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# FIELD EVALUATION OF BURLEY LINES CONTAINING ALLELES MINIMIZING NICOTINE TO NORNICOTINE CONVERSION

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THESIS

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A thesis submitted in partial fulfillment of the
Requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment
at the University of Kentucky

By
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Lexington, Kentucky
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ABSTRACT OF THESIS

# FIELD EVALUATION OF BURLEY LINES CONTAINING ALLELES MINIMIZING NICOTINE TO NORNICOTINE CONVERSION

CYP82e4, CYP82e5, and CYP82e10 mutant alleles that minimize the conversion of nicotine to nornicotine have been introgressed into numerous existing low converting (LC) burley varieties and parental lines developed by the Kentucky-Tennessee tobacco breeding program. A backcross breeding protocol was utilized, with the objective being the creation of "e3" varieties that differed from their LC counterparts only for nornicotine and nitroso-nornicotine content. Field studies were conducted in Kentucky and Tennessee during the 2013 growing season, with 17 prospective parental lines and 20 prospective commercial varieties grown and compared to their original counterparts. Most of the e3 lines were not morphologically equivalent to their LC counterparts; several were also lacking in black shank resistance. Selections and/or backcrosses to the appropriate LC counterparts were made in 2013 in an attempt to improve the e3 lines to make them more comparable to the LC versions. The improved parental lines and hybrids were re-evaluated in 2014. The comparative performance of e3 versus LC lines was substantially improved in the 2014 trials. After making selections and/or one or two backcrosses, the plant type and black shank resistance were improved for all e3 lines. The reduction in nicotine to nornicotine conversion was successful, with the e3 lines having conversion rates ranging from 0.48 to 0.66 percent, compared to a range of 2.35 to 4.86 percent for the LC lines. With a lower rate of conversion to nornicotine, the nitroso-nornicotine amounts were also reduced; values for the e3 materials ranged from 0.06 to 0.12 ppm, compared to a range of 0.27 to 0.61 ppm for the LC materials. All data for disease resistance, agronomic characteristics, and yield are presented.

KEYWORDS: Nicotine to Nornicotine Conversion, nitroso-nornicotine, TSNA reduction

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## Chapter One: Introduction

Burley tobacco (*Nicotiana tabacum*) production is facing a great deal of scrutiny due to potentially negative health effects. In recent years, one of the primary objectives of the Kentucky-Tennessee Tobacco Improvement Initiative (KTTII) has been breeding for improved chemical characteristics that will lead to less harmful tobacco products. The Food and Drug Administration (FDA) gained the ability to regulate tobacco products when the Family Smoking Prevention and Tobacco Control Act (Tobacco Control Act) was signed in 2009. This development, coupled with the World Health Organization (WHO) Framework Convention on Tobacco Control, makes it likely that the importance of breeding for altered chemical composition will increase in the future.

Although the Tobacco Control Act does not allow the FDA to require reductions of nicotine levels to zero, the FDA can establish maximum content if deemed necessary. As a result, in recent years KTTII has focused on reducing levels of alkaloids and compounds that are believed to be carcinogens in tobacco varieties. The reduction of nornicotine levels has been of particular interest. Nornicotine is a secondary alkaloid produced by N-demethylation of nicotine by the enzyme nicotine N-demethylase (Fig. 1) (Siminszky et al., 2005). High levels of nornicotine are undesirable in tobacco products due to its detrimental effects on smoke flavor. Even low levels of nornicotine in tobacco have recently become a concern because nornicotine is a precursor of nitroso-nornicotine, which is one of the most harmful tobacco specific nitrosamines (TSNA). TSNA content, specifically N'-nitrosonornicotine (NNN) and 4-methylnitrosoamino-1-(3-pyridyl)-1-butanone (NNK), is of major concern due to being among the most prevalent tobacco compounds that have been shown to be carcinogenic in laboratory animals (Siminszky et al., 2005).


Figure 1. Structures of nicotine, nornicotine, and NNN. (Siminszky et al., 2005)

The first step KTTII took to address TSNA content in tobacco varieties was to develop low nicotine to nornicotine converting, commonly referred to as “LC”, strains of existing KTTII varieties. Cultivars can be “cleaned-up” by eliminating converter plants from foundation seed lots. This involves screening field grown plants for nornicotine formation. To be classified as a low converter line, there must be less than three percent conversion of nicotine to nornicotine in cured leaves when calculated as [nornicotine / (nicotine + nornicotine)] x 100 according to the LC Protocol (Jack and Bush, 2007). Plants are tagged and numbered in a field nursery, then single leaves are collected, ethylene-cured, and screened for nicotine and nornicotine content via gas chromatography. As a check, known high converter (HC) plants, greater than 90 percent conversion, are grown and analyzed simultaneously. This process results in the production of new LC cultivars having low nicotine conversion. Low conversion KTTII burley cultivars TN 90LC, TN 86LC, TN 97LC, KT 200LC, and KY 907LC were re-released in 2004. Percent nornicotine in these LC varieties typically ranges from 2-5%, compared to 12-18% for their original counterparts

More recent efforts to reduce TSNA levels have involved the introgression of three recessive mutant alleles that minimize the conversion of nicotine to nornicotine, developed by researchers at North Carolina State University, into existing KTTII commercial varieties. In plants that are homozygous for all three of these mutant alleles, nornicotine and NNN levels are dramatically reduced, ultimately reducing levels of NNN in tobacco products. These three alleles have subsequently been introgressed into existing KTTII parental lines and hybrid varieties. The objective of this thesis

research was to evaluate the effect of these introgressed alleles on the agronomic and disease resistance characteristics of the recipient parental lines and hybrids in comparison to their original LC counterparts.

## Chapter Two: Literature Review

### Mutation Breeding

According to Forster and Shu (2012) mutagenesis is defined as "the process by which genetic information of an organism is changed in a stable manner." Mutations occur as the result of several types or combinations of errors that occur during the replication of deoxyribonucleic acid (DNA). Damage ranges from aberrations at the DNA level of individual chromosomes (breaking the chemical bonds in DNA molecules, deleting or adding nucleotides, and/or by substituting one nucleotide for the other) to gross chromosomal breakages and rearrangements (Mba, 2013).

Mutagenesis has become an important technique to help develop new varieties of plants by creating additional genetic variability (Patial et al., 2015). Using mutations as a source of new genetic material to add variety to germplasm has been a part of breeding programs for many years. Originally, the only source of mutations was through natural occurrence, typically caused by environmental factors or genetic errors. As science moved into the 20$^{th}$ century, chemical and physical mutagens were discovered and processes were refined to induce mutations (Kharkwal, 2012). This induction serves a complementary approach for improving the genetics of crops (Sikder et al., 2015). It has been used to overcome yield plateaus, increase disease resistance, and manipulate plant height and other agronomic traits. This is possible because induced mutations can be in the form of knock-out, knock-down, gain of function, or alteration of function mechanisms, depending upon the chemical or physical method used (Shu et al., 2012). In an induced mutation breeding program, mutations are deliberately initiated by treating seeds with either a chemical or physical mutagen. Mutagens are scored based on their efficiency and effectiveness. Mutagenic efficiency is the proportion of the desirable mutation frequency in relation to damages associated with mutation (Konzak

et al., 1965) and effectiveness is based on the dose or concentration and its specificity to act on gene and genetic-makeup (Blixt, 1970).

The first released commercial variety developed using induced mutagenesis was the tobacco variety “Chlorina” in 1936. It was mutated via the use of X-rays, which was the method of choice at the time. Within the following ten years chemically induced mutations would be confirmed, but the first variety created with this method, Luther barley, would not be released until 1966 (Forster and Shu, 2012). According to the International Atomic Energy Agency’s Mutant Variety Database, there are 3,222 mutagenic crop varieties that have now been registered. Mustard gas was the first confirmed chemical to be consistently mutagenic in 1940 after more than 20 years of testing other chemicals and metals (Auerbach et al., 1947). This encouraged the search to continue with hopes of finding a substance that was less destructive than mustard gas.

Ethyl methanesulfonate (EMS) is the most commonly used chemical mutagen in plants due to its potency and ease of use (Bhat et al., 2007 and Talebi et al., 2012). It has proved to be the most efficient and effective chemical mutagen as well as the most frequently and universally used (Harten, 1998). EMS is a monofunctional ethylating agent that is a colorless liquid at room temperature and induces mutations in viruses, bacteria, fungi, plants and mammals. It is formed from the reaction of methanesulfonic anhydride with ethyl alcohol and is not known to occur naturally (Sega, 1984). EMS, along with other chemical mutagens, cause point and segment mutations (Rhaese and Boetker, 1973). EMS induces chemical modification of nucleotides, which results in mispairing and base changes that cause many inversion or deletion point mutations randomly distributed throughout the genome (Hoffman, 1980). As a result, EMS mutagenesis can be used not only to search for loss, or gain, of function mutants but also to understand the role of specific amino acid residues in protein function. The frequency of mutation depends on the position of the gene in the genome and the treatment conditions, such as the length of exposure to concentration of EMS, during the mutagenesis process. This makes EMS useful for inducing mis-sense and nonsense

mutations that provide change-of-function by alkylating guanine bases and resulting in primarily G/C to A/T transitions (Bhat et al., 2007) but also A/T to G/C transitions (Sega, 1984). EMS can be used to cause general variability with no direct goal in mind, or to rectify a simply inherited defect in an otherwise agronomically superior cultivar. Using the latter approach allows for the functional analysis of various genes (Patial et al., 2015).

Fast-neutron (FN) irradiation is also an effective way to generate gene deletion mutants in plants. FN-induced chromosomal deletions range from 1 bp-30 kb, with most deletions at 1-4 kb. The dose of FN and, consequently, the number of deletions per genome can be controlled (Li and Zhang, 2002). FN mutants can be screened for a gene or a set of genes that are responsible for a particular phenotype. The mutants can also be used to identify specific gene deletions using high throughput PCR, and subsequently used to alter a phenotype by genomic modification.

The generalized scheme for induced mutagenesis as a crop breeding strategy is straight forward and involves the sequential steps of the exposure of seeds to pre-determined doses of a mutagen, the identification of stable mutants amongst the progeny, and the incorporation of the desirable mutants into breeding programs. Seeds of a selected genotype are treated by the selected mutagen, followed by several cycles of selection. The best genetic background to be used is an elite homozygous genotype that is deficient for only the desired new trait or characteristic. The generations of mutants are generally referred to as $M_0$, $M_1$, $M_2$, etc. $M_0$ refers to the original non-mutated line, $M_1$ refers to the first generation of plants that is grown from mutagenized seed, $M_2$ refers to the progeny seed collected from $M_1$ plants, $M_3$ refers to the progeny seed collected from the $M_2$ population, etc.

The first step in a mutation breeding program involves growing $M_1$ plants from the seed treated with the mutagen, then selecting seed from mutant plants. Some plants will show obvious mutations, even to the point that many plants will never flower; however, other plants may look relatively normal. This is because most mutant alleles are

recessive, so mutant traits may not be seen until the $M_2$ generation, when lethality and infertility will often significantly affect the plants in the population. From this point forward in the breeding process, mutation breeding is similar to any other type of plant breeding strategy.

Low levels of efficiency for the induction and detection of mutation events, and the necessity of producing and evaluating large mutant populations, constitute significant obstacles to the routine application of induced mutations in plant breeding (Mba, 2013). Most induced mutations are predominantly recessive. As a result, the expression of the desired phenotype is masked if the alleles are in a heterozygous state. Attaining homozygosity at the mutated alleles so that the desired phenotypes can be visibly detected normally requires several cycles of selfing, followed by selection. Fortunately, in tobacco totipotency (the ability of individual single plant cells to regenerate a whole organism) can easily be exploited to produce genetically stable doubled haploid (DH) breeding lines from plants displaying desirable traits in the $M_2$ breeding population. Even recessive traits that are not visible in mutant plants used to initiate the DH process will be visibly expressed in the homozygous DH progeny lines.

**Mutation Breeding in Tobacco**

The primary requirement for a successful tobacco breeding effort is the identification of genetic variability for traits of interest, and the ability to incorporate those traits into commercial varieties that have agronomic characteristics needed by growers. Most modern cultivated crops, and especially tobacco, have a very narrow genetic base. Years of breeding efforts to maximize yield potential, disease resistance, and leaf quality have resulted in the development of new varieties that are far superior to those utilized even 30 years ago. However, as a result of intensive breeding efforts most modern burley varieties are extremely closely related and offer little potential for variability for desired traits. If desired traits cannot be identified in other tobacco varieties, Tobacco Introductions, or closely related species, then genetic engineering and/or mutation

breeding techniques may be necessary to create genetic variability that may provide the desired trait.

As mentioned earlier, the first released commercial variety developed using induced mutagenesis was the tobacco variety “Chlorina” in 1936. Mutation breeding has also been used to develop the virgin A mutant (VAM) source of potato virus Y (PVY) resistance in tobacco (Koelle, 1961). For many years, the use of VAM for PVY resistance in tobacco was unsuccessful due to genetic linkage to a trait that reduced normal leaf trichome exudates, resulting in extensive insect damage. TN 86 was the first released tobacco variety in which the linkage between desirable VAM PVY resistance and the tightly linked undesirable non-secreting trichome trait was successfully broken (Miller, 1987). Since that breakthrough, many new burley varieties developed by numerous breeding programs, including most of the varieties developed by KTTII, possess this mutated allele. More recently, mutation breeding techniques have been used to produce tobacco lines that have altered chemical or physiological traits in comparison to traditional tobacco (Julio, et.al. 2008).

With the advance in molecular genetics techniques, traits of interest can be moved from one species into another to develop “transgenic” varieties that can possess novel characteristics impossible to achieve through traditional breeding methods. Genetic engineering techniques have been used successfully in tobacco to reduce nornicotine content (Gavilano, et. al., 2006; Lewis, et.al., 2008 ) and to alter leaf surface chemistry (Wagner and Kroumova 2008; Kroumova and Wagner, 2009). Although both mutation and genetic engineering methodologies have been successfully utilized in tobacco breeding, transgenic varieties are not currently acceptable within the tobacco industry. An alternative approach is to combine mutation and molecular genetics breeding techniques to reach a desirable target. Transgenic varieties containing traits of interest can be developed as a preliminary step; once the relevant genes are identified and sequenced, individual plants in a mutation breeding population can be screened for mutant alleles having the same genetic sequence as the target transgenes. The mutant alleles can then be moved into commercial varieties via traditional breeding methods. A

highly saturated microsatellite marker based linkage map of tobacco has recently been published (Bindler, et.al., 2007), which makes the possibility of being able to use mutated plants to select alleles having the same genetic sequence as the transgenes much more likely than it was prior to the advent of molecular genetics breeding techniques.

**Nicotine to Nornicotine Conversion in Tobacco**

Nicotine is the dominant pyridine alkaloid found in modern cultivated tobacco (*Nicotiana tabacum L.)* (Benowitz, 2008). It is primarily formed in the roots and translocated throughout the plant in the xylem (Guthrie et al., 1962). Genetics, agricultural practices, and environmental conditions affect both nicotine and nornicotine production. Increased nitrogen fertilizer, decreased topping height, increased length of photoperiod, decreased soil temperature, increased time between topping and harvest and increased sucker control all increase nicotine accumulation and were most likely the result of increased leaf yield (Bush, 1999). The same holds true for nornicotine, but it only makes up about five percent of the total alkaloid content in most green, actively growing plants (Lewis et al., 2010; Carvalho et al., 2014). Nornicotine formation comes primarily from nicotine demethylation by the enzyme *nicotine N-demethylase* (Bush, 2001; Hao and Yeoman, 1998). Nornicotine content increases when the plants reach maturity and have the apical meristem removed (topped) and begin to senesce, and continues after the plants are harvested and the leaves are cured with the vast majority of nornicotine production occurring during senescence and curing of mature leaves (Xu et al., 2007; Lewis et al., 2010). Up to 98 percent of leaf nicotine can be converted to nornicotine (Jack and Bush, 2007).

Researchers at North Carolina State University (NCSU) and the University of Kentucky (UK) determined that nicotine demethylation is mediated by three CYP450 genes (Siminszky et.al, 2005; Gavilano et.al, 2006; Lewis et.al, 2008; Lewis et.al, 2010). Control of nicotine demethylation by these genes has been demonstrated by producing plants with RNAi silenced versions of each of the three CYP450 genes; this reduces the amount

of genetically controlled nicotine demethylation (Dewey and Xie, 2013). This research will be discussed in more detail later in this thesis. A second study by Hung et al. (2013) that manipulated tobacco methylenetetrahydrofolate reductase (MTHFR), which regulates the expression of one of these genes, also reduced nicotine demethylation.

**Appeal of Reducing Nornicotine Formation**

Tobacco specific nitrosamines (TSNAs) are produced by the nitrosation of tobacco pyridine alkaloids nicotine, nornicotine, anatabine and anabasine. TSNA are absent or in negligible concentration in green tobacco, but are produced primarily during the curing of leaves (Bush et al., 2001). Their levels also fluctuate due to changes in alkaloid amounts during topping and harvesting (Cai et al. 2013). Nornicotine has been identified as the precursor for N-nitrosonornicotine (NNN) which is considered one of the most carcinogenic TSNAs (Dewey and Xie, 2013). TSNAs have been labeled as contributors to increased risk for cancer of the upper digestive tract for smokeless tobacco users and cancer of the respiratory tract and pancreas for smokers (Brunnemann et al., 1996). Following the LC protocol, levels of TSNAs have been lowered by ensuring no more than three percent nicotine conversion occurs in foundation seed lots (Jack and Bush, 2007). This does not permanently maintain lower levels, however, due to the possibility of spontaneous formation of high nornicotine producing converter plants (Lewis et al., 2010).

**Identification of Three Genes Involved in Nicotine Conversion**

As mentioned earlier, researchers at North Carolina State University (NCSU) and the University of Kentucky (UK) identified three CYP 450 genes that control nicotine to nornicotine conversion by mediating nicotine demethylase (NND) enzymes (Siminszky et.al, 2005; Gavilano et.al, 2006; Lewis et.al, 2008; Lewis et.al, 2010). These three genes were designated as CYP82E4 (E4), CYP82E5v2 (E5) and CYP82E10 (E10). For simplification, the dominant versions of these alleles will be referred to as E4, E5, and E10 and the recessive versions as e4, e5, and e10 throughout the remainder of this thesis. Siminszky and Xu separately discovered E4 through microarray-based expression

profiling and DNA chip identification, respectively (Siminszky et al., 2005; Xu et al., 2007). Later, Gavilano and Siminszky, independently from Lewis, identified E5 and E10. The three genes all act differently, indicating the role of each in nicotine demethylation. E4 expression is strongly induced during senescence and in response to the senescence associated hormone ethylene, specifically in converter plants. E5 is expressed in green, non-senescent leaf tissue in both converter and non-converter plants, but at lower levels than E4. E10 is also expressed at levels lower than E4 in both converter and non-converter plants, but primarily in root tissue (Dewey and Xie, 2013).

Following the discovery of the E4, E5, and E10 NND genes, transgenic lines of burley tobacco carrying an RNA interference (RNAi) construct designed to inhibit the expression of the genes were developed (Siminszky et.al, 2005; Gavilano et.al, 2006; Lewis et.al, 2008; Lewis et.al, 2010). Selected transgenic lines containing the three silenced NND genes exhibited a six-fold decrease in nornicotine content relative to untransformed controls, with a commensurate decrease in NNN and total TSNAs. Because these transgenic lines were not acceptable by the tobacco industry, EMS chemical mutagenesis was subsequently used by NCSU researchers to produce a mutagenic breeding population. From this population, knockout mutations for all three tobacco NND genes were identified utilizing the genetic sequence of the RNAi transgenes. With all three of these recessive genes present in a homozygous state, nornicotine levels were shown to be drastically lowered when testing samples of plants grown in growth chambers and greenhouses. In these mutated lines, the nornicotine levels were of approximately 0.5 percent of total alkaloid content. Incorporation of these three mutated alleles into tobacco varieties is proving to be an effective non-transgenic strategy for lowering the levels of both nicotine and NNN in tobacco products (Lewis et al., 2010).

## Chapter 3: Materials And Methods

### Introgression of Mutant Alleles into KTTII Breeding Materials

All of the mutant alleles were introgressed into KTTII breeding lines via backcross breeding, with the presence of the desired alleles in segregating generations verified by dCAPS markers developed by KTTII molecular geneticist Dr. Dandan Li (Li, et.al, 2012). In the initial stages of this project, KTTII worked with Philip Morris International (PMI) researchers to transfer the e4 and e5 mutant alleles into existing KTTII cultivars TN 90LC and TN 86LC, as well as the male and female parental lines for hybrid cultivars KT 204LC, KT 206LC, KT 209LC, KT 210LC, KT 212LC, and KY 14 X L8LC. Traditional backcross breeding techniques were utilized in this phase of the project, which was terminated in 2013. These homozygous KTTII lines that contain only the e4 and e5 alleles, but are lacking e10, will be referred to as "e2" lines in this thesis. At the point of termination, e2 versions of parental lines TKF 2002, TKS 2002, TKF 4028, TKF 4024, and TKF 6400 were in the $BC_6$ generation, while e2 versions of KY 14 and L8 were only in the $BC_3$ generation.

During this phase of the project, KTTII did not have intellectual property rights to work with the e10 allele. As a result, researchers at Altria and NCSU were working simultaneously to introgress all three mutant alleles into KTTII parental lines, which had been provided by KTTII. The time required for the introgression process was significantly shortened by utilizing an early flowering FT (Flowering Locus T) trait, which was transferred from *Arabidopsis thaliana* gene into tobacco using transgenic breeding procedures (Lewis and Kernodle, 2009). For most tobacco varieties, insertion of the FT trait will result in flowering in approximately 38-45 days after seeding, compared to approximately 125 – 140 days for greenhouse or field grown plants. However, the use of the FT trait eliminates the ability to make visual selection for agronomic type during the backcrossing process. NCSU took the lead in transferring the three alleles into TN 90, ms TN 90, TN 86, ms TN 86, KY 14, ms KY 14, and L8. Altria transferred the alleles into KTTII parental lines TKF 2002, TKS 2002, TKF 4028, TKF 4024, and TKF 6400. Altria

also produced several experimental versions of KTTII hybrids that were homozygous for all three alleles. Varieties and parental lines which are homozygous for all three alleles will be referred to as "e3" lines in this thesis.

**2013 Field Evaluations of e2 and e3 Lines/Varieties in Comparison to Their Original LC Counterparts**

The e3 parental lines and self-pollinated varieties, and hybrid varieties comprised from the appropriate parental lines, were returned to KTTII by NCSU and Altria for field evaluation in 2013. All of the parental lines and self-pollinated varieties were in the backcross 6 ($BC_6$) generation, which should statistically be 98% homozygous when dealing with a single gene (Fehr, 2001). Field trials were conducted in Lexington and Versailles, Kentucky and Greeneville, Tennessee to compare the experimental e2 and e3 parental lines, and the e3 hybrid varieties, to their LC counterparts. However, due to high amounts of rainfall, the plots in Greeneville were lost to drowning, erosion, and fertility losses. All fields were prepared and managed based on recommendations in the 2013-2014 Kentucky and Tennessee Tobacco Production Guide (Seebold et. al, 2013).

Varying numbers of parental lines within each family were available for testing in yield and black shank resistance trials. For parental lines developed by Altria, 15 versions of TKS 2002, three versions of TKF 2002, eight versions of TKF 4024, six versions of TKF 4028, and six versions of TKF 6400 were provided. Because there was not room to put all versions of the parental lines provided by Altria in the yield trials, three e3 parental lines were randomly chosen and compared to the original LC parental line, plus one e2 line developed by KTTII that was also randomly chosen. NCSU provided only one version of parental lines KY 14 and L8; the single version of the e3 lines was compared to their e2 and LC counterparts. All test entries were seeded in a greenhouse in mid- March, with the Lexington yield trials transplanted on May 21 and the Versailles yield trials transplanted on May 29. The lines were randomized in groups, referred to as "family groups," which included the LC commercial line between the experimental parental lines. They were grown side by side in order to provide visual comparisons of leaf

orientation, color and shape, as well as time to maturity. Each group was replicated in a randomized complete block design with three replications at all locations. Plot size was 32 plants, spaced 53 cm within the row and 107 cm between rows. Within each replication, ten plants were randomly chosen for evaluation of agronomic traits. Data were collected for plant height, leaf number, leaf internode length, and length and width of the 5$^{th}$ leaf from the tip after topping. Teams of three people would walk the plots with one person recording data as another measured the stalk height in centimeters from the ground to the tip and counted the total number of leaves and the third person measured, in centimeters, the 5$^{th}$ leaf's length and width. Within each variety family, bloom counts were taken on each plot; individual lines were topped based on an average of 25% flowering across the three replications. After removal of the border plants, 30 plants were harvested from each plot, with five plants placed on each stick. Where weather allowed, plants were allowed to field wilt for 2-3 days before being hung on rail wagons and transported to curing barns. After air-curing, all plants were taken out of the barn and leaves were stripped from the stalks and separated into four grades: flyings, cutters, leaf, and tips in order from lower to upper stalk position. Each leaf grade was weighed independently, then weights were combined to give a total for the number of tobacco plants harvested. The total weight was divided by the number of harvested plants per plot, then multiplied by 7,200 to estimate yield per acre based on a plant population of 7,200 plants. Data for each family group were analyzed independently using SAS version 9.3. Both the ANOVA and LSD analyses used a PR > F of 0.05 to identify significant differences among the entries within a given family.

All of the parental lines from Altria and NCSU were evaluated for resistance to black shank in two black shank nurseries, one located in Greeneville, Tennessee and the second in Frankfort, Kentucky. Both nurseries contained both race 0 and race 1 black shank. The Greeneville nursery was transplanted on May 30, and the Frankfort nursery was transplanted on June 12. A completely randomized design with three replications was utilized; plot size was 20 plants spaced 53 cm within the row and 107 cm between rows. Black shank resistance was evaluated based on percent survival of each

replication of each line. Initial stand counts were done to establish the number of plants that survived transplanting. The plots were then reevaluated on a three week schedule to determine how many plants were infected or dead from black shank.

For the commercial variety evaluations, Altria provided four versions of hybrid varieties KT 204, KT 206, KT 209, KT 210 and KT 212, while NCSU provided one version of varieties TN 86, TN 90, and KY 14 x L8. For yield and quality evaluation trials, the four versions of the e3 hybrid lines from each variety family were compared to its original LC hybrid variety; for varieties provided by NCSU, the single version of each variety family was compared to its LC counterpart. For the yield trials, each independent hybrid family was evaluated in a randomized complete block design with three replications. All of the e3 varieties from Altria and NCSU were compared to their LC counterparts for resistance to black shank in the Kentucky and Tennessee black shank nurseries. For the nursery evaluations, a completely randomized design with three replications was utilized. For both the yield and black shank trials, transplant dates, plot layout and management, and data collection and analyses were as described above for the parental lines evaluations.

**2014 Field Evaluations of e2 and e3 Lines/Varieties in Comparison to Their Original LC Counterparts**

Field evaluations of the e3 parental lines and varieties were repeated in 2014. However, for the parental line trials, only the best parental line within each family, developed through selection and/or backcrossing in 2013 as described in the 2013 Results and Discussion section later in this thesis, was compared with its LC and/or e2 counterpart. Parental line families evaluated included TKF 2002, TKS 2002, TKF 4024, TKF 4028, and TKF 6400. Because parental lines KY 14e3 and L8e3 were extremely off-type in the 2013 trials and on-going efforts for their improvement were being made by NCSU researchers, the L8 and KY 14 families were not evaluated in 2014.

In the 2014 variety trials, families evaluated included TN 86, TN 90, KT 204, KT 209, KT 210, and KT 212; because one or both parental lines were significantly off-type in the

2013 trials, hybrid varieties ms KY 14 X L8 and KT 206 were not evaluated in 2014. For open pollinated varieties TN 86 and TN 90, both the male sterile and male fertile e3 versions were compared to their LC counterpart. For hybrid varieties, only the hybrid combination using the best parental line combination, also as described in the 2013 Results and Discussion section, was compared with its LC counterpart.

The 2014 agronomic trials were located in Greeneville and Springfield, TN and Lexington and Versailles, KY. Due to an extremely severe hail storm, however, 100 percent of Lexington's crop was lost. All parental lines and varieties were also evaluated for black shank resistance in the Tennessee and Kentucky nurseries used in 2013. All seeding was completed March 18-20 except for the Franklin County black shank nursery, which was seeded April 1. All transplanting in Lexington was May 19-20 and Versailles was May 28-29. The experimental design, data collection methods, and data analyses were the same as described for the 2013 studies.

**Chemical Analysis of Cured Leaf Samples**

A composite sample of leaves from each stalk position was collected from each entry in the Greeneville 2014 parental line and variety trials. The stems were removed from each leaf, due to higher levels of nicotine and nornicotine and their lack of use in consumer products. The lamina samples were placed in brown paper bags and allowed to dry at room temperature near a dehumidifier. Once dried, they were crushed by hand and then run through a plant material grinding machine with a 1 mm screen. A sub-sample of each composite sample was then taken to the lab for alkaloid and TSNA analyses. The laboratory analysis procedures were as published in a collaborative effort between R.J. Reynolds, U.S. Smokeless, and the University of Kentucky (Morgan et. al, 2004).

**Chapter 4: Results And Discussion**

**2013 Field Evaluation**

For the 2013 field season, there was considerable variation among the individual e3 lines for agronomic type within each hybrid and parental line group at both Lexington

and Versailles; there was also considerable variability for level of resistance in the black shank nurseries. Although some of the differences were primarily cosmetic in the replicated field trials, for some of the hybrid varieties and parental lines differences were quite noticeable. For several parental line families, all of the e3 lines were visibly detectable, and in many cases were inferior in comparison to the original LC materials. As would be expected, the differences among the parental lines within some families resulted in differences among the hybrid varieties comprised from those respective parental lines. Morphological problems that seemed to be more or less consistent across all e3 materials included wider leaf internodes (particularly in the top third of a plant) and a tendency for upper leaves to be "rolled" or "cupped". The severity of these symptoms varied among lines and individual plants for a given variety family, which suggests that careful plant selection and/or additional backcrossing, followed by self-pollination and selection, may eliminate these problems.

**2013 Parental Lines Evaluations**

The TKF 2002 family group contained only three e3 versions. TKF 2002LC is the pollinator line for the TKS 2002LC, which is the female parent for all KT hybrid varieties. In order for all of the e3 hybrids to remain true to type and comparable to the original LC hybrids, TKS 2002LC and TKS 2002e3 must be nearly identical. As TKS 2002e3 is maintained through pollination by TKF 2002e3 for seed increase, it will eventually become virtually identical to TKF 2002e3; as a result, TKF 2002e3 is the most important line among the e3 populations. For the yield trials, there were no significant differences among the three e3, one e2, and original LC version of TKF 2002 for any trait at either location (Tables 1a and 1b). All e3 lines and the e2 version performed as well or better than the LC in the yield trials. Although differences were non-significant, entry e3-A was the highest yielding line at both locations, but it had the lowest level of black shank resistance. The e2 version had the highest level of black shank resistance and also had similar yields to the TKF 2002LC, with 80 pounds less in Lexington and 264 pounds more in Versailles.

Table 1a. 2013 TKF 2002 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| Lexington | TKF 2002e3-A | 127.09 | 16.21 | 7.84 | 28.56 |
| | TKF 2002e3-B | 127.75 | 16.01 | 7.98 | 27.08 |
| | TKF 2002LC | 123.75 | 15.92 | 7.77 | 27.06 |
| | TKF 2002e3-C | 128.67 | 16.00 | 8.04 | 27.05 |
| | TKF 2002e2 | 123.46 | 15.84 | 7.79 | 27.90 |
| | **PR>F** | **0.50** | **0.88** | **0.16** | **0.33** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |
| Versailles | TKF 2002e3-A | 166.25 | 20.05 | 8.29 | 30.59 |
| | TKF 2002e3-B | 165.63 | 20.04 | 8.26 | 26.25 |
| | TKF 2002LC | 152.50 | 19.21 | 7.94 | 27.46 |
| | TKF 2002e3-C | 163.79 | 19.33 | 8.47 | 27.96 |
| | TKF 2002e2 | 158.50 | 19.92 | 7.96 | 28.33 |
| | **PR>F** | **0.34** | **0.44** | **0.21** | **0.08** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |

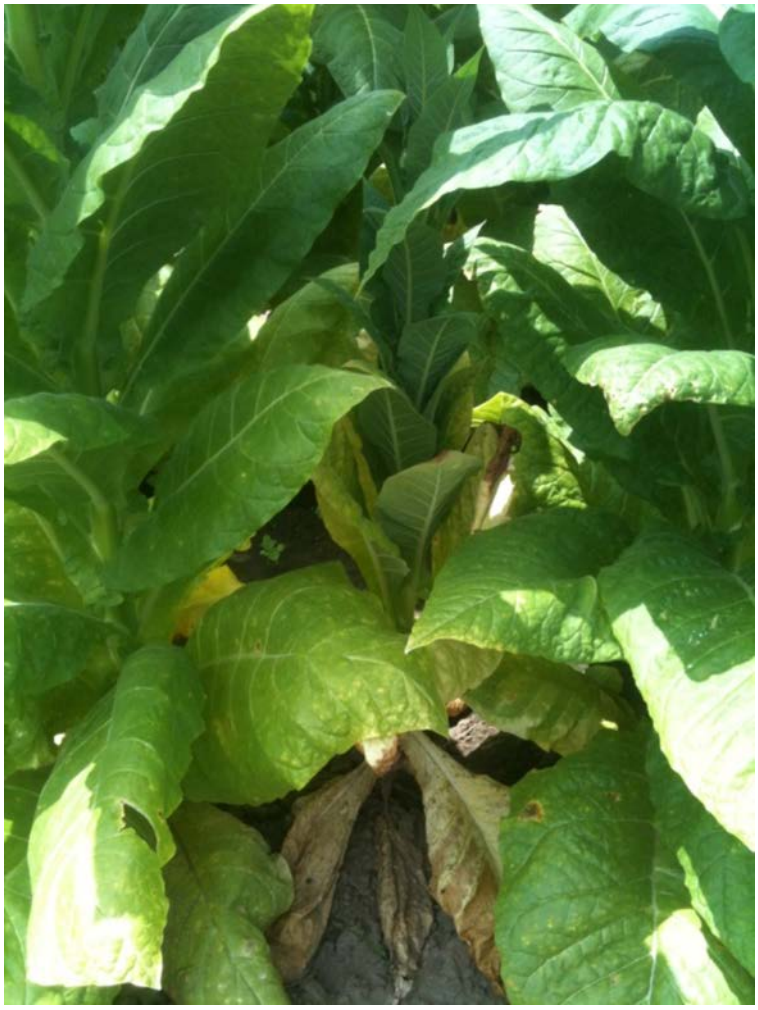
Table 1b. 2013 TKF 2002 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Leaf Length (cm) | Yield (kg/ha) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|
| Lexington | TKF 2002e3-A | 61.01 | 2865.28 | 70.00 | 92.00b |
| | TKF 2002e3-B | 58.85 | 2243.12 | 77.00 | 89.60b |
| | TKF 2002LC | 59.21 | 2610.81 | 92.90 | 98.50a |
| | TKF 2002e3-C | 60.29 | 2418.00 | 85.00 | 95.50ab |
| | TKF 2002e2 | 60.06 | 2521.13 | 97.40 | 100.00a |
| | **PR>F** | **0.11** | **0.08** | **0.48** | **0.03** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **5.95** |
| Versailles | TKF 2002e3-A | 62.58 | 3543.48 | | |
| | TKF 2002e3-B | 58.73 | 2762.14 | | |
| | TKF 2002LC | 60.06 | 2692.64 | | |
| | TKF 2002e3-C | 63.02 | 2889.94 | | |
| | TKF 2002e2 | 62.15 | 2988.59 | | |
| | **PR>F** | **0.49** | **0.12** | | |
| | **LSD(.05)** | **ns** | **ns** | | |

Although the data revealed no significant differences for any trait, there were, however, some extreme phenotypic differences observed within and between some of the family lines. One such example would be the off-type plant in the TKF 2002e3-C line shown in Photo 1. Among the e3 versions, entry C had the highest level of black shank resistance, but it had the greatest internode length (Table 1b). Although the leaf number and internode length of entry C were not significantly different from TKF 2002LC in the topped agronomic trials, the differences were visibly detectable in the un-topped black shank nursery evaluations (Photos 2 and 3). However, because of the relatively low level of black shank resistance contained in entries TKF 2002e3-A and TKF 2002e3-B, selection C was chosen as the best e3 line for use in further backcrosses to improve TKF 2002e3 to the point that it is comparable to TKF 2002LC and TKF 2002e2. To do this, TKF 2002e3-C was crossed directly to TKF 2002e2. The $F_1$ hybrid was homozygous for e4e5, but heterozygous for e10. This cross was then self-pollinated in the greenhouse during the winter of 2013-2014 to get to the $S_1$ generation, which was homozygous for e4e5, but segregating for e10 (1/4 E10/E10; 1/2E10/e10; 1/4 desired e10/e10). In the Spring of 2014, the segregating $S_1$ plants were planted in float trays in the greenhouse. By using the co-dominant dCaps markers for e4, e5, and e10, plants that were homozygous for all three recessive e alleles were identified and provided to the UK Foundation Seed (UKFS) project for production of foundation seed of TKF 2002e3 and TKS 2002e3. As a contingency plan in case more than one backcross would ultimately be needed to improve TKF 2002e3 to the point that it was comparable to TKF 2002e2 and TKF 2002LC, $F_1$ plants from the summer cross between TKF 2002e3-C and TKF 2002e2 plants were also backcrossed a second time to TKF 2002e2.

TKS 2002 is the female parent for KT 204, KT 206, KT 209, KT 210, and KT 212. Fifteen versions of TKS 2002e3 were provided for field observation by Altria. Entries A, E, and M were randomly selected for evaluation in the agronomic trials, with the remaining versions evaluated only for black shank resistance. For the agronomic trials, the TKS 2002 family group had statistically significant differences for leaf number and leaf width at the Lexington location (Tables 2a and 2b). However, this was due to entry TKF

Photo 1. Extremely Off Type Plant within TKF 2002e3-C



Photos 2 and 3. Morphological Characteristics of TKF2002e2 and TKF 2002e3-C

TKF 2002e2; 98.7% BS Survival

25.0 Leaves Not Topped

TKF 2002e3-C; 90.3% BS Survival

21.9 Leaves Not Topped

Table 2a. 2013 TKS 2002 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| Lexington | TKS 2002e3-A | 135.34 | 16.79a | 8.06 | 27.19ab |
| | TKS 2002e3-E | 133.63 | 16.34b | 8.18 | 28.15a |
| | TKS 2002LC | 136.38 | 16.96a | 8.04 | 26.69abc |
| | TKS 2002e3-M | 134.17 | 16.42ab | 8.17 | 25.29bc |
| | TKS 2002e2 | 120.92 | 15.75b | 7.68 | 24.75c |
| | **PR>F** | **0.06** | **0.04** | **0.41** | **0.03** |
| | **LSD(.05)** | **ns** | **0.74** | **ns** | **2.12** |
| Versailles | TKS 2002e3-A | 170.59 | 20.25 | 8.42a | 31.38a |
| | TKS 2002e3-E | 169.13 | 20.09 | 8.42a | 29.48b |
| | TKS 2002LC | 156.21 | 20.09 | 7.78bc | 27.58c |
| | TKS 2002e3-M | 164.21 | 20.5 | 8.01ab | 29.69b |
| | TKS 2002e2 | 148.46 | 20.42 | 7.27c | 26.36c |
| | **PR>F** | **0.13** | **0.98** | **0.01** | **0.01** |
| | **LSD(.05)** | **ns** | **ns** | **0.58** | **1.64** |

Table 2b. 2013 TKS 2002 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Leaf Length (cm) | Yield (kg/ha) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|
| Lexington | TKS 2002e3-A | 59.59 | 2744.21 | 93.10a | 94.00 |
| | TKS 2002e3-E | 60.52 | 2534.58 | 67.80c | 88.60 |
| | TKS 2002LC | 58.27 | 2545.79 | 97.80a | 100.00 |
| | TKS 2002e3-M | 58.94 | 2630.99 | 83.40abc | 90.50 |
| | TKS 2002e2 | 56.75 | 2448.26 | 100.00a | 98.50 |
| | **PR>F** | **0.19** | **0.61** | **0.04** | **0.06** |
| | **LSD(.05)** | **ns** | **ns** | **19.65** | **ns** |
| Versailles | TKS 2002e3-A | 63.90a | 3680.24a | | |
| | TKS 2002e3-E | 63.73a | 3076.02bc | | |
| | TKS 2002LC | 59.80b | 2662.38c | | |
| | TKS 2002e3-M | 64.17a | 3467.25ab | | |
| | TKS 2002e2 | 58.85b | 2996.43c | | |
| | **PR>F** | **0.01** | **0.01** | | |
| | **LSD(.05)** | **3.24** | **419** | | |
| | | | | | |
| | TKS 2002e3-B | | | 95.20a | 98.60 |
| | TKS 2002e3-C | | | 97.80a | 98.50 |
| | TKS 2002e3-D | | | 81.00abc | 91.40 |
| | TKS 2002e3-F | | | 95.50a | 97.30 |
| **Evaluated** | TKS 2002e3-G | | | 85.70ab | 87.60 |
| **Only for** | TKS 2002e3-H | | | 85.80ab | 92.70 |
| **Black** | TKS 2002e3-I | | | 93.20a | 95.10 |
| **Shank** | TKS 2002e3-J | | | 83.40abc | 89.40 |
| **Resistance** | TKS 2002e3-K | | | 64.30c | 92.20 |
| | TKS 2002e3-L | | | 90.50a | 94.70 |
| | TKS 2002e3-N | | | 92.90a | 95.70 |
| | TKS 2002e3-O | | | 97.60a | 98.30 |

2002e2 having significantly fewer and narrower leaves than TKS 2002LC, rather than any of the three e3 entries being statistically different from TKS 2002LC. At the Versailles location, statistically significant differences were detected for leaf internode length, leaf width, leaf length, and yield. All three of the e3 entries had greater internode lengths, wider leaves, longer leaves, and higher yields than either TKF 2002 LC or TKF 2002e2. In general, the overall agronomic performance of all three e3 entries was actually superior to TKS 2002LC or TKS 2002e2, particularly at the Versailles location. Although this would normally be desirable, in order for all of the e3 hybrids to remain true to type and comparable to the original LC hybrids, TKS 2002LC and TKS 2002e3 must be nearly identical, not only for agronomic characteristics but also for disease resistance. TKS 2002LC and TKS 2002e2 were very similar, both for agronomic characteristics and black shank resistance. Although it was not included in the agronomic field trials, after careful visual observation it was determined that TKS 2002e3 entry O most closely resembled TKF 2002LC and TKF 2002e2 for agronomic type and level of black shank resistance (Photos 4 and 5). Although the internode length was slightly greater than for TKS 2002LC, TKS 2002e3-O would be an acceptable e3 version of TKS 2002LC once a better TKF 2002e3 pollinator line was developed. As a result, female line TKS 2002e3-O was used as the female parent to re-make all of the KT e3 hybrid varieties for further testing in 2014. However, to further improve the TKS 2002e3-O, an additional backcross was made to TKF 2002e2 in an attempt to increase the leaf number and decrease the internode length of TKS 2002e3. This initial backcross would be homozygous for the e4 and e5 alleles, but heterozygous for the e10 allele. However, in order to produce homozygous foundation seed of TKS 2002e3, both fertile and sterile parents must be homozygous for all three e alleles. A second backcross with the final TKF 2002e3 selection that is homozygous for all three recessive e alleles will be necessary before a final improved, homozygous TKS 2002e3 parental line can be obtained.

TKF 4024LC is the male parent for KTTII hybrid variety KT 209LC. Eight versions of TKF 4024e3 were provide to KTTII by Altria researchers. Entries, TKF 4024e3 -A, - B, and -C were randomly selected for evaluation in the agronomic trials, while all entries were

Photos 4 and 5. Morphological Characteristics of TKS 2002e2 and TKS 2002e3-O

TKS 2002e2; 99.3% BS Survival

25.6 Leaves Not Topped

TKS 2002e3-O; 98.0% BS Survival

23.9 Leaves Not topped

evaluated in the black shank nurseries. For the agronomic trials, the TKF 4024 family group showed no significant differences for any trait at either location (Tables 3a and 3b). All three of the e3 versions evaluated in the agronomic trials were comparable to the LC and e2 versions. There was some phenotypic variation among the lines, but selection of the best plants from the best lines would result in acceptable e3 parental lines. Based on agronomic characteristics and level of disease resistance observed in the black shank nurseries, it was determined that selection F most closely resembled TKF 4024LC. Although the degree of similarity between TKF 4024LC and TKF 4024e3-F was great enough that it was anticipated that no further backcrosses were necessary, one additional backcross to TKF 4024e2 was made as a contingency plan. TKF 2002e3-F was also crossed onto TKS 2002e3 selection O to make the version of KT 209e3 that was to be entered into 2014 variety trials. The TKF 4024e3-F selection was also used to produce foundation seed of the TKF 4024e3 parental line.

TKF 4028LC is the male parental line for KT 206LC. Six versions of TKF 4028e3 were provided to KTTII by Altria; entries A, B, and C were randomly chosen for inclusion in the agronomic trials, while all six e3 entries were evaluated in the black shank nurseries. Although statistically significant differences were detected in the agronomic trials only for leaf width and length at the Versailles location, all six of the e3 lines were inferior to TKF 4028LC and TKF 4028e2 for either agronomic type or level of black shank resistance (Table 4b and Photo 6). Although not significantly significant, TKF 4028LC yielded approximately 500 lbs/A higher than TKF 4028e3-A at the Versailles location. In addition, none of the six TKF 4028e3 lines that were evaluated in the black shank nurseries had a level of resistance comparable to TKF 4028e2. During the initial KTTII/ PMI breeding effort to introgress e4 and e5 into TKF 4028, the last two backcross cycles of TKF 4028e2 were done in a black shank nursery having very high disease pressure, and only the most resistant plants were utilized. As a result, the level of black shank resistance was substantially increased in comparison to the original TKF 4028LC. It is highly desirable to maintain this added level of black shank resistance in the development of TKF 4028e3 if at all possible. However, because of the degree of

Table 3a. 2013 TKF 4024 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| Lexington | TKF 4024e3-A | 127.96 | 16.04 | 7.98 | 33.29 |
| | TKF 4024e3-B | 133.71 | 17.38 | 7.69 | 25.11 |
| | TKF 4024LC | 129.05 | 17.21 | 7.50 | 22.23 |
| | TKF 4024e3-C | 128.79 | 16.92 | 7.61 | 24.15 |
| | TKF 4024e2 | 134.25 | 16.75 | 8.01 | 25.08 |
| | **PR>F** | **0.22** | **0.17** | **0.28** | **0.34** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |
| Versailles | TKF 4024e3-A | 144.84 | 19.17 | 7.56 | 27.36 |
| | TKF 4024e3-B | 141.21 | 19.25 | 7.34 | 25.27 |
| | TKF 4024LC | 143.13 | 19.92 | 7.19 | 25.31 |
| | TKF 4024e3-C | 139.71 | 19.34 | 7.22 | 26.25 |
| | TKF 4024e2 | 142.21 | 18.8 | 7.56 | 26.92 |
| | **PR>F** | **0.34** | **0.12** | **0.07** | **0.55** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |

Table 3b. 2013 TKF 4024 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Leaf Length (cm) | Yield (kg/ha) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|
| Lexington | TKF 4024e3-A | 41.79 | 1974.08 | 95.10 | 100.00a |
| | TKF 4024e3-B | 53.36 | 2439.30 | 88.10 | 95.50a |
| | TKF 4024LC | 48.77 | 2129.90 | 90.90 | 98.00a |
| | TKF 4024e3-C | 53.92 | 1808.17 | 84.00 | 100.00a |
| | TKF 4024e2 | 50.98 | 2081.70 | 91.70 | - |
| | **PR>F** | **0.34** | **0.13** | **0.66** | **0.001** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **5.49** |
| Versailles | TKF 4024e3-A | 58.46 | 2869.76 | | |
| | TKF 4024e3-B | 53.88 | 2555.88 | | |
| | TKF 4024LC | 53.11 | 2663.50 | | |
| | TKF 4024e3-C | 56.23 | 2699.37 | | |
| | TKF 4024e2 | 55.40 | 2484.14 | | |
| | **PR>F** | **0.11** | **0.39** | | |
| | **LSD(.05)** | **ns** | **ns** | | |
| | | | | | |
| Evaluated Only for Black Shank Resistance | TKF 4024e3-D | | | 100.00 | 94.30b |
| | TKF 4024e3-E | | | 95.30 | 95.80a |
| | TKF 4024e3-F | | | 95.20 | 95.40a |
| | TKF 4024e3-G | | | 97.60 | 92.70b |
| | TKF 4024e3-H | | | 57.10 | 95.20a |

Table 4a. 2013 TKF 4028 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| **Lexington** | TKF 4028e3-A | 121.34 | 15.38 | 7.89 | 25.73 |
| | TKF 4028e3-B | 125.71 | 15.96 | 7.88 | 23.19 |
| | TKF 4028LC | 127.01 | 17.09 | 7.43 | 25.88 |
| | TKF 4028e3-C | 125.3 | 16.13 | 7.77 | 24.44 |
| | TKF 4028e2 | 122.54 | 15.59 | 7.86 | 26.88 |
| | **PR>F** | **0.88** | **0.15** | **0.75** | **0.08** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |
| **Versailles** | TKF 4028e3-A | 164.50 | 20.96 | 7.85 | 22.56b |
| | TKF 4028e3-B | 162.42 | 21.46 | 7.57 | 22.92b |
| | TKF 4028LC | 167.75 | 21.46 | 7.82 | 25.67a |
| | TKF 4028e3-C | 164.75 | 21.68 | 7.60 | 27.40a |
| | TKF 4028e2 | 161.25 | 21.88 | 7.37 | 26.67a |
| | **PR>F** | **0.68** | **0.82** | **0.43** | **0.01** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **1.94** |

Table 4b. 2013 TKF 4028 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Leaf Length (cm) | Yield (kg/ha) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|
| Lexington | TKF 4028e3-A | 57.98 | 2409.03 | 4.80b | 63.90ab |
| | TKF 4028e3-B | 54.77 | 2253.21 | 2.30b | 63.90ab |
| | TKF 4028LC | 60.06 | 2555.88 | 7.40b | 18.90c |
| | TKF 4028e3-C | 57.04 | 1992.02 | 0.00b | 62.40ab |
| | TKF 4028e2 | 60.77 | 2375.40 | 62.40a | 82.40a |
| | **PR>F** | **0.26** | **0.15** | **0.01** | **0.01** |
| | **LSD(.05)** | **ns** | **ns** | **23.36** | **23.52** |
| Versailles | TKF 4028e3-A | 58.17bc | 2636.59 | | |
| | TKF 4028e3-B | 56.13c | 2793.53 | | |
| | TKF 4028LC | 61.09ab | 3183.64 | | |
| | TKF 4028e3-C | 63.32a | 3053.60 | | |
| | TKF 4028e2 | 63.23a | 3185.88 | | |
| | **PR>F** | **0.01** | **0.12** | | |
| | **LSD(.05)** | **4.29** | **ns** | | |
| | | | | | |
| Evaluated Only for Black Shank Resistance | TKF 4028e3-D | | | 0.00b | 42.70b |
| | TKF 4028e3-E | | | 0.00b | 59.00ab |
| | TKF 4028e3-F | | | 0.00b | 64.60ab |
| | | | | | |
| | | | | | |

Photo 6. Morphological Characteristics of TKF 4028LC and TKF 4028e3-C

inferiority observed in the TKF 4028e3 selections evaluated in 2013, more than one backcross to TKF 4028e2 will be needed in order to obtain a satisfactory TKF 4028e3 line. Due to the inferiority of all of the TKF 4028e3 lines, no seed of KT 206e3 was made for testing in 2014. Based on agronomic type in the absence of black shank, the best TKF 4028e3 line was entry F; this line was back-crossed with TKF 4028e2. The $F_1$ seed, which was homozygous for e4e5 but heterozygous for e10/E10, was backcrossed a second time to TKF 4028e2 in the greenhouse during the winter of 2013. The $BC_2$ line will have to be self-pollinated to produce a population that can be utilized to select plants that are homozygous for all three recessive e alleles.

TKF 6400LC is the male parent of hybrid variety KT 210LC. Six versions of TKF 6400e3 were available for evaluation in 2013 filed trials; entries A, B, and C were compared with TKF 6400LC and TKF 6400e2 in the agronomic trials, with all six e3 entries evaluated in the black shank nurseries. The TKF 6400 family group only showed significant differences for internode length at the Versailles location (Tables 5a and 5b). There were, however, obvious differences observed within some lines **(**Photo 7.) Although there was variability within the lines, selections of the best plants within each entry resulted in acceptable e3 parental lines. All three of the e3 versions evaluated in the agronomic trials were also comparable to the LC and e2 versions. However, when selections were made in the black shank nurseries, it was determined that selection B most closely resembled TKF 6400LC when agronomic type and race 1 black shank resistance were considered. The similarity between TKF 6400e3-B and TKF 6400LC was close enough that no further backcrosses were deemed to be necessary. Selection B was therefore crossed onto selection TKS 2002e3 selection O to make the version of KT 210e3 that was to be evaluated in the 2014 field trials. Entry B was also used to produce foundation seed of TKF 6400e3.

The male sterile version of KY 14LC is the female parent for the commercial hybrid variety ms KY 14 X L8. Only one version of KY 14e3 was provided to KTTII by NCSU; in addition, NCSU researchers indicated that KY 14e3 was visibly off-type in comparison to KY 14LC. In the agronomic trials, the KY 14 family group showed statistically significant

Table 5a. 2013 TKF 6400 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| Lexington | TKF 6400e3-A | 119.33 | 16.96 | 7.04 | 27.58 |
| | TKF 6400e3-B | 123.75 | 16.75 | 7.39 | 26.29 |
| | TKF 6400LC | 119.63 | 16.46 | 7.27 | 25.90 |
| | TKF 6400e3-C | 130.25 | 17.04 | 7.64 | 27.19 |
| | TKF 6400e2 | 107.29 | 15.21 | 7.05 | 25.10 |
| | **PR>F** | **0.14** | **0.59** | **0.20** | **0.31** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |
| Versailles | TKF 6400e3-A | 165.84 | 25.13 | 6.60b | 27.15 |
| | TKF 6400e3-B | 168.42 | 24.67 | 6.83b | 27.23 |
| | TKF 6400LC | 160.54 | 23.38 | 6.87b | 26.44 |
| | TKF 6400e3-C | 155.38 | 21.25 | 7.31a | 28.75 |
| | TKF 6400e2 | 158.88 | 23.67 | 6.71b | 26.29 |
| | **PR>F** | **0.51** | **0.10** | **0.03** | **0.48** |
| | **LSD(.05)** | **ns** | **ns** | **0.39** | **ns** |

Table 5b. 2013 TKF 6400 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Leaf Length (cm) | Yield (kg/ha) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|
| Lexington | TKF 6400e3-A | 59.44 | 2495.35 | 85.80 | 95.80 |
| | TKF 6400e3-B | 55.04 | 2412.39 | 67.30 | 91.40 |
| | TKF 6400LC | 56.42 | 2488.62 | 77.20 | 90.20 |
| | TKF 6400e3-C | 56.06 | 2663.50 | 56.40 | 94.60 |
| | TKF 6400e2 | 55.59 | 2300.29 | 95.50 | 97.20 |
| | **PR>F** | **0.14** | **0.46** | **0.39** | **0.06** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |
| Versailles | TKF 6400e3-A | 58.56 | 2762.14 | | |
| | TKF 6400e3-B | 55.42 | 3068.18 | | |
| | TKF 6400LC | 55.09 | 2873.12 | | |
| | TKF 6400e3-C | 56.63 | 2957.20 | | |
| | TKF 6400e2 | 57.50 | 2977.38 | | |
| | **PR>F** | **0.38** | **0.73** | | |
| | **LSD(.05)** | **ns** | **ns** | | |
| | | | | | |
| Evaluated Only for Black Shank Resistance | TKF 6400e3-D | | | 90.50 | 97.20 |
| | TKF 6400e3-E | | | 62.90 | 86.80 |
| | TKF 6400e3-F | | | 76.90 | 76.70 |
| | | | | | |
| | | | | | |

Photo 7. Three Distinct Phenotypes within TKF 6400e3-C in Versailles, KY 2013

differences only for leaf length at Lexington, and for leaf width at Versailles (Tables 6a and 6b). This is very misleading, however, due to the extreme differences between KY 14e3 and KY 14LC for plant type. There were obvious visible, though non-significant, differences between KY 14 LC and KY 14e3 for plant height (P>F values of 0.12 and 0.07 for Lexington and Versailles respectively) and leaf number (P>F values of 0.06 and 0.17 in Lexington and Versailles, respectively). In general KY 14e3 produced shorter plants that had fewer but larger leaves in comparison to KY 14LC; in addition the growth habit of KY 14LC was much more erect than KY 14e3 (Photo 8). This resulted in lines that were clearly distinguishable in the field, even though their yields were very similar. However, because the fertile and male sterile KY 14e3 lines were developed by NCSU and attempts to improve both lines were still ongoing, no attempt was made by KTTII in 2013 to improve either of these lines.

Breeding line L8LC is the male parent of commercial hybrid variety ms KY 14 X L8. Only one e3 version of L8e3 was provided by NCSU. In the agronomic trials, L8LC was noticeably different from both L8e3 and L8e2, with L8LC being shorter and having shorter leaf internodes than the latter two. However, statistically significant differences for plant type were observed only between L8LC and L8e2 for leaf width and leaf length at Lexington (Tables 7a and 7b). There were substantial differences among the three L8 lines for yield, with L8e2 having the highest and L8e3 having the lowest yields. The fact that L8e2 was significantly different from L8LC for several traits was not surprising since L8e2 was only in the $BC_3$ generation. L8LC is a notoriously inferior breeding line due to a recessive “physiological breakdown” gene that is expressed in the homozygous breeding line, but is not a factor when the line is used in hybrid combinations. In developing a true L8e3 version of the breeding line, each succeeding backcross actually reduces the vigor of the line as the non-reciprocal parent has less impact on plant type. Because of the poor agronomic type of L8LC, researchers at NCSU who developed L8e3 felt that it was not necessary to compare L8e3 to the original L8LC in replicated field trials. Although both L8LC and L8e3 are very off-type in comparison to other burley tobacco breeding lines, the two L8 lines were easily distinguishable in the field (Photo 9).

Table 6a. 2013 KY 14 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| Lexington | KY 14e2 | 139.46 | 18.29 | 7.62 | 25.44 |
| | KY 14LC | 145.88 | 19.09 | 7.64 | 24.54 |
| | KY 14e3 | 127.09 | 16.71 | 7.61 | 27.42 |
| | **PR>F** | **0.12** | **0.06** | **0.97** | **0.17** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |
| Versailles | KY 14e2 | 156.38 | 20.63 | 7.58 | 31.81a |
| | KY 14LC | 165.75 | 22.63 | 7.32 | 28.69c |
| | KY 14e3 | 150.63 | 19.75 | 7.63 | 30.15b |
| | **PR>F** | **0.07** | **0.17** | **0.59** | **0.01** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **0.89** |

Table 6b. 2013 KY 14 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Leaf Length (cm) | Yield (kg/ha) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|
| Lexington | KY 14e2 | 50.25b | 2373.16 | 0.00 | 0.00 |
| | KY 14LC | 53.83b | 2781.20 | 0.00 | 0.00 |
| | KY 14e3 | 60.77a | 2711.70 | 0.00 | 0.00 |
| | **PR>F** | **0.01** | **0.35** | **n/a** | **n/a** |
| | **LSD(.05)** | **4.21** | **ns** | **ns** | **ns** |
| Versailles | KY 14e2 | 61.07 | 2988.59 | | |
| | KY 14LC | 59.11 | 3154.49 | | |
| | KY 14e3 | 63.08 | 3166.83 | | |
| | **PR>F** | **0.13** | **0.34** | | |
| | **LSD(.05)** | **ns** | **ns** | | |

Photo 8. Morphological Characteristics of KY 14LC and KY 14e3

Table 7a. 2013 L8 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| Lexington | L8e2 | 108.59 | 13.01 | 8.35 | 29.73a |
| | L8LC | 98.67 | 12.58 | 7.84 | 25.40b |
| | L8e3 | 105.33 | 12.67 | 8.31 | 25.59b |
| | **PR>F** | **0.10** | **0.43** | **0.09** | **0.05** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **3.70** |
| Versailles | L8e2 | 115.59a | 14.59 | 7.92 | 29.06 |
| | L8LC | 100.63b | 14.17 | 7.10 | 25.61 |
| | L8e3 | 106.13ab | 14.25 | 7.45 | 24.98 |
| | **PR>F** | **0.05** | **0.55** | **0.28** | **0.26** |
| | **LSD(.05)** | **10.98** | **ns** | **ns** | **ns** |

Table 7b. 2013 L8 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Leaf Length (cm) | Yield (kg/ha) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|
| Lexington | L8e2 | 68.02a | 2593.99a | 0.00 | 0.00 |
| | L8LC | 57.65b | 1826.11b | 0.00 | 0.00 |
| | L8e3 | 55.21b | 1549.22c | 0.00 | 0.00 |
| | **PR>F** | **0.02** | **0.01** | **n/a** | **n/a** |
| | **LSD(.05)** | **8.42** | **168** | **ns** | **ns** |
| Versailles | L8e2 | 62.00 | 2370.92 | | |
| | L8LC | 60.23 | 1898.97 | | |
| | L8e3 | 55.83 | 1563.80 | | |
| | **PR>F** | **0.22** | **0.07** | | |
| | **LSD(.05)** | **ns** | **ns** | | |

Photo 9. Morphological Characteristics of L8e3 and L8LC

In addition to the obvious phenotypic differences between L8LC and L8e3, the e3 version had a substantially reduced yield in comparison to the original L8LC parental line. However, because L8e3 was being developed by NCSU researchers, KTTII made no further attempt to improve the L8e3 line.

**2013 Commercial Variety Evaluations**

Although TN 86LC is a fertile cross pollinated variety, in order to protect intellectual property only a male sterile version of TN 86e3 will eventually be released for commercial production. For TN 86, only one fertile and one male sterile e3 selection was provided by NCSU; both versions were evaluated for black shank resistance, while only the male sterile version was compared to TN 86LC in the agronomic trials. Only cosmetic visual differences were noted between TN 86LC, TN 86e3, and ms TN 86e3 in 2013 field evaluations. In the agronomic trials, significant differences between ms TN 86e3 and TN 86LC were noted only for leaf length at Versailles (Tables 8a and 8b). Black shank resistance was also relatively comparable for all three lines. Because differences between the three TN 86 lines were very minor, foundation seed of both ms TN 86e3 and TN 86e3 that was produced in 2013 is satisfactory for use for commercial seed production of TN 86e3.

TN 90LC is unique among the materials evaluated in this study because it is grown not only as a fertile open pollinated commercial variety, but it is also the male parent of hybrid variety KT 204LC. Similar to TN 86e3, only the male sterile version would be released for commercial production of an e3 version of TN 90, but the male fertile TN 90e3 line would be used as the male parent of KT 204e3. Only one fertile and one male sterile e3 selection were provided by NCSU for comparison to the original TN 90LC. Although there were visual agronomic differences between these two lines in comparison to TN 90LC, these differences were primarily cosmetic. For the agronomic trials comparing ms TN 90e3 to TN 90LC, statistically significant differences between the two lines were noted only for plant height and leaf width at Lexington, and for internode length at Versailles (Tables 9a and 9b). Morphologically, there was enough

Table 8a. 2013 TN 86 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| **Lexington** | TN 86LC | 121.73 | 20.0 | 6.07 | 26.73 |
| | msTN 86e3 | 123.07 | 19.5 | 6.30 | 28.07 |
| | **PR>F** | **0.62** | **0.20** | **0.25** | **0.22** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |
| **Versailles** | TN 86LC | 149.67 | 20.87 | 7.19 | 27.07 |
| | msTN 86e3 | 154.71 | 21.42 | 7.27 | 28.27 |
| | **PR>F** | **0.26** | **0.09** | **0.69** | **0.52** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |

Table 8b. 2013 TN 86 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Leaf Length (cm) | Yield (kg/ha) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|
| **Lexington** | TN 86LC | 58.63 | 2320.47 | 45.50 | 83.30a |
| | msTN 86e3 | 60.38 | 2160.17 | 52.40 | 61.60b |
| | **PR>F** | **0.07** | **0.11** | **0.24** | **0.05** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **20.99** |
| **Versailles** | TN 86LC | 58.69b | 2734.12 | | |
| | msTN 86e3 | 61.38a | 3034.55 | | |
| | **PR>F** | **0.03** | **0.42** | | |
| | **LSD(.05)** | **1.86** | **ns** | | |

Table 9a. 2013 TN 90 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| **Lexington** | TN 90LC | 148.38a | 19.92 | 7.47 | 25.88b |
| | ms TN 90e3 | 140.88b | 18.92 | 7.43 | 27.13a |
| | **PR>F** | **0.04** | **0.06** | **0.84** | **0.01** |
| | **LSD(.05)** | **6.96** | **ns** | **ns** | **0.63** |
| **Versailles** | TN 90LC | 162.88 | 19.90 | 8.17b | 29.27 |
| | ms TN 90e3 | 163.75 | 19.27 | 8.53a | 28.80 |
| | **PR>F** | **0.87** | **0.37** | **0.01** | **0.68** |
| | **LSD(.05)** | **ns** | **ns** | **0.14** | **ns** |

Table 9b. 2013 TN 90 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Leaf Length (cm) | Yield (kg/ha) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|
| **Lexington** | TN 90LC | 57.59 | 2438.18 | 81.00 | 73.90 |
| | ms TN 90e3 | 57.42 | 2393.34 | 27.60 | 57.60 |
| | **PR>F** | **0.44** | **0.77** | **0.20** | **0.14** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |
| **Versailles** | TN 90LC | 61.93 | 2845.10 | | |
| | ms TN 90e3 | 59.53 | 2688.16 | | |
| | **PR>F** | **0.06** | **0.36** | | |
| | **LSD(.05)** | **ns** | **ns** | | |

similarity between TN 90LC and the fertile and sterile e3 versions that ms TN 90e3 and TN 90e3 could be released to seed companies for commercial seed production without further improvement. However, by the end of the growing season, it was apparent that there was a substantial difference for black shank resistance between TN 90LC and the fertile and sterile e3 versions (Table 9b and Photos 10, 11, and 12). Utilizing plants in the 2013 black shank nurseries, an additional backcross was made to both TN 90e3 and ms TN 90e3, using TN 90LC as the pollinator. During the winter of 2013-14, the fertile (TN 90e3 X TN 90LC)$F_1$ plants were self-pollinated to get to the $BC_1S_1$ generation, and also backcrossed a second time to TN 90LC. The $BC_1S_1$ and $BC_2$ populations were planted in 2014 black shank nurseries to determine how much improvement was made for black shank resistance. If the $BC_1S_1$ population proved to have black shank resistance equivalent to TN 90LC, molecular markers could be utilized to select fertile TN 90 plants that are homozygous for all three e alleles. Pollen from these plants would then be used to cross onto the ms TN 90$BC_1$ generation; homozygous ms e3 plants could then be identified via marker analyses in the next generation. However, if the TN 90e3 $BC_2$ population displays significantly higher black shank resistance in comparison to the $BC_1S_1$ population, further self-pollinated generations would be required to develop a satisfactory fertile TN 90e3 line that could be used to not only regenerate the ms TN 90e3 line, but also as the male parental line for production of hybrid variety KT 204LC.

As mentioned in the Materials and Methods section, Altria provided four versions of all KTTII hybrid varieties for comparison to their original LC hybrid counterparts. However, the exact combination of versions of the appropriate parental lines used to create the hybrid varieties was not known. Because Altria provided 15 versions of TKS 2002e3, the female parental line used for all of the KT e3 hybrid varieties, and varying numbers of each of the male parental lines used in the respective hybrids, it is impossible to match up the performance of the e3 hybrid varieties with the performance of their respective e3 parental lines. For the KT 204 family, there were only subtle visible differences within the KT 204 family, but significant differences did appear for some agronomic traits (Table 10a and 10b). At the Lexington site, significant differences were detected

Photos 10, 11, and 12. Relative Black Shank Resistance of TN 90LC, ms TN 90e, and TN 90e3

TN 90LC Mean BS Survival = 81.0%

ms TN 90e3 Mean BS Survival = 27.6%

TN 90e3 Mean BS Survival =41.1%

Table 10a. 2013 KT 204 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| Lexington | KT 204e3-A | 136.58 | 19.20c | 7.07 | 26.77 |
| | KT 204e3-B | 144.23 | 19.60bc | 7.37 | 24.97 |
| | KT 204LC | 141.00 | 20.10ab | 7.03 | 26.47 |
| | KT 204e3-C | 139.27 | 19.43bc | 7.17 | 27.13 |
| | KT 204e3-D | 144.17 | 20.57a | 7.00 | 26.63 |
| | **PR>F** | **0.29** | **0.03** | **0.21** | **0.26** |
| | **LSD(.05)** | **ns** | **0.81** | **ns** | **ns** |
| Versailles | KT 204e3-A | 163.07a | 19.70ab | 8.28 | 31.03 |
| | KT 204e3-B | 163.63a | 19.23b | 8.50 | 29.82 |
| | KT 204LC | 166.49a | 20.30a | 8.21 | 29.20 |
| | KT 204e3-C | 163.07a | 19.73ab | 8.26 | 30.86 |
| | KT 204e3-D | 154.73b | 19.27b | 8.04 | 28.75 |
| | **PR>F** | **0.01** | **0.04** | **0.06** | **0.32** |
| | **LSD(.05)** | **3.97** | **0.70** | **ns** | **ns** |

Table 10b. 2013 KT 204 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Leaf Length (cm) | Yield (kg/ha) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|
| **Lexington** | KT 204E3-A | 62.03a | 2311.50cd | 75.90a | 93.90 |
| | KT 204E3-B | 59.77abc | 2205.01d | 66.40b | 86.90 |
| | KT 204LC | 58.38c | 2403.42bc | 75.50a | 78.30 |
| | KT 204E3-C | 59.10bc | 2487.50bc | 64.30b | 88.70 |
| | KT 204E3-D | 60.93ab | 2656.77a | 52.40c | 85.90 |
| | **PR>F** | **0.05** | **0.01** | **0.002** | **0.35** |
| | **LSD(.05)** | **2.44** | **146.3** | **6.23** | **ns** |
| **Versailles** | KT 204E3-A | 68.23a | 3059.21a | | |
| | KT 204E3-B | 67.17ab | 2690.40b | | |
| | KT 204LC | 61.83c | 2974.01a | | |
| | KT 204E3-C | 64.17bc | 3102.93a | | |
| | KT 204E3-D | 63.02c | 2990.83a | | |
| | **PR>F** | **0.01** | **0.02** | | |
| | **LSD(.05)** | **3.33** | **201.5** | | |

among the five entries for leaf number, leaf length, and yield. In comparison to KT 204LC, e3 entry C had significantly fewer leaves, and entries A and D had longer leaves. For yield, entry D had significantly higher and entry B had significantly lower values in comparison to KT 204LC. At Versailles, significant differences were detected among the five entries for plant height, leaf number, leaf length, and yield. Among the e3 entries, A and D were significantly taller than KT 204LC, B and D had fewer leaves than KT 204LC, and A and D had longer leaves. Entry D produced significantly higher and entry B produced significantly lower yield in comparison to KT 204LC. Surprisingly, even though male parental line TN 90e3 had substantially lower black shank resistance than TN 90LC, none of the e3 versions, with the possible exception of KT 204e3-D, had substantial differences for black shank resistance compared to KT 204LC (Table 10b). Although an additional backcross had been made to improve KT 204e3 pollinator TN 90e3, TKS 2002e3-O was crossed with the original NCSU TN 90e3. This was done to ensure that the version of KT 204e3 used for further testing in 2014 would be homozygous for all three e3 alleles.

For KT 206, there were noticeable differences among the five entries evaluated; this was likely due to the variability and inferiority of TKF 4028e3 male parental lines used for these hybrids. At Lexington, significant differences were detected for plant height and yield. All of the e3 lines were taller than KT 206LC, although the differences were significant only for entry D (Tables 11a and 11b). Although the differences were non-significant ($P>F = 0.06$), all of the e3 entries also had a greater leaf internode length in comparison to KT 206LC. Only KT 206e3-A had a significantly lower yield than KT 206LC at Lexington. At Versailles, statistically significant differences were noted for leaf number and leaf internode length; all e3 entries had lower leaf numbers and wider internode lengths in comparison to KT 206LC. There was considerable variability for level of black shank resistance, with some e3 varieties appearing to have higher resistance in comparison to KT 206LC, while others had slightly lower resistance. Because all of the TKF 4028e3 parental lines were significantly off-type and needed

Table 11a. 2013 KT 206 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| Lexington | KT 206e3-A | 134.0c | 19.54 | 6.86 | 22.34 |
| | KT 206e3-B | 142.77ab | 20.59 | 6.93 | 24.92 |
| | KT 206LC | 135.59bc | 20.96 | 6.47 | 24.83 |
| | KT 206e3-C | 139.07bc | 20.67 | 6.73 | 22.88 |
| | KT 206e3-D | 148.57a | 20 | 7.43 | 24.92 |
| | **PR>F** | **0.03** | **0.35** | **0.06** | **0.30** |
| | **LSD(.05)** | **8.50** | **ns** | **ns** | **ns** |
| Versailles | KT 206e3-A | 160.57 | 19.77b | 8.13a | 28.98 |
| | KT 206e3-B | 159.20 | 19.43b | 8.23a | 27.79 |
| | KT 206LC | 162.17 | 21.07a | 7.73b | 29.15 |
| | KT 206e3-C | 161.97 | 19.83b | 8.17a | 29.34 |
| | KT 206e3-D | 159.40 | 19.40b | 8.27a | 27.90 |
| | **PR>F** | **0.69** | **0.01** | **0.04** | **0.42** |
| | **LSD(.05)** | **ns** | **0.86** | **0.34** | **ns** |

Table 11b. 2013 KT 206 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Leaf Length (cm) | Yield (kg/ha) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|
| **Lexington** | KT 206e3-A | 55.63 | 1972.96 | 71.40 | 73.80 |
| | KT 206e3-B | 58.88 | 2468.44 | 42.90 | 75.10 |
| | KT 206LC | 58.59 | 2364.19 | 59.60 | 65.30 |
| | KT 206e3-C | 56.84 | 2234.15 | 38.10 | 73.10 |
| | KT 206e3-D | 59.42 | 2591.75 | 65.70 | 76.90 |
| | **PR>F** | **0.61** | **0.05** | **0.44** | **0.93** |
| | **LSD(.05)** | **ns** | **355.60** | **ns** | **ns** |
| **Versailles** | KT 206e3-A | 66.19 | 2864.16 | | |
| | KT 206e3-B | 64.6 | 2778.96 | | |
| | KT 206LC | 63.02 | 3110.78 | | |
| | KT 206e3-C | 64.44 | 2866.40 | | |
| | KT 206e3-D | 63.48 | 2789.05 | | |
| | **PR>F** | **0.52** | **0.44** | | |
| | **LSD(.05)** | **ns** | **ns** | | |

additional backcrosses to increase their similarity to TKF 4028LC, hybrid variety KT 206e3 was not re-made for testing in 2014.

For the KT 209 family, no significant differences were detected for any trait at the Lexington location, but there were differences for plant height, leaf number and yield at Versailles (Tables 12a and 12b). In comparison to KT 209LC, e3 entries C and D were shorter, and all e3 entries had fewer leaves. With the exception of entry A, all e3 entries produced significantly lower yields at Versailles in comparison to KT 209LC. In the Tennessee black shank nursery, which has much higher disease pressure than does the Kentucky nursery, e3 entries A and C appeared to have a low level of resistance compared to e3 entry B and KT 209LC. Overall, all of the e3 versions of KT 209LC appeared to be inferior to KT 209LC for yield and/or level of black shank resistance. Because KT 204e3 parental line TKF 4024e3-F was deemed to be comparable to TKF 4024LC in the 2013 parental lines trials, a cross was made between TKS 2002e3-O and TKF 4024e3-F to reformulate KT 204e3 for further evaluation in 2014.

Although the KT210 family had some visible differences among the LC and e3 versions, a statistically significant difference was detected only for leaf internode length at Lexington; e3 entries A and B had a wider leaf spacing in comparison to KT 210LC (Tables 13a and 13b). All of the e3 entries were lower yielding than KT 210LC at both locations, but the differences were not statistically significant. For black shank resistance, KT 210e3-C had virtually the same percentage of survival as KT 210LC in both disease nurseries. Entries KT 210e3-B and KT 210-D appeared to have a somewhat lower level of resistance, particularly in the Tennessee nursery. The similarity between KT 210 e3 male parental line TKF 6400e3-B and TKF 6400LC was close enough that no further backcrosses were deemed to be necessary for further improvement. TKF 6400e3-B was therefore crossed onto female parental line TKS 2002e3-O to make the version of KT 210e3 for evaluation 2014 field trials.

KT 212LC is a hybrid cross between female parental line TKS 2002LC and male parental breeding line L8LC. The KT212 family had highly significant differences for leaf

Table 12a. 2013 KT 209 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| Lexington | KT 209e3-A | 114.17 | 17.92 | 6.37 | 27.88 |
| | KT 209e3-B | 119.21 | 18.79 | 6.34 | 29.50 |
| | KT 209LC | 119.54 | 19.52 | 6.12 | 27.93 |
| | KT 209e3-C | 110.25 | 17.70 | 6.23 | 29.52 |
| | KT 209e3-D | 115.05 | 17.92 | 6.42 | 30.06 |
| | **PR>F** | **0.44** | **0.23** | **0.09** | **0.46** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |
| Versailles | KT 209e3-A | 157.17ab | 19.20b | 8.18 | 30.36 |
| | KT 209e3-B | 157.73a | 19.70b | 7.67 | 29.33 |
| | KT 209LC | 158.34a | 20.70a | 7.66 | 30.21 |
| | KT 209e3-C | 147.17c | 18.97b | 7.76 | 29.06 |
| | KT 209e3-D | 150.53bc | 19.50b | 7.72 | 28.38 |
| | **PR>F** | **0.02** | **0.01** | **0.06** | **0.57** |
| | **LSD(.05)** | **6.70** | **0.80** | **ns** | **ns** |

Table 12b. 2013 KT 209 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Leaf Length (cm) | Yield (kg/ha) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|
| **Lexington** | KT 209e3-A | 60.92 | 2058.16 | 78.60 | 97.00 |
| | KT 209e3-B | 64.17 | 2504.31 | 100.00 | 98.60 |
| | KT 209LC | 62.09 | 2550.28 | 92.90 | 92.20 |
| | KT 209e3-C | 62.56 | 2154.56 | 68.90 | 94.40 |
| | KT 209e3-D | 64.6 | 2397.82 | 83.40 | 100.00 |
| | **PR>F** | **0.36** | **0.08** | **0.38** | **0.30** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |
| **Versailles** | KT 209e3-A | 65.9 | 3061.45ab | | |
| | KT 209e3-B | 64.62 | 2659.01c | | |
| | KT 209LC | 61.9 | 3124.23ab | | |
| | KT 209e3-C | 63.96 | 2624.26c | | |
| | KT 209e3-D | 62.94 | 2716.18bc | | |
| | **PR>F** | **0.42** | **0.04** | | |
| | **LSD(.05)** | **ns** | **329.6** | | |

Table 13a. 2013 KT 210 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| Lexington | KT 210e3-A | 140.75 | 21.59 | 6.53a | 24.34 |
| | KT 210e3-B | 136.67 | 21.60 | 6.34ab | 23.02 |
| | KT 210LC | 134.88 | 22.46 | 6.00c | 24.96 |
| | KT 210e3-C | 134.54 | 21.54 | 6.23abc | 23.71 |
| | KT 210e3-D | 136.80 | 22.21 | 6.17bc | 22.54 |
| | **PR>F** | **0.93** | **0.78** | **0.05** | **0.23** |
| | **LSD(.05)** | **ns** | **ns** | **0.33** | **ns** |
| Versailles | KT 210e3-A | 157.38 | 20.96 | 7.51 | 28.61 |
| | KT 210e3-B | 157.63 | 21.04 | 7.49ab | 26.36 |
| | KT 210LC | 161.75 | 21.59 | 7.49 | 28.34 |
| | KT 210e3-C | 154.04 | 21.04 | 7.32 | 29.17 |
| | KT 210e3-D | 159.09 | 21.63 | 7.36 | 28.06 |
| | **PR>F** | **0.19** | **0.86** | **0.80** | **0.52** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |

Table 13b. 2013 KT 210 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Leaf Length (cm) | Yield (kg/ha) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|
| **Lexington** | KT 210e3-A | 58.98 | 2548.03 | 77.30 | 88.40 |
| | KT 210e3-B | 56.71 | 2372.04 | 69.10 | 93.90 |
| | KT 210LC | 58.84 | 2628.75 | 87.90 | 92.20 |
| | KT 210e3-C | 58.50 | 2540.19 | 85.90 | 89.50 |
| | KT 210e3-D | 55.59 | 2333.92 | 64.30 | 91.30 |
| | **PR>F** | **0.24** | **0.46** | **0.72** | **0.95** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |
| **Versailles** | KT 210e3-A | 63.59 | 3111.90 | | |
| | KT 210e3-B | 59.85 | 2803.62 | | |
| | KT 210LC | 62.00 | 3139.92 | | |
| | KT 210e3-C | 63.77 | 2880.97 | | |
| | KT 210e3-D | 61.85 | 2785.69 | | |
| | **PR>F** | **0.06** | **0.72** | | |
| | **LSD(.05)** | **ns** | **ns** | | |

internode length at both Lexington and Versailles, with all e3 entries having a greater internode length than KT 212LC (Tables 14a and 14b). This was the result of the e3 lines being taller, although differences were not statistically different, while having fewer leaves, with a significant difference in leaf number seen at Versailles. This was primarily due to the male parental line L8 that was discussed earlier. Conversely, most of the e3 lines tended to have larger leaves in comparison to KT 212LC, with significant differences detected for leaf width and leaf length at Versailles. The increased leaf size appeared to compensate for the reduced leaf number in the e3 lines; although there were visible differences in appearance, there were no significant difference for yield among the five entries in the KT 212 family. Although the male parental line of KT 212e3, (L8e3) was significantly off- type in the 2013 trials, the original NCSU L8e3 was crossed onto female parental line TKS 2002e3-O to allow further evaluation of KT 212e3 in 2014 trials.

L8LC is also the male parent for hybrid variety 14 X L8LC. Only one version of KY 14 x L8e3 was provided by NCSU, and it was noted to be off-type compared to KY 14 X L8LC. The morphological differences between the e3 and LC versions were very apparent in the 2013 field trials, which was as expected since the L8e3 and KY 14e3 parental lines were distinctly different from the LC counterparts as discussed earlier in the parental lines section of 2013 Field Trials. The most obvious differences between KY 14 X L8e3 and KY 14 X L8LC were for leaf number and internode length, which were significant at Lexington (Tables 15a and 15b), resulting in distinctly different agronomic type. Although the differences were not significant, the e3 version was visibly taller, but had fewer leaves with a significant difference observed at Lexington. Similar to the KT 212 family, the reduction in leaf number appeared to be off-set by larger leaves in the e3 version, resulting in very similar yields for KY 14 X L8e3 and KY 14 X L8LC. Because the parental lines KY 14e3, ms KY 14e3, and L8e3 were developed by NCSU, KTTII made no attempt to improve these lines, which also left KY 14 x L8e3 unchanged moving forward to 2014.

Table 14a. 2013 KT 212 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| Lexington | KT 212e3-A | 137.96 | 17.75 | 7.77a | 28.42 |
| | KT 212e3-B | 139.25 | 17.79 | 7.80a | 27.88 |
| | KT 212LC | 136.00 | 19.04 | 7.13b | 25.63 |
| | KT 212e3-C | 146.46 | 18.17 | 8.07a | 28.71 |
| | KT 212e3-D | 137.34 | 17.54 | 7.83a | 27.71 |
| | **PR>F** | **0.19** | **0.17** | **0.01** | **0.40** |
| | **LSD(.05)** | **ns** | **ns** | **0.34** | **ns** |
| Versailles | KT 212e3-A | 161.22 | 18.47ab | 8.73b | 32.70a |
| | KT 212e3-B | 161.47 | 18.33b | 8.80ab | 30.93b |
| | KT 212LC | 158.29 | 18.90a | 8.37c | 31.03b |
| | KT 212e3-C | 159.03 | 17.80c | 8.93a | 30.60b |
| | KT 212e3-D | 164.95 | 18.63ab | 8.87ab | 33.27a |
| | **PR>F** | **0.17** | **0.05** | **0.01** | **0.01** |
| | **LSD(.05)** | **ns** | **0.45** | **0.17** | **1.07** |

Table 14b. 2013 KT 212 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Leaf Length (cm) | Yield (kg/ha) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|
| **Lexington** | KT 212e3-A | 64.04 | 2502.07 | 16.70c | 66.80 |
| | KT 212e3-B | 64.55 | 2205.01 | 40.50b | 79.30 |
| | KT 212LC | 64.08 | 2477.41 | 61.60ab | 74.30 |
| | KT 212e3-C | 64.84 | 2543.55 | 62.60ab | 83.60 |
| | KT 212e3-D | 62.50 | 2166.89 | 76.20a | 74.20 |
| | **PR>F** | **0.66** | **0.14** | **0.01** | **0.58** |
| | **LSD(.05)** | **ns** | **ns** | **23.73** | **ns** |
| **Versailles** | KT 212e3-A | 69.40b | 2558.12 | | |
| | KT 212e3-B | 64.40d | 2250.97 | | |
| | KT 212LC | 66.33c | 2451.63 | | |
| | KT 212e3-C | 66.93c | 2626.50 | | |
| | KT 212e3-D | 71.13a | 2813.71 | | |
| | **PR>F** | **0.01** | **0.21** | | |
| | **LSD(.05)** | **1.50** | **ns** | | |

Table 15a. 2013 KY 14 X L8 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| **Lexington** | KY 14 X L8LC | 132.97 | 19.07a | 6.97b | 28.37 |
| | KY 14 X L8e3 | 139.47 | 16.73b | 8.33a | 28.47 |
| | **PR>F** | **0.12** | **0.02** | **0.02** | **0.97** |
| | **LSD(.05)** | **ns** | **1.52** | **0.80** | **ns** |
| **Versailles** | KY 14 X L8LC | 145.54 | 17.88 | 8.14 | 31.42 |
| | KY 14 X L8e3 | 151.84 | 16.92 | 8.97 | 32.17 |
| | **PR>F** | **0.22** | **0.13** | **0.13** | **0.78** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |

Table 15b. 2013 KY 14 X L8 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Leaf Length (cm) | Yield (kg/ha) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|
| **Lexington** | KY 14 X L8LC | 59.83 | 2412.39 | 0.00 | 0.00 |
| | KY 14 X L8e3 | 60.92 | 2301.41 | 0.00 | 0.00 |
| | **PR>F** | **0.8** | **0.29** | **n/a** | **n/a** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** |
| **Versailles** | KY 14 X L8LC | 65.81 | 2842.86 | | |
| | KY 14 X L8e3 | 71.04 | 2846.22 | | |
| | **PR>F** | **0.13** | **0.37** | | |
| | **LSD(.05)** | **ns** | **ns** | | |

**Discussion of 2013 Trials**

There was a large amount of variation observed in the field between the LC and e3 versions for both parental lines and commercial varieties in 2013. When receiving the e3 versions of parental lines, it was thought they would be identical, or nearly identical, to their original LC counterparts due to undergoing six backcrosses. However, with all e3 selections made using early flowering techniques that only allowed the plants to grow to approximately 12 inches tall, genetic markers were utilized to ensure the three mutated alleles were still present, but no phenotypic evaluations were conducted. After testing in multiple locations, it was apparent that more backcrossing and field selections were needed to bring the e3 lines back to the original phenotypic characteristics. Some of this variation could be explained by linkage drag from the non-recurrent mutated parent (NRP). According to Peng et al. (2013) the reduction of NRP material can be greatly reduced by either increasing the population size in early generations or increasing the selection intensity. The selections also need to be tested for not only the presence of the mutated alleles, but also the amount of recurrent parent germplasm (RP) and NRP germplasm. In order to recover the performance of the elite line, multiple versions of the altered lines must be yield tested (Mumm and Walters, 2001). This ensures that the selections made are relative to all other characteristics of the target line. A more intense approach would have been to test the linkage drag by using a marker on either side of the target gene in the chromosomal region, as well as markers elsewhere in the genome to test the RP germplasm recovery (Frisch, 2005). After our field evaluations in 2013, selections were made based on performance and phenotype, as well as markers for the CYP82 genes. Further backcrossing to the RP was undertaken where needed in preparation for the 2014 field trials.

**2014 Field Evaluations**

The e3 parental lines and variety trials were conducted at Versailles, KY and Greeneville and Springfield, TN in 2014. However, it is important to note that for all of the parental lines and variety families except TN 86, the e3 entries tested in 2014 were different from those tested in 2013. Because many of the parental lines evaluated in 2013 were

inferior for either agronomic type and/or level of black shank resistance, new versions of the parental lines were developed through selection and/or backcrossing in 2013. Although parental lines that were improved simply by selecting the best e3 line within a family are genetically stable, for families where the best e3 parental line needed further backcrossing, the 2014 versions are heterozygous for the e10 allele. These lines will need additional self-pollination and selection to develop homozygous lines that will eventually serve as foundation seed lots. The hybrid varieties evaluated in 2014 were re-made using parental lines that were improved in 2013, and are therefore also not in the final version that will eventually be released. For example, all of the hybrid varieties evaluated in 2014 used TKS 2002e3-O as the female parent, even though that parental line was visibly somewhat off-type compared to TKS 2002LC. One additional backcross was made to TKS 2002e3-O using TKF 2002e2 as the pollinator; an additional backcross using an improved strain of TKF 2002e3 will be made, followed by a self-pollinated generation to identify homozygosity for all three alleles, in order to develop the eventual foundation seed lot for TKS 2002e3. Because the parental lines and varieties evaluated in 2014 are not in their final state, data from the 2014 trials should be considered as a measure of breeding progress rather than a definitive measure of eventual variety performance. The 2014 data demonstrated that efforts in 2013 to improve the parental lines and resultant hybrids were generally successful as a lower number of significant differences for the measurable agronomic traits and black shank resistance was observed in 2014 compared to 2013. Many of the e3 lines/hybrids regained the phenotypic appearance of their LC counterparts and were not considered as being significantly off-type.

**2014 Parental Line Evaluations**

As noted in the 2013 Parental Lines Results and Discussion, the best TKF 2002e3 version evaluated in 2013 was entry C. However, it was off-type for leaf number and internode length compared to TKF 2002LC and TKF 2002e2. As a result, TKF 2002e3-C was crossed with TKF 2002e2. The resulting cross was self-pollinated in the greenhouse during the

winter of 2013-2014 to obtain the $BC_1S_1$ line designated as (TKF 2002e3-C SGe10); this line was homozygous for the e4 and e5 alleles, but heterozygous for the e10 /E10 alleles. TKF 2002e3-C SGe10 was compared with TKF 2002LC, TKF 2002e2, and the original e3 line, TKF 2002e3-C, in 2014 field trials. As can be seen in Tables 16a and 16b, TKF 2002e3-C SGe10 was much more comparable to TKF 2002LC than the original TKF 2002e3-C parental line. No differences were detected between TKF 2002e3-C SGe10 and TKF 2002LC for any trait at any of the three test locations; conversely, significant differences were detected between TKF 2002e3-C and TKF 2002LC for leaf internode length at Greeneville, and for yield at Versailles. TKF 2002e3-C was the lowest yielding entry evaluated at all three locations, although the differences were not significant at Greeneville and Springfield. Based on the similarity between TKF 2002e3-C SGe10 and TKF 2002LC for agronomic traits and disease resistance, it was determined that one additional backcross to TKF 2002e2 was sufficient in identifying a usable e3 version of TKF 2002e3. Samples were taken from individual plants in the TKF 2002e3-C SGe10 population to allow identification of plants that were homozygous for all three e alleles. $BC_2S_2$ seed was collected from these homozygous plants and designated as breeder seed of TKF 2002e3.

Although the black shank resistance for TKS 2002e3-O was very similar to TKS 2002LC and TKF 2002e2 in the 2013 field trials, it had fewer leaves and wider leaf spacing in comparison to the latter two lines. An additional backcross was made to TKF 2002e2 in an attempt to eventually increase the leaf number and decrease the internode length of TKS 2002e3. This backcross, which was homozygous for the e4 and e5 alleles but homozygous for the e10 allele, was designated as TKS 2002e3-O BCe10 and compared to TKS 2002LC in 2014. The agronomic performance of the two lines was very similar, with the only significant difference detected being internode length in Versailles; however, the LC version actually had a slightly greater internode length than the e3 version (Tables 17a and 17b). It was decided that the single backcross to TKF 2002e2 was sufficient to eliminate differences observed between TKS 2002LC and TKS 2002e3-O in 2013. As a result, TKS 2002e3-O BCe10 was increased using pollen from the TKF

Table 16a. 2014 TKF 2002 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Line | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) | Leaf Length (cm) |
|---|---|---|---|---|---|---|
| **Greeneville** | TKF 2002LC | 126.00 | 19.28 | 6.54b | 29.00 | 64.94 |
| | TKF 2002e3-C SGe10 | 135.67 | 19.39 | 7.00b | 28.89 | 66.89 |
| | TKF 2002e2-A | 136.33 | 19.44 | 7.01b | 28.06 | 64.11 |
| | TKF 2002e3-C | 136.94 | 18.11 | 7.56a | 31.44 | 69.28 |
| | **PR>F** | **0.27** | **0.07** | **0.03** | **0.25** | **0.33** |
| | **LSD(.05)** | **ns** | **ns** | **0.55** | **ns** | **ns** |
| **Springfield** | TKF 2002LC | 120.72 | 20.94 | 5.77 | 21.89 | 53.78 |
| | TKF 2002e3-C SGe10 | 112.72 | 20.56 | 5.49 | 21.06 | 51.50 |
| | TKF 2002e2-A | 125.89 | 22.11 | 5.71 | 20.28 | 50.44 |
| | TKF 2002e3-C | 119.44 | 21.06 | 5.67 | 21.72 | 51.50 |
| | **PR>F** | **0.09** | **0.58** | **0.42** | **0.33** | **0.67** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Versailles** | TKF 2002LC | 137.83 | 17.33 | 7.95 | 29.06 | 63.89 |
| | TKF 2002e3-C SGe10 | 131.22 | 16.56 | 7.96 | 27.56 | 65.50 |
| | TKF 2002e2-A | 124.83 | 15.94 | 7.85 | 28.44 | 61.89 |
| | TKF 2002e3-C | 126.89 | 16.06 | 7.89 | 26.44 | 62.61 |
| | **PR>F** | **0.22** | **0.52** | **0.97** | **0.52** | **0.66** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |

Table 16b. 2014 TKF 2002 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Line | | Grade Index | Yield (kg/ha) | Revenue ($) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|---|
| **Greeneville** | TKF 2002LC | 36.70 | 2888.82 | 3815 | 83.60 | 92.90 |
| | TKF 2002e3-C SGe10 | 43.00 | 3081.63 | 4414 | 88.90 | 97.20 |
| | TKF 2002e2-A | 40.20 | 2852.95 | 3933 | 90.00 | 95.40 |
| | TKF 2002e3-C | 46.50 | 2681.43 | 3879 | 90.40 | 93.10 |
| | **PR>F** | **0.72** | **0.54** | **0.60** | **0.92** | **0.85** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Springfield** | TKF 2002LC | 47.10 | 2633.23 | 3926 | | |
| | TKF 2002e3-C SGe10 | 47.60 | 2586.15 | 3947 | | |
| | TKF 2002e2-A | 57.50 | 2638.83 | 4300 | | |
| | TKF 200e3-C | 57.30 | 2486.38 | 4078 | | |
| | **PR>F** | **0.10** | **0.96** | **0.90** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |
| **Versailles** | TKF 2002LC | 32.70 | 3716.12a | 4724 | | |
| | TKF 2002e3-C SGe10 | 35.60 | 3417.93a | 4553 | | |
| | TKF 2002e2-A | 33.00 | 3665.67a | 4599 | | |
| | TKF 2002e3-C | 49.40 | 2784.56b | 4149 | | |
| | **PR>F** | **0.10** | **0.02** | **0.62** | | |
| | **LSD(.05)** | **ns** | **492.26** | **ns** | | |

Table 17a. 2014 TKS 2002 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) | Leaf Length (cm) |
|---|---|---|---|---|---|---|
| **Greeneville** | TKS 2002LC | 131.22 | 19.89 | 6.61 | 28.89 | 63.17 |
| | TKS 2002e3-O BCe10 | 129.22 | 18.94 | 6.82 | 30.50 | 66.00 |
| | **PR>F** | **0.50** | **0.20** | **0.37** | **0.25** | **0.23** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Springfield** | TKS 2002LC | 114.11 | 19.61 | 5.83 | 21.94 | 50.56 |
| | TKS 2002e3-O BCe10 | 119.67 | 20.78 | 5.75 | 23.44 | 52.61 |
| | **PR>F** | **0.73** | **0.65** | **0.81** | **0.58** | **0.60** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Versailles** | TKS 2002LC | 125.95 | 15.72 | 8.01 | 30.00 | 66.78 |
| | TKS 2002e3-O BCe10 | 128.89 | 16.83 | 7.66 | 34.72 | 66.17 |
| | **PR>F** | **0.51** | **0.09** | **0.04** | **0.54** | **0.89** |
| | **LSD(.05)** | **ns** | **ns** | **0.32** | **ns** | **ns** |

Table 17b. 2014 TKS 2002 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Grade Index | Yield (kg/ha) | Revenue ($) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|---|
| **Greeneville** | TKS 2002LC | 33.80 | 3022.22 | 3868 | 83.30 | 95.80 |
| | TKS 2002e3-O BCe10 | 32.30 | 2661.25 | 3363 | 86.10 | 98.50 |
| | **PR>F** | **0.74** | **0.40** | **0.49** | **0.93** | **0.18** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Springfield** | TKS 2002LC | 53.50 | 2370.92 | 3687 | | |
| | TKS 2002e3-O BCe10 | 50.70 | 2453.87 | 3651 | | |
| | **PR>F** | **0.84** | **0.86** | **0.96** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |
| **Versailles** | TKS 2002LC | 38.0 | 3253.14 | 4382 | | |
| | TKS 2002e3-O BCe10 | 32.3 | 3786.74 | 4779 | | |
| | **PR>F** | **0.33** | **0.10** | **0.47** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |

2002e3 plants that were determined via molecular markers to be homozygous for all three e alleles as described in the previous paragraph. Using molecular markers, TKS plants that are determined to be homozygous for all three alleles will be pollinated with the homozygous TKF 2002e3 line to establish breeder seed of TKS 2002e3.

Although the degree of similarity between TKF 4024LC and TKF 4024e3-F was great enough that it was anticipated that no further backcrosses were necessary, one additional backcross to TKF 4024e2 was made as a contingency plan. This line which, was designated as TKF 4024e3-F SGe3, was homozygous for the e4 and e5 alleles, but heterozygous for e10/E10. In the 2014 trials, The TKF 4024 family group did not show any statistically significant differences for any traits (Tables 18a and 18b) at any of the three test locations. Although TKF 4024e3-F SGe3 displayed slightly higher black shank resistance, the genetically stable TKF 4024e3-F was deemed to comparable to TKF 4024LC without the additional backcross. Since foundation seed was also produced from TKF 4024e3-F in 2014, this e3 parental line was considered to be completed.

All six of the e3 lines in TKF 4028 family group were inferior to TKF 4028LC and TKF 4028e2, either for type or black shank resistance, in the 2013 trials. Based on agronomic type in the absence of black shank, the best TKF 4028e3 line was entry F; this line was back-crossed with TKF 4028e2. The $F_1$ seed, which was homozygous for e4e5 but heterozygous for e10/E10, was backcrossed a second time to TKF 4028e2 in the greenhouse during the winter of 2013. The $BC_2$ line, which continued to be Heterozygous for the e10 allele, was designated as TKF 4028e3-F SGe10 and compared with TKF 4028e2 and TKF 4028e3-F in the 2014 field trials. No statistically significant differences were detected among the three lines for any trait and any of the test locations (Tables 19a and 19b). However, the yield and black shank resistance of TKF 4028e3-F SGe10 was substantially improved over the original TK 4028e3, and was fairly comparable to TKF 4028e2. $BC_2S_1$ seed was saved from TKF 4028e3-F SGe10 and grown in a greenhouse to allow the molecular marker identification of plants that are

Table 18a. 2014 TKF 4024 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) | Leaf Length (cm) |
|---|---|---|---|---|---|---|
| **Greeneville** | TKF 4024LC | 136.17 | 21.94 | 6.22 | 57.94 | 25.00 |
| | TKF 4024e3-F SGe3 | 128.28 | 21.17 | 6.07 | 59.72 | 25.44 |
| | TKF 4024e3-F | 130.83 | 20.50 | 6.38 | 63.56 | 27.72 |
| | **PR>F** | **0.44** | **0.26** | **0.53** | **0.21** | **0.37** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Springfield** | TKF 4024LC | 128.00 | 24.78 | 5.17 | 47.28 | 19.94 |
| | TKF 4024e3-F SGe3 | 123.22 | 24.06 | 5.13 | 47.33 | 20.17 |
| | TKF 4024e3-F | 124.72 | 24.11 | 5.18 | 49.5 | 21.67 |
| | **PR>F** | **0.77** | **0.90** | **0.97** | **0.79** | **0.57** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Versailles** | TKF 4024LC | 120.56 | 16.50 | 7.32 | 60.78 | 25.45 |
| | TKF 4024e3-F SGe3 | 117.89 | 15.67 | 7.53 | 59.28 | 24.56 |
| | TKF 4024e3-F | 127.05 | 16.89 | 7.53 | 59.44 | 25.05 |
| | **PR>F** | **0.52** | **0.50** | **0.83** | **0.52** | **0.66** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |

Table 18b. 2014 TKF 4024 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Grade Index | Yield (kg/ha) | Revenue ($) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|---|
| **Greeneville** | TKF 4024LC | 39.80 | 3473.98 | 4735 | 82.30 | 96.90 |
| | TKF 4024e3-F SGe3 | 39.60 | 3173.55 | 4293 | 91.20 | 95.90 |
| | TKF 4024e3-F | 36.20 | 3396.63 | 4460 | 84.10 | 88.80 |
| | **PR>F** | **0.54** | **0.63** | **0.54** | **0.11** | **0.12** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Springfield** | TKF 4024LC | 64.70 | 2865.28 | 4961 | | |
| | TKF 4024e3-F SGe3 | 63.70 | 2766.63 | 4760 | | |
| | TKF 4024e3-F | 63.20 | 2950.47 | 5097 | | |
| | **PR>F** | **0.87** | **0.80** | **0.70** | | |
| | **LSD(.05)** | **Ns** | **ns** | **ns** | | |
| **Versailles** | TKF 4024LC | 69.60 | 3082.75 | 5378 | | |
| | TKF 4024e3-F SGe3 | 63.00 | 2611.93 | 4459 | | |
| | TKF 4024e3-F | 56.50 | 2783.44 | 4521 | | |
| | **PR>F** | **0.46** | **0.36** | **0.30** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |

Table 19a. 2014 TKF 4028 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) | Leaf Length (cm) |
|---|---|---|---|---|---|---|
| **Greeneville** | TKF 4028e3-F SGe10 | 140.67 | 20.89 | 6.74 | 27.78 | 64.72 |
| | TKF 4028e3-F | 137.67 | 19.94 | 6.91 | 26.56 | 63.44 |
| | TKF 4028e2 | 142.00 | 21.50 | 6.61 | 28.06 | 64.00 |
| | **PR>F** | **0.49** | **0.22** | **0.46** | **0.64** | **0.83** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Springfield** | TKF 4028e3-F SGe10 | 135.00 | 24.11 | 5.60 | 20.56 | 53.67 |
| | TKF 4028e3-F | 131.11 | 23.56 | 5.57 | 19.83 | 52.22 |
| | TKF 4028e2 | 134.94 | 23.11 | 5.84 | 20.72 | 55.11 |
| | **PR>F** | **0.89** | **0.18** | **0.72** | **0.84** | **0.81** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Versailles** | TKF 4028e3-F SGe10 | 133.67 | 16.50 | 8.10 | 24.94 | 66.17 |
| | TKF 4028e3-F | 140.22 | 17.06 | 8.23 | 22.22 | 60.00 |
| | TKF 4028e2 | 144.55 | 17.72 | 8.17 | 24.28 | 62.56 |
| | **PR>F** | **0.26** | **0.44** | **0.83** | **0.014** | **0.33** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |

Table 19b. 2014 TKF 4028 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Grade Index | Yield (kg/ha) | Revenue ($) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|---|
| **Greeneville** | TKF 4028e3-F SGe10 | 35.90 | 2939.26 | 3866 | 14.40 | 52.20 |
| | TKF 4028e3-F | 36.10 | 2553.64 | 3348 | 2.70 | 25.30 |
| | TKF 4028e2 | 35.40 | 3132.07 | 4077 | 24.00 | 51.60 |
| | **PR>F** | **0.68** | **0.11** | **0.11** | **0.06** | **0.46** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Springfield** | TKF 4028e3-F SGe10 | 52.30 | 2618.66 | 4148 | | |
| | TKF 4028e3-F | 58.60 | 2384.37 | 4022 | | |
| | TKF 4028e2 | 60.60 | 3156.74 | 5377 | | |
| | **PR>F** | **0.67** | **0.20** | **0.31** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |
| **Versailles** | TKF 4028e3-F SGe10 | 55.20 | 2502.07 | 3888 | | |
| | TKF 4028e3-F | 56.20 | 2822.68 | 4426 | | |
| | TKF 4028e2 | 52.60 | 2812.59 | 4276 | | |
| | **PR>F** | **0.95** | **0.55** | **0.26** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |

homozygous for all three e3 alleles. These plants will be used to collect breeder seed of TKF 4028e3.

In the 2013 parental lines trial, within the TKF 6400 family it was determined that TKF 6400e3-B most closely resembled TKF 6400LC when agronomic type and race 1 black shank resistance were considered. The similarity between TKF 6400e3-B and TKF 6400LC was close enough that no further backcrosses were deemed to be necessary. The acceptability of TKF 6400e3-B was confirmed in the 2014 field trials as it was not significantly different form TKF 6400LC for any trait at any of the three test locations (Tables 20a and 20b). The level of black shank resistance was also nearly identical for the two lines. Foundation seed was produced from TKF 6400e3-B in 2014; this seed stock will be maintained in the future as the strain of TKF 6400e3 used to produce hybrid variety KT 210e3.

**2014 Commercial Variety Evaluations**

As mentioned above, the TN 86 family was the only variety family that was unchanged from 2013 to 2014. No significant differences were detected among TN 86LC, TN 86e3, and ms TN 86e3 any of the 2014 test locations (Tables 21a and 21b). Although it was still possible to visually distinguish TN 86LC from the fertile e3 versions when planted in adjacent rows, based on the two years of data showing no meaningful differences for agronomic traits or black shank resistance, msTN 86e3 was released as a potential commercial variety in March, 2015.

For the TN 90 family, the original fertile and male sterile e3 lines provided by NCSU in 2013 were compared with TN 90LC; in addition, a fourth line that had one additional backcross to TN 90LC (TN 90e3 X TN90LC) was included in the trials. In the agronomic trials, no significant differences were detected for any measured trait at any location (Tables 22a and 22b). TN 90e3 was phenotypically very similar to TN 90LC (Photo 13). This was not surprising since the primary problem with the e3 lines that was detected in 2013 was black shank resistance, rather than agronomic traits. Differences in the level of black shank resistance between TN 90LC versus TN 90e3 and ms TN 90 e3 were again

Table 20a. 2014 TKF 6400 Agronomic Measurements, ANOVA PR>F and LSD

| Location/Variety | | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) | Leaf Length (cm |
|---|---|---|---|---|---|---|
| **Greeneville** | TKF 6400LC | 128.00 | 22.78 | 5.63 | 27.22 | 63.00 |
| | TKF 6400e3-B | 138.28 | 22.56 | 6.14 | 26.33 | 62.11 |
| | **PR>F** | **0.12** | **0.84** | **0.21** | **0.58** | **0.80** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Springfield** | TKF 6400LC | 133.56 | 24.67 | 5.41 | 22.06 | 51.94 |
| | TKF 6400e3-B | 133.06 | 24.94 | 5.33 | 23.44 | 52.89 |
| | **PR>F** | **0.97** | **0.73** | **0.86** | **0.24** | **0.76** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Versailles** | TKF 6400LC | 132.39 | 17.89 | 7.44 | 26.22 | 64.22 |
| | TKF 6400e3-B | 133.78 | 19.22 | 7.01 | 26.33 | 64.11 |
| | **PR>F** | **0.92** | **0.61** | **0.36** | **0.96** | **0.96** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |

Table 20b. 2014 TKF 6400 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location/Variety | | Grade Index | Yield (kg/ha) | Revenue ($) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|---|
| **Greeneville** | TKF 6400LC | 36.40 | 3281.17 | 4062 | 68.90 | 84.70 |
| | TKF 6400e3-B | 35.80 | 3311.43 | 4338 | 69.70 | 81.80 |
| | **PR>F** | **0.57** | **0.91** | **0.97** | **0.59** | **0.37** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Springfield** | TKF 6400LC | 57.30 | 2934.78 | 4884 | | |
| | TKF 6400e3-B | 55.30 | 2850.70 | 4547 | | |
| | **PR>F** | **0.75** | **0.76** | **0.51** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |
| **Versailles** | TKF 6400LC | 35.30 | 3254.26 | 4141 | | |
| | TKF 6400e3-B | 42.30 | 3108.53 | 4595 | | |
| | **PR>F** | **0.24** | **0.66** | **0.51** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |

Table 21a. 2014 TN 86 Agronomic Measurements, ANOVA PR>F and LSD

| Location | Variety | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) | Leaf Length (cm) |
|---|---|---|---|---|---|---|
| **Greeneville** | TN 86LC | 131.39 | 20.83 | 6.31 | 28.89 | 65.83 |
| | TN 86e3 | 133.50 | 20.67 | 6.46 | 30.94 | 65.61 |
| | msTN 86e3 | 129.78 | 20.61 | 6.30 | 28.94 | 69.00 |
| | **PR>F** | **0.85** | **0.97** | **0.60** | **0.36** | **0.27** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Springfield** | TN 86LC | 124.11 | 23.61 | 5.27 | 19.83 | 46.83 |
| | TN 86e3 | 125.94 | 24.22 | 5.20 | 20.44 | 50.72 |
| | msTN 86e3 | 130.06 | 23.61 | 5.51 | 20.00 | 49.06 |
| | **PR>F** | **0.85** | **0.47** | **0.80** | **0.95** | **0.80** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Versailles** | TN 86LC | 128.66 | 16.72 | 7.69 | 27.78 | 62.33 |
| | TN 86e3 | 130.33 | 16.72 | 7.79 | 27.44 | 62.17 |
| | msTN 86e3 | 118.94 | 15.16 | 7.90 | 28.50 | 64.50 |
| | **PR>F** | **0.60** | **0.56** | **0.41** | **0.66** | **0.59** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |

Table 21b. 2014 TN 86 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location | Variety | Grade Index | Yield (kg/ha) | Revenue ($) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|---|
| **Greeneville** | TN 86LC | 36.70 | 3737.41 | 5015 | 35.20 | 74.30 |
| | TN 86e3 | 36.20 | 3702.66 | 4909 | 15.90 | 78.50 |
| | msTN 86e3 | 39.90 | 3480.71 | 4784 | 26.90 | 62.90 |
| | **PR>F** | **0.44** | **0.55** | **0.85** | **0.10** | **0.54** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Springfield** | TN 86LC | 56.00 | 2699.37 | 4397 | | |
| | TN 86e3 | 56.30 | 2793.53 | 4656 | | |
| | msTN 86e3 | 54.10 | 2894.42 | 4713 | | |
| | **PR>F** | **0.94** | **0.78** | **0.83** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |
| **Versailles** | TN 86LC | 64.00 | 3922.38 | 6731 | | |
| | TN 86e3 | 63.30 | 3597.29 | 5847 | | |
| | msTN 86e3 | 59.70 | 2951.59 | 5017 | | |
| | **PR>F** | **0.95** | **0.14** | **0.13** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |

Table 22a. 2014 TN 90 Agronomic Measurements, ANOVA PR>F and LSD

| Location | Variety | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) | Leaf Length (cm) |
|---|---|---|---|---|---|---|
| Greeneville | TN 90LC | 141.00 | 19.83 | 7.12 | 30.89 | 65.22 |
| | TN 90e3 | 138.56 | 18.83 | 7.37 | 29.22 | 63.72 |
| | msTN 90e3 | 138.72 | 19.66 | 7.06 | 31.05 | 65.78 |
| | TN 90LC x TN 90e3 | 145.70 | 19.70 | 7.40 | 28.20 | 57.60 |
| | **PR>F** | **0.90** | **0.51** | **0.70** | **0.12** | **0.45** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| Springfield | TN 90LC | 135.67 | 20.33 | 6.67 | 24.94 | 57.11 |
| | TN 90e3 | 129.56 | 18.78 | 6.89 | 23.94 | 53.61 |
| | msTN 90e3 | 129.28 | 19.11 | 6.77 | 24.84 | 54.56 |
| | TN 90LC x TN 90e3 | 130.80 | 20.20 | 6.48 | 22.80 | 53.90 |
| | **PR>F** | **0.67** | **0.14** | **0.75** | **0.86** | **0.73** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| Versailles | TN 90LC | 123.34 | 16.22 | 7.61 | 26.22 | 58.17 |
| | TN 90e3 | 127.67 | 16.89 | 7.57 | 27.67 | 60.50 |
| | msTN 90e3 | 123.56 | 16.17 | 7.65 | 27.5 | 58.28 |
| | TN 90LC x TN 90e3 | 113.30 | 15.90 | 7.14 | 26.20 | 57.60 |
| | **PR>F** | **0.61** | **0.46** | **0.97** | **0.45** | **0.56** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |

Table 22b. 2014 TN 90 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location | Variety | Grade Index | Yield (kg/ha) | Revenue ($) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|---|
| Greeneville | TN 90LC | 40.80 | 3472.86 | 4764 | 50.67a | 36.90ab |
| | TN 90e3 | 55.40 | 3102.93 | 4918 | 19.40b | 45.80ab |
| | msTN 90e3 | 53.00 | 3342.82 | 5212 | 34.22ab | 24.60b |
| | TN 90LC x TN 90e3 | 48.10 | 3355.15 | 4975 | 34.30ab | 54.80a |
| | **PR>F** | **0.43** | **0.09** | **0.77** | **0.01** | **0.05** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **26.13** | **21.43** |
| Springfield | TN 90LC | 50.80 | 3204.94 | 5049 | | |
| | TN 90e3 | 55.10 | 2602.96 | 4187 | | |
| | msTN 90e3 | 62.70 | 2364.19 | 3962 | | |
| | TN 90LC x TN 90e3 | 59.20 | 2931.42 | 4693 | | |
| | **PR>F** | **0.65** | **0.21** | **0.63** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |
| Versailles | TN 90LC | 66.00 | 2930.29 | 4934 | | |
| | TN 90e3 | 58.60 | 3376.45 | 5497 | | |
| | msTN 90e3 | 66.70 | 3071.54 | 5285 | | |
| | TN 90LC x TN 90e3 | 70.30 | 2986.34 | 5205 | | |
| | **PR>F** | **0.66** | **0.21** | **0.63** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |

Photos 13 and 14. TN 90LC (left) and TN 90e3 (right) Show Very Little Phenotypic Difference

readily apparent in 2014 (Table 22b). One additional backcross to TN 90LC improved the level of resistance in the fertile TN 90e3, but TN 90LC still displayed a higher level of resistance In the TN nursery that had high disease pressure. Because of the importance of TN 90 as both a variety and as the male parent of KT 204, another line having two backcrosses to TN 90LC will be used to derive the eventual TN 90e3 parental line.

The KT 204e3 hybrid evaluated in 2014 was a cross between TKS 2002e3-O and the original TN 90e3 parental line provided by NCSU. No significant differences were detected between KT 204LC and KT 204e3 for any agronomic trait at any of the test locations (Tables 23a and 23b). There was only a slight difference between the two lines with regard to black shank resistance; this was surprising because TN 90e3 has substantially lower resistance in comparison to TN 90LC. Because both parental lines of KT 204e3 will have undergone an additional cycle of backcrossing to their LC counterparts, followed by selection for homozygosity for the three e alleles, the version of KT 204e3 that will eventually be available for commercial production is expected to essentially be phenotypically identical to KT 204LC.

The version of KT 209e3 that was evaluated in 2014 was a cross between TKS 2002e3-O and TKF 4024e3 selection F. No significant differences were detected between KT 209LC and KT 209E3 at any of the 2014 test locations, and the level of black shank resistance for the two lines was virtually identical (Tables 24a and 24b). This was as expected because the two parental lines for KT 209LC and KT 209e3 were very similar in the 2013 trials. Foundation seed of TKF 4024e3-F was made in 2014, and will be used for future production of KT 209e3. One additional backcross was made to TKS 2002e3-O to in an effort to increase leaf number. Once this backcrossed version of TKF 2002e3-0 is stabilized (as described in the 2013 parental line discussion), it will become the female parental line not only for KT 209e3, but all of the new KTTII e3 hybrid varieties.

The version of KT 210e3 that was evaluated in 2014 was a cross between TKS 2002e3-O and TKF 6400e3 selection B. No significant differences were detected between KT 210LC and KT 210e3 at any of the 2014 test locations, and the level of black shank

Table 23a. 2014 KT 204 Agronomic Measurements, ANOVA PR>F and LSD

| Location | Variety | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) | Leaf Length (cm) |
|---|---|---|---|---|---|---|
| Greeneville | KT 204LC | 141.00 | 20.72 | 6.81 | 32.83 | 66.44 |
| | KT 204e3 | 141.67 | 19.89 | 7.14 | 32.94 | 70.95 |
| | **PR>F** | **0.79** | **0.42** | **0.25** | **0.89** | **0.06** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| Springfield | KT 204LC | 137.83 | 19.83 | 6.96 | 25.50 | 56.67 |
| | KT 204e3 | 138.56 | 19.39 | 7.14 | 26.33 | 58.00 |
| | **PR>F** | **0.86** | **0.45** | **0.54** | **0.57** | **0.50** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| Versailles | KT 204LC | 124.11 | 16.45 | 7.57 | 29.11 | 64.28 |
| | KT 204e3 | 117.11 | 15.67 | 7.47 | 31.28 | 64.67 |
| | **PR>F** | **0.19** | **0.41** | **0.73** | **0.21** | **0.88** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |

Table 23b. 2014 KT 204 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location | Variety | Grade Index | Yield (kg/ha) | Revenue ($) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|---|
| Greeneville | KT 204LC | 37.40 | 3799.07 | 5137 | 69.50 | 78.90 |
| | KT 204e3 | 37.50 | 3417.93 | 4623 | 58.80 | 81.50 |
| | **PR>F** | **0.74** | **0.29** | **0.28** | **0.13** | **0.79** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| Springfield | KT 204LC | 68.00 | 3327.13 | 5768 | | |
| | KT 204e3 | 51.50 | 2766.63 | 4124 | | |
| | **PR>F** | **0.98** | **0.70** | **0.96** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |
| Versailles | KT 204LC | 56.90 | 3512.09 | 5759 | | |
| | KT 204e3 | 57.20 | 3407.84 | 5558 | | |
| | **PR>F** | **0.98** | **0.70** | **0.96** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |

Table 24a. 2014 KT 209 Agronomic Measurements, ANOVA PR>F and LSD

| Location | Variety | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) | Leaf Length (cm) |
|---|---|---|---|---|---|---|
| **Greeneville** | KT 209LC | 124.61 | 20.45 | 6.13 | 31.06 | 68.01 |
| | KT 209e3 | 140.22 | 20.50 | 6.84 | 30.83 | 67.89 |
| | **PR>F** | **0.08** | **0.94** | **0.19** | **0.81** | **0.97** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Springfield** | KT 209LC | 137.61 | 21.61 | 6.39 | 23.89 | 53.83 |
| | KT 209e3 | 129.78 | 19.94 | 6.51 | 23.50 | 54.72 |
| | **PR>F** | **0.89** | **0.31** | **0.76** | **0.77** | **0.82** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Versailles** | KT 209LC | 118.34 | 15.56 | 7.63 | 30.61 | 66.28 |
| | KT 209e3 | 125.39 | 16.45 | 7.62 | 27.89 | 63.22 |
| | **PR>F** | **0.16** | **0.39** | **0.38** | **0.31** | **0.61** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |

Table 24b. 2014 KT 209 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location | Variety | Grade Index | Yield (kg/ha) | Revenue ($) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|---|
| **Greeneville** | KT 209LC | 36.30 | 3667.91 | 4887 | 83.80 | 91.70 |
| | KT 209e3 | 36.50 | 3661.19 | 4837 | 86.30 | 91.70 |
| | **PR>F** | **0.95** | **0.97** | **0.96** | **0.70** | **0.99** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Springfield** | KT 209LC | 56.70 | 2789.05 | 4429 | | |
| | KT 209e3 | 59.70 | 2702.73 | 4444 | | |
| | **PR>F** | **0.12** | **0.16** | **0.48** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |
| **Versailles** | KT 209LC | 50.50 | 3452.68 | 5380 | | |
| | KT 209e3 | 63.10 | 2958.32 | 4923 | | |
| | **PR>F** | **0.85** | **0.20** | **0.44** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |

resistance for the two lines was very similar (Tables 25a and 25b). Like the KT 209 family, this was as expected for the KT 201 family because the two parental lines for KT 210LC and KT 210e3 were also very similar in the 2013 trials. Foundation seed of TKF 6400e3-B was made in 2014, and will be used for future production of KT 210e3.

The version of KT 212e3 evaluated in 2014 was a cross between TKS 2002e3-O and the original L8e3 line provided by NCSU. No significant differences were detected between KT 212LC and KT 212e3 for any trait at any of the three test locations (Tables 26a and 26b). KT 212e3 also had slightly higher black shank resistance in comparison to KT 212LC. Phenotypic differences could be detected between the two lines, primarily with regard to the relative erectness of the two varieties. This is likely due to the L8e3 parent, which displayed a distinctly different phenotype in comparison to L8LC. NCSU is still attempting to improve the L8e3 parental line; if they are successful, it is anticipated that the final version of KT 212 e3 will bear a closer resemblance to KT 212LC than did the version evaluated in 2014.

**Chemical Analyses of Selected Entries in 2014 Field Trials**

Samples for chemical analyses were taken from all three replications of selected parental lines and varieties at the Greeneville test location in 2014. Data for parental line and variety entries are presented in Tables 27 and 28, respectively. Conversion of nicotine to nornicotine ranged from 2.35 – 4.86% for the LC lines and varieties that were evaluated. This is typical for LC burley lines. Even though foundation seed plants must have a conversion level of 3.0% or lower, the LC lines are genetically unstable, with a variable small percentage of plants reverting to converters each generation of seed increase; composite samples taken from plants produced from commercial seed of LC varieties will typically have conversion rates ranging from 3.5 – 5.0%.

The necessity of incorporating all three e3 alleles into KTTII varieties to minimize nornicotine and NNN levels can be seen in the TKF 2002 family. In parental line TKF 2002e3-C SGe10, alleles e4 and e5 are homozygous, while e10 is heterozygous. The effect of recessive e10 in reducing conversion is not expressed because it is in a

Table 25a. 2014 KT 210 Agronomic Measurements, ANOVA PR>F and LSD

| Location | Variety | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) | Leaf Length (cm) |
|---|---|---|---|---|---|---|
| Greeneville | KT 210LC | 144.28 | 21.78 | 6.63 | 31.67 | 70.33 |
| | KT 210e3 | 138.89 | 20.55 | 6.78 | 31.67 | 68.11 |
| | **PR>F** | **0.06** | **0.27** | **0.51** | **1.00** | **0.13** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| Springfield | KT 210LC | 136.33 | 21.28 | 6.40 | 24.05 | 54.95 |
| | KT 210e3 | 139.34 | 20.94 | 6.67 | 24.00 | 53.56 |
| | **PR>F** | **0.76** | **0.82** | **0.38** | **0.98** | **0.70** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| Versailles | KT 210LC | 112.11 | 16.17 | 6.90 | 30.56 | 66.33 |
| | KT 210e3 | 128.39 | 16.94 | 7.58 | 28.56 | 64.78 |
| | **PR>F** | **0.35** | **0.65** | **0.22** | **0.20** | **0.64** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |

Table 25b. 2014 KT 210 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location | Variety | Grade Index | Yield (kg/ha) | Revenue ($) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|---|
| Greeneville | KT 210LC | 47.30 | 3922.38 | 5786 | 91.40 | 94.40 |
| | KT 210e3 | 40.30 | 3714.99 | 5147 | 88.60 | 94.30 |
| | **PR>F** | **0.48** | **0.65** | **0.52** | **0.21** | **0.96** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| Springfield | KT 210LC | 42.50 | 2660.13 | 3545 | | |
| | KT 210e3 | 48.60 | 2626.50 | 3911 | | |
| | **PR>F** | **0.16** | **0.87** | **0.97** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |
| Versailles | KT 210LC | 46.90 | 3264.35 | 4997 | | |
| | KT 210e3 | 43.40 | 3364.12 | 4965 | | |
| | **PR>F** | **0.16** | **0.87** | **0.97** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |

Table 26a. 2014 KT 212 Agronomic Measurements, ANOVA PR>F and LSD

| Location | Variety | Plant Height (cm) | Leaf Number | Internode (cm) | Leaf Width (cm) | Leaf Length (cm) |
|---|---|---|---|---|---|---|
| **Greeneville** | KT 212LC | 134.44 | 18.39 | 7.31 | 30.00 | 65.50 |
| | KT 212e3 | 132.78 | 17.67 | 7.51 | 27.94 | 68.05 |
| | **PR>F** | **0.63** | **0.08** | **0.13** | **0.22** | **0.01** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **1.19** |
| **Springfield** | KT 212LC | 127.17 | 17.33 | 7.36 | 26.45 | 54.11 |
| | KT 212e3 | 128.83 | 16.72 | 7.70 | 25.89 | 58.39 |
| | **PR>F** | **0.71** | **0.51** | **0.21** | **0.82** | **0.54** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |
| **Versailles** | KT 212LC | 126.78 | 15.72 | 8.06 | 29.39 | 66.61 |
| | KT 212e3 | 125.89 | 15.67 | 8.04 | 29.61 | 67.33 |
| | **PR>F** | **0.86** | **0.88** | **0.91** | **0.83** | **0.62** |
| | **LSD(.05)** | **ns** | **ns** | **ns** | **ns** | **ns** |

Table 26b. 2014 KT 212 Agronomic Measurements, ANOVA PR>F, LSD and Percent Black Shank Survival

| Location | Variety | Grade Index | Yield (kg/ha) | Revenue ($) | Black Shank Survival TN (%) | Black Shank Survival KY (%) |
|---|---|---|---|---|---|---|
| **Greeneville** | KT 212LC | 46.50 | 3333.85 | 4916 | 42.60 | 52.00 |
| | KT 212e3 | 37.20 | 2724.03 | 3667 | 54.60 | 54.90 |
| | **PR>F** | **0.47** | **0.11** | **0.06** | **0.80** | **0.82** |
| | **LSD(.05)** | **Ns** | **ns** | **ns** | **ns** | **ns** |
| **Springfield** | KT 212LC | 60.60 | 2822.68 | 4764 | | |
| | KT 212e3 | 43.40 | 2727.39 | 3755 | | |
| | **PR>F** | **0.63** | **0.46** | **0.32** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |
| **Versailles** | KT 212LC | 53.20 | 3485.19 | 5618 | | |
| | KT 212e3 | 47.60 | 3342.82 | 4955 | | |
| | **PR>F** | **0.63** | **0.46** | **0.32** | | |
| | **LSD(.05)** | **ns** | **ns** | **ns** | | |

Table 27. Effectiveness of "e" Alleles in Reducing Nornicotine and Nitroso-nornicotine in Parental Breeding Lines

| Entry | Nicotine %DM | Nornicotine %DM | Conversion % | Nitroso-nornicotine ppm | Total Nitrosamines ppm |
|---|---|---|---|---|---|
| | | | | | |
| TKF 2002LC | 5.18 | 0.13 | 2.35 | 0.27 | 0.73 |
| TKF 2002e2 | 5.21 | 0.09 | 1.68 | 0.19 | 0.63 |
| TKF 2002e3-C SGe10 | 5.53 | 0.08 | 1.34 | 0.19 | 0.78 |
| TKF 2002e3 | 5.63 | 0.03 | 0.52 | 0.07 | 0.69 |
| | | | | | |
| TKF 4024LC | 6.01 | 0.22 | 3.55 | 0.27 | 0.73 |
| TKF 4024e3 | 6.99 | 0.05 | 0.66 | 0.11 | 0.87 |
| | | | | | |
| TKF 6400LC | 6.04 | 0.17 | 2.80 | 0.61 | 1.36 |
| TKF 6400e3 | 6.61 | 0.03 | 0.48 | 0.11 | 0.81 |
| | | | | | |

Table 28. Effectiveness of “e” Alleles in Reducing Nornicotine and Nitroso-nornicotine in Commercial Burley Varieties

| Entry | Nicotine %DM | Nornicotine %DM | Conversion % | Nitroso-nornicotine ppm | Total Nitrosamines ppm |
|---|---|---|---|---|---|
| | | | | | |
| TN 86LC | 6.66 | 0.23 | 3.31 | 0.61 | 1.24 |
| ms TN 86e3 | 6.02 | 0.04 | 0.58 | 0.06 | 0.44 |
| TN 86e3 | 6.09 | 0.03 | 0.52 | 0.07 | 0.53 |
| | | | | | |
| TN 90LC | 6.24 | 0.25 | 3.91 | 0.51 | 0.95 |
| ms TN 90e3 | 7.09 | 0.04 | 0.54 | 0.12 | 0.92 |
| TN 90e3 | 6.82 | 0.04 | 0.53 | 0.08 | 0.66 |
| | | | | | |
| KT 204LC | 5.67 | 0.17 | 2.99 | 0.39 | 0.91 |
| KT 204e3 | 6.09 | 0.03 | 0.48 | 0.07 | 0.79 |
| | | | | | |
| KT 209LC | 4.71 | 0.24 | 4.86 | 0.50 | 0.88 |
| KTH 209e3 | 5.26 | 0.03 | 0.64 | 0.09 | 0.70 |
| | | | | | |
| KT 210LC | 5.39 | 0.17 | 3.07 | 0.51 | 1.08 |
| KTH 210e3 | 5.45 | 0.03 | 0.57 | 0.08 | 0.78 |
| | | | | | |
| KT 212LC | 4.69 | 0.18 | 3.65 | 0.38 | 0.77 |
| KTH 212e3 | 4.74 | 0.02 | 0.48 | 0.07 | 0.49 |

heterozygous state. As a result, the overall levels of nornicotine and NNN are very similar to that observed in TKF 2002e2, with both lines being intermediate to TKF 2002LC and TKF 2002e3. When all three of the recessive e3 alleles are present in a homozygous state, conversion of nicotine to nornicotine is reduced by 80-90% in comparison to LC varieties. This was observed in all of the e3 parental lines and varieties evaluated in 2014 (Tables 27 and 28). As would be expected, the reduction in nornicotine content in the e3 parental lines and varieties leads to an associated reduction in levels of NNN. Total TSNA levels also show a substantial reduction, but the percentage reduction is somewhat lower because the e3 levels affect only NNN, having no impact on other TSNA components.

**Chapter 5. Conclusions**

Reduction of nicotine to nornicotine conversion and TSNA formation are two of the leading goals within the tobacco industry, but they have also been among the most difficult to achieve due to the effect of weather conditions throughout the tobacco plant's life cycle and curing. Genetic control, however, can minimize the effect of these environmental factors and establish a more stable baseline than the LC protocol currently offers (Tables 27 and 28). We now have the knowledge and ability to effectively and consistently regulate chemical formation in tobacco plants. This would not have been possible without being able to evaluate the effects of mutated genes. Induced mutations allowed researchers to make changes to the genome that could be monitored by genetic markers by confirming the presence or absence of the mutations. This greatly decreases the time required to make selections and provides a very definitive evaluation step.

This study has, however, reiterated the importance of field evaluations of crops to ensure plant performance, especially when using early flowering techniques. When the plants are growing for a few short weeks in greenhouses and only reaching about 15% of production plant height before flowering, it is only possible to speculate about the growth habit, disease resistance and yield of a field production plant. For this project, it

was assumed that after making up to six backcrosses, all of the e3 lines would be nearly identical to the original LC line or variety. It quickly became apparent that there were vast differences in disease resistance, as well as agronomic characteristics and yield. With field selections, genetic markers and additional backcrossing, great progress was made to bring all lines back to their true type and performance level. Table 29 illustrates the success of the steps that were taken and the improvements that were made to the black shank resistance of the e3 lines.

One of the strongest points of this thesis is the importance, and effectiveness, of a well-rounded and equipped breeding program. With the available lab equipment and genome mapping, selections can be made before plants are ever put into a field trial, saving time and resources, but these practices cannot be used alone to release new varieties. Field trials must still be undertaken to allow plants to be evaluated based on their type, growth habit, quantitatively inherited disease resistance, and yield. Most researchers are not proficient in all stages of the process, which reinforces multiple disciplines being a part of the same program, as well as collaborations between researchers, both of which have been integral parts of this research.

Table 29. Comparison of LC and e3 Parental Lines for Race 1 Black Shank Resistance

| Entry | 2013 Mean | 2014 Mean | 2015 Mean |
|---|---|---|---|
| TN 90LC | 79 | 36 | 71 |
| ms TN 90LC | 84 | | |
| ms TN 90e3 | 38 | 26* | 85** |
| TN 90e3 | 41.1 | 34* | 76** |
| TKF 2002LC FS | 93 | 87 | 93 |
| TKF 2002Ze3 | 85 | 92* | 93 |
| TKS 2002LC FS | 97.6 | 88 | 92 |
| TKS 2002Ze3 | 98 | 90* | 90 |
| TKF 4024LC | 95 | 87 | 96 |
| TKF 4024e3 FS | 95 | 93* | 97 |
| TKF 4028LC | 37 | 33 | 38 |
| TKF 4028e3 Bd Sd | 0 | 27** | 33** |
| TKF 6400LC | 77.2 | 74 | 93 |
| TKF 6400e3 FS | 67 | 74 | 94 |

*One additional backcross to LC parental line

**Two additional backcrosses to LC parental line

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