	M36	134.9	<.0001	0.6749
DI	M37	141.2	<.0001	0.6781
DI	M38	141.2	<.0001	0.6781
DI	M39	141.2	<.0001	0.6781
DI	M40	96.01	<.0001	0.5853
DI	M41	138.9	<.0001	0.6713
DI	M42	138.9	<.0001	0.6713
DI	M43	144.1	<.0001	0.6793
DI	M44	138.1	<.0001	0.6733
DI	M45	131.9	<.0001	0.6732
DI	M46	131.9	<.0001	0.6732

[a]EPS, End Percent Survival; [b]APS, Average Percent Survival;
[c]arcsin relative Area Under Disease Progress Curve; [d]Area Under Disease Progress Curve;
[e]Disease Index

Table 2.9. Disease measure means for field evaluation at 6 North Carolina environments of a WZ x NC 55 DH population.

Disease Measure	Marker	Genotype	Mean	Standard Error
EPS[a]	M1	0	26.87	1.79
EPS	M1	1	65.19	2.22
EPS	M2	0	25.91	1.65
EPS	M2	1	65.15	1.98
EPS	M3	0	25.91	1.67
EPS	M3	1	65.20	2.04
EPS	M4	0	25.91	1.67
EPS	M4	1	65.20	2.04
EPS	M5	0	25.91	1.64
EPS	M5	1	66.63	1.90
EPS	M6	0	25.91	1.64
EPS	M6	1	66.63	1.90
EPS	M7	0	26.66	1.79
EPS	M7	1	66.12	2.04
EPS	M8	0	25.91	1.65
EPS	M8	1	65.55	1.95
EPS	M9	0	25.91	1.98
EPS	M9	1	64.48	2.16
EPS	M10	0	26.84	1.76
EPS	M10	1	66.10	2.03
EPS	M11	0	25.91	1.64
EPS	M11	1	66.00	1.84
EPS	M12	0	25.91	1.64
EPS	M12	1	66.00	1.84
EPS	M13	0	25.91	1.64
EPS	M13	1	66.00	1.84
EPS	M14	0	25.91	1.64
EPS	M14	1	66.00	1.84
EPS	M15	0	25.91	1.64
EPS	M15	1	65.76	1.87
EPS	M16	0	25.91	1.64
EPS	M16	1	65.76	1.87
EPS	M17	0	25.91	1.64
EPS	M17	1	65.76	1.87

Table 2.9. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
EPS	M18	0	25.91	1.64
EPS	M18	1	65.76	1.87
EPS	M19	0	25.91	1.64
EPS	M19	1	66.00	1.84
EPS	M20	0	26.76	1.68
EPS	M20	1	66.00	1.81
EPS	M21	0	25.72	1.66
EPS	M21	1	66.00	1.84
EPS	M22	0	25.91	1.64
EPS	M22	1	66.00	1.84
EPS	M23	0	25.91	1.64
EPS	M23	1	66.00	1.84
EPS	M24	0	25.91	1.64
EPS	M24	1	66.00	1.84
EPS	M25	0	25.91	1.64
EPS	M25	1	66.00	1.84
EPS	M26	0	26.97	1.81
EPS	M26	1	65.92	2.09
EPS	M27	0	65.64	2.11
EPS	M27	1	26.44	1.72
EPS	M28	0	64.79	1.99
EPS	M28	1	25.68	1.85
EPS	M29	0	66.04	2.06
EPS	M29	1	26.57	1.69
EPS	M30	0	66.04	2.06
EPS	M30	1	26.57	1.69
EPS	M31	0	66.58	2.12
EPS	M31	1	27.46	1.79
EPS	M32	0	66.46	1.93
EPS	M32	1	26.57	1.67
EPS	M33	0	66.46	1.93
EPS	M33	1	26.57	1.67
EPS	M34	0	65.76	1.87
EPS	M34	1	25.91	1.64
EPS	M35	0	65.76	1.87
EPS	M35	1	25.91	1.64
EPS	M36	0	66.46	1.90

Table 2.9. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
EPS	M36	1	27.45	1.71
EPS	M37	0	66.46	1.94
EPS	M37	1	26.41	1.70
EPS	M38	0	66.46	1.94
EPS	M38	1	26.41	1.70
EPS	M39	0	66.46	1.94
EPS	M39	1	26.41	1.70
EPS	M40	0	64.56	2.24
EPS	M40	1	27.06	2.00
EPS	M41	0	66.46	1.93
EPS	M41	1	26.57	1.67
EPS	M42	0	66.46	1.93
EPS	M42	1	26.57	1.67
EPS	M43	0	25.91	1.64
EPS	M43	1	66.00	1.84
EPS	M44	0	65.62	1.86
EPS	M44	1	25.91	1.63
EPS	M45	0	25.91	1.63
EPS	M45	1	66.31	1.96
EPS	M46	0	25.91	1.63
EPS	M46	1	66.31	1.96
APS[b]	M1	0	54.82	1.47
APS	M1	1	79.23	1.83
APS	M2	0	54.27	1.43
APS	M2	1	79.13	1.72
APS	M3	0	54.27	1.44
APS	M3	1	79.20	1.77
APS	M4	0	54.27	1.44
APS	M4	1	79.20	1.77
APS	M5	0	54.27	1.41
APS	M5	1	79.97	1.64
APS	M6	0	54.27	1.41
APS	M6	1	79.97	1.64
APS	M7	0	54.61	1.46
APS	M7	1	79.75	1.67
APS	M8	0	54.27	1.43

Table 2.9. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
APS	M8	1	79.40	1.68
APS	M9	0	54.46	1.63
APS	M9	1	78.60	1.78
APS	M10	0	54.78	1.44
APS	M10	1	79.80	1.66
APS	M11	0	54.27	1.40
APS	M11	1	79.64	1.57
APS	M12	0	54.27	1.40
APS	M12	1	79.64	1.57
APS	M13	0	54.27	1.40
APS	M13	1	79.64	1.57
APS	M14	0	54.27	1.40
APS	M14	1	79.64	1.57
APS	M15	0	54.27	1.41
APS	M15	1	79.47	1.61
APS	M16	0	54.27	1.41
APS	M16	1	79.47	1.61
APS	M17	0	54.27	1.41
APS	M17	1	79.47	1.61
APS	M18	0	54.27	1.41
APS	M18	1	79.47	1.61
APS	M19	0	54.27	1.40
APS	M19	1	79.64	1.57
APS	M20	0	55.13	1.40
APS	M20	1	79.64	1.51
APS	M21	0	53.98	1.41
APS	M21	1	79.64	1.57
APS	M22	0	54.27	1.40
APS	M22	1	79.64	1.57
APS	M23	0	54.27	1.40
APS	M23	1	79.64	1.57
APS	M24	0	54.27	1.40
APS	M24	1	79.64	1.57
APS	M25	0	54.27	1.40
APS	M25	1	79.64	1.57
APS	M26	0	54.98	1.48
APS	M26	1	79.54	1.71

Table 2.9. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
APS	M27	0	79.48	1.77
APS	M27	1	54.39	1.45
APS	M28	0	78.87	1.63
APS	M28	1	54.01	1.52
APS	M29	0	79.75	1.74
APS	M29	1	54.66	1.43
APS	M30	0	79.75	1.74
APS	M30	1	54.66	1.43
APS	M31	0	80.14	1.70
APS	M31	1	55.15	1.43
APS	M32	0	79.96	1.62
APS	M32	1	54.66	1.40
APS	M33	0	79.96	1.62
APS	M33	1	54.66	1.40
APS	M34	0	79.47	1.61
APS	M34	1	54.27	1.41
APS	M35	0	79.47	1.61
APS	M35	1	54.27	1.41
APS	M36	0	79.96	1.56
APS	M36	1	55.53	1.40
APS	M37	0	79.96	1.62
APS	M37	1	54.39	1.42
APS	M38	0	79.96	1.62
APS	M38	1	54.39	1.42
APS	M39	0	79.96	1.62
APS	M39	1	54.39	1.42
APS	M40	0	78.59	1.80
APS	M40	1	55.10	1.60
APS	M41	0	79.96	1.62
APS	M41	1	54.66	1.40
APS	M42	0	79.96	1.62
APS	M42	1	54.66	1.40
APS	M43	0	54.27	1.40
APS	M43	1	79.64	1.57
APS	M44	0	79.39	1.60
APS	M44	1	54.27	1.41
APS	M45	0	54.27	1.42

Table 2.9. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
APS	M45	1	79.81	1.71
APS	M46	0	54.27	1.42
APS	M46	1	79.81	1.71
arAUDPC[c]	M1	0	1.00	0.01
arAUDPC	M1	1	1.23	0.02
arAUDPC	M2	0	0.99	0.01
arAUDPC	M2	1	1.23	0.02
arAUDPC	M3	0	0.99	0.01
arAUDPC	M3	1	1.23	0.02
arAUDPC	M4	0	0.99	0.01
arAUDPC	M4	1	1.23	0.02
arAUDPC	M5	0	0.99	0.01
arAUDPC	M5	1	1.24	0.02
arAUDPC	M6	0	0.99	0.01
arAUDPC	M6	1	1.24	0.02
arAUDPC	M7	0	1.00	0.01
arAUDPC	M7	1	1.24	0.02
arAUDPC	M8	0	0.99	0.01
arAUDPC	M8	1	1.24	0.02
arAUDPC	M9	0	1.00	0.02
arAUDPC	M9	1	1.23	0.02
arAUDPC	M10	0	1.00	0.01
arAUDPC	M10	1	1.24	0.02
arAUDPC	M11	0	0.99	0.01
arAUDPC	M11	1	1.24	0.02
arAUDPC	M12	0	0.99	0.01
arAUDPC	M12	1	1.24	0.02
arAUDPC	M13	0	0.99	0.01
arAUDPC	M13	1	1.24	0.02
arAUDPC	M14	0	0.99	0.01
arAUDPC	M14	1	1.24	0.02
arAUDPC	M15	0	0.99	0.01
arAUDPC	M15	1	1.24	0.02
arAUDPC	M16	0	0.99	0.01
arAUDPC	M16	1	1.24	0.02
arAUDPC	M17	0	0.99	0.01

Table 2.9. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
arAUDPC	M17	1	1.24	0.02
arAUDPC	M18	0	0.99	0.01
arAUDPC	M18	1	1.24	0.02
arAUDPC	M19	0	0.99	0.01
arAUDPC	M19	1	1.24	0.02
arAUDPC	M20	0	1.00	0.01
arAUDPC	M20	1	1.24	0.01
arAUDPC	M21	0	0.99	0.01
arAUDPC	M21	1	1.24	0.01
arAUDPC	M22	0	0.99	0.01
arAUDPC	M22	1	1.24	0.02
arAUDPC	M23	0	0.99	0.01
arAUDPC	M23	1	1.24	0.02
arAUDPC	M24	0	0.99	0.01
arAUDPC	M24	1	1.24	0.02
arAUDPC	M25	0	0.99	0.01
arAUDPC	M25	1	1.24	0.02
arAUDPC	M26	0	1.00	0.01
arAUDPC	M26	1	1.24	0.02
arAUDPC	M27	0	1.24	0.02
arAUDPC	M27	1	1.00	0.01
arAUDPC	M28	0	1.23	0.02
arAUDPC	M28	1	0.99	0.01
arAUDPC	M29	0	1.24	0.02
arAUDPC	M29	1	1.00	0.01
arAUDPC	M30	0	1.24	0.02
arAUDPC	M30	1	1.00	0.01
arAUDPC	M31	0	1.24	0.02
arAUDPC	M31	1	1.00	0.01
arAUDPC	M32	0	1.24	0.02
arAUDPC	M32	1	1.00	0.01
arAUDPC	M33	0	1.24	0.02
arAUDPC	M33	1	1.00	0.01
arAUDPC	M34	0	1.24	0.02
arAUDPC	M34	1	0.99	0.01
arAUDPC	M35	0	1.24	0.02
arAUDPC	M35	1	0.99	0.01

Table 2.9. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
arAUDPC	M36	0	1.24	0.01
arAUDPC	M36	1	1.01	0.01
arAUDPC	M37	0	1.24	0.02
arAUDPC	M37	1	1.00	0.01
arAUDPC	M38	0	1.24	0.02
arAUDPC	M38	1	1.00	0.01
arAUDPC	M39	0	1.24	0.02
arAUDPC	M39	1	1.00	0.01
arAUDPC	M40	0	1.23	0.02
arAUDPC	M40	1	1.00	0.02
arAUDPC	M41	0	1.24	0.02
arAUDPC	M41	1	1.00	0.01
arAUDPC	M42	0	1.24	0.02
arAUDPC	M42	1	1.00	0.01
arAUDPC	M43	0	0.99	0.01
arAUDPC	M43	1	1.24	0.02
arAUDPC	M44	0	1.24	0.02
arAUDPC	M44	1	0.99	0.01
arAUDPC	M45	0	0.99	0.01
arAUDPC	M45	1	1.24	0.02
arAUDPC	M46	0	0.99	0.01
arAUDPC	M46	1	1.24	0.02
AUDPC[d]	M1	0	6647.65	112.58
AUDPC	M1	1	8391.85	139.64
AUDPC	M2	0	6609.91	110.17
AUDPC	M2	1	8381.77	132.41
AUDPC	M3	0	6609.91	111.23
AUDPC	M3	1	8389.88	136.23
AUDPC	M4	0	6609.91	111.23
AUDPC	M4	1	8389.88	136.23
AUDPC	M5	0	6609.91	108.86
AUDPC	M5	1	8436.05	126.24
AUDPC	M6	0	6609.91	108.86
AUDPC	M6	1	8436.05	126.24
AUDPC	M7	0	6631.44	111.91
AUDPC	M7	1	8425.77	127.59

Table 2.9. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
AUDPC	M8	0	6609.91	109.89
AUDPC	M8	1	8402.21	129.69
AUDPC	M9	0	6629.09	124.01
AUDPC	M9	1	8344.83	135.48
AUDPC	M10	0	6642.94	109.17
AUDPC	M10	1	8432.67	126.06
AUDPC	M11	0	6609.91	108.05
AUDPC	M11	1	8416.49	121.19
AUDPC	M12	0	6609.91	108.05
AUDPC	M12	1	8416.49	121.19
AUDPC	M13	0	6609.91	108.05
AUDPC	M13	1	8416.49	121.19
AUDPC	M14	0	6609.91	108.05
AUDPC	M14	1	8416.49	121.19
AUDPC	M15	0	6609.91	108.67
AUDPC	M15	1	8405.99	123.90
AUDPC	M16	0	6609.91	108.67
AUDPC	M16	1	8405.99	123.90
AUDPC	M17	0	6609.91	108.67
AUDPC	M17	1	8405.99	123.90
AUDPC	M18	0	6609.91	108.67
AUDPC	M18	1	8405.99	123.90
AUDPC	M19	0	6609.91	108.05
AUDPC	M19	1	8416.49	121.19
AUDPC	M20	0	6677.57	107.83
AUDPC	M20	1	8416.49	116.21
AUDPC	M21	0	6583.52	108.42
AUDPC	M21	1	8416.49	120.04
AUDPC	M22	0	6609.91	108.05
AUDPC	M22	1	8416.49	121.19
AUDPC	M23	0	6609.91	108.05
AUDPC	M23	1	8416.49	121.19
AUDPC	M24	0	6609.91	108.05
AUDPC	M24	1	8416.49	121.19
AUDPC	M25	0	6609.91	108.05
AUDPC	M25	1	8416.49	121.19
AUDPC	M26	0	6661.25	113.45

Table 2.9. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
AUDPC	M26	1	8408.25	131.00
AUDPC	M27	0	8405.83	135.85
AUDPC	M27	1	6614.03	110.92
AUDPC	M28	0	8364.47	124.31
AUDPC	M28	1	6587.47	115.60
AUDPC	M29	0	8426.13	133.89
AUDPC	M29	1	6638.57	110.00
AUDPC	M30	0	8426.13	133.89
AUDPC	M30	1	6638.57	110.00
AUDPC	M31	0	8455.99	129.66
AUDPC	M31	1	6670.09	109.05
AUDPC	M32	0	8438.50	124.83
AUDPC	M32	1	6638.57	108.11
AUDPC	M33	0	8438.50	124.83
AUDPC	M33	1	6638.57	108.11
AUDPC	M34	0	8405.99	123.90
AUDPC	M34	1	6609.91	108.67
AUDPC	M35	0	8405.99	123.90
AUDPC	M35	1	6609.91	108.67
AUDPC	M36	0	8438.50	119.60
AUDPC	M36	1	6706.72	107.69
AUDPC	M37	0	8438.50	123.82
AUDPC	M37	1	6613.59	108.60
AUDPC	M38	0	8438.50	123.82
AUDPC	M38	1	6613.59	108.60
AUDPC	M39	0	8438.50	123.82
AUDPC	M39	1	6613.59	108.60
AUDPC	M40	0	8340.36	136.30
AUDPC	M40	1	6670.43	121.52
AUDPC	M41	0	8438.50	124.83
AUDPC	M41	1	6638.57	108.11
AUDPC	M42	0	8438.50	124.83
AUDPC	M42	1	6638.57	108.11
AUDPC	M43	0	6609.91	108.05
AUDPC	M43	1	8416.49	121.19
AUDPC	M44	0	8400.22	123.61
AUDPC	M44	1	6609.91	108.41

Table 2.9. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
AUDPC	M45	0	6609.91	110.00
AUDPC	M45	1	8427.55	132.20
AUDPC	M46	0	6609.91	110.00
AUDPC	M46	1	8427.55	132.20
DI[e]	M1	0	44.27	1.46
DI	M1	1	20.65	1.84
DI	M2	0	44.82	1.40
DI	M2	1	20.44	1.69
DI	M3	0	44.82	1.42
DI	M3	1	20.37	1.74
DI	M4	0	44.82	1.42
DI	M4	1	20.37	1.74
DI	M5	0	44.82	1.39
DI	M5	1	19.61	1.61
DI	M6	0	44.82	1.39
DI	M6	1	19.61	1.61
DI	M7	0	44.48	1.44
DI	M7	1	19.85	1.64
DI	M8	0	44.82	1.40
DI	M8	1	20.18	1.65
DI	M9	0	44.60	1.61
DI	M9	1	21.01	1.75
DI	M10	0	44.33	1.41
DI	M10	1	19.78	1.62
DI	M11	0	44.82	1.38
DI	M11	1	19.96	1.55
DI	M12	0	44.82	1.38
DI	M12	1	19.96	1.55
DI	M13	0	44.82	1.38
DI	M13	1	19.96	1.55
DI	M14	0	44.82	1.38
DI	M14	1	19.96	1.55
DI	M15	0	44.82	1.39
DI	M15	1	20.11	1.58
DI	M16	0	44.82	1.39
DI	M16	1	20.11	1.58

Table 2.9. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
DI	M17	0	44.82	1.39
DI	M17	1	20.11	1.58
DI	M18	0	44.82	1.39
DI	M18	1	20.11	1.58
DI	M19	0	44.82	1.38
DI	M19	1	19.96	1.55
DI	M20	0	44.02	1.39
DI	M20	1	19.96	1.49
DI	M21	0	45.10	1.39
DI	M21	1	19.96	1.54
DI	M22	0	44.82	1.38
DI	M22	1	19.96	1.55
DI	M23	0	44.82	1.38
DI	M23	1	19.96	1.55
DI	M24	0	44.82	1.38
DI	M24	1	19.96	1.55
DI	M25	0	44.82	1.38
DI	M25	1	19.96	1.55
DI	M26	0	44.13	1.46
DI	M26	1	20.05	1.68
DI	M27	0	20.09	1.73
DI	M27	1	44.75	1.41
DI	M28	0	20.71	1.61
DI	M28	1	45.06	1.49
DI	M29	0	19.83	1.71
DI	M29	1	44.44	1.40
DI	M30	0	19.83	1.71
DI	M30	1	44.44	1.40
DI	M31	0	19.44	1.67
DI	M31	1	43.97	1.40
DI	M32	0	19.63	1.59
DI	M32	1	44.44	1.38
DI	M33	0	19.63	1.59
DI	M33	1	44.44	1.38
DI	M34	0	20.11	1.58
DI	M34	1	44.82	1.39
DI	M35	0	20.11	1.58

Table 2.9. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
DI	M35	1	44.82	1.39
DI	M36	0	19.63	1.54
DI	M36	1	43.64	1.38
DI	M37	0	19.63	1.59
DI	M37	1	44.71	1.39
DI	M38	0	19.63	1.59
DI	M38	1	44.71	1.39
DI	M39	0	19.63	1.59
DI	M39	1	44.71	1.39
DI	M40	0	20.95	1.76
DI	M40	1	44.03	1.57
DI	M41	0	19.63	1.59
DI	M41	1	44.44	1.38
DI	M42	0	19.63	1.59
DI	M42	1	44.44	1.38
DI	M43	0	44.82	1.38
DI	M43	1	19.96	1.55
DI	M44	0	20.20	1.57
DI	M44	1	44.82	1.38
DI	M45	0	44.82	1.40
DI	M45	1	19.76	1.68
DI	M46	0	44.82	1.40
DI	M46	1	19.76	1.68

[a]EPS, End Percent Survival; [b]APS, Average Percent Survival;
[c]arcsin relative Area Under Disease Progress Curve; [d]Area Under Disease Progress Curve;
[e]Disease Index

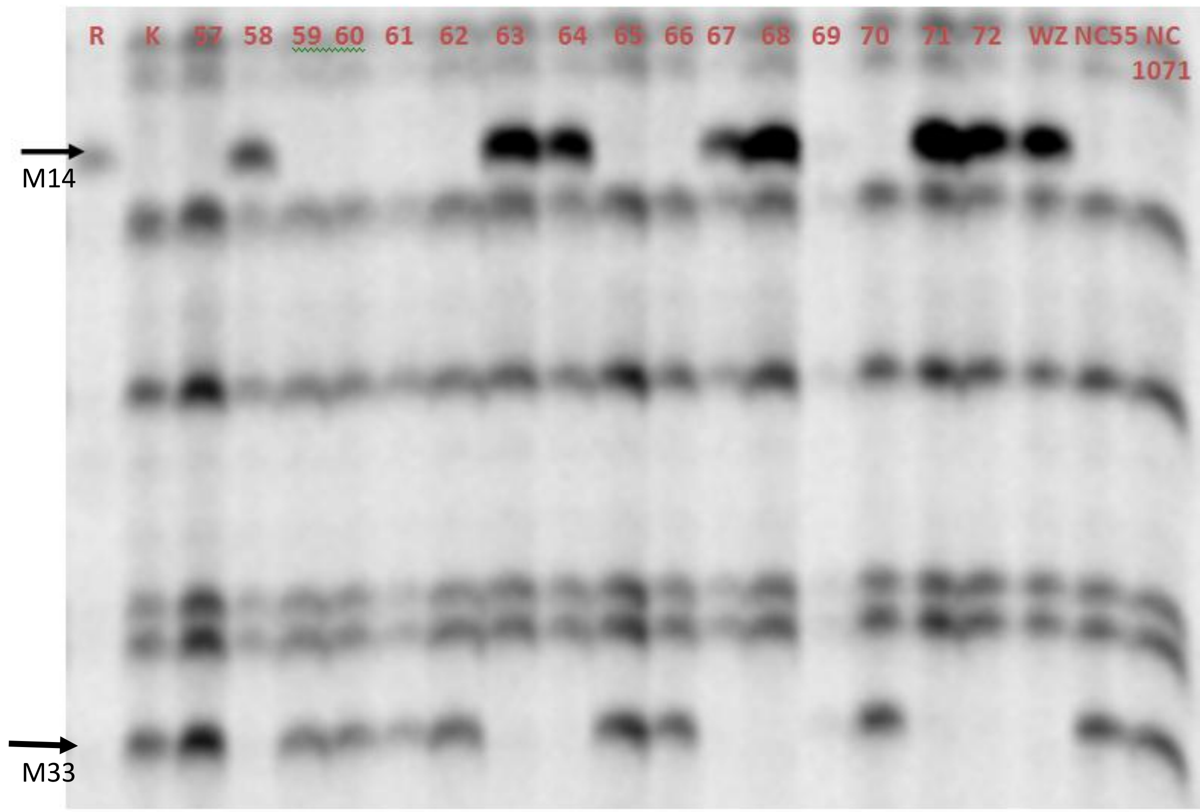

Figure 2.6. Gel image of M14 marker (coupling phase) and M33 marker (repulsion phase) on WZ x NC 55 DH plants and controls.

(R=*N. rustica*, K=Kutsaga 51)

Table 2.10. Means for disease measures for WZ x NC 55 DH lines, parental lines, and NC 1071 after inoculation with race 0 *P. nicotianae*.

Genotype	Entry	EPS[a]	APS[b]	arAUDPC[c]	AUDPC[d]	DI[e]
DH09-339-1	1	5.56	43.33	0.8247	1847.22	56.67
DH09-339-3	2	0.00	34.45	0.7291	1555.55	65.56
DH09-339-4	3	77.78	95.56	1.4837	3422.22	4.44
DH09-339-7	4	94.44	97.78	1.4956	3441.67	2.22
DH09-339-10	5	94.44	96.67	1.4732	3402.78	3.33
DH09-339-17	6	100.00	100.00	1.5708	3500	0.00
DH09-339-20	7	22.22	51.11	0.877	2061.11	48.89
DH09-339-22	8	100.00	100.00	1.5708	3500	0.00
DH09-339-24	9	0.00	41.11	0.7966	1788.89	58.89
DH09-339-26	10	100.00	100.00	1.5708	3500	0.00
DH09-339-27	11	5.56	46.67	0.8487	1963.89	53.33
DH09-339-30	12	16.67	48.89	0.8601	2002.78	51.11
DH09-339-32	14	94.44	98.89	1.5276	3480.56	1.11
DH09-339-36	15	100.00	100.00	1.5708	3500	0.00
DH09-339-38	16	77.78	92.22	1.4306	3305.56	7.78
DH09-339-39	17	22.22	67.78	1.0537	2644.44	32.22
DH09-339-43	18	0.00	36.67	0.7512	1633.33	63.33
DH09-339-45	19	72.22	83.33	1.3369	3013.89	16.67
DH09-339-48	20	88.89	93.33	1.3756	3305.55	6.67
DH09-339-52	21	0.00	11.11	0.4699	738.89	88.89
DH09-339-56	22	0.00	6.67	0.4172	583.34	93.33
DH09-339-57	23	33.33	65.56	1.1058	2527.78	34.44
DH09-339-58	24	100.00	100.00	1.5708	3500	0.00
DH09-339-59	25	88.89	96.67	1.4837	3422.22	3.33
DH09-339-62	26	55.56	71.11	1.1568	2644.44	28.89
DH09-339-63	27	16.67	56.67	0.9388	2275	43.33
DH09-339-65	28	66.67	86.67	1.3157	3150	13.33
DH09-339-66	29	5.56	46.67	0.8469	1963.89	53.33
DH09-339-67	30	100.00	100.00	1.5708	3500	0.00
DH09-339-68	31	0.00	27.78	0.659	1322.22	72.22

Table 2.10. Continued.

Genotype	Entry	EPS[a]	APS[b]	arAUDPC[c]	AUDPC[d]	DI[e]
DH09-339-70	32	11.11	41.11	0.7862	1750	58.89
DH09-339-71	33	5.56	47.78	0.8585	2002.78	52.22
DH09-339-74	34	0.00	26.67	0.6498	1283.34	73.33
DH09-339-75	35	77.78	91.11	1.4162	3266.67	8.89
DH09-339-79	36	0.00	31.11	0.6916	1438.89	68.89
DH09-339-81	37	100.00	100.00	1.5708	3500	0.00
DH09-339-89	38	0.00	45.56	0.8412	1944.45	54.44
DH09-339-90	39	100.00	100.00	1.5708	3500	0.00
DH09-339-92	40	11.11	54.44	0.927	2216.67	45.56
DH09-339-94	41	0.00	28.89	0.6721	1361.11	71.11
DH09-339-96	42	100.00	100.00	1.5708	3500	0.00
DH09-339-97	43	94.44	98.89	1.5276	3480.56	1.11
DH09-339-99	44	94.44	98.89	1.5276	3480.56	1.11
DH09-339-100	45	77.78	91.11	1.4162	3266.67	8.89
DH09-339-103	46	0.00	12.22	0.4843	777.78	87.78
DH09-339-107	47	0.00	35.56	0.7407	1594.44	64.44
DH09-339-108	48	100.00	100.00	1.5708	3500	0.00
DH09-339-111	49	100.00	100.00	1.5708	3500	0.00
DH09-339-122	50	0.00	10.00	0.4607	700	90.00
DH09-339-124	51	0.00	18.89	0.5589	1011.11	81.11
DH09-339-126	52	16.67	63.33	1.012	2508.33	36.67
DH09-339-128	53	94.44	98.89	1.5276	3480.56	1.11
DH09-339-135	54	0.00	4.44	0.3864	505.56	95.56
DH09-339-139	55	0.00	22.22	0.5952	1127.78	77.78
DH09-339-140	56	11.11	53.33	0.9149	2177.78	46.67
DH09-339-142	57	22.22	52.22	0.9002	2100	47.78
DH09-339-145	58	83.33	91.11	1.3731	3247.22	8.89
DH09-339-146	59	0.00	28.89	0.6706	1361.11	71.11
DH09-339-149	60	72.22	92.22	1.3861	3325	7.78
DH09-339-150	61	0.00	18.89	0.5646	1011.11	81.11
DH09-339-151	62	0.00	47.78	0.8654	2022.22	52.22
DH09-339-155	63	100.00	100.00	1.5708	3500	0.00

Table 2.10. Continued.

Genotype	Entry	EPS[a]	APS[b]	arAUDPC[c]	AUDPC[d]	DI[e]
DH09-339-34	64	94.44	97.78	1.4956	3441.67	2.22
DH05B-114-26	65	0.00	30.00	0.6805	1400	70.00
DH05B-114-31	66	11.11	55.56	0.9341	2255.56	44.44
DH05B-114-34	67	100.00	100.00	1.5708	3500	0.00
DH05B-114-8	68	100.00	100.00	1.5708	3500	0.00
DH05B-114-28	69	16.67	54.44	0.9266	2197.22	45.56
DH05B-114-15	70	5.56	31.11	0.689	1419.45	68.89
DH05B-114-24	71	100.00	100.00	1.5708	3500	0.00
DH05B-114-13	72	94.44	98.89	1.5276	3480.56	1.11
WZ	73	83.33	95.56	1.4732	3402.78	4.44
NC55	74	33.33	67.78	1.0913	2605.56	32.22
NC 1071	75	100.00	100.00	1.5708	3500	0.00
LSD[f]		29.16	20.27	0.25	637.82	20.27

[a]EPS, End Percent Survival; [b]APS, Average Percent Survival;
[c]arcsin relative Area Under Disease Progress Curve; [d]Area Under Disease Progress Curve;
[e]Disease Index; [f]Least Significant Difference

Tablel 2.11. Means for disease measures of WZ x NC 55 DH lines, parental lines, and NC 1071 after inoculation with race 1 *P. nicotianae*.

Genotype	Entry	EPS[a]	APS[b]	arAUDPC[c]	AUDPC[d]	DI[e]
DH09-339-1	1	88.89	91.11	1.3384	3227.78	8.89
DH09-339-3	2	72.22	90.00	1.3138	3247.22	10.00
DH09-339-4	3	100.00	100.00	1.5708	3500.00	0.00
DH09-339-7	4	100.00	100.00	1.5708	3500.00	0.00
DH09-339-10	5	100.00	100.00	1.5708	3500.00	0.00
DH09-339-17	6	100.00	100.00	1.5708	3500.00	0.00
DH09-339-20	7	50.00	56.67	0.9192	2158.33	43.33
DH09-339-22	8	100.00	100.00	1.5708	3500.00	0.00
DH09-339-24	9	100.00	100.00	1.5708	3500.00	0.00
DH09-339-26	10	94.44	97.78	1.4956	3441.67	2.22
DH09-339-27	11	72.22	78.89	1.132	2858.33	21.11
DH09-339-30	12	72.22	82.22	1.2417	2975.00	17.78
DH09-339-32	14	100.00	100.00	1.5708	3500.00	0.00
DH09-339-36	15	100.00	100.00	1.5708	3500.00	0.00
DH09-339-38	16	100.00	100.00	1.5708	3500.00	0.00
DH09-339-39	17	72.22	88.89	1.333	3208.33	11.11
DH09-339-43	18	94.44	96.67	1.4732	3402.78	3.33
DH09-339-45	19	100.00	100.00	1.5708	3500.00	0.00
DH09-339-48	20	83.33	96.67	1.4956	3441.67	3.33
DH09-339-52	21	0.00	26.67	0.6369	1283.33	73.33
DH09-339-56	22	27.78	44.45	0.8021	1808.33	55.56
DH09-339-57	23	83.33	87.78	1.3051	3130.55	12.22
DH09-339-58	24	100.00	100.00	1.5708	3500.00	0.00
DH09-339-59	25	94.44	97.78	1.4956	3441.67	2.22
DH09-339-62	26	100.00	100.00	1.5708	3500.00	0.00
DH09-339-63	27	94.44	96.67	1.4732	3402.78	3.33
DH09-339-65	28	88.89	90.00	1.3899	3188.89	10.00
DH09-339-66	29	77.78	87.78	1.3776	3150.00	12.22
DH09-339-67	30	100.00	100.00	1.5708	3500.00	0.00
DH09-339-68	31	22.22	42.22	0.7854	1750.00	57.78
DH09-339-70	32	55.56	64.44	1.0056	2411.11	35.56
DH09-339-71	33	94.44	96.67	1.4732	3402.78	3.33

Table 2.11. Continued.

Genotype	Entry	EPS[a]	APS[b]	arAUDPC[c]	AUDPC[d]	DI[e]
DH09-339-74	34	50.00	72.22	1.1277	2702.78	27.78
DH09-339-75	35	100.00	100.00	1.5708	3500.00	0.00
DH09-339-79	36	77.78	84.44	1.3426	3033.33	15.56
DH09-339-81	37	100.00	100.00	1.5708	3500.00	0.00
DH09-339-89	38	66.67	74.45	1.0919	2722.22	25.56
DH09-339-90	39	100.00	100.00	1.5708	3500.00	0.00
DH09-339-92	40	100.00	100.00	1.5708	3500.00	0.00
DH09-339-94	41	38.89	64.44	0.9984	2469.44	35.56
DH09-339-96	42	100.00	100.00	1.5708	3500.00	0.00
DH09-339-97	43	100.00	100.00	1.5708	3500.00	0.00
DH09-339-99	44	100.00	100.00	1.5708	3500.00	0.00
DH09-339-100	45	100.00	100.00	1.5708	3500.00	0.00
DH09-339-103	46	16.67	31.11	0.6695	1380.56	68.89
DH09-339-107	47	55.56	65.56	1.0837	2450.00	34.44
DH09-339-108	48	100.00	100.00	1.5708	3500.00	0.00
DH09-339-111	49	100.00	100.00	1.5708	3500.00	0.00
DH09-339-122	50	11.11	25.56	0.615	1205.56	74.44
DH09-339-124	51	55.56	75.56	1.1194	2800.00	24.44
DH09-339-126	52	100.00	100.00	1.5708	3500.00	0.00
DH09-339-128	53	100.00	100.00	1.5708	3500.00	0.00
DH09-339-135	54	5.56	30.00	0.6767	1380.55	70.00
DH09-339-139	55	5.56	28.89	0.6543	1341.67	71.11
DH09-339-140	56	94.44	97.78	1.4956	3441.67	2.22
DH09-339-142	57	72.22	87.78	1.2594	3169.44	12.22
DH09-339-145	58	94.44	94.44	1.4382	3325.00	5.56
DH09-339-146	59	61.11	74.45	1.1726	2741.67	25.56
DH09-339-149	60	100.00	100.00	1.5708	3500.00	0.00
DH09-339-150	61	11.11	43.33	0.8082	1827.78	56.67
DH09-339-151	62	77.78	90.00	1.2958	3227.78	10.00
DH09-339-155	63	94.44	98.89	1.5276	3480.56	1.11
DH09-339-34	64	94.44	98.89	1.5276	3480.56	1.11
DH05B-114-26	65	44.44	62.22	1.0227	2372.22	37.78
DH05B-114-31	66	66.67	76.67	1.2017	2800.00	23.33

Table 2.11. Continued.

Genotype	Entry	EPS[a]	APS[b]	arAUDPC[c]	AUDPC[d]	DI[e]
DH05B-114-34	67	100.00	100.00	1.5708	3500.00	0.00
DH05B-114-8	68	100.00	100.00	1.5708	3500.00	0.00
DH05B-114-28	69	100.00	100.00	1.5708	3500.00	0.00
DH05B-114-15	70	77.78	87.78	1.3118	3150.00	12.22
DH05B-114-24	71	100.00	100.00	1.5708	3500.00	0.00
DH05B-114-13	72	100.00	100.00	1.5708	3500.00	0.00
WZ	73	100.00	100.00	1.5708	3500.00	0.00
NC55	74	94.44	95.56	1.4546	3363.89	4.44
NC 1071	75	22.22	31.11	0.6638	1361.11	68.89
LSD[f]		26.39	21.83	0.27	686.54	21.83

[a]EPS, End Percent Survival; [b]APS, Average Percent Survival;
[c]arcsin relative Area Under Disease Progress Curve; [d]Area Under Disease Progress Curve;
[e]Disease Index; [f]Least Significant Difference

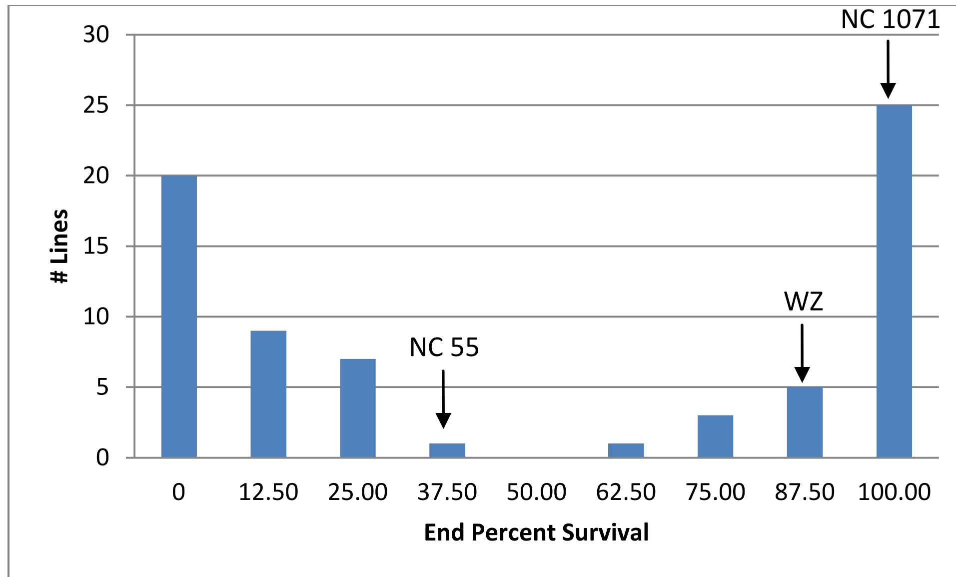

Figure 2.7. Frequency histogram for end percent survival for DH population inoculated with race 0 *P. nicotianae*.

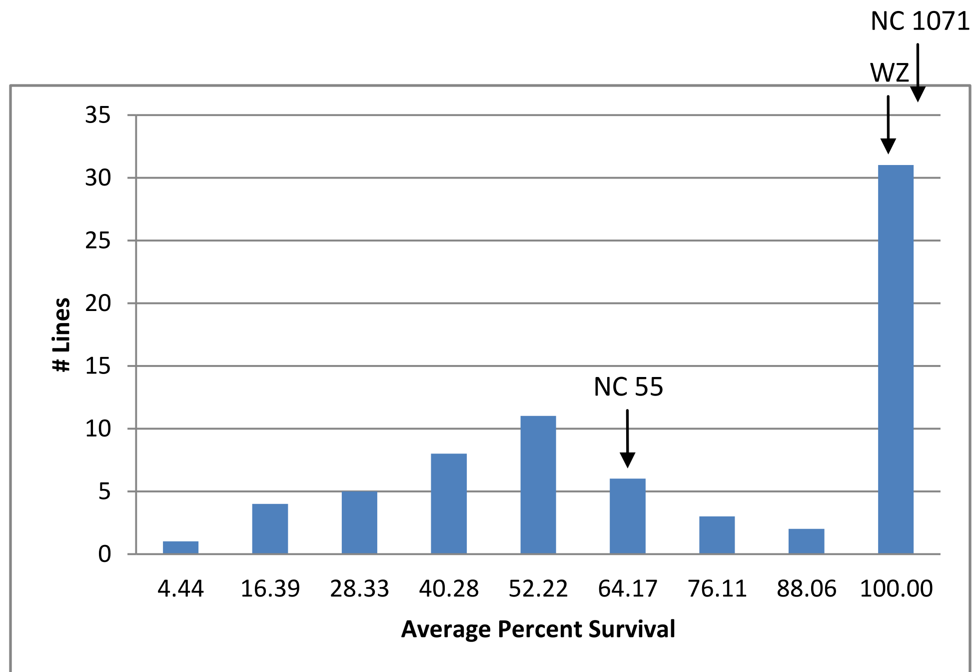

Figure 2.8. Frequency histogram for average percent survival for DH population inoculated with race 0 *P. nicotianae*.

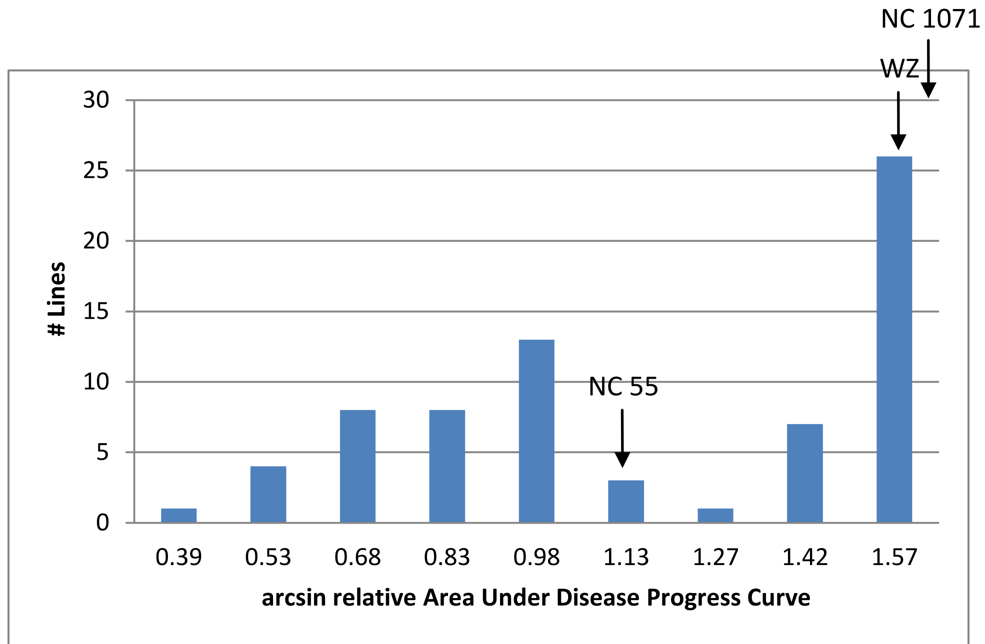

Figure 2.9. Frequency histogram for arcsin relative area under disease progress curve for DH population inoculated with race 0 *P. nicotianae*.

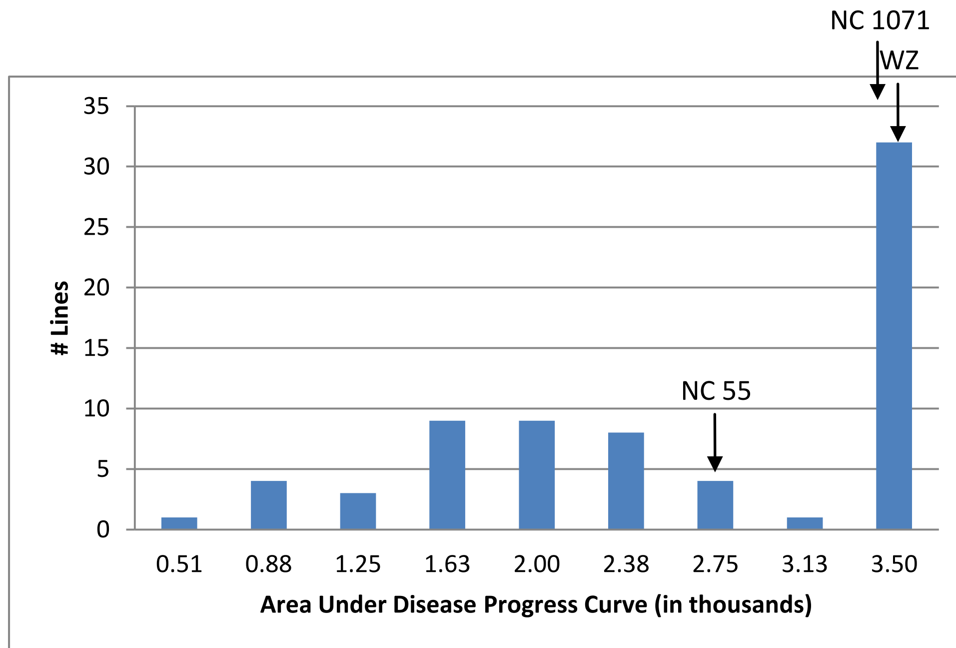

Figure 2.10. Frequency histogram for area under disease progress curve for DH population inoculated with race 0 *P. nicotianae*.

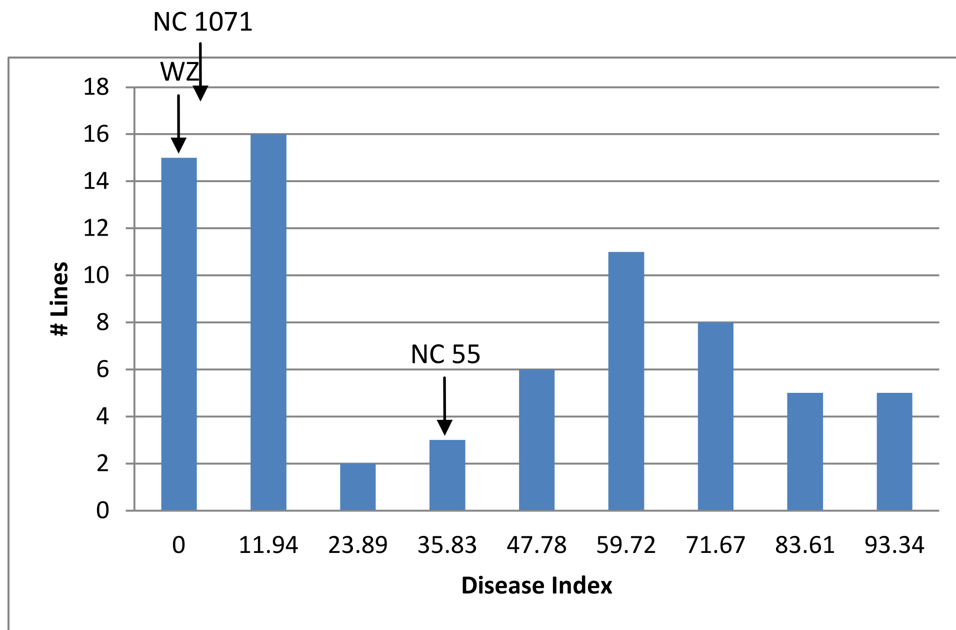

Figure 2.11. Frequency histogram for disease index for DH population inoculated with race 0 *P. nicotianae*.

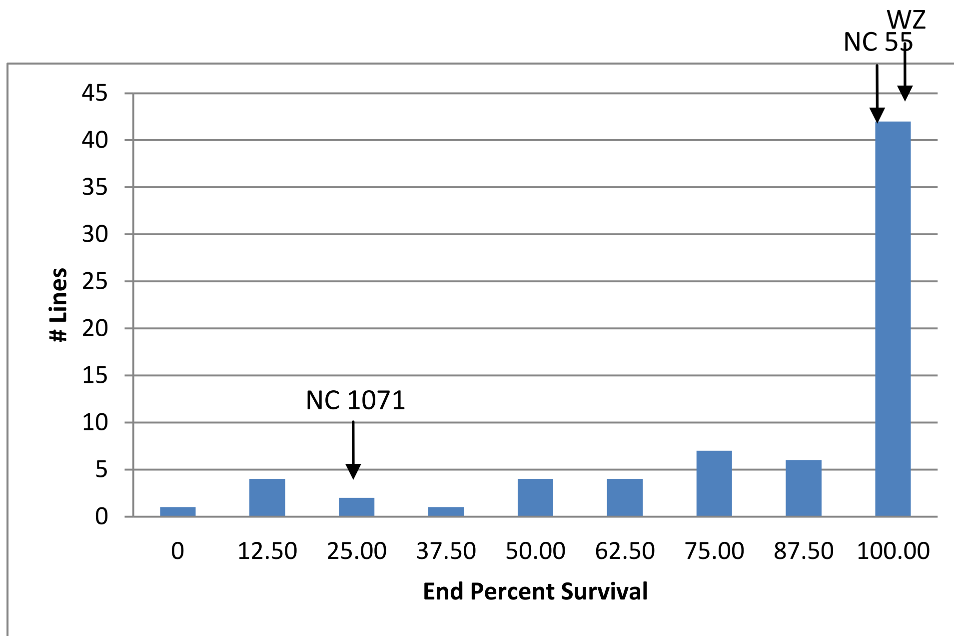

Figure 2.12. Frequency histogram for end percent survival for DH population inoculated with race 1 *P. nicotianae*.

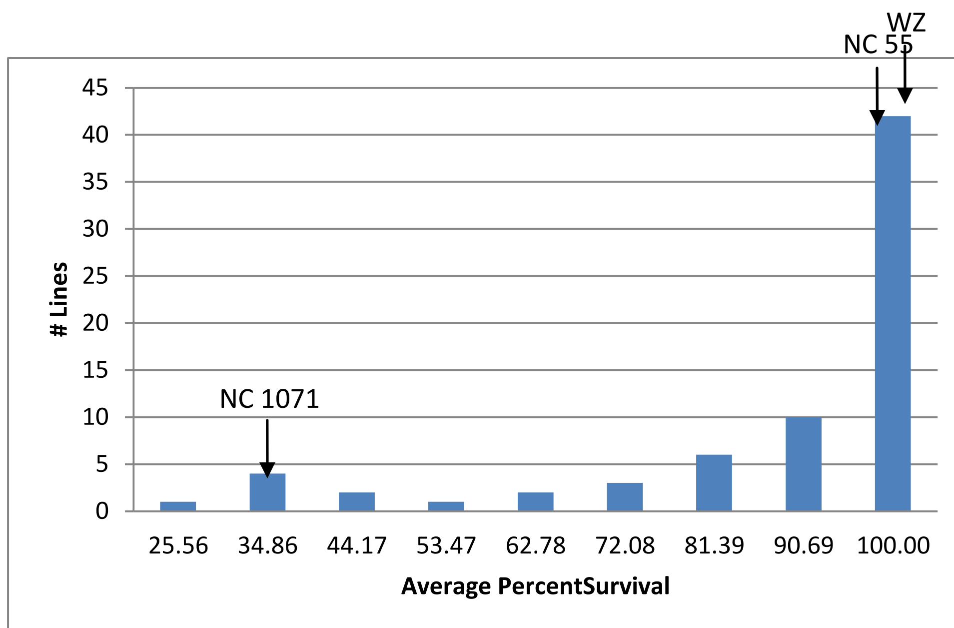

Figure 2.13. Frequency histogram for average percent survival for DH population inoculated with race 1 *P. nicotianae*.

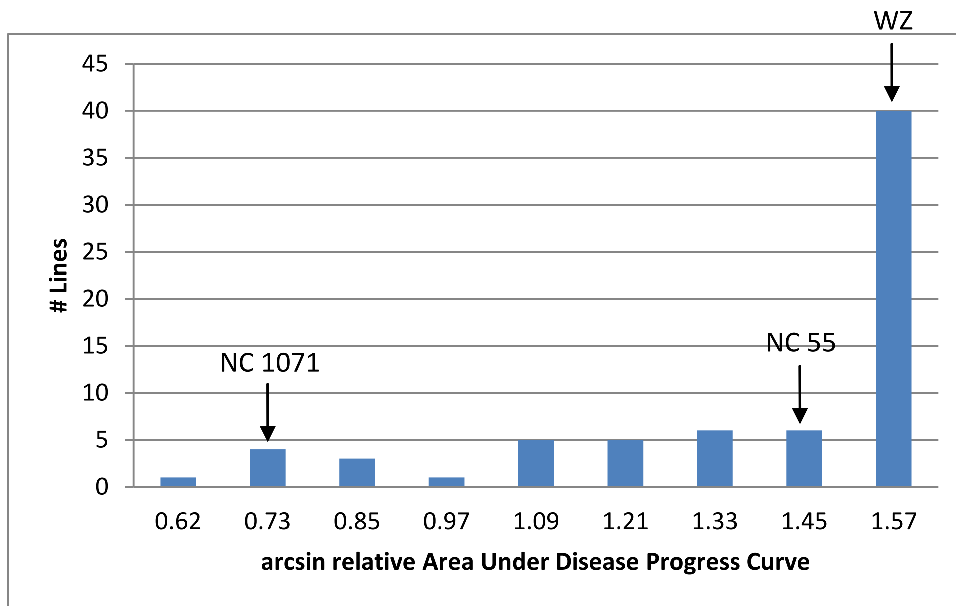

Figure 2.14. Frequency histogram for arcsin relative area under disease progress curve for DH population inoculated with race 1 *P. nicotianae*.

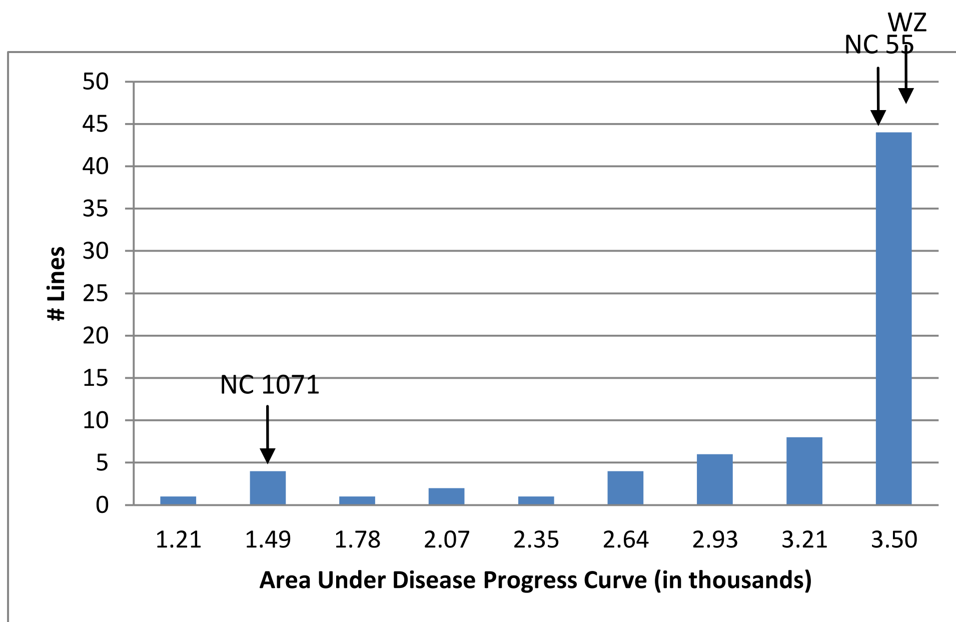

Figure 2.15. Frequency histogram for area under disease progress curve for DH population inoculated with race 1 *P. nicotianae*.

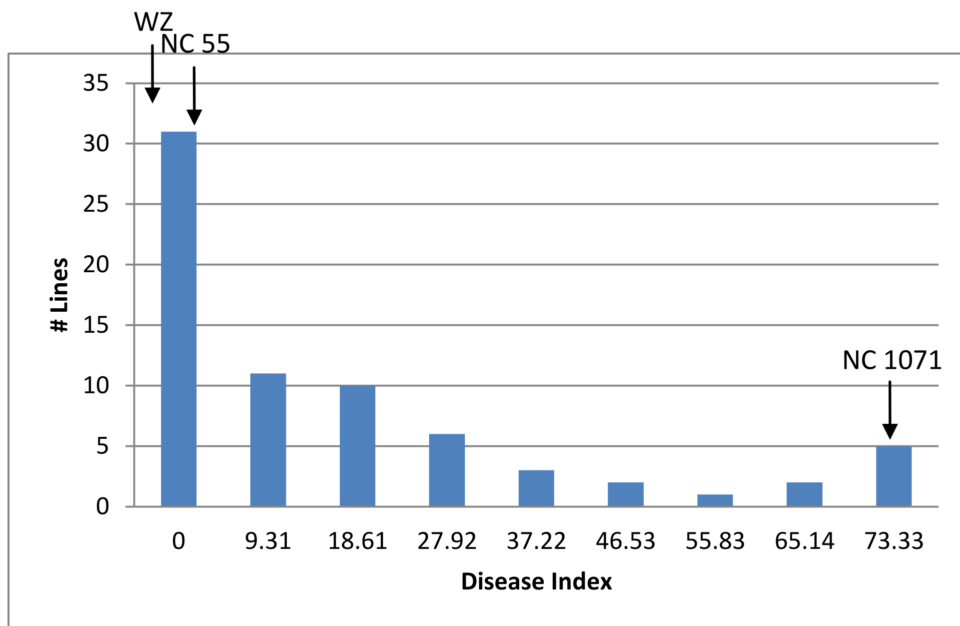

Figure 2.16. Frequency histogram for disease index for DH population inoculated with race 1 *P. nicotianae*.

Table 2.12. ANOVA for end percent survival for DH population growth chamber data.

Source	DF[a]	SS[b]	MS[c]	F-Value	P-Value
Rep	2	75.30	37.65	0.13	0.8811
Race	1	95824.64	95824.64	24.95	0.0378
Rep*Race	2	7682.69	3841.34	12.92	<.0001
Entry	74	475608.20	6427.13	21.62	<.0001
Entry*Race	74	149823.11	2024.63	6.81	<.0001
Error	296	8798.68	297.24	-	-

R^2 = 0.8923, C.V. = 27.25
[a]DF, Degrees of Freedom; [b]SS, Sum of Squares; [c]MS, Mean Square

Table 2.13. ANOVA for average percent survival for DH population growth chamber data.

Source	DF[a]	SS[b]	MS[c]	F-Value	P-Value
Rep	2	2045.51	1022.75	6	0.0028
Race	1	34322.09	34322.09	13.24	0.0679
Rep*Race	2	5185.02	2592.51	15.21	<.0001
Entry	74	278179.32	3759.18	22.05	<.0001
Entry*Race	74	62754.12	848.028	4.97	<.0001
Error	296	50465.91	170.49	-	-

R^2 = 0.8834, C.V. = 17.21
[a]DF, Degrees of Freedom; [b]SS, Sum of Squares; [c]MS, Mean Square

Table 2.14. ANOVA for arcsin relative area under disease progress curve for DH population growth chamber data.

Source	DF[a]	SS[b]	MS[c]	F-Value	P-Value
Rep	2	0.28	0.14	5.38	0.0051
Race	1	4.82	4.82	12.08	0.0738
Rep*Race	2	0.79	0.39	14.84	<.0001
Entry	74	46.41	0.62	23.27	<.0001
Entry*Race	74	9.18	0.12	4.61	<.0001
Error	296	7.97	0.03	-	-

R^2 = 0.8852, C.V. = 13.33
[a]DF, Degrees of Freedom; [b]SS, Sum of Squares; [c]MS, Mean Square

Table 2.15. ANOVA for area under disease progress curve for DH population growth chamber data.

Source	DF[a]	SS[b]	MS[c]	F-Value	P-Value
Rep	2	2464622	1232311	7.31	0.0008
Race	1	29167801	29167801	11.84	0.0751
Rep*Race	2	4927056	2463528	14.61	<.0001
Entry	74	260000000	3508728	20.8	<.0001
Entry*Race	74	56483506	763290	4.53	<.0001
Error	296	49922469	168657	-	-

R^2 = 0.8760, C.V. = 14.75
[a]DF, Degrees of Freedom; [b]SS, Sum of Squares; [c]MS, Mean Square

Table 2.16. ANOVA for disease index for DH population growth chamber data.

Source	DF[a]	SS[b]	MS[c]	F-Value	P-Value
Rep	2	2045.50	1022.75	6	0.0028
Race	1	34322.02	34322.02	13.24	0.0679
Rep*Race	2	5185.00	2592.50	15.21	<.0001
Entry	74	278179.63	3759.18	22.05	<.0001
Entry*Race	74	62753.97	848.02	4.97	<.0001
Error	296	50465.7	170.49	-	-

R^2 = 0.8834, C.V. = 54.05

[a]DF, Degrees of Freedom; [b]SS, Sum of Squares; [c]MS, Mean Square

Table 2.17. Marker F-tests for disease measures for WZ x NC 55 DH population inoculated with race 0 *P. nicotianae*.

Dependent Variable	Marker	F-Value	P-Value	R^2
EPS[a]	M1	287.01	<.0001	0.8200
EPS	M2	494.87	<.0001	0.8855
EPS	M3	475.08	<.0001	0.8829
EPS	M4	475.08	<.0001	0.8829
EPS	M5	578.73	<.0001	0.8976
EPS	M6	578.73	<.0001	0.8976
EPS	M7	389.18	<.0001	0.8531
EPS	M8	514.67	<.0001	0.8879
EPS	M9	388.16	<.0001	0.8547
EPS	M10	341.98	<.0001	0.8341
EPS	M11	573.49	<.0001	0.8940
EPS	M12	573.49	<.0001	0.8940
EPS	M13	573.49	<.0001	0.8940
EPS	M14	573.49	<.0001	0.8940
EPS	M15	553.98	<.0001	0.8921
EPS	M16	553.98	<.0001	0.8921
EPS	M17	553.98	<.0001	0.8921
EPS	M18	553.98	<.0001	0.8921
EPS	M19	573.49	<.0001	0.8940
EPS	M20	529.84	<.0001	0.8907
EPS	M21	591.27	<.0001	0.8982
EPS	M22	573.49	<.0001	0.8940
EPS	M23	573.49	<.0001	0.8940
EPS	M24	573.49	<.0001	0.8940
EPS	M25	573.49	<.0001	0.8940
EPS	M26	544.69	<.0001	0.8890
EPS	M27	351.95	<.0001	0.8482
EPS	M28	327.36	<.0001	0.8301
EPS	M29	377.36	<.0001	0.8531
EPS	M30	377.36	<.0001	0.8531
EPS	M31	278.34	<.0001	0.8037
EPS	M32	422.47	<.0001	0.8614
EPS	M33	422.47	<.0001	0.8614

Table 2.17. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
EPS	M34	553.98	<.0001	0.8921
EPS	M35	553.98	<.0001	0.8921
EPS	M36	390.17	<.0001	0.8572
EPS	M37	429.04	<.0001	0.8649
EPS	M38	429.04	<.0001	0.8649
EPS	M39	429.04	<.0001	0.8649
EPS	M40	270.61	<.0001	0.7992
EPS	M41	422.47	<.0001	0.8614
EPS	M42	422.47	<.0001	0.8614
EPS	M43	573.49	<.0001	0.8940
EPS	M44	553.74	<.0001	0.8921
EPS	M45	509.43	<.0001	0.8884
EPS	M46	509.43	<.0001	0.8884
APS[b]	M1	140.79	<.0001	0.6909
APS	M2	189.82	<.0001	0.7479
APS	M3	181.9	<.0001	0.7428
APS	M4	181.9	<.0001	0.7428
APS	M5	213.83	<.0001	0.7641
APS	M6	213.83	<.0001	0.7641
APS	M7	179.16	<.0001	0.7278
APS	M8	197.75	<.0001	0.7526
APS	M9	164.42	<.0001	0.7136
APS	M10	171.76	<.0001	0.7164
APS	M11	221.18	<.0001	0.7648
APS	M12	221.18	<.0001	0.7648
APS	M13	221.18	<.0001	0.7648
APS	M14	221.18	<.0001	0.7648
APS	M15	213.43	<.0001	0.7611
APS	M16	213.43	<.0001	0.7611
APS	M17	213.43	<.0001	0.7611
APS	M18	213.43	<.0001	0.7611
APS	M19	221.18	<.0001	0.7648
APS	M20	219.87	<.0001	0.7718
APS	M21	229.39	<.0001	0.7739
APS	M22	221.18	<.0001	0.7648

Table 2.17. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
APS	M23	221.18	<.0001	0.7648
APS	M24	221.18	<.0001	0.7648
APS	M25	221.18	<.0001	0.7648
APS	M26	216.96	<.0001	0.7614
APS	M27	152.55	<.0001	0.7077
APS	M28	156.47	<.0001	0.7002
APS	M29	162.39	<.0001	0.7141
APS	M30	162.39	<.0001	0.7141
APS	M31	145.8	<.0001	0.6819
APS	M32	182.33	<.0001	0.7284
APS	M33	182.33	<.0001	0.7284
APS	M34	213.43	<.0001	0.7611
APS	M35	213.43	<.0001	0.7611
APS	M36	179.24	<.0001	0.7339
APS	M37	187.31	<.0001	0.7365
APS	M38	187.31	<.0001	0.7365
APS	M39	187.31	<.0001	0.7365
APS	M40	121.18	<.0001	0.6406
APS	M41	182.33	<.0001	0.7284
APS	M42	182.33	<.0001	0.7284
APS	M43	221.18	<.0001	0.7648
APS	M44	213.23	<.0001	0.7609
APS	M45	191.62	<.0001	0.7496
APS	M46	191.62	<.0001	0.7496
arAUDPC[c]	M1	169.81	<.0001	0.7294
arAUDPC	M2	244.2	<.0001	0.7923
arAUDPC	M3	233.79	<.0001	0.7877
arAUDPC	M4	233.79	<.0001	0.7877
arAUDPC	M5	276.86	<.0001	0.8075
arAUDPC	M6	276.86	<.0001	0.8075
arAUDPC	M7	232.6	<.0001	0.7764
arAUDPC	M8	254.61	<.0001	0.7966
arAUDPC	M9	203.77	<.0001	0.7553
arAUDPC	M10	207.42	<.0001	0.7531
arAUDPC	M11	284.71	<.0001	0.8072

Table 2.17. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
arAUDPC	M12	284.71	<.0001	0.8072
arAUDPC	M13	284.71	<.0001	0.8072
arAUDPC	M14	284.71	<.0001	0.8072
arAUDPC	M15	274.87	<.0001	0.8040
arAUDPC	M16	274.87	<.0001	0.8040
arAUDPC	M17	274.87	<.0001	0.8040
arAUDPC	M18	274.87	<.0001	0.8040
arAUDPC	M19	284.71	<.0001	0.8072
arAUDPC	M20	282.37	<.0001	0.8129
arAUDPC	M21	298.53	<.0001	0.8167
arAUDPC	M22	284.71	<.0001	0.8072
arAUDPC	M23	284.71	<.0001	0.8072
arAUDPC	M24	284.71	<.0001	0.8072
arAUDPC	M25	284.71	<.0001	0.8072
arAUDPC	M26	283.19	<.0001	0.8064
arAUDPC	M27	190.88	<.0001	0.7519
arAUDPC	M28	194.32	<.0001	0.7436
arAUDPC	M29	203.83	<.0001	0.7582
arAUDPC	M30	203.83	<.0001	0.7582
arAUDPC	M31	173.99	<.0001	0.7190
arAUDPC	M32	228.87	<.0001	0.7709
arAUDPC	M33	228.87	<.0001	0.7709
arAUDPC	M34	274.87	<.0001	0.8040
arAUDPC	M35	274.87	<.0001	0.8040
arAUDPC	M36	224.24	<.0001	0.7753
arAUDPC	M37	236.85	<.0001	0.7795
arAUDPC	M38	236.85	<.0001	0.7795
arAUDPC	M39	236.85	<.0001	0.7795
arAUDPC	M40	148.32	<.0001	0.6856
arAUDPC	M41	228.87	<.0001	0.7709
arAUDPC	M42	228.87	<.0001	0.7709
arAUDPC	M43	284.71	<.0001	0.8072
arAUDPC	M44	274.29	<.0001	0.8037
arAUDPC	M45	247.12	<.0001	0.7943
arAUDPC	M46	247.12	<.0001	0.7943

Table 2.17. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
AUDPC[d]	M1	122.07	<.0001	0.6596
AUDPC	M2	160.17	<.0001	0.7145
AUDPC	M3	153.51	<.0001	0.7090
AUDPC	M4	153.51	<.0001	0.7090
AUDPC	M5	179.78	<.0001	0.7315
AUDPC	M6	179.78	<.0001	0.7315
AUDPC	M7	154.35	<.0001	0.6973
AUDPC	M8	166.84	<.0001	0.7196
AUDPC	M9	140.71	<.0001	0.6807
AUDPC	M10	149.23	<.0001	0.6870
AUDPC	M11	186.64	<.0001	0.7330
AUDPC	M12	186.64	<.0001	0.7330
AUDPC	M13	186.64	<.0001	0.7330
AUDPC	M14	186.64	<.0001	0.7330
AUDPC	M15	180.07	<.0001	0.7288
AUDPC	M16	180.07	<.0001	0.7288
AUDPC	M17	180.07	<.0001	0.7288
AUDPC	M18	180.07	<.0001	0.7288
AUDPC	M19	186.64	<.0001	0.7330
AUDPC	M20	186.69	<.0001	0.7417
AUDPC	M21	193.48	<.0001	0.7428
AUDPC	M22	186.64	<.0001	0.7330
AUDPC	M23	186.64	<.0001	0.7330
AUDPC	M24	186.64	<.0001	0.7330
AUDPC	M25	186.64	<.0001	0.7330
AUDPC	M26	183.63	<.0001	0.7298
AUDPC	M27	130.87	<.0001	0.6750
AUDPC	M28	135.74	<.0001	0.6695
AUDPC	M29	138.99	<.0001	0.6814
AUDPC	M30	138.99	<.0001	0.6814
AUDPC	M31	127.73	<.0001	0.6526
AUDPC	M32	156.11	<.0001	0.6966
AUDPC	M33	156.11	<.0001	0.6966
AUDPC	M34	180.07	<.0001	0.7288
AUDPC	M35	180.07	<.0001	0.7288

Table 2.17. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
AUDPC	M36	154.43	<.0001	0.7038
AUDPC	M37	160.47	<.0001	0.7055
AUDPC	M38	160.47	<.0001	0.7055
AUDPC	M39	160.47	<.0001	0.7055
AUDPC	M40	104.96	<.0001	0.6068
AUDPC	M41	156.11	<.0001	0.6966
AUDPC	M42	156.11	<.0001	0.6966
AUDPC	M43	186.64	<.0001	0.7330
AUDPC	M44	179.97	<.0001	0.7287
AUDPC	M45	161.44	<.0001	0.7161
AUDPC	M46	161.44	<.0001	0.7161
DI[e]	M1	140.79	<.0001	0.6909
DI	M2	189.82	<.0001	0.7479
DI	M3	181.9	<.0001	0.7428
DI	M4	181.9	<.0001	0.7428
DI	M5	213.83	<.0001	0.7641
DI	M6	213.83	<.0001	0.7641
DI	M7	179.16	<.0001	0.7278
DI	M8	197.75	<.0001	0.7526
DI	M9	164.42	<.0001	0.7136
DI	M10	171.76	<.0001	0.7164
DI	M11	221.18	<.0001	0.7649
DI	M12	221.18	<.0001	0.7649
DI	M13	221.18	<.0001	0.7649
DI	M14	221.18	<.0001	0.7649
DI	M15	213.43	<.0001	0.7611
DI	M16	213.43	<.0001	0.7611
DI	M17	213.43	<.0001	0.7611
DI	M18	213.43	<.0001	0.7611
DI	M19	221.18	<.0001	0.7649
DI	M20	219.87	<.0001	0.7718
DI	M21	229.39	<.0001	0.7739
DI	M22	221.18	<.0001	0.7649
DI	M23	221.18	<.0001	0.7649
DI	M24	221.18	<.0001	0.7649

Table 2.17. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
DI	M25	221.18	<.0001	0.7649
DI	M26	216.96	<.0001	0.7614
DI	M27	152.55	<.0001	0.7077
DI	M28	156.47	<.0001	0.7002
DI	M29	162.39	<.0001	0.7141
DI	M30	162.39	<.0001	0.7141
DI	M31	145.8	<.0001	0.6819
DI	M32	182.33	<.0001	0.7284
DI	M33	182.33	<.0001	0.7284
DI	M34	213.43	<.0001	0.7611
DI	M35	213.43	<.0001	0.7611
DI	M36	179.24	<.0001	0.7339
DI	M37	187.31	<.0001	0.7365
DI	M38	187.31	<.0001	0.7365
DI	M39	187.31	<.0001	0.7365
DI	M40	121.18	<.0001	0.6406
DI	M41	182.33	<.0001	0.7284
DI	M42	182.33	<.0001	0.7284
DI	M43	221.18	<.0001	0.7649
DI	M44	213.23	<.0001	0.7609
DI	M45	191.62	<.0001	0.7496
DI	M46	191.62	<.0001	0.7496

[a]EPS, End Percent Survival; [b]APS, Average Percent Survival;
[c]arcsin relative Area Under Disease Progress Curve; [d]Area Under Disease Progress Curve;
[e]Disease Index; [f]Least Significant Difference

Table 2.18. Marker F-tests for WZ x NC 55 DH population inoculated with race 1 *P. nicotianae* disease measures.

Dependent Variable	Marker	F-Value	P-Value	R^2
EPS[a]	M1	30.06	<.0001	0.3230
EPS	M2	34.87	<.0001	0.3527
EPS	M3	33.45	<.0001	0.3468
EPS	M4	33.45	<.0001	0.3468
EPS	M5	37.72	<.0001	0.3637
EPS	M6	37.72	<.0001	0.3637
EPS	M7	40.31	<.0001	0.3757
EPS	M8	36.3	<.0001	0.3583
EPS	M9	28.38	<.0001	0.3007
EPS	M10	36.77	<.0001	0.3509
EPS	M11	40.58	<.0001	0.3737
EPS	M12	40.58	<.0001	0.3737
EPS	M13	40.58	<.0001	0.3737
EPS	M14	40.58	<.0001	0.3737
EPS	M15	39.15	<.0001	0.3688
EPS	M16	39.15	<.0001	0.3688
EPS	M17	39.15	<.0001	0.3688
EPS	M18	39.15	<.0001	0.3688
EPS	M19	40.58	<.0001	0.3737
EPS	M20	41.5	<.0001	0.3897
EPS	M21	41.2	<.0001	0.3808
EPS	M22	40.58	<.0001	0.3737
EPS	M23	40.58	<.0001	0.3737
EPS	M24	40.58	<.0001	0.3737
EPS	M25	40.58	<.0001	0.3737
EPS	M26	36.77	<.0001	0.3509
EPS	M27	31.4	<.0001	0.3326
EPS	M28	32.09	<.0001	0.3238
EPS	M29	32.74	<.0001	0.3350
EPS	M30	32.74	<.0001	0.3350
EPS	M31	33.38	<.0001	0.3293
EPS	M32	36.77	<.0001	0.3509
EPS	M33	36.77	<.0001	0.3509

Table 2.18. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
EPS	M34	39.15	<.0001	0.3688
EPS	M35	39.15	<.0001	0.3688
EPS	M36	37.35	<.0001	0.3649
EPS	M37	37.25	<.0001	0.3573
EPS	M38	37.25	<.0001	0.3573
EPS	M39	37.25	<.0001	0.3573
EPS	M40	25.6	<.0001	0.2735
EPS	M41	36.77	<.0001	0.3509
EPS	M42	36.77	<.0001	0.3509
EPS	M43	40.58	<.0001	0.3737
EPS	M44	39.15	<.0001	0.3688
EPS	M45	34.87	<.0001	0.3527
EPS	M46	34.87	<.0001	0.3527
APS[b]	M1	26.13	<.0001	0.2931
APS	M2	29.97	<.0001	0.3189
APS	M3	28.79	<.0001	0.3137
APS	M4	28.79	<.0001	0.3137
APS	M5	32.32	<.0001	0.3287
APS	M6	32.32	<.0001	0.3287
APS	M7	34.82	<.0001	0.3420
APS	M8	31.14	<.0001	0.3239
APS	M9	25.33	<.0001	0.2773
APS	M10	31.69	<.0001	0.3179
APS	M11	34.67	<.0001	0.3377
APS	M12	34.67	<.0001	0.3377
APS	M13	34.67	<.0001	0.3377
APS	M14	34.67	<.0001	0.3377
APS	M15	33.49	<.0001	0.3333
APS	M16	33.49	<.0001	0.3333
APS	M17	33.49	<.0001	0.3333
APS	M18	33.49	<.0001	0.3333
APS	M19	34.67	<.0001	0.3377
APS	M20	35.07	<.0001	0.3504
APS	M21	35.03	<.0001	0.3433
APS	M22	34.67	<.0001	0.3377

Table 2.18. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
APS	M23	34.67	<.0001	0.3377
APS	M24	34.67	<.0001	0.3377
APS	M25	34.67	<.0001	0.3377
APS	M26	31.69	<.0001	0.3179
APS	M27	27.48	<.0001	0.3037
APS	M28	27.04	<.0001	0.2875
APS	M29	28.35	<.0001	0.3037
APS	M30	28.35	<.0001	0.3037
APS	M31	29	<.0001	0.2990
APS	M32	31.69	<.0001	0.3179
APS	M33	31.69	<.0001	0.3179
APS	M34	33.49	<.0001	0.3333
APS	M35	33.49	<.0001	0.3333
APS	M36	31.88	<.0001	0.3291
APS	M37	31.96	<.0001	0.3230
APS	M38	31.96	<.0001	0.3230
APS	M39	31.96	<.0001	0.3230
APS	M40	20.59	<.0001	0.2325
APS	M41	31.69	<.0001	0.3179
APS	M42	31.69	<.0001	0.3179
APS	M43	34.67	<.0001	0.3377
APS	M44	33.49	<.0001	0.3333
APS	M45	29.97	<.0001	0.3189
APS	M46	29.97	<.0001	0.3189
arAUDPC[c]	M1	37.02	<.0001	0.3701
arAUDPC	M2	43.26	<.0001	0.4033
arAUDPC	M3	41.51	<.0001	0.3972
arAUDPC	M4	41.51	<.0001	0.3972
arAUDPC	M5	46.78	<.0001	0.4148
arAUDPC	M6	46.78	<.0001	0.4148
arAUDPC	M7	52.12	<.0001	0.4375
arAUDPC	M8	45.02	<.0001	0.4092
arAUDPC	M9	37.41	<.0001	0.3618
arAUDPC	M10	45.22	<.0001	0.3994
arAUDPC	M11	50.31	<.0001	0.4252

Table 2.18. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
arAUDPC	M12	50.31	<.0001	0.4252
arAUDPC	M13	50.31	<.0001	0.4252
arAUDPC	M14	50.31	<.0001	0.4252
arAUDPC	M15	48.54	<.0001	0.4201
arAUDPC	M16	48.54	<.0001	0.4201
arAUDPC	M17	48.54	<.0001	0.4201
arAUDPC	M18	48.54	<.0001	0.4201
arAUDPC	M19	50.31	<.0001	0.4252
arAUDPC	M20	51.37	<.0001	0.4414
arAUDPC	M21	50.14	<.0001	0.4280
arAUDPC	M22	50.31	<.0001	0.4252
arAUDPC	M23	50.31	<.0001	0.4252
arAUDPC	M24	50.31	<.0001	0.4252
arAUDPC	M25	50.31	<.0001	0.4252
arAUDPC	M26	45.22	<.0001	0.3994
arAUDPC	M27	39.11	<.0001	0.3830
arAUDPC	M28	40.13	<.0001	0.3746
arAUDPC	M29	40.3	<.0001	0.3827
arAUDPC	M30	40.3	<.0001	0.3827
arAUDPC	M31	40.78	<.0001	0.3749
arAUDPC	M32	45.22	<.0001	0.3994
arAUDPC	M33	45.22	<.0001	0.3994
arAUDPC	M34	48.54	<.0001	0.4201
arAUDPC	M35	48.54	<.0001	0.4201
arAUDPC	M36	45.84	<.0001	0.4136
arAUDPC	M37	45	<.0001	0.4018
arAUDPC	M38	45	<.0001	0.4018
arAUDPC	M39	45	<.0001	0.4018
arAUDPC	M40	30.69	<.0001	0.3110
arAUDPC	M41	45.22	<.0001	0.3994
arAUDPC	M42	45.22	<.0001	0.3994
arAUDPC	M43	50.31	<.0001	0.4252
arAUDPC	M44	48.54	<.0001	0.4201
arAUDPC	M45	43.26	<.0001	0.4033
arAUDPC	M46	43.26	<.0001	0.4033

Table 2.18. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
AUDPC[d]	M1	25.38	<.0001	0.2872
AUDPC	M2	29.06	<.0001	0.3122
AUDPC	M3	27.93	<.0001	0.3071
AUDPC	M4	27.93	<.0001	0.3071
AUDPC	M5	31.32	<.0001	0.3218
AUDPC	M6	31.32	<.0001	0.3218
AUDPC	M7	33.78	<.0001	0.3352
AUDPC	M8	30.19	<.0001	0.3171
AUDPC	M9	24.72	<.0001	0.2725
AUDPC	M10	30.74	<.0001	0.3113
AUDPC	M11	33.58	<.0001	0.3306
AUDPC	M12	33.58	<.0001	0.3306
AUDPC	M13	33.58	<.0001	0.3306
AUDPC	M14	33.58	<.0001	0.3306
AUDPC	M15	32.45	<.0001	0.3263
AUDPC	M16	32.45	<.0001	0.3263
AUDPC	M17	32.45	<.0001	0.3263
AUDPC	M18	32.45	<.0001	0.3263
AUDPC	M19	33.58	<.0001	0.3306
AUDPC	M20	33.84	<.0001	0.3424
AUDPC	M21	33.91	<.0001	0.3360
AUDPC	M22	33.58	<.0001	0.3306
AUDPC	M23	33.58	<.0001	0.3306
AUDPC	M24	33.58	<.0001	0.3306
AUDPC	M25	33.58	<.0001	0.3306
AUDPC	M26	30.74	<.0001	0.3113
AUDPC	M27	26.72	<.0001	0.2978
AUDPC	M28	26.15	<.0001	0.2807
AUDPC	M29	27.52	<.0001	0.2975
AUDPC	M30	27.52	<.0001	0.2975
AUDPC	M31	28.18	<.0001	0.2930
AUDPC	M32	30.74	<.0001	0.3113
AUDPC	M33	30.74	<.0001	0.3113
AUDPC	M34	32.45	<.0001	0.3263
AUDPC	M35	32.45	<.0001	0.3263

Table 2.18. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
AUDPC	M36	30.82	<.0001	0.3217
AUDPC	M37	30.99	<.0001	0.3162
AUDPC	M38	30.99	<.0001	0.3162
AUDPC	M39	30.99	<.0001	0.3162
AUDPC	M40	19.77	<.0001	0.2253
AUDPC	M41	30.74	<.0001	0.3113
AUDPC	M42	30.74	<.0001	0.3113
AUDPC	M43	33.58	<.0001	0.3306
AUDPC	M44	32.45	<.0001	0.3263
AUDPC	M45	29.06	<.0001	0.3122
AUDPC	M46	29.06	<.0001	0.3122
DI[e]	M1	26.13	<.0001	0.2931
DI	M2	29.97	<.0001	0.3189
DI	M3	28.79	<.0001	0.3137
DI	M4	28.79	<.0001	0.3137
DI	M5	32.32	<.0001	0.3287
DI	M6	32.32	<.0001	0.3287
DI	M7	34.82	<.0001	0.3420
DI	M8	31.14	<.0001	0.3239
DI	M9	25.33	<.0001	0.2773
DI	M10	31.69	<.0001	0.3179
DI	M11	34.67	<.0001	0.3377
DI	M12	34.67	<.0001	0.3377
DI	M13	34.67	<.0001	0.3377
DI	M14	34.67	<.0001	0.3377
DI	M15	33.49	<.0001	0.3333
DI	M16	33.49	<.0001	0.3333
DI	M17	33.49	<.0001	0.3333
DI	M18	33.49	<.0001	0.3333
DI	M19	34.67	<.0001	0.3377
DI	M20	35.07	<.0001	0.3504
DI	M21	35.03	<.0001	0.3433
DI	M22	34.67	<.0001	0.3377
DI	M23	34.67	<.0001	0.3377
DI	M24	34.67	<.0001	0.3377

Table 2.18. Continued.

Dependent Variable	Marker	F-Value	P-Value	R^2
DI	M25	34.67	<.0001	0.3377
DI	M26	31.69	<.0001	0.3179
DI	M27	27.48	<.0001	0.3037
DI	M28	27.04	<.0001	0.2875
DI	M29	28.35	<.0001	0.3037
DI	M30	28.35	<.0001	0.3037
DI	M31	29.00	<.0001	0.2990
DI	M32	31.69	<.0001	0.3179
DI	M33	31.69	<.0001	0.3179
DI	M34	33.49	<.0001	0.3333
DI	M35	33.49	<.0001	0.3333
DI	M36	31.89	<.0001	0.3291
DI	M37	31.96	<.0001	0.3230
DI	M38	31.96	<.0001	0.3230
DI	M39	31.96	<.0001	0.3230
DI	M40	20.59	<.0001	0.2325
DI	M41	31.69	<.0001	0.3179
DI	M42	31.69	<.0001	0.3179
DI	M43	34.67	<.0001	0.3377
DI	M44	33.49	<.0001	0.3333
DI	M45	29.97	<.0001	0.3189
DI	M46	29.97	<.0001	0.3189

[a]EPS, End Percent Survival; [b]APS, Average Percent Survival;
[c]arcsin relative Area Under Disease Progress Curve; [d]Area Under Disease Progress Curve;
[e]Disease Index; [f]Least Significant Difference

Table 2.19. Marker group means for WZ x NC 55 DH population inoculated with race 0 *P. nicotianae*.

Disease Measure	Marker	Genotype	Mean	Standard Error
EPS[a]	M1	0	11.94	2.92
EPS	M1	1	91.78	3.70
EPS	M2	0	9.69	2.35
EPS	M2	1	91.56	2.83
EPS	M3	0	9.69	2.37
EPS	M3	1	91.24	2.90
EPS	M4	0	9.69	2.37
EPS	M4	1	91.24	2.90
EPS	M5	0	9.69	2.27
EPS	M5	1	93.49	2.64
EPS	M6	0	9.69	2.27
EPS	M6	1	93.49	2.64
EPS	M7	0	11.82	2.70
EPS	M7	1	92.59	3.08
EPS	M8	0	9.69	2.34
EPS	M8	1	91.87	2.76
EPS	M9	0	8.41	2.78
EPS	M9	1	89.43	3.03
EPS	M10	0	11.94	2.84
EPS	M10	1	92.04	3.27
EPS	M11	0	9.69	2.30
EPS	M11	1	92.29	2.57
EPS	M12	0	9.69	2.30
EPS	M12	1	92.29	2.57
EPS	M13	0	9.69	2.30
EPS	M13	1	92.29	2.57
EPS	M14	0	9.69	2.30
EPS	M14	1	92.29	2.57
EPS	M15	0	9.69	2.31
EPS	M15	1	92.22	2.64
EPS	M16	0	9.69	2.31
EPS	M16	1	92.22	2.64
EPS	M17	0	9.69	2.31

Table 2.19. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
EPS	M17	1	92.22	2.64
EPS	M18	0	9.69	2.31
EPS	M18	1	92.22	2.64
EPS	M19	0	9.69	2.30
EPS	M19	1	92.29	2.57
EPS	M20	0	10.19	2.43
EPS	M20	1	92.29	2.61
EPS	M21	0	9.06	2.29
EPS	M21	1	92.29	2.54
EPS	M22	0	9.69	2.30
EPS	M22	1	92.29	2.57
EPS	M23	0	9.69	2.30
EPS	M23	1	92.29	2.57
EPS	M24	0	9.69	2.30
EPS	M24	1	92.29	2.57
EPS	M25	0	9.69	2.30
EPS	M25	1	92.29	2.57
EPS	M26	0	10.83	2.32
EPS	M26	1	93.52	2.68
EPS	M27	0	92.09	3.33
EPS	M27	1	11.54	2.72
EPS	M28	0	89.41	3.21
EPS	M28	1	10.21	2.98
EPS	M29	0	92.39	3.22
EPS	M29	1	11.39	2.65
EPS	M30	0	92.39	3.22
EPS	M30	1	11.39	2.65
EPS	M31	0	92.53	3.62
EPS	M31	1	13.55	3.05
EPS	M32	0	92.78	2.99
EPS	M32	1	11.39	2.59
EPS	M33	0	92.78	2.99
EPS	M33	1	11.39	2.59
EPS	M34	0	92.22	2.64
EPS	M34	1	9.69	2.31
EPS	M35	0	92.22	2.64
EPS	M35	1	9.69	2.31

Table 2.19. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
EPS	M36	0	92.78	3.04
EPS	M36	1	12.01	2.74
EPS	M37	0	92.78	2.97
EPS	M37	1	10.83	2.61
EPS	M38	0	92.78	2.97
EPS	M38	1	10.83	2.61
EPS	M39	0	92.78	2.97
EPS	M39	1	10.83	2.61
EPS	M40	0	89.78	3.54
EPS	M40	1	11.68	3.16
EPS	M41	0	92.78	2.99
EPS	M41	1	11.39	2.59
EPS	M42	0	92.78	2.99
EPS	M42	1	11.39	2.59
EPS	M43	0	9.69	2.30
EPS	M43	1	92.29	2.57
EPS	M44	0	92.04	2.63
EPS	M44	1	9.69	2.31
EPS	M45	0	9.69	2.34
EPS	M45	1	92.18	2.81
EPS	M46	0	9.69	2.34
EPS	M46	1	92.18	2.81
APS[b]	M1	0	41.94	2.83
APS	M1	1	96.00	3.57
APS	M2	0	40.46	2.58
APS	M2	1	96.13	3.11
APS	M3	0	40.46	2.60
APS	M3	1	95.98	3.19
APS	M4	0	40.46	2.60
APS	M4	1	95.98	3.19
APS	M5	0	40.46	2.53
APS	M5	1	97.16	2.94
APS	M6	0	40.46	2.53
APS	M6	1	97.16	2.94
APS	M7	0	41.74	2.71
APS	M7	1	96.74	3.09
APS	M8	0	40.46	2.57

Table 2.19. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
APS	M8	1	96.27	3.03
APS	M9	0	39.79	2.86
APS	M9	1	94.05	3.12
APS	M10	0	41.94	2.72
APS	M10	1	96.44	3.14
APS	M11	0	40.46	2.51
APS	M11	1	96.56	2.82
APS	M12	0	40.46	2.51
APS	M12	1	96.56	2.82
APS	M13	0	40.46	2.51
APS	M13	1	96.56	2.82
APS	M14	0	40.46	2.51
APS	M14	1	96.56	2.82
APS	M15	0	40.46	2.53
APS	M15	1	96.48	2.88
APS	M16	0	40.46	2.53
APS	M16	1	96.48	2.88
APS	M17	0	40.46	2.53
APS	M17	1	96.48	2.88
APS	M18	0	40.46	2.53
APS	M18	1	96.48	2.88
APS	M19	0	40.46	2.51
APS	M19	1	96.56	2.82
APS	M20	0	41.60	2.52
APS	M20	1	96.56	2.72
APS	M21	0	39.80	2.51
APS	M21	1	96.56	2.78
APS	M22	0	40.46	2.51
APS	M22	1	96.56	2.82
APS	M23	0	40.46	2.51
APS	M23	1	96.56	2.82
APS	M24	0	40.46	2.51
APS	M24	1	96.56	2.82
APS	M25	0	40.46	2.51
APS	M25	1	96.56	2.82
APS	M26	0	41.22	2.50
APS	M26	1	97.41	2.88

Table 2.19. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
APS	M27	0	96.28	3.43
APS	M27	1	41.62	2.80
APS	M28	0	94.41	3.14
APS	M28	1	40.78	2.92
APS	M29	0	96.42	3.31
APS	M29	1	41.75	2.72
APS	M30	0	96.42	3.31
APS	M30	1	41.75	2.72
APS	M31	0	96.59	3.39
APS	M31	1	43.17	2.85
APS	M32	0	96.70	3.08
APS	M32	1	41.75	2.66
APS	M33	0	96.70	3.08
APS	M33	1	41.75	2.66
APS	M34	0	96.48	2.88
APS	M34	1	40.46	2.53
APS	M35	0	96.48	2.88
APS	M35	1	40.46	2.53
APS	M36	0	96.70	2.98
APS	M36	1	42.97	2.69
APS	M37	0	96.70	3.05
APS	M37	1	41.14	2.68
APS	M38	0	96.70	3.05
APS	M38	1	41.14	2.68
APS	M39	0	96.70	3.05
APS	M39	1	41.14	2.68
APS	M40	0	93.91	3.48
APS	M40	1	42.56	3.10
APS	M41	0	96.70	3.08
APS	M41	1	41.75	2.66
APS	M42	0	96.70	3.08
APS	M42	1	41.75	2.66
APS	M43	0	40.46	2.51
APS	M43	1	96.56	2.82
APS	M44	0	96.44	2.88
APS	M44	1	40.46	2.53
APS	M45	0	40.46	2.58

Table 2.19. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
APS	M45	1	96.38	3.11
APS	M46	0	40.46	2.58
APS	M46	1	96.38	3.11
arAUDPC[c]	M1	0	0.81	0.03
arAUDPC	M1	1	1.49	0.04
arAUDPC	M2	0	0.79	0.03
arAUDPC	M2	1	1.50	0.03
arAUDPC	M3	0	0.79	0.03
arAUDPC	M3	1	1.49	0.04
arAUDPC	M4	0	0.79	0.03
arAUDPC	M4	1	1.49	0.04
arAUDPC	M5	0	0.79	0.03
arAUDPC	M5	1	1.51	0.03
arAUDPC	M6	0	0.79	0.03
arAUDPC	M6	1	1.51	0.03
arAUDPC	M7	0	0.80	0.03
arAUDPC	M7	1	1.51	0.03
arAUDPC	M8	0	0.79	0.03
arAUDPC	M8	1	1.50	0.03
arAUDPC	M9	0	0.78	0.03
arAUDPC	M9	1	1.47	0.04
arAUDPC	M10	0	0.81	0.03
arAUDPC	M10	1	1.50	0.04
arAUDPC	M11	0	0.79	0.03
arAUDPC	M11	1	1.50	0.03
arAUDPC	M12	0	0.79	0.03
arAUDPC	M12	1	1.50	0.03
arAUDPC	M13	0	0.79	0.03
arAUDPC	M13	1	1.50	0.03
arAUDPC	M14	0	0.79	0.03
arAUDPC	M14	1	1.50	0.03
arAUDPC	M15	0	0.79	0.03
arAUDPC	M15	1	1.50	0.03
arAUDPC	M16	0	0.79	0.03
arAUDPC	M16	1	1.50	0.03
arAUDPC	M17	0	0.79	0.03
arAUDPC	M17	1	1.50	0.03

Table 2.19. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
arAUDPC	M18	0	0.79	0.03
arAUDPC	M18	1	1.50	0.03
arAUDPC	M19	0	0.79	0.03
arAUDPC	M19	1	1.50	0.03
arAUDPC	M20	0	0.80	0.03
arAUDPC	M20	1	1.50	0.03
arAUDPC	M21	0	0.78	0.03
arAUDPC	M21	1	1.50	0.03
arAUDPC	M22	0	0.79	0.03
arAUDPC	M22	1	1.50	0.03
arAUDPC	M23	0	0.79	0.03
arAUDPC	M23	1	1.50	0.03
arAUDPC	M24	0	0.79	0.03
arAUDPC	M24	1	1.50	0.03
arAUDPC	M25	0	0.79	0.03
arAUDPC	M25	1	1.50	0.03
arAUDPC	M26	0	0.80	0.03
arAUDPC	M26	1	1.52	0.03
arAUDPC	M27	0	1.50	0.04
arAUDPC	M27	1	0.80	0.03
arAUDPC	M28	0	1.48	0.04
arAUDPC	M28	1	0.79	0.03
arAUDPC	M29	0	1.50	0.04
arAUDPC	M29	1	0.80	0.03
arAUDPC	M30	0	1.50	0.04
arAUDPC	M30	1	0.80	0.03
arAUDPC	M31	0	1.50	0.04
arAUDPC	M31	1	0.82	0.03
arAUDPC	M32	0	1.51	0.04
arAUDPC	M32	1	0.80	0.03
arAUDPC	M33	0	1.51	0.04
arAUDPC	M33	1	0.80	0.03
arAUDPC	M34	0	1.50	0.03
arAUDPC	M34	1	0.79	0.03
arAUDPC	M35	0	1.50	0.03
arAUDPC	M35	1	0.79	0.03
arAUDPC	M36	0	1.51	0.03

Table 2.19. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
arAUDPC	M36	1	0.82	0.03
arAUDPC	M37	0	1.51	0.03
arAUDPC	M37	1	0.80	0.03
arAUDPC	M38	0	1.51	0.03
arAUDPC	M38	1	0.80	0.03
arAUDPC	M39	0	1.51	0.03
arAUDPC	M39	1	0.80	0.03
arAUDPC	M40	0	1.47	0.04
arAUDPC	M40	1	0.81	0.04
arAUDPC	M41	0	1.51	0.04
arAUDPC	M41	1	0.80	0.03
arAUDPC	M42	0	1.51	0.04
arAUDPC	M42	1	0.80	0.03
arAUDPC	M43	0	0.79	0.03
arAUDPC	M43	1	1.50	0.03
arAUDPC	M44	0	1.50	0.03
arAUDPC	M44	1	0.79	0.03
arAUDPC	M45	0	0.79	0.03
arAUDPC	M45	1	1.50	0.03
arAUDPC	M46	0	0.79	0.03
arAUDPC	M46	1	1.50	0.03
AUDPC[d]	M1	0	1776.25	90.51
AUDPC	M1	1	3388.78	114.49
AUDPC	M2	0	1732.05	84.00
AUDPC	M2	1	3394.14	100.95
AUDPC	M3	0	1732.05	84.63
AUDPC	M3	1	3390.06	103.66
AUDPC	M4	0	1732.05	84.63
AUDPC	M4	1	3390.06	103.66
AUDPC	M5	0	1732.05	82.39
AUDPC	M5	1	3423.56	95.54
AUDPC	M6	0	1732.05	82.39
AUDPC	M6	1	3423.56	95.54
AUDPC	M7	0	1769.44	87.17
AUDPC	M7	1	3411.85	99.39
AUDPC	M8	0	1732.05	83.38
AUDPC	M8	1	3397.92	98.40

Table 2.19. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
AUDPC	M9	0	1713.21	91.96
AUDPC	M9	1	3328.76	100.46
AUDPC	M10	0	1776.25	87.20
AUDPC	M10	1	3403.43	100.69
AUDPC	M11	0	1732.05	81.57
AUDPC	M11	1	3406.54	91.49
AUDPC	M12	0	1732.05	81.57
AUDPC	M12	1	3406.54	91.49
AUDPC	M13	0	1732.05	81.57
AUDPC	M13	1	3406.54	91.49
AUDPC	M14	0	1732.05	81.57
AUDPC	M14	1	3406.54	91.49
AUDPC	M15	0	1732.05	82.16
AUDPC	M15	1	3404.07	93.68
AUDPC	M16	0	1732.05	82.16
AUDPC	M16	1	3404.07	93.68
AUDPC	M17	0	1732.05	82.16
AUDPC	M17	1	3404.07	93.68
AUDPC	M18	0	1732.05	82.16
AUDPC	M18	1	3404.07	93.68
AUDPC	M19	0	1732.05	81.57
AUDPC	M19	1	3406.54	91.49
AUDPC	M20	0	1770.53	81.45
AUDPC	M20	1	3406.54	87.77
AUDPC	M21	0	1711.11	81.70
AUDPC	M21	1	3406.54	90.45
AUDPC	M22	0	1732.05	81.57
AUDPC	M22	1	3406.54	91.49
AUDPC	M23	0	1732.05	81.57
AUDPC	M23	1	3406.54	91.49
AUDPC	M24	0	1732.05	81.57
AUDPC	M24	1	3406.54	91.49
AUDPC	M25	0	1732.05	81.57
AUDPC	M25	1	3406.54	91.49
AUDPC	M26	0	1754.86	81.02
AUDPC	M26	1	3431.95	93.56
AUDPC	M27	0	3397.54	110.44

Table 2.19. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
AUDPC	M27	1	1766.45	90.17
AUDPC	M28	0	3341.41	100.55
AUDPC	M28	1	1741.59	93.51
AUDPC	M29	0	3401.34	106.82
AUDPC	M29	1	1771.39	87.77
AUDPC	M30	0	3401.34	106.82
AUDPC	M30	1	1771.39	87.77
AUDPC	M31	0	3406.80	107.89
AUDPC	M31	1	1813.55	90.74
AUDPC	M32	0	3409.91	99.13
AUDPC	M32	1	1771.39	85.85
AUDPC	M33	0	3409.91	99.13
AUDPC	M33	1	1771.39	85.85
AUDPC	M34	0	3404.07	93.68
AUDPC	M34	1	1732.05	82.16
AUDPC	M35	0	3404.07	93.68
AUDPC	M35	1	1732.05	82.16
AUDPC	M36	0	3409.91	95.55
AUDPC	M36	1	1812.01	86.04
AUDPC	M37	0	3409.91	98.40
AUDPC	M37	1	1751.99	86.30
AUDPC	M38	0	3409.91	98.40
AUDPC	M38	1	1751.99	86.30
AUDPC	M39	0	3409.91	98.40
AUDPC	M39	1	1751.99	86.30
AUDPC	M40	0	3322.49	111.01
AUDPC	M40	1	1798.86	98.97
AUDPC	M41	0	3409.91	99.13
AUDPC	M41	1	1771.39	85.85
AUDPC	M42	0	3409.91	99.13
AUDPC	M42	1	1771.39	85.85
AUDPC	M43	0	1732.05	81.57
AUDPC	M43	1	3406.54	91.49
AUDPC	M44	0	3403.43	93.67
AUDPC	M44	1	1732.05	82.15
AUDPC	M45	0	1732.05	84.00

Table 2.19. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
AUDPC	M45	1	3400.62	100.95
AUDPC	M46	0	1732.05	84.00
AUDPC	M46	1	3400.62	100.95
DI[e]	M1	0	58.06	2.83
DI	M1	1	4.00	3.57
DI	M2	0	59.54	2.58
DI	M2	1	3.87	3.11
DI	M3	0	59.54	2.60
DI	M3	1	4.02	3.19
DI	M4	0	59.54	2.60
DI	M4	1	4.02	3.19
DI	M5	0	59.54	2.53
DI	M5	1	2.84	2.94
DI	M6	0	59.54	2.53
DI	M6	1	2.84	2.94
DI	M7	0	58.26	2.71
DI	M7	1	3.26	3.09
DI	M8	0	59.54	2.57
DI	M8	1	3.73	3.03
DI	M9	0	60.21	2.86
DI	M9	1	5.95	3.12
DI	M10	0	58.06	2.72
DI	M10	1	3.56	3.14
DI	M11	0	59.54	2.51
DI	M11	1	3.44	2.82
DI	M12	0	59.54	2.51
DI	M12	1	3.44	2.82
DI	M13	0	59.54	2.51
DI	M13	1	3.44	2.82
DI	M14	0	59.54	2.51
DI	M14	1	3.44	2.82
DI	M15	0	59.54	2.53
DI	M15	1	3.52	2.88
DI	M16	0	59.54	2.53
DI	M16	1	3.52	2.88
DI	M17	0	59.54	2.53

Table 2.19. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
DI	M17	1	3.52	2.88
DI	M18	0	59.54	2.53
DI	M18	1	3.52	2.88
DI	M19	0	59.54	2.51
DI	M19	1	3.44	2.82
DI	M20	0	58.40	2.52
DI	M20	1	3.44	2.72
DI	M21	0	60.20	2.51
DI	M21	1	3.44	2.78
DI	M22	0	59.54	2.51
DI	M22	1	3.44	2.82
DI	M23	0	59.54	2.51
DI	M23	1	3.44	2.82
DI	M24	0	59.54	2.51
DI	M24	1	3.44	2.82
DI	M25	0	59.54	2.51
DI	M25	1	3.44	2.82
DI	M26	0	58.78	2.50
DI	M26	1	2.59	2.88
DI	M27	0	3.72	3.43
DI	M27	1	58.38	2.80
DI	M28	0	5.59	3.14
DI	M28	1	59.22	2.92
DI	M29	0	3.58	3.31
DI	M29	1	58.25	2.72
DI	M30	0	3.58	3.31
DI	M30	1	58.25	2.72
DI	M31	0	3.41	3.39
DI	M31	1	56.83	2.85
DI	M32	0	3.30	3.08
DI	M32	1	58.25	2.66
DI	M33	0	3.30	3.08
DI	M33	1	58.25	2.66
DI	M34	0	3.52	2.88
DI	M34	1	59.54	2.53
DI	M35	0	3.52	2.88
DI	M35	1	59.54	2.53

Table 2.19. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
DI	M36	0	3.30	2.98
DI	M36	1	57.03	2.69
DI	M37	0	3.30	3.05
DI	M37	1	58.86	2.68
DI	M38	0	3.30	3.05
DI	M38	1	58.86	2.68
DI	M39	0	3.30	3.05
DI	M39	1	58.86	2.68
DI	M40	0	6.09	3.48
DI	M40	1	57.44	3.10
DI	M41	0	3.30	3.08
DI	M41	1	58.25	2.66
DI	M42	0	3.30	3.08
DI	M42	1	58.25	2.66
DI	M43	0	59.54	2.51
DI	M43	1	3.44	2.82
DI	M44	0	3.56	2.88
DI	M44	1	59.54	2.53
DI	M45	0	59.54	2.58
DI	M45	1	3.62	3.11
DI	M46	0	59.54	2.58
DI	M46	1	3.62	3.11

[a]EPS, End Percent Survival; [b]APS, Average Percent Survival;
[c]arcsin relative Area Under Disease Progress Curve; [d]Area Under Disease Progress Curve;
[e]Disease Index; [f]Least Significant Difference

Table 2.20. Marker group means for WZ x NC 55 DH population inoculated with race 1 *P. nicotianae*.

Disease Measure	Marker	Genotype	Mean	Standard Error
EPS[a]	M1	0	63.89	3.88
EPS	M1	1	98.22	4.91
EPS	M2	0	62.96	3.83
EPS	M2	1	98.35	4.61
EPS	M3	0	62.96	3.86
EPS	M3	1	98.29	4.73
EPS	M4	0	62.96	3.86
EPS	M4	1	98.29	4.73
EPS	M5	0	62.96	3.77
EPS	M5	1	98.47	4.38
EPS	M6	0	62.96	3.77
EPS	M6	1	98.47	4.38
EPS	M7	0	62.82	3.73
EPS	M7	1	98.70	4.25
EPS	M8	0	62.96	3.80
EPS	M8	1	98.41	4.49
EPS	M9	0	63.66	4.08
EPS	M9	1	95.88	4.46
EPS	M10	0	63.89	3.74
EPS	M10	1	98.52	4.32
EPS	M11	0	62.96	3.72
EPS	M11	1	98.57	4.17
EPS	M12	0	62.96	3.72
EPS	M12	1	98.57	4.17
EPS	M13	0	62.96	3.72
EPS	M13	1	98.57	4.17
EPS	M14	0	62.96	3.72
EPS	M14	1	98.57	4.17
EPS	M15	0	62.96	3.75
EPS	M15	1	98.52	4.27
EPS	M16	0	62.96	3.75
EPS	M16	1	98.52	4.27
EPS	M17	0	62.96	3.75
EPS	M17	1	98.52	4.27

Table 2.20. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
EPS	M18	0	62.96	3.75
EPS	M18	1	98.52	4.27
EPS	M19	0	62.96	3.72
EPS	M19	1	98.57	4.17
EPS	M20	0	65.28	3.51
EPS	M20	1	98.57	3.79
EPS	M21	0	62.43	3.77
EPS	M21	1	98.57	4.18
EPS	M22	0	62.96	3.72
EPS	M22	1	98.57	4.17
EPS	M23	0	62.96	3.72
EPS	M23	1	98.57	4.17
EPS	M24	0	62.96	3.72
EPS	M24	1	98.57	4.17
EPS	M25	0	62.96	3.72
EPS	M25	1	98.57	4.17
EPS	M26	0	63.89	3.74
EPS	M26	1	98.52	4.32
EPS	M27	0	98.29	4.80
EPS	M27	1	63.53	3.92
EPS	M28	0	96.18	4.28
EPS	M28	1	63.06	3.98
EPS	M29	0	98.35	4.65
EPS	M29	1	63.89	3.82
EPS	M30	0	98.35	4.65
EPS	M30	1	63.89	3.82
EPS	M31	0	98.47	4.46
EPS	M31	1	64.77	3.75
EPS	M32	0	98.52	4.32
EPS	M32	1	63.89	3.74
EPS	M33	0	98.52	4.32
EPS	M33	1	63.89	3.74
EPS	M34	0	98.52	4.27
EPS	M34	1	62.96	3.75
EPS	M35	0	98.52	4.27
EPS	M35	1	62.96	3.75
EPS	M36	0	98.52	3.93

Table 2.20. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
EPS	M36	1	66.22	3.54
EPS	M37	0	98.52	4.33
EPS	M37	1	63.39	3.80
EPS	M38	0	98.52	4.33
EPS	M38	1	63.39	3.80
EPS	M39	0	98.52	4.33
EPS	M39	1	63.39	3.80
EPS	M40	0	95.70	4.49
EPS	M40	1	65.24	4.01
EPS	M41	0	98.52	4.32
EPS	M41	1	63.89	3.74
EPS	M42	0	98.52	4.32
EPS	M42	1	63.89	3.74
EPS	M43	0	62.96	3.72
EPS	M43	1	98.57	4.17
EPS	M44	0	98.52	4.27
EPS	M44	1	62.96	3.75
EPS	M45	0	62.96	3.83
EPS	M45	1	98.35	4.61
EPS	M46	0	62.96	3.83
EPS	M46	1	98.35	4.61
APS[b]	M1	0	74.83	2.98
APS	M1	1	99.38	3.77
APS	M2	0	74.19	2.95
APS	M2	1	99.42	3.54
APS	M3	0	74.19	2.97
APS	M3	1	99.40	3.64
APS	M4	0	74.19	2.97
APS	M4	1	99.40	3.64
APS	M5	0	74.19	2.90
APS	M5	1	99.46	3.37
APS	M6	0	74.19	2.90
APS	M6	1	99.46	3.37
APS	M7	0	74.05	2.86
APS	M7	1	99.67	3.26
APS	M8	0	74.19	2.93
APS	M8	1	99.44	3.45

Table 2.20. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
APS	M9	0	74.59	3.11
APS	M9	1	97.74	3.39
APS	M10	0	74.83	2.87
APS	M10	1	99.48	3.31
APS	M11	0	74.19	2.86
APS	M11	1	99.50	3.21
APS	M12	0	74.19	2.86
APS	M12	1	99.50	3.21
APS	M13	0	74.19	2.86
APS	M13	1	99.50	3.21
APS	M14	0	74.19	2.86
APS	M14	1	99.50	3.21
APS	M15	0	74.19	2.88
APS	M15	1	99.48	3.29
APS	M16	0	74.19	2.88
APS	M16	1	99.48	3.29
APS	M17	0	74.19	2.88
APS	M17	1	99.48	3.29
APS	M18	0	74.19	2.88
APS	M18	1	99.48	3.29
APS	M19	0	74.19	2.86
APS	M19	1	99.50	3.21
APS	M20	0	76.02	2.70
APS	M20	1	99.50	2.91
APS	M21	0	73.83	2.91
APS	M21	1	99.50	3.22
APS	M22	0	74.19	2.86
APS	M22	1	99.50	3.21
APS	M23	0	74.19	2.86
APS	M23	1	99.50	3.21
APS	M24	0	74.19	2.86
APS	M24	1	99.50	3.21
APS	M25	0	74.19	2.86
APS	M25	1	99.50	3.21
APS	M26	0	74.83	2.87
APS	M26	1	99.48	3.31
APS	M27	0	99.40	3.68

Table 2.20. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
APS	M27	1	74.50	3.00
APS	M28	0	97.71	3.29
APS	M28	1	74.35	3.06
APS	M29	0	99.42	3.57
APS	M29	1	74.83	2.93
APS	M30	0	99.42	3.57
APS	M30	1	74.83	2.93
APS	M31	0	99.46	3.41
APS	M31	1	75.45	2.87
APS	M32	0	99.48	3.31
APS	M32	1	74.83	2.87
APS	M33	0	99.48	3.31
APS	M33	1	74.83	2.87
APS	M34	0	99.48	3.29
APS	M34	1	74.19	2.88
APS	M35	0	99.48	3.29
APS	M35	1	74.19	2.88
APS	M36	0	99.48	3.00
APS	M36	1	76.67	2.70
APS	M37	0	99.48	3.32
APS	M37	1	74.50	2.91
APS	M38	0	99.48	3.32
APS	M38	1	74.50	2.91
APS	M39	0	99.48	3.32
APS	M39	1	74.50	2.91
APS	M40	0	97.10	3.45
APS	M40	1	76.10	3.08
APS	M41	0	99.48	3.31
APS	M41	1	74.83	2.87
APS	M42	0	99.48	3.31
APS	M42	1	74.83	2.87
APS	M43	0	74.19	2.86
APS	M43	1	99.50	3.21
APS	M44	0	99.48	3.29
APS	M44	1	74.19	2.88
APS	M45	0	74.19	2.95
APS	M45	1	99.42	3.54

Table 2.20. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
APS	M46	0	74.19	2.95
APS	M46	1	99.42	3.54
arAUDPC[c]	M1	0	1.18	0.04
arAUDPC	M1	1	1.55	0.05
arAUDPC	M2	0	1.17	0.04
arAUDPC	M2	1	1.55	0.04
arAUDPC	M3	0	1.17	0.04
arAUDPC	M3	1	1.55	0.05
arAUDPC	M4	0	1.17	0.04
arAUDPC	M4	1	1.55	0.05
arAUDPC	M5	0	1.17	0.04
arAUDPC	M5	1	1.56	0.04
arAUDPC	M6	0	1.17	0.04
arAUDPC	M6	1	1.56	0.04
arAUDPC	M7	0	1.17	0.04
arAUDPC	M7	1	1.56	0.04
arAUDPC	M8	0	1.17	0.04
arAUDPC	M8	1	1.55	0.04
arAUDPC	M9	0	1.18	0.04
arAUDPC	M9	1	1.53	0.04
arAUDPC	M10	0	1.18	0.04
arAUDPC	M10	1	1.56	0.04
arAUDPC	M11	0	1.17	0.04
arAUDPC	M11	1	1.56	0.04
arAUDPC	M12	0	1.17	0.04
arAUDPC	M12	1	1.56	0.04
arAUDPC	M13	0	1.17	0.04
arAUDPC	M13	1	1.56	0.04
arAUDPC	M14	0	1.17	0.04
arAUDPC	M14	1	1.56	0.04
arAUDPC	M15	0	1.17	0.04
arAUDPC	M15	1	1.56	0.04
arAUDPC	M16	0	1.17	0.04
arAUDPC	M16	1	1.56	0.04
arAUDPC	M17	0	1.17	0.04
arAUDPC	M17	1	1.56	0.04
arAUDPC	M18	0	1.17	0.04

Table 2.20. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
arAUDPC	M18	1	1.56	0.04
arAUDPC	M19	0	1.17	0.04
arAUDPC	M19	1	1.56	0.04
arAUDPC	M20	0	1.19	0.03
arAUDPC	M20	1	1.56	0.04
arAUDPC	M21	0	1.17	0.04
arAUDPC	M21	1	1.56	0.04
arAUDPC	M22	0	1.17	0.04
arAUDPC	M22	1	1.56	0.04
arAUDPC	M23	0	1.17	0.04
arAUDPC	M23	1	1.56	0.04
arAUDPC	M24	0	1.17	0.04
arAUDPC	M24	1	1.56	0.04
arAUDPC	M25	0	1.17	0.04
arAUDPC	M25	1	1.56	0.04
arAUDPC	M26	0	1.18	0.04
arAUDPC	M26	1	1.56	0.04
arAUDPC	M27	0	1.55	0.05
arAUDPC	M27	1	1.18	0.04
arAUDPC	M28	0	1.53	0.04
arAUDPC	M28	1	1.17	0.04
arAUDPC	M29	0	1.55	0.05
arAUDPC	M29	1	1.18	0.04
arAUDPC	M30	0	1.55	0.05
arAUDPC	M30	1	1.18	0.04
arAUDPC	M31	0	1.56	0.04
arAUDPC	M31	1	1.19	0.04
arAUDPC	M32	0	1.56	0.04
arAUDPC	M32	1	1.18	0.04
arAUDPC	M33	0	1.56	0.04
arAUDPC	M33	1	1.18	0.04
arAUDPC	M34	0	1.56	0.04
arAUDPC	M34	1	1.17	0.04
arAUDPC	M35	0	1.56	0.04
arAUDPC	M35	1	1.17	0.04
arAUDPC	M36	0	1.56	0.04
arAUDPC	M36	1	1.20	0.03

Table 2.20. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
arAUDPC	M37	0	1.56	0.04
arAUDPC	M37	1	1.18	0.04
arAUDPC	M38	0	1.56	0.04
arAUDPC	M38	1	1.18	0.04
arAUDPC	M39	0	1.56	0.04
arAUDPC	M39	1	1.18	0.04
arAUDPC	M40	0	1.53	0.04
arAUDPC	M40	1	1.20	0.04
arAUDPC	M41	0	1.56	0.04
arAUDPC	M41	1	1.18	0.04
arAUDPC	M42	0	1.56	0.04
arAUDPC	M42	1	1.18	0.04
arAUDPC	M43	0	1.17	0.04
arAUDPC	M43	1	1.56	0.04
arAUDPC	M44	0	1.56	0.04
arAUDPC	M44	1	1.17	0.04
arAUDPC	M45	0	1.17	0.04
arAUDPC	M45	1	1.55	0.04
arAUDPC	M46	0	1.17	0.04
arAUDPC	M46	1	1.55	0.04
AUDPC[d]	M1	0	2745.56	90.96
AUDPC	M1	1	3484.45	115.05
AUDPC	M2	0	2726.21	90.11
AUDPC	M2	1	3485.60	108.29
AUDPC	M3	0	2726.21	90.82
AUDPC	M3	1	3485.04	111.23
AUDPC	M4	0	2726.21	90.82
AUDPC	M4	1	3485.04	111.23
AUDPC	M5	0	2726.21	88.73
AUDPC	M5	1	3486.59	102.90
AUDPC	M6	0	2726.21	88.73
AUDPC	M6	1	3486.59	102.90
AUDPC	M7	0	2721.72	87.48
AUDPC	M7	1	3492.87	99.75
AUDPC	M8	0	2726.21	89.41
AUDPC	M8	1	3486.11	105.52
AUDPC	M9	0	2737.99	94.72

Table 2.20. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
AUDPC	M9	1	3435.39	103.48
AUDPC	M10	0	2745.56	87.55
AUDPC	M10	1	3487.04	101.10
AUDPC	M11	0	2726.21	87.42
AUDPC	M11	1	3487.46	98.05
AUDPC	M12	0	2726.21	87.42
AUDPC	M12	1	3487.46	98.05
AUDPC	M13	0	2726.21	87.42
AUDPC	M13	1	3487.46	98.05
AUDPC	M14	0	2726.21	87.42
AUDPC	M14	1	3487.46	98.05
AUDPC	M15	0	2726.21	88.07
AUDPC	M15	1	3487.04	100.41
AUDPC	M16	0	2726.21	88.07
AUDPC	M16	1	3487.04	100.41
AUDPC	M17	0	2726.21	88.07
AUDPC	M17	1	3487.04	100.41
AUDPC	M18	0	2726.21	88.07
AUDPC	M18	1	3487.04	100.41
AUDPC	M19	0	2726.21	87.42
AUDPC	M19	1	3487.46	98.05
AUDPC	M20	0	2782.18	82.47
AUDPC	M20	1	3487.46	88.87
AUDPC	M21	0	2715.57	88.85
AUDPC	M21	1	3487.46	98.37
AUDPC	M22	0	2726.21	87.42
AUDPC	M22	1	3487.46	98.05
AUDPC	M23	0	2726.21	87.42
AUDPC	M23	1	3487.46	98.05
AUDPC	M24	0	2726.21	87.42
AUDPC	M24	1	3487.46	98.05
AUDPC	M25	0	2726.21	87.42
AUDPC	M25	1	3487.46	98.05
AUDPC	M26	0	2745.56	87.55
AUDPC	M26	1	3487.04	101.10
AUDPC	M27	0	3485.04	112.37
AUDPC	M27	1	2735.18	91.75

Table 2.20. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
AUDPC	M28	0	3433.16	100.46
AUDPC	M28	1	2731.68	93.43
AUDPC	M29	0	3485.60	108.99
AUDPC	M29	1	2745.56	89.55
AUDPC	M30	0	3485.60	108.99
AUDPC	M30	1	2745.56	89.55
AUDPC	M31	0	3486.59	104.18
AUDPC	M31	1	2763.96	87.62
AUDPC	M32	0	3487.04	101.10
AUDPC	M32	1	2745.56	87.55
AUDPC	M33	0	3487.04	101.10
AUDPC	M33	1	2745.56	87.55
AUDPC	M34	0	3487.04	100.41
AUDPC	M34	1	2726.21	88.07
AUDPC	M35	0	3487.04	100.41
AUDPC	M35	1	2726.21	88.07
AUDPC	M36	0	3487.04	91.75
AUDPC	M36	1	2801.58	82.62
AUDPC	M37	0	3487.04	101.48
AUDPC	M37	1	2735.68	89.00
AUDPC	M38	0	3487.04	101.48
AUDPC	M38	1	2735.68	89.00
AUDPC	M39	0	3487.04	101.48
AUDPC	M39	1	2735.68	89.00
AUDPC	M40	0	3413.44	105.48
AUDPC	M40	1	2785.04	94.04
AUDPC	M41	0	3487.04	101.10
AUDPC	M41	1	2745.56	87.55
AUDPC	M42	0	3487.04	101.10
AUDPC	M42	1	2745.56	87.55
AUDPC	M43	0	2726.21	87.42
AUDPC	M43	1	3487.46	98.05
AUDPC	M44	0	3487.04	100.41
AUDPC	M44	1	2726.21	88.07
AUDPC	M45	0	2726.21	90.11
AUDPC	M45	1	3485.60	108.29
AUDPC	M46	0	2726.21	90.11

Table 2.20. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
AUDPC	M46	1	3485.60	108.29
DI[e]	M1	0	25.17	2.98
DI	M1	1	0.62	3.77
DI	M2	0	25.81	2.95
DI	M2	1	0.58	3.54
DI	M3	0	25.81	2.97
DI	M3	1	0.60	3.64
DI	M4	0	25.81	2.97
DI	M4	1	0.60	3.64
DI	M5	0	25.81	2.90
DI	M5	1	0.54	3.37
DI	M6	0	25.81	2.90
DI	M6	1	0.54	3.37
DI	M7	0	25.95	2.86
DI	M7	1	0.33	3.26
DI	M8	0	25.81	2.93
DI	M8	1	0.56	3.45
DI	M9	0	25.41	3.11
DI	M9	1	2.26	3.39
DI	M10	0	25.17	2.87
DI	M10	1	0.52	3.31
DI	M11	0	25.81	2.86
DI	M11	1	0.50	3.21
DI	M12	0	25.81	2.86
DI	M12	1	0.50	3.21
DI	M13	0	25.81	2.86
DI	M13	1	0.50	3.21
DI	M14	0	25.81	2.86
DI	M14	1	0.50	3.21
DI	M15	0	25.81	2.88
DI	M15	1	0.52	3.29
DI	M16	0	25.81	2.88
DI	M16	1	0.52	3.29
DI	M17	0	25.81	2.88
DI	M17	1	0.52	3.29
DI	M18	0	25.81	2.88
DI	M18	1	0.52	3.29

Table 2.20. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
DI	M19	0	25.81	2.86
DI	M19	1	0.50	3.21
DI	M20	0	23.98	2.70
DI	M20	1	0.50	2.91
DI	M21	0	26.17	2.91
DI	M21	1	0.50	3.22
DI	M22	0	25.81	2.86
DI	M22	1	0.50	3.21
DI	M23	0	25.81	2.86
DI	M23	1	0.50	3.21
DI	M24	0	25.81	2.86
DI	M24	1	0.50	3.21
DI	M25	0	25.81	2.86
DI	M25	1	0.50	3.21
DI	M26	0	25.17	2.87
DI	M26	1	0.52	3.31
DI	M27	0	0.60	3.68
DI	M27	1	25.50	3.00
DI	M28	0	2.29	3.29
DI	M28	1	25.65	3.06
DI	M29	0	0.58	3.57
DI	M29	1	25.17	2.93
DI	M30	0	0.58	3.57
DI	M30	1	25.17	2.93
DI	M31	0	0.54	3.41
DI	M31	1	24.55	2.87
DI	M32	0	0.52	3.31
DI	M32	1	25.17	2.87
DI	M33	0	0.52	3.31
DI	M33	1	25.17	2.87
DI	M34	0	0.52	3.29
DI	M34	1	25.81	2.88
DI	M35	0	0.52	3.29
DI	M35	1	25.81	2.88
DI	M36	0	0.52	3.00
DI	M36	1	23.33	2.70
DI	M37	0	0.52	3.32

Table 2.20. Continued.

Disease Measure	Marker	Genotype	Mean	Standard Error
DI	M37	1	25.50	2.91
DI	M38	0	0.52	3.32
DI	M38	1	25.50	2.91
DI	M39	0	0.52	3.32
DI	M39	1	25.50	2.91
DI	M40	0	2.90	3.45
DI	M40	1	23.90	3.08
DI	M41	0	0.52	3.31
DI	M41	1	25.17	2.87
DI	M42	0	0.52	3.31
DI	M42	1	25.17	2.87
DI	M43	0	25.81	2.86
DI	M43	1	0.50	3.21
DI	M44	0	0.52	3.29
DI	M44	1	25.81	2.88
DI	M45	0	25.81	2.95
DI	M45	1	0.58	3.54
DI	M46	0	25.81	2.95
DI	M46	1	0.58	3.54

[a]EPS, End Percent Survival; [b]APS, Average Percent Survival;
[c]arcsin relative Area Under Disease Progress Curve; [d]Area Under Disease Progress Curve;
[e]Disease Index; [f]Least Significant Difference

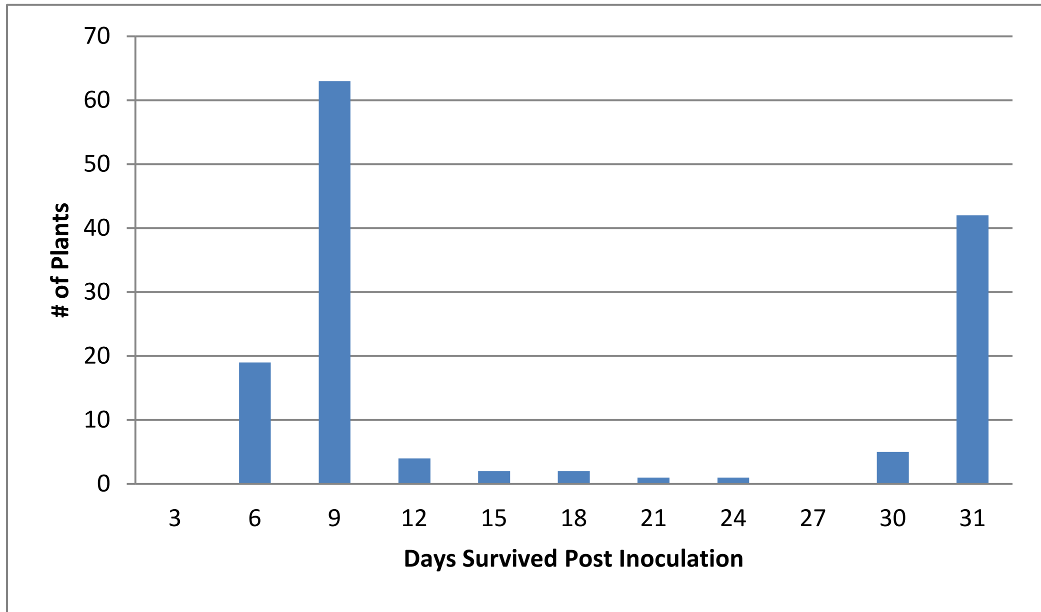

Figure 2.17. Frequency histogram for days survived post inoculation for (WZ x Hicks) x Hicks BC_1F_1 population inoculated with race 0 *P. nicotianae*.

Figure 2.18. WZ plants (back) and Hicks plants (front) 31 days after inoculation with a *P. nicotianae* race 0 ioslate.

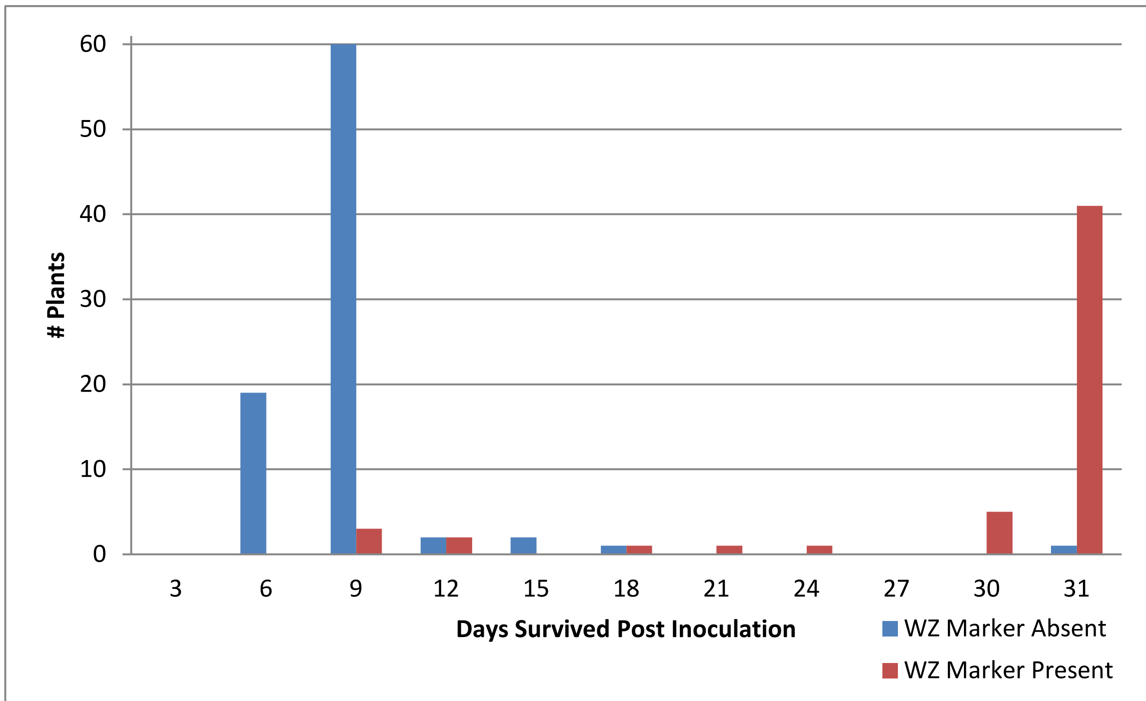

Figure 2.19. Frequency histogram for days survived post inoculation for (WZ x Hicks) x Hicks BC1F1 population inoculated with race 0 P. nicotianae with WZ marker group data. Seven coupling phase markers were used for this analysis.

Table 2.21. T-tests for days survived post inoculation for (WZ x Hicks) x Hicks BC_1F_1 population inoculated with race 0 *P. nicotianae* for seven AFLP markers.

Marker	Class	N[a]	Mean	StdDev[b]	Variances	t-Value	DF[c]	P-Value
M19	0	84	7.5119	3.1297	Unequal	-21.3	69.4	<.0001
	1	55	28.218	6.7486		.	.	.
	Diff (1-2)	_	-20.71	4.8873		.	.	.
M20	0	83	7.506	3.1483	Unequal	-21.28	69.7	<.0001
	1	55	28.218	6.7486		.	.	.
	Diff (1-2)	_	-20.71	4.9051		.	.	.
M14	0	85	7.5059	3.1115	Unequal	-20.97	67.3	<.0001
	1	54	28.167	6.8011		.	.	.
	Diff (1-2)	_	-20.66	4.8816		.	.	.
M21	0	85	7.5059	3.1115	Unequal	-20.97	67.3	<.0001
	1	54	28.167	6.8011		.	.	.
	Diff (1-2)	_	-20.66	4.8816		.	.	.
M43	0	84	7.8095	4.0404	Unequal	-16.45	56.7	<.0001
	1	44	27.568	7.4127		.	.	.
	Diff (1-2)	_	-19.76	5.4319		.	.	.
M45	0	85	7.5059	3.1115	Unequal	-19.86	62.2	<.0001
	1	51	28	6.9656		.	.	.
	Diff (1-2)	_	-20.49	4.9167		.	.	.
M46	0	85	7.5059	3.1115	Unequal	-19.86	62.2	<.0001
	1	51	28	6.9656		.	.	.
	Diff (1-2)	-	-20.49	4.9167		.	.	.

[a]Differences in N for each marker are due to missing data where a marker was not amplified in a specific plant
[b]StdDev, Standard Deviation; [c]DF, Degrees of Freedom

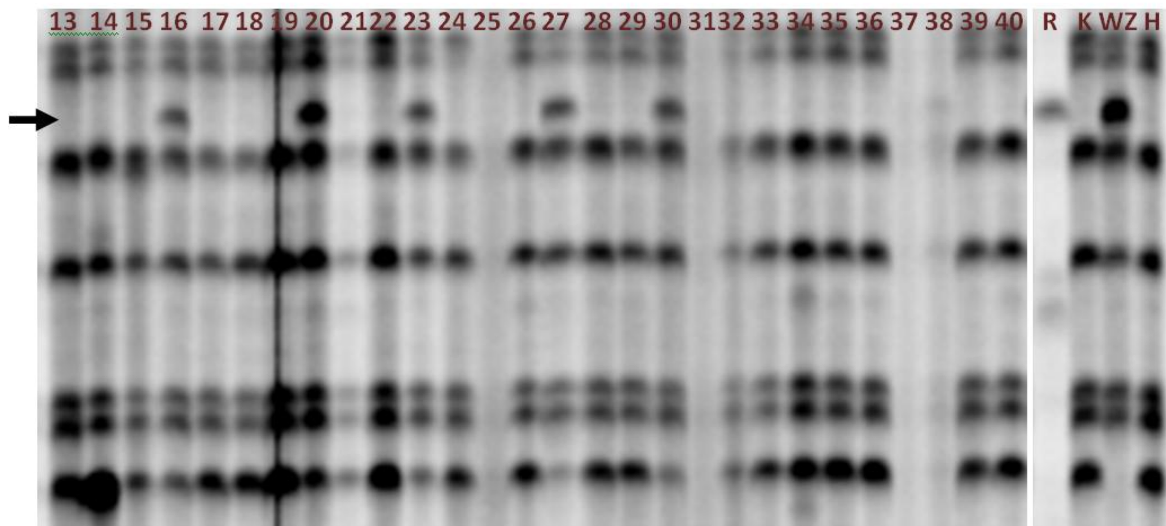

Figure 2.20. Gel image of M14 marker for WZ x Hicks BC_1F_1 plants and controls.

(R=*N. rustica*, K=Kutsaga 51, H=Hicks)

Table 2.22. Marker results of progeny from the (WZ x NC 61) x Hicks cross, where SOPZ-5 is a *Ph* SCAR marker.

Plant	SOPZ-5	WZ-AFLP
1	+	+
2		
3	+	
4	+	
5	+	
6		+
7		
8	+	+
9		
10		
11		
12	+	+
13		+
14	+	
15	+	+
16		
17		
18		+
19	+	+
20		+
21	+	+
22	+	+
23		+
24	+	+
25		+
26	+	+
27	+	+
28		
29		+
30		+
31		
32		+
33	+	+
34		+
35	+	+
36	+	+

Chapter 3: Evaluation of a Transgenic Approach for Reducing Sucker Growth in Flue-Cured Tobacco

ABSTRACT

Like many plant species, tobacco (*Nicotiana tabacum* L.) exhibits exceptionally strong apical dominance. Molecular signals from the shoot apical meristem mediate a hormonal environment that effectively inhibits outgrowth of lateral buds. Upon removal of the apical meristem (topping), this signal is lost, enabling the formation of new shoots at one or more leaf axils. Because of negative relationships between development of axillary meristems and tobacco leaf quality, chemicals such as maleic hydrazide (MH) and flumetralin are administered to topped tobacco plants to prevent outgrowth of axillary meristems, or suckers. The development of tobacco cultivars without the genetic capacity to form suckers upon topping would be desirable, as (1) it would provide a reduction in the levels of potentially hazardous chemical residues on cured leaf, and (2) it would provide a reduction in costs and labor associated with tobacco production. In *Arabidopsis thaliana*, the gene *BRANCHED1* was previously shown to be involved in the suppression of lateral branching. A similar gene has the same effect in tomato. A transgenic approach involving over-expression of the *A. thaliana* TCP transcription factor, *BRANCHED1* was investigated for its potential to suppress sucker growth. Plants of cultivar 'K326' were transformed with the *BRANCHED1* gene under the control of the CaMV 35S constitutive promoter. Field testing of R_1 plants did not reveal the desired phenotype of reduced suckering after topping. Additional strategies to reduce axillary meristem development in *N. tabacum* should be studied for the possible development of non-suckering tobacco cultivars.

INTRODUCTION

The plant architecture of *Nicotiana tabacum* L. is comprised of a single erect stalk with a terminal meristem. Tobacco demonstrates apical dominance so that when the primary inflorescence is intact, growth of axillary shoots is suppressed through hormonal activity (Tso, 1999). In conventional tobacco production, however, the terminal meristem is removed early in the flowering stage in a process known as 'topping' (Fisher et al., 2011). This decapitation diverts physiological resources to the leaves rather than to reproductive development by stimulating root growth and slowing the rate of decline in net photosynthesis in the remaining leaves (Tso, 1999). It also relieves stress on the plant, which results in plants with a greater leaf area and leaf mass (Papenfus, 1987). In addition to contributing to increased yields, topping also increases the concentration of desired compounds such as alkaloids in the cured leaf, as well as most of the volatile constituents associated with desirable smoke character (Weeks and Seltmann, 1986).

While topping has many positive effects on yield, cured leaf quality, and chemistry, it also leads to growth of lateral meristems (referred to in tobacco production as suckers) that develop within the leaf axils after topping. These lateral meristems serve as a substitute for the lost terminal inflorescence of the plant (Papenfus, 1987) and act as a sink for water, light, and nutrients. Suckers negatively affect leaf quality and chemistry if they are not removed or controlled (Weeks and Seltmann, 1986).

Chemical control is currently the most popular method used by growers to control suckers. A combination of fatty alcohols, contact-local systemics, and systemics is typically used to control sucker growth in conventional production. In addition, most growers often

supplement control by hand removal of suckers not controlled through chemical application (Peedin, 1999). Sucker control is one of the most significant economic inputs in flue-cured tobacco production. An effective program that combines chemical applications with hand suckering can cost a grower $300 an acre, which corresponds to approximately 10 % of the total expected costs associated with production (Fisher et al., 2011). In addition to contributing to high production costs, chemical sucker control raises concerns associated with pesticide residues. Maleic hydrazide (MH) is a systemic chemical that inhibits plant cell division, thus preventing growth of new suckers without retarding the growth of mature leaves (Meyer et al., 1987). It is the only true systemic chemical labeled for sucker control in tobacco and is very effective, making it popular with growers (Taylor et al., 2007). MH is metabolically stable within the plant, however, and is not degraded by ultraviolet radiation or extreme temperatures associated with curing. Cured leaves from plants treated with MH therefore usually contain MH residues (Collins and Hawks, 1993).

Because of high residues on cured leaves, MH has been scrutinized by political and governmental agencies throughout the world. In 1978, the Federal Republic of Germany enacted a law that required all pesticides approved for use meet tolerance levels established for foods. Under this law, tobacco was considered a food product (Hunt et al., 1977) and the residue levels of MH on cured leaf could not exceed 80 ppm (Wittekindt, 1978). Since this law was enacted, several other countries in the European Union have established a residue tolerance of 80 ppm. In response, a number of major tobacco exporting countries such as Brazil, Canada, and Zimbabwe, have prohibited the use of MH (Peedin, 1999). United States growers must be willing to ensure they are producing a product that has no more than 80 ppm

MH residue in order to continue to remain a leading exporter of tobacco. Manufacturing companies are beginning to take pesticide residues very seriously, and in 2011 one US purchaser bought only MH free tobacco (Fisher et al., 2011). Therefore, it is essential that the tobacco industry continue to find ways to reduce pesticide residues on the cured leaf.

A genetic approach for reducing sucker growth is attractive because it could eliminate or greatly reduce the need for chemical control, thus increasing the efficiency of tobacco growing and the production of cured leaf free of MH residues. Shoot branching (suckering) is a complex process within plants and involves a number of genes and transcription factors (Schumacher et al., 1999; Schmits et al, 2002; Gerb et al., 2003; Komatsu et al, 2003; Keller et al., 2006; Dun et al., 2009; Martin-Trillo et al., 2011). Branching in plants is dependent upon growth of axillary buds in leaf axils. Branching does not occur if development at meristematic tissues in these areas is not initiated, or if the meristem remain dormant (Gerb et al., 2003). When the main shoot exerts control over the outgrowth of the lateral buds, the plant is under apical dominance. Topping of the plant leads to a loss of apical dominance and growth of lateral buds is stimulated. The apical dominance phenomenon is mediated by plant hormones. Auxins, for example, have been shown to inhibit lateral bud outgrowth while cytokinins have been shown to promote it (Cline, 1997).

One gene that plays a role in lateral branching in *Arabidopsis thaliana* is *BRANCHED1* (*BRC1*). *BRC1* acts to integrate hormone signals in the bud to suppress branching in this species (Aguilar-Martinez et al., 2007). Before flowering, when axillary meristems (AMs) were not yet initiated, *BRC1* transcripts were not detectable. After flowering when the AMs became visible, *BRC1* transcripts were reported in all cell layers of

the meristems. At the time of bud outgrowth, *BRC1* became down-regulated. *BRANCHED1* mutants exhibited a significantly higher degree of branching relative to wild-type individuals, demonstrating that expression of *BRC1* is inversely correlated with branching. The same group also reported that auxin and the *MORE AXILLARY GROWTH* (*MAX*) genes act through *BRC1* to promote bud arrest and that *BRC1* also responds to environmental and endogenous signals that influence bud outgrowth. These findings suggest that *BRC1* acts as a local integrator of the pathways controlling bud outgrowth.

Martín-Trillo et al. (2011) identified two *BRC1* like paralogs in tomato: *SlBRC1a* and *SlBRC1b*. Like the *Arabidopsis BRC1* gene, both tomato paralogs were expressed in arrested axillary buds and down-regulated upon bud activation. However, *SlBRC1b* transcript levels were much higher than transcript levels for *SlBRC1a*. The authors finding of two *BRC1*-like genes in tomato suggests that a duplication of *BRC1* occurred after the separation of the Brassicaceae and Solanaceae, probably in a whole-genome duplication event. The authors reported that *SlBRC1b* retained the ancestral *BRC1*-like gene function in suppression of shoot branching (Martín-Trillo et al., 2011). Since the second copy of the gene was not lost during evolution, *SlBRC1a* may have evolved to produce an alternative, yet to be identified, function. Knowledge of *BRC1* information might be used in a genetic engineering-based strategy to suppress sucker growth in tobacco plants. The objectives of this study were to (1) produce transgenic tobacco plants constitutively overexpressing the *A. thaliana BRC1* gene, and (2) to investigate the effect of this transgene on the phenotype of tobacco plants after removal of the terminal inflorescense.

MATERIALS AND METHODS

Generation of Transgenic Plant Material

Two pUC19-based plasmid constructs (Fig 3.1) into which *BRC1* had been cloned were obtained from Dr. Ralph Dewey (NCSU, Raleigh, NC). The two constructs were identical with the exception that one (pUC19-F2BRC1genomicSeqR1 (*BRC1*R1)), had an optimal start codon context, while the other (pUC-19F2BRC1genomicSeqR2 (*BRC1*R2)), did not. *Escherichia coli* competent cells (NEB, Ipswich, MA) were transformed with the two pUC19-*BRC1* clones. The *BRC1* fragments (1835 base pairs) were then isolated by digestion with restriction enzymes *Xba*I and *Sac*I. The plant expression vector, pBI121, was also digested with *Xba*I and *Sac*I to remove the GUS cassette (Figure 3.2). The *BRC1* fragments were then ligated into the pBI121 vector which contained the CaMV 35S promoter and the selectable marker neomycin phosphotransferase II (*npt*II) to allow for the selection of transformed plants in the presence of kanamycin. This vector was then transformed into *Agrobacterium tumefaciens* strain LBA4404 (Invitrogen, Grand Island, NY) by electroporation according to the procedure of Wen-Jun et al. (1989).

Ten plants of tobacco cultivar ‘K326’ were used as sources of leaf explants for transformation with the *BRC1*R1 and *BRC1*R2 constructs using the *A. tumefaciens* method of An et al. (1986). Two sterilized leaves from each K326 plant were cut into approximately 1 cm^2 discs, wounded with a scalpel, and inoculated with *A. tumefaciens*. The inoculated discs were placed on a solid MS culture medium (Murashige and Skoog, 1962). After two days of co-cultivation, the inoculated leaf discs were transferred to shoot regeneration medium comprised of MS inorganic salts supplemented with indole acetic acid (IAA), kinetin,

sucrose, and agar as described by Murashige and Skoog (1962). In addition, 100 mg L^{-1} kanamycin was added to the medium to select for transformed cells, and 250 mg L^{-1} cefotaxime and 100 mg L^{-1} timentin were added to eliminate any contaminating bacteria. Plates were kept in a lighted growth chamber, and all discs were transferred to fresh media every 14 to 21 days. Regenerated shoots were transferred to rooting medium consisting of MS inorganic salts plus sucrose and agar (Murashige and Skoog, 1962). Rooted plants were transferred to soil-filled, 7.5 cm pots and designated as R_0 transformants.

Molecular Analysis

A total of 49 R_0 plants were regenerated (24 from *BRC1*R1 transformations and 25 from *BRC1*R2 transformations). Leaf tissue samples (two to three small leaves) were collected from R_0 transformants approximately three weeks after transplanting to soil and DNA was isolated using a modified (Johnson et al., 1995) cetyltrimethylammonium bromide (cTAB) procedure (Afandor et al., 1993), with the exception that a BIO 101 FastPrep machine (BIO 101, Holbrook, N.Y.) was used to grind leaf samples. DNA was quantified using a Hoefer fluorometer (Hoefer Scientific Instruments, San Francisco, CA) according to product specifications. After quantification, DNA samples were diluted with water to 50 ng μL^{-1} and stored at -20°C.

Polymerase chain reaction (PCR) analysis was then performed using *BRC1*-specific primers (Table 3.1) to test for presence of the transgene. In addition, Northern blots were performed to determine expression levels of the *BRC1* transgene. Total RNA was isolated using TRIzol (Invitrogen, Grand Island, NY) based on a protocol developed by Chomczynski

and Sacchi (1987). Northern blots were then performed according to the protocol described by Sambrook and Russell (2001).

Field Evaluation

Each of the 49 R_0 plants were transplanted to 25 cm pots and grown under natural light conditions in a greenhouse. Only one or two transformants per leaf disc were selected in order to minimize the number of possible clones that might have been evaluated. Mature R_0 plants were self-pollinated to generate 49 R_1 lines.

Eight *BRC1*R1 and 10 *BRC1*R2 R_1 lines derived from R_0 plants exhibiting strong *BRC1* transgene expression were evaluated along with non-transformed K326 in a field experiment conducted at the Central Crops Research Station (Clayton, NC) during 2010. Alternative topping heights were evaluated to determine the potential effect of *BRC1* transgene expression on sucker growth potential. The experimental design was a split-plot design with three replications. The main-plot factor was topping height and the sub-plot factor was entry. Three topping treatments were evaluated: plants topped at 10 leaves, plants topped at 20 leaves, and plants that were not topped. Notes were taken regarding the phenotypes of each line. In addition, plants that were of interest were self-pollinated to produce R_2 seed. R_2 seeds were germinated and resulting plants were grown in a greenhouse where the phenotypes of single R_2 plants were again visually evaluated.

RESULTS

All 49 R_0 plants screened for the presence of the *BRC1* transgene by PCR tested positive. Of these 49 plants, only 9 *BRC1*R1 and 19 *BRC1*R2 plants were found to be strongly expressing the transgene based upon Northern blot results (Table 3.2).

Field evaluation of the R_1 families revealed a range of interesting phenotypes, but no plants were observed that had a decrease or complete elimination of suckers. As the desired phenotype of non-suckering after topping was not observed, no quantitative data were collected on the R_1 plants for plant height, sucker proliferation, etc. Only visual observations were made.

Immediately after seeding, some differences in germination were observed. Most of the R_1 families had normal germination rates, while seed of some families either failed to germinate or had rates as low as 5%. In the field, phenotypic variation was observed for plant height, leaf size and shape, flowering time, and number of suckers. The height of the un-topped plants ranged from 65 to 160 cm and was variable both among and within families (Figure 3.3). Some plants had elongated internodes while others had very compact internodes (Figure 3.4). Leaf shape ranged from thin and lanceolate to curled and deformed (Figure 3.5). Some plants flowered very early on in the season while others had not yet flowered in the field at the end of the growing season (Figure 3.6). Some plants suckered prolifically while others had sucker pressure similar to K326 (Figure 3.7). No plants were found to lack suckers, however. Visual comparisons of the phenotypes of the R_1 plants to K326 indicated that the majority of the plants had phenotypes very similar to the untransformed check. Some plants exhibited dramatically altered phenotypes, however.

Plants transformed with either the *BRC1*R1 and *BRC1*R2 construct displayed unusual phenotypes. Plants with interesting phenotypes (e.g., curled leaves, prolific suckering, short stature) were self-pollinated and their progeny were grown in the greenhouse. Like the R_1 parental plants, the R_2 progeny also exhibited mutant phenotypes varying in height, leaf

shape, flowering time, and number of suckers (Figure 3.8). None were non-suckering, however.

DISCUSSION

Although *BRC1* expression has been found to be negatively correlated with branching in *A. thaliana*, over-expression of this gene did not eliminate sucker growth in *N. tabacum*. Suckering was observed to be more prolific in some families carrying *BRC1* as compared to the non-transformed check, K326, while in others, there were few visual differences. There were no visual differences in number of suckers observed between the three topping treatments. As the desired phenotype of a reduction or elimination of suckering was not observed, no quantitative measurements or molecular data were collected for R_1 individuals. Much variation for plant types within R_1 families could probably be attributed to transgene segregation. We did not test for transgenic copy number in the R_0 transformants, but under the assumption of integration of a single T-DNA locus, 75% of R_1 individuals would have been expected to possess the transgene.

No plants in any R_1 family were non-suckering. This indicates that constitutive overexpression of *BRC1* failed to repress branching in *N. tabacum*. It may be the case that the *A. thaliana* BRC1 transcription factor has diverged sufficiently to negatively affect interactions with *N. tabacum* molecular components that are required to suppress branching in this species. The effect of transgene expression was not totally innocuous in all cases, however. Some plants from some families exhibited deformed phenotypes. This could be due to the complexity of the genetic system that controls branching. Interruption of this complex network with over-expression of *BRC1* may have contributed to phenotypes with reduced

germination rates, shorter heights, and elongation of leaves. These observations suggest that *BRC1*-like sequences likely play additional roles in plant development outside of lateral branching. There is little published research on the control of lateral branching in *Nicotiana*. Further research may provide insight on the results observed in our experiments.

Observations of increased suckering in some plants in some families suggests the possibility that transgenic expression of *BRC1* may have led to cosuppression of orthologous genes in *N. tabacum* that play a role in suppressing sucker development. Cosuppression is a form of RNA interference (RNAi) triggered by double-stranded RNA that leads to post-transcriptional silencing of genes with a very high degree of nucleotide similarity to the transgene being expressed (Fire et al., 1998). In a number of other species, excess shoot branching has been seen in plants with a loss of function of *BRC1*-like genes (Doebley et al., 1997; Takeda et al., 2003; Aguilar-Martinez et al., 2007; Finlayson, 2007; Minakuchi et al., 2010). Many examples of the over-expression of a transgene leading to cosuppression can be found in the literature (Jorgensen, 1995; Baulcombe, 1996; Pal-Bhadra et al., 1997; Elmayan, 1998).

All lines in the field were derived from R_0 plants with strong expression levels of *BRC1* as determined by Northern blots, indicating a lack of cosuppression in the R_0 generation. In addition, there were no unusual phenotypes observed among the R_0 plants. Cosuppression may have occurred in some R_1 plants because self pollination of R_0 plants heterozygous for *BRC1* would have resulted in many R_1 progeny carrying *BRC1* in the homozygous condition. An increase in homozygosity can lead to an increase in the expression of transcript levels (Beaujean et al., 1998), which, in turn, could be great enough

to trigger silencing. It has been demonstrated that silencing occurs more often in plants that contain multiple transgene copies per locus (Hobbs et al., 1990; Niebel et al., 1995). The possible role of cosuppression in our experiments is speculation at this point in time, however, as transgene expression data were not collected for R_1 plants exhibiting an increase in suckering.

Two reasons for the over-expression of *BRC1* not contributing to the desired phenotype of a reduction in suckering in *N. tabacum* were presented. Yet, it must be pointed out that these two theories do not support each other. The first suggests no difference in phenotype between the transgenic plants and check were observed because *BRC1* has reduced effectiveness for affecting branching in *N. tabacum* due to taxonomic divergence. The second suggests the *A. thaliana BRC1* gene and a *BRC1*-like gene in *N. tabacum* are so similar in sequence that over-expression of *BRC1* triggers silencing of both genes. Both are presented as possibilities, but no further data were collected to confirm either because we did not observe the commercially desired non-suckering phenotype.

CONCLUSIONS

Overexpression of the *A. thaliana* gene *BRC1* did not result in the desired elimination of shoot branching in tobacco. A number of speculations were drawn as to why the desired outcome was not observed, but no firm conclusions can be made as limited molecular data were collected to try to understand the obviously complex system of factors that might control branching in *N. tabacum*. Since overexpression of *BRC1* did not decrease sucker growth, other genetic methods to reduce suckers warrant investigation. Potential alternative gene candidates to target for modified expression in tobacco might include orthologues of

LATERAL SUPPRESSOR, the *MORE AXILLARY GROWTH* genes, or the *REGULATOR OF AXILLARY MERISTEMS* genes.

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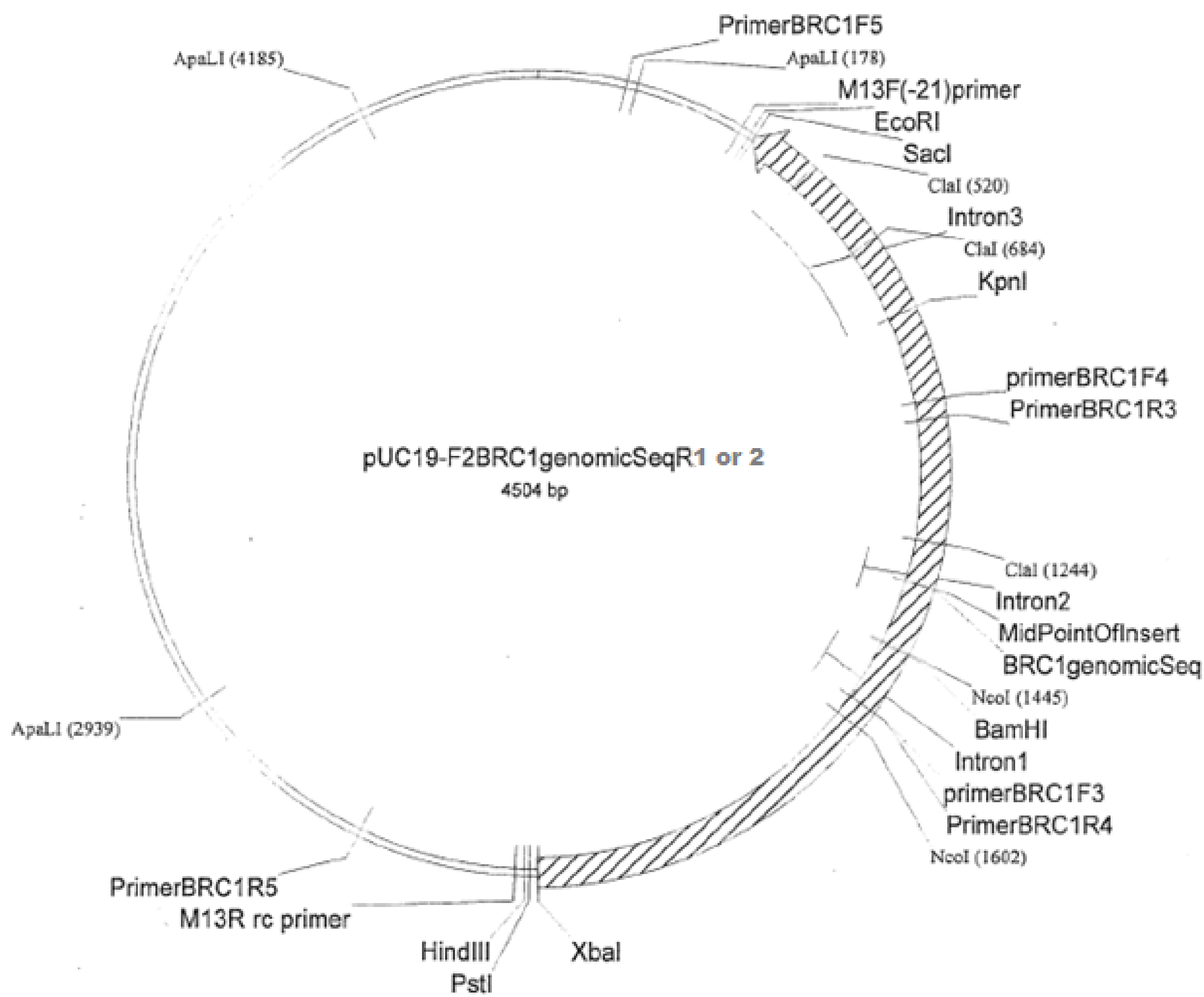

Figure 3.1. pUC19-F2BRC1 feature map.

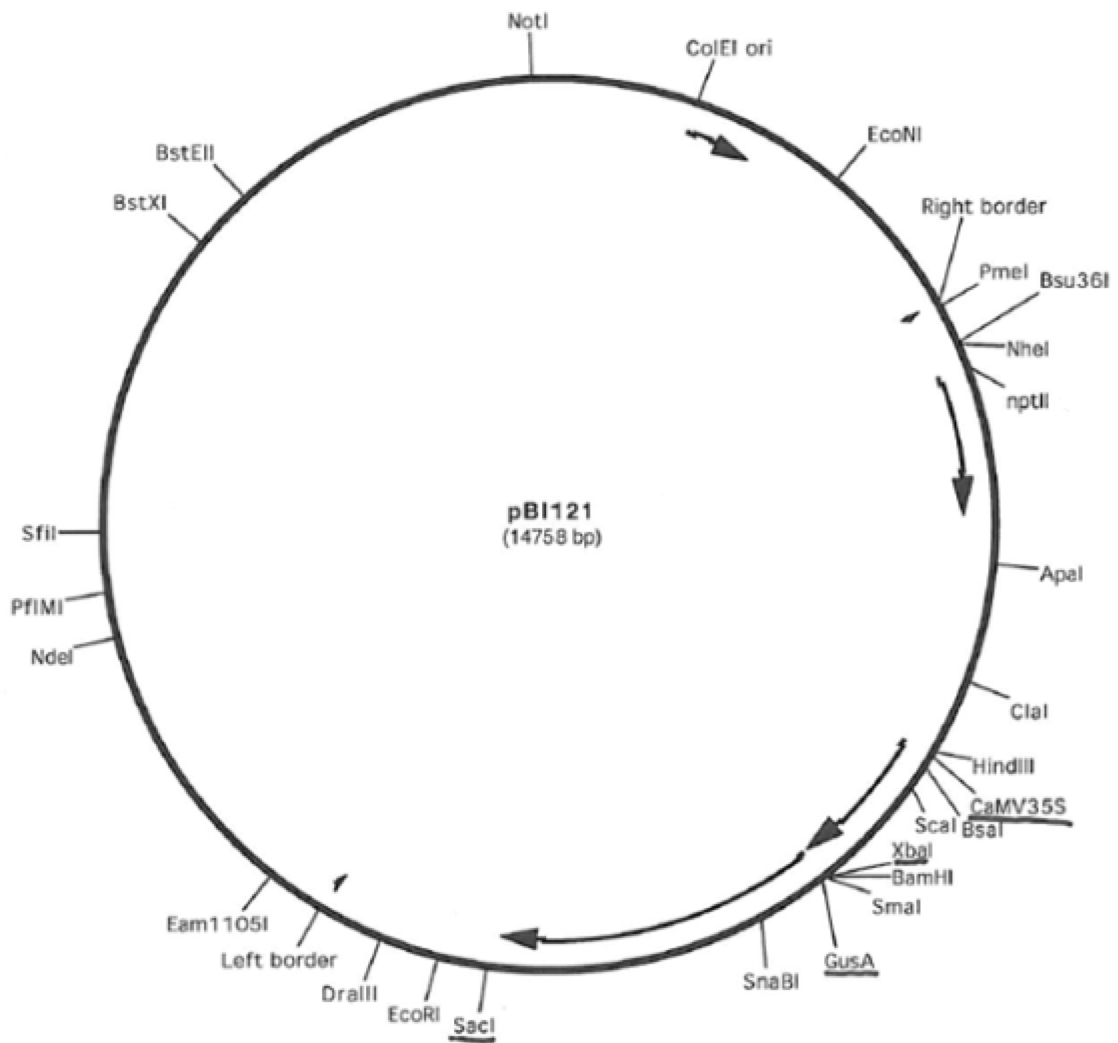

Figure 3.2. Feature map of the expression vector pBI121.

Table 3.1. Primers used for PCR testing of *Branched1* transgenic plants.

Primer	Sequence	Tm (^{o}C)
*Brc1*Forward	CACAACCATTGCTCCCTTTT	63.7
*Brc1*Reverse	TTGATCTTGCTGTGCCTGTC	64.1

Table 3.2. R_o plants tested for presence of *BRC1* using PCR and for transgene expression using Northern blots.

Plant	Construct	PCR	Northern Blot	Plant	Construct	PCR	Northern Blot
770	R1	+	weak	715	R2	+	none
771	R1	+	weak	718	R2	+	strong
773	R1	+	weak	728*	R2	+	strong
775	R1	+	weak	730	R2	+	none
777	R1	+	none	732	R2	+	strong
778	R1	+	weak	734	R2	+	none
779*	R1	+	strong	736*	R2	+	strong
781*	R1	+	strong	738	R2	+	strong
783	R1	+	weak	741*	R2	+	strong
786*	R1	+	strong	742	R2	+	strong
787	R1	+	weak	745*	R2	+	strong
790*	R1	+	strong	746	R2	+	none
792*	R1	+	strong	748	R2	+	strong
812	R1	+	strong	831	R2	+	strong
814	R1	+	none	833*	R2	+	strong
816	R1	+	none	835	R2	+	strong
817	R1	+	none	837	R2	+	strong
819*	R1	+	strong	838*	R2	+	strong
821	R1	+	none	840*	R2	+	strong
823	R1	+	weak	842*	R2	+	strong
824*	R1	+	strong	845	R2	+	none
825	R1	+	None	847	R2	+	weak
854	R1	+	none	848*	R2	+	strong
856*	R1	+	strong	850	R2	+	strong
				852*	R2	+	strong

*Plants self-pollinated to produce R_1 seed for field-testing.

Figure 3.3. Within BRC1 R1 family variation for plant height.

Figure 3.4. Variation in distances between internodes of *BRC1* R_1 plants.

(Left: Elongated distance between axils; Right: Compact distance between axils)

Figure 3.5. Examples of the high degree of variation for leaf size and shape of selected *BRC1* R_1 plants.

Figure 3.6. Variation in flowering time among of *BRC1* R_1 plants.

(Left: Early flowering; Right: Plant that hasn't flowered at the end of the season)

Figure 3.7. Variation in sucker growth for selected *BRC1* R_1 plants.

(Top Left: Prolific suckering; Top Right: Ground suckers; Bottom Left: Large number of suckers; Bottom Right: Fewer suckers)

Figure 3.8. ***BRC1*** **R_2 plants exhibing abnormal phenotypes.**

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