Inheritance of Resistance to Tobacco Cyst Nematode *Globodera tabacum solanacearum*

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(ABSTRACT)

The tobacco cyst nematode [*Globodera tabacum solanacearum* (Miller & Gray, 1972) Behrens, 1975] is an important pathogen affecting flue-cured tobacco (*Nicotiana tabacum* L.) in Virginia, North Carolina, and Maryland. The resistant cultivars Coker 371 Gold and Kutsaga 110 were evaluated during 1999 and 2000 in the greenhouse to determine the mode of inheritance of resistance to the tobacco cyst nematode (TCN). Each cultivar was crossed to the susceptible cultivar K 326 and F_1 progeny were backcrossed to each parent. Plants from each parent and F_1, F_2, BC_1P_s, and BC_1P_r progeny were evaluated for TCN resistance. Six-week-old transplants were inoculated with 6000 TCN eggs from crushed cysts. Eight weeks after inoculation, a 1-g sample of fibrous root was stained and vermiform, swollen, pyriform, and adult nematodes were counted. The number of cysts and eggs per 400 cm^3 of soil were counted from each transplant. Generation means analyses were performed. Additive and dominance gene action play an important role in resistance to TCN in Coker 371 Gold and Kutsaga 110. F_2 generation data from the Coker 371 Gold cross fit a 3:1 (resistant:susceptible) segregation ratio and BC_1P_s generation data fit a 1:1 segregation ratio, indicating that resistance to TCN is conferred by a single dominant gene. A continuous range of variation was observed among the F_2 progeny for the K 326 X Kutsaga 110 cross, indicating resistance in Kutsaga 110 is quantitative. TCN resistance in Coker 371 Gold and Kutsaga 110 may be derived from different sources.

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Tobacco Cyst Nematode

Tobacco cyst nematode (TCN) is a serious soil-borne pest of flue-cured tobacco (*Nicotiana tabacum* L.) in the Southern Piedmont region of Virginia and some parts of North Carolina and Maryland. *Globodera tabacum solanacearum* (Miller & Gray, 1972) Behrens, 1975 was first observed in 1961 parasitizing the roots of 'Hicks' in Amelia County, Virginia (Komm et al., 1983). W. W. Osborne made the discovery (Osborne, 1961) and the nematode became referred to as Osborne's cyst nematode. TCN was described as a species of the genus *Heterodera* until Behrens reclassified it in the genus *Globodera* (Behrens, 1975). It was thought that *G. t. solanacearum* only plagued flue-cured tobacco in Virginia until 1991 when it was identified in a flue-cured tobacco field in Warren County, North Carolina (Melton et al., 1991) and then recently detected in 1995 on Maryland-type tobacco in Maryland (Johnson, 1998). *Globodera tabacum solanacearum* parasitizes most commercially grown flue-cured tobacco cultivars, certain cultivars of other tobacco types, horsenettle (*Solanum carolinense* L.), and some cultivars of tomato (*Lycopersicon* spp. L.), eggplant (*Solanum melongena* L.), and sweet peppers (*Capsicum* spp. L.) (Harrison and Miller, 1969; Adams et al., 1982).

Stone (1983) evaluated the *Globodera tabacum* (Lownsbery and Lownsbery, 1954) Behrens, 1975 species complex and concluded that three subspecies exist, *Globodera tabacum tabacum* (Lownsbery and Lownsbery, 1954) Behrens, 1975, *Globodera t. virginiae* (Miller and Gray, 1968) Behrens, 1975, and *G. t. solanacearum*. All three subspecies will mature on tobacco and horsenettle (Miller and Gray, 1968), but differ in terms of host preference. *Globodera tabacum tabacum* is found in New York, Massachusetts, and Connecticut (Miller and Gray, 1972) where it primarily parasitizes shade tobacco. *Globodera tabacum virginiae* occurs in Virginia (Miller and Gray, 1972) and North Carolina (Johnson, 1998). *Globodera tabacum virginiae* does not reproduce on flue-cured tobacco, but reproduces slowly on burley tobacco (Johnson, 1998).

Harrison and Miller (1969) first reported eggplant and sweet pepper as hosts of *G. t. solanacearum*. Morphological differences between the three subspecies are very subtle. *Globodera tabacum solanacearum* closely resembles *G. t. virginiae* and *G. t. tabacum*. However, the three subspecies differ morphologically from each other in cyst shape, cyst ridge patterning, Granek's ratio, shape of stylet knobs, and fenestra shape (Miller and Gray, 1972; Stone, 1983; Mota and Eisenback, 1993).

Losses due to TCN reported on 339 ha of infested land amounted to an estimated $700,000 in 1982 compared with $13,000 in 1980 (Komm et al., 1983). Continuous tobacco production is the main factor leading to TCN-induced crop losses (Johnson et al., 1989). The number of farms infested with TCN increased by 18% annually between 1961 and 1982 and yield of tobacco grown on infested land was reduced by an average of 15% (Komm et al., 1983). TCN infestation reduced Virginia's flue-cured tobacco crop by an estimated 3% in 1998. This amounted to $4,000,000 in crop losses. An estimated $1,000,000 were spent for nematicides that year, resulting in $5,000,000 in total expenses and losses to Virginia tobacco farmers (C. S. Johnson, personal communication, 2000). The main management tactic used for nematode control is the application of nematicides to susceptible cultivars at an average cost of $250/ha (C. S. Johnson, personal communication, 2000). Many Virginia flue-cured tobacco producers spend as much as $337/ha to control tobacco cyst nematodes (C. S. Johnson, personal communication, 2000).

Effects on Tobacco

Tobacco plants parasitized by TCN have greatly reduced root systems and tend to wilt in the hottest part of the day (Baalawy and Fox, 1971). *Globodera tabacum tabacum* suppresses the growth of shade tobacco and increases the incidence and severity of Fusarium wilt of broadleaf tobacco (LaMondia, 1988).

Adult tobacco cyst nematode females are globular and become filled with hundreds of eggs. After the female dies, these eggs are protected by the body wall and

can remain viable in the soil for many years. Second-stage juveniles, the infective stage, use their stylet to pierce and rupture cell walls of the root epidermis and migrate through the root cortex causing cellular damage (Williamson and Hussey, 1996). Damage caused by nematodes feeding directly on the roots reduces the rate of root extension and tends to decrease rates of nutrient uptake and leaf content of macro-nutrients and water (Trudgill, 1991). The mechanical damage caused by cyst nematode invasion increases the formation of abscisic acid and substantially reduces the photosynthetic rate per unit area of leaf (Trudgill, 1991). For this reason, serious decreases in yield are experienced when tobacco is grown on infested land.

Control

The tobacco cyst nematode is a difficult pest to control for several reasons. It has a high reproductive potential, producing approximately 300 eggs per cyst in 6 to 8 wk. The survival stage, unhatched juveniles within the cyst, can live without a host for at least 11 yr. The cyst also provides a protective covering that makes it difficult to kill eggs with a nematicide (Johnson, 1998). Shortly after the discovery of the tobacco cyst nematode, Osborne (1969) suggested controlling TCN with crop rotation, plowing out stubble in the fall, and planting resistant cultivars. Osborne (1969) considered these control practices to be supplements to a chemical control method. Nematicides should always be used in conjunction with resistance, rotation, and early root and stalk destruction.

Today, chemical control is the prominent form of control for TCN. In 1988, more than 60% of the flue-cured tobacco crop was being treated with a chemical (Rich et al., 1989). Chemicals are applied as a fumigant or non-fumigant. According to Rich et al. (1989), the major fumigant nematicides included chloropicrin, 1,3-dichloropropene (1,3-D), ethylene dibromide, 1,3-D-methyl isothiocyanate mixtures, metham-sodium, and methyl bromide. Methyl bromide is also currently used as a multipurpose chemical for transplant bed treatments. As of 1989, the major nonfumigant nematicides included aldicarb, carbofuran, ethoprop, fenamiphos, and fensulfothion (Rich et al., 1989).

Current fumigants that provide good control for TCN include Chlor-O-Pic, Chloropicrin 100, Telone II [1,3-dichloropropene (94%)], Telone C-17 [1,3-dichloropropene (77.9%) + chloropicrin (16.5%)], Terr-O-Gas 67 [methyl bromide (66%) + chloropicrin (33%)], and Tri-Con 67/33 [methyl bromide (66%) + chloropicrin (33%)]. Nemacur 3SC (fenamiphos) and Temik 15G (aldicarb) are non-fumigant nematicides that offer good TCN control (Reed et al., 1999; CPR, 1996). Therefore, the phasing out of methyl bromide will remove many effective nematicides from the market.

Elliott et al. (1986) investigated continuous cropping of the resistant flue-cured tobacco breeding line VA 81 and the resistant flue-cured tobacco cultivar PD 4 as a method of controlling TCN. The results of the study showed that the reproductive potential of *G. t. solanacearum* differed under continuous cropping of resistant and susceptible tobacco cultivars. Elliott et al. (1986) recommended continuous cropping of the resistant breeding line VA 81 and cultivar PD 4 since loss of resistance was not demonstrated during the study. Elliott et al. (1986) attributed the maintenance of resistance to the presumed multigenic nature of resistance in VA 81 and PD 4.

Johnson et al. (1989) found post-harvest TCN population densities to be reduced when fenamiphos was used with a TCN resistant cultivar (NC 567) relative to susceptible cultivars (K 326 or McNair 944). Using NC 567 with fenamiphos also reduced pre-plant TCN population densities in the next growing season. Treatments involving fenamiphos and/or NC 567 in 1984 and 1985 resulted in higher economic returns in 1986 than did treatments using a susceptible cultivar without fenamiphos in both previous years (Johnson et al., 1989). While TCN resistant cultivars have been shown to reduce TCN densities, the yield and quality of TCN resistant cultivars, even when treated with a nematicide, are not significantly greater than yield and quality of agronomically superior TCN susceptible cultivars treated with the same nematicide (Johnson et al., 1989).

Johnson (1995) investigated the use of fosthiazate to control TCN in flue-cured tobacco. Fosthiazate was shown to reduce nematode population densities and was associated with increases in flue-cured tobacco yield and quality. These results were as

great or greater than those resulting from the use of aldicarb, fenamiphos, methyl bromide, or 1,3-dichloropropene (Johnson, 1995). However, fosthiazate is not registered for use on tobacco. Therefore, it is important to develop TCN resistant lines that are equivalent to agronomically superior TCN susceptible cultivars. 'Coker 371 Gold', NC 567, and 'Speight G-80' reduce TCN populations, but require the use of a recommended nematicide to produce acceptable yield and quality because they are hypersensitive to nematode invasion (Reed et al., 1999).

Planting resistant cultivars is one method of managing nematodes. However, according to Elliot et al. (1986), several researchers have found that continuous cropping of resistant cultivars results in selective pressure that leads to development of new nematode biotypes adapted to these cultivars. Elliot et al. (1986) found that the reproductive potential of *G. t. solanacearum* was low on VA 81 and PD 4 over three years of continuous cropping. This finding suggests that multiple genes may confer resistance in these two tobacco lines.

Rideout et al. (2000b) investigated the development of TCN isolates on K 326 (susceptible) and NC 567 (resistant) to determine if there were differences among isolates from different geographic locations in Virginia, Maryland, and North Carolina. Consistent TCN reproduction was found among 15 nematode isolates on the resistant cultivar NC 567, suggesting that few differences in virulence exist among the isolates studied. No differences were observed among TCN isolates in development and reproduction on a resistant and a susceptible flue-cured tobacco cultivar (Rideout et al., 2000b). These results suggest that different TCN biotypes do not currently exist. Therefore, single isolates of TCN can reliably be used to screen tobacco germplasm for resistance.

Rideout et al. (2000a) evaluated soil from six locations that were not infested with TCN and one TCN-infested location as a control. The samples were selected based on geographic location and soil type differences to determine the possible effects of soil pasteurization and edaphic factors on TCN parasitism. Three treatment combinations

were evaluated: non-pasteurized soil, non-inoculated; pasteurized soil inoculated with TCN; and non-pasteurized soil inoculated with TCN. Nematode reproduction was found to be similar to or lower in pasteurized soil than in non-pasteurized soil, suggesting the absence of TCN-suppressive parasites in the soils under investigation. TCN were shown to reproduce in the various soil types tested, illustrating the ability of TCN to spread to other flue-cured tobacco growing regions. The study also concluded that soil edaphic factors had little influence over TCN reproduction, but that further testing was necessary (Rideout et al., 2000a).

Johnson (1990) investigated alternate planting of TCN resistant flue-cured tobacco cultivars (NC 567 or Speight G-80) or TCN susceptible K 326. Planting a resistant cultivar reduced TCN population densities compared with continuous planting of K 326. Using a resistant cultivar prior to planting K 326 results in higher economic yields than if K 326 had been planted continuously (Johnson, 1990).

Resistance

Using resistant cultivars can provide an effective means of controlling nematode populations in infested fields. Resistance describes the effects of host genes that restrict or prevent nematode multiplication in a host species (Trudgill, 1991). Dalmasso et al. (1992) describes resistance as "an active mechanism which inhibits, restricts, retards or alters nematode development". Resistance to nematodes does not usually protect plants from invasion damage. Resistance to nematodes usually occurs after the plant has been invaded, indicating the lack of a mechanical barrier to the entry of nematodes (Baalawy and Fox, 1971). Johnson et al. (1989) reported that planting TCN resistant flue-cured tobacco cultivar NC 567 in severely infested fields effectively reduced TCN population densities at the end of one growing season and at the beginning of the following growing season.

It is important that resistant cultivars also be tolerant. Tolerance is related to the ability of the host genotype to withstand or recover from the damaging effects of

nematode attack (Trudgill, 1991). Thus, tolerant plants retain the ability to yield well even under conditions of high nematode infestation. Intolerant resistant cultivars will suffer damage if grown in heavily infested soil. Tolerant cultivars that are not resistant will allow nematode population densities to increase to dangerously high levels (Trudgill, 1991). Fox and Spasoff (1976) concluded that the ability of the host to inhibit the development of the nematode (resistance) was genetically independent of the host's ability to grow in the presence of the nematode (tolerance). However, it is common for plants to possess some combination of tolerance and resistance.

The genetics of plant resistance to nematodes is greatly influenced by several factors. These factors include the nematode race or pathotype being tested, the genetic background of the resistant line, the effect of the environment on expression of resistance, and the criteria used for assessing resistance (Fassuliotis, 1987). Baalawy and Fox (1971) performed some of the earliest research on resistance to *G. t. solanacearum* by comparing several wild *Nicotiana* species. *Nicotiana glutinosa* L., *N. paniculata* L., *N. plumbaginifolia* Viviani, and *N. longiflora* Cavanilles exhibited differing forms of resistance in greenhouse tests. While all the parental materials were resistant to *G. t. solanacearum*, hybrids derived from *N. plumbaginifolia* were the most resistant and had the most succulent and extensive root systems (Baalawy and Fox, 1971). Spasoff et al. (1971) evaluated the inheritance of resistance to TCN in a cross between the TCN resistant burley tobacco breeding line BVA 523 and the TCN susceptible flue-cured cultivar NC 2326. The F_2 and the F_3 exhibited a continuous range of variation in TCN females per pot, suggesting that resistance to TCN is multigenic (Spasoff et al., 1971). A subsequent study by Miller et al. (1972) involving inheritance of resistance in the dark fire-cured breeding line DVA 606 supported the evidence that resistance to TCN is of a multigenic nature.

Komm and Terrill (1982) reported the use of wildfire resistance as a possible method for quickly and inexpensively determining resistance to *G. t. solanacearum* in tobacco cultivars containing *N. longiflora* germplasm. In the same study, a new flue-cured cultivar, PD 4, was reported as having resistance to *G. t. solanacearum* as well as

several sun-cured and Virginia dark fire-cured cultivars (Komm and Terrill, 1982). Hayes et al. (1997) evaluated a wide array of tobacco accessions to determine the reliability of using wildfire resistance as a screening tool for TCN resistance. Twenty-one accessions were demonstrated to be resistant to TCN, including previously unreported resistance in *N. miersii* Remy, *N. cordifolia* Philippi, 'TN 90', 'Burley 21', 'Burley 49', 'Burley 64', 'MD 40', 'Pennbell 69', 'Pennlan', TI 1597, and TI 1625. Tobacco introduction 551 and 'KY 190' were found to be resistant to wildfire, but susceptible to TCN, and *N. miersii* was resistant to TCN, but susceptible to wildfire. Wildfire resistance was found to be highly correlated with TCN resistance, but the genes responsible for resistance to these two pathogens may not be as closely linked as previously thought. Some accessions evaluated (TI 551, KY 190, and *N. miersii*) did not possess a linkage between the two pathogens. Therefore, wildfire resistance may not be a reliable indicator of TCN resistance (Hayes et al., 1997).

Herrero et al. (1996) observed low levels of TCN reproduction in the accessions Burley 21, Speight G-80, NC 567, 'Kutsaga Mammoth 10', Cyst 913, 9025-1, PD 4, 'Kutsaga 110', and VA 81. Hayes et al. (1995) used a diallel analysis to study resistance of eight tobacco accessions to TCN. Six resistant accessions, PD 4, Burley 64, Pennbell 69, MD 40, TI 1597, and Kutsaga 110, and two susceptible accessions, 040-1 and K 394, were evaluated in a half-diallel design. Burley 64, Kutsaga 110, and TI 1597 were found to significantly contribute to increased resistance to TCN based on general combining ability (GCA) effects. General coming ability evaluates the additive portion of the genetic effects. GCA effects were found to account for the majority of variation observed among crosses, suggesting that additive gene action plays a significant role in the inheritance of resistance to TCN. Specific combining ability effects, which correspond to non-additive gene action, were not significant in this study (Hayes et al., 1995).

The nature of inheritance of resistance to *G. t. solanacearum* in cultivar PD 4 and breeding line VA 81 has not been genetically evaluated (Herrero et al., 1996). LaMondia (1988) tested PD 4 and VA 81 for resistance to *G. t. tabacum*. LaMondia (1988) reported

a decline in population densities of *G. t. tabacum* by 72% to 80% in naturally infested field soil when planted with PD 4 and VA 81 and an increase in population densities by 144% under CT86-4, a susceptible check. LaMondia (1988) observed that those juveniles that invade VA 81 and PD 4 either leave roots, die, or have little chance of developing into adult females. These findings lead LaMondia (1991) to investigate the genetics of tobacco resistance to *G. t. tabacum* using VA 81 and PD 4 as resistant parents. Crosses with either susceptible Connecticut shade or broadleaf tobacco lines indicated that resistance to *G. t. tabacum* is conferred by a single dominant gene (LaMondia, 1991). However, resistance to *G. t. solanacearum* in tobacco has been described as multigenically inherited.

Tobacco resistance to both *G. t. tabacum* and *G. t. solanacearum* may involve some of the same genes. Apparently, the multigenic nature of resistance to *G. t. solanacearum* was based on the burley and dark fire-cured breeding lines BVA 523, DVA 606, and hybrids between these lines and flue-cured tobacco. However, VA 81 and PD 4 were not evaluated. It is possible that a single gene for resistance was selected from several in the development of VA 81 and PD 4 (LaMondia, 1991). If resistance is inherited as a single dominant gene segregating in a diploid manner, incorporation of resistance into adapted tobacco types can be quickly achieved.

Resistant cultivars provide a low-labor, cost-effective means of controlling nematodes. The use of resistant cultivars requires little or no technology, allows for shorter crop rotations, and does not leave toxic residues (Trudgill, 1991). Resistance, however, should be used as part of an integrated pest management program to be most effective and to reduce the risk of selection for resistance breaking biotypes of nematodes. The inevitable phasing out of methyl bromide as a chemical control for TCN has left growers with a strong need for an alternative method of control. Breeding nematode resistance into an agronomically acceptable cultivar will be a valuable component of an integrated pest management program.

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Chapter 2: Inheritance of Resistance to Tobacco Cyst Nematode *Globodera tabacum solanacearum*

ABSTRACT

The tobacco cyst nematode [*Globodera tabacum solanacearum* (Miller & Gray, 1972) Behrens, 1975] is an important pathogen affecting flue-cured tobacco (*Nicotiana tabacum* L.) in Virginia and some regions of North Carolina and Maryland. The resistant cultivars Coker 371 Gold (P_r) and Kutsaga 110 (P_r) were evaluated during 1999 and 2000 in the greenhouse to determine the mode of inheritance of resistance to the tobacco cyst nematode (TCN). Each cultivar was crossed to the susceptible cultivar K 326 (P_s) and F_1 progeny were backcrossed to each parent. Plants from each parent and F_1, F_2, BC_1P_s, and BC_1P_r progeny were evaluated for TCN resistance. Six-week-old transplants were inoculated with 6000 TCN eggs from crushed cysts. Approximately eight weeks after inoculation, a 1-g sample of fibrous root was stained and vermiform, swollen, pyriform, and adult nematodes were counted. The number of adult cysts and eggs per 400 cm^3 of soil were counted from each transplant. Generation means analyses were performed. Additive and dominance gene action play an important role in resistance to TCN in Coker 371 Gold and Kutsaga 110. F_2 generation data from the Coker 371 Gold cross fit a 3:1 (resistant:susceptible) segregation ratio and BC_1P_s generation data fit a 1:1 segregation ratio, indicating that resistance to *G. t. solanacearum* is conferred by a single dominant gene. A continuous range of variation was observed among the F_2 progeny for the K 326 X Kutsaga 110 cross, indicating resistance in Kutsaga 110 is quantitative. Differences in inheritance of TCN resistance between Coker 371 Gold and Kutsaga 110 suggest that resistance may be derived from different sources for these two cultivars.

INTRODUCTION

The tobacco cyst nematode (TCN), *Globodera tabacum solanacearum* (Miller & Gray, 1972) Behrens, 1975, is a serious soil-borne pest of flue-cured tobacco, *Nicotiana tabacum* L., in the Southern Piedmont region of Virginia and some areas of North Carolina and Maryland. Three subspecies of *Globodera tabacum* (Lownsbery and Lownsbery, 1954) Behrens, 1975 exist, *Globodera tabacum tabacum* (Lownsbery and Lownsbery, 1954) Behrens, 1975, *Globodera t. virginiae* (Miller and Gray, 1968) Behrens, 1975, and *G. t. solanacearum* (Stone, 1983). *Globodera tabacum tabacum* parasitizes Connecticut shade tobacco and occurs in New York, Massachusetts, and Connecticut (Miller and Gray, 1972). *Globodera tabacum virginiae* occurs in Virginia (Miller and Gray, 1972) and North Carolina (Johnson, 1998). *Globodera tabacum solanacearum* parasitizes most commercially grown flue-cured tobacco cultivars, certain cultivars of other tobacco types, horsenettle, and some cultivars of tomato (*Lycopersicon* spp. L.), eggplant (*Solanum melongena* L.), and sweet peppers (*Capsicum* spp. L.) (Harrison and Miller, 1969; Adams et al., 1982).

TCN currently infests approximately one-third of all flue-cured tobacco acreage in Virginia, costing farmers an estimated $5,000,000 annually in crop losses and pesticide expenses (C. S. Johnson, personal communication, 2000). TCN infested fields average 15% yield reduction annually (Komm et al., 1983). The primary TCN management tactic is the application of nematicides at an average cost of $250/ha (C. S. Johnson, personal communication, 2000). Other control measures for TCN include crop rotation, sanitation, and planting resistant cultivars (Reed et al., 1999).

Using resistant cultivars can provide an effective means of controlling nematode populations in infested fields. However, there has been limited success in finding or developing tobacco cultivars that are both resistant to TCN and have acceptable agronomic quality. The mode of inheritance of resistance to TCN in most resistant cultivars and breeding lines is poorly understood, which makes breeding for resistance more difficult.

Flue-cured tobacco cultivars such as Coker 371 Gold, Speight G-80, and NC 567 can reduce TCN population densities, but yield losses may occur when these cultivars are planted in highly infested fields. Early sources of TCN resistance were reported in *N. longiflora* Cavanilles, *N. glutinosa* L., and *N. plumbaginifolia* Viviani by Baalawy and Fox (1971). Additional sources of resistance include older dark fire-cured (DVA 606) and burley (BVA 523) tobacco breeding lines, which both appear to be multigenic in nature (Spasoff et al., 1971; Miller et al., 1972). In 1988, LaMondia tested flue-cured tobacco breeding line VA 81 and flue-cured cultivar PD 4 for resistance to *G. t. tabacum*. LaMondia (1988) demonstrated resistance and in a subsequent study (1991), determined that resistance to *G. t. tabacum* is conferred by a single dominant gene. Breeding line VA 81 and cultivar PD 4 are also resistant to *G. t. solanacearum.* However, the nature of inheritance of resistance to *G. t. solanacearum* in PD 4 and VA 81 has not been genetically evaluated (Herrero et al., 1996).

Herrero et al. (1996) observed low levels of TCN reproduction in the accessions 'Burley 21', Speight G-80, NC 567, 'Kutsaga Mammoth 10', Cyst 913, 9025-1, PD 4, 'Kutsaga 110', and VA 81. Hayes et al. (1995) used diallel analysis to study resistance of eight tobacco accessions to TCN. Six resistant accessions, PD 4, 'Burley 64', 'Pennbell 69', 'MD 40', TI 1597, and Kutsaga 110, and two susceptible accessions, 040-1 and 'K 394', were evaluated in a half-diallel design. Burley 64, Kutsaga 110, and TI 1597 were found to significantly contribute to increased resistance to TCN based on general combining ability (GCA) effects. General combining ability evaluates the additive portion of the genetic effects. GCA effects were found to account for the majority of variation observed among crosses, suggesting that additive gene action plays a significant role in the inheritance of resistance to TCN. Specific combining ability effects, which correspond to non-additive gene action, were not significant in this study (Hayes et al., 1995).

Komm and Terrill (1982) reported the use of wildfire resistance as a possible method for quickly and inexpensively identifying resistance to *G. t. solanacearum* in

tobacco cultivars containing *N. longiflora* germplasm. Hayes et al. (1997) evaluated several tobacco accessions to determine the reliability of using wildfire resistance as a screening tool for TCN resistance. Twenty-one accessions were demonstrated to be resistant to TCN, including previously unreported resistance in *N. miersii* Remy, *N. cordifolia* Philippi, 'TN 90', Burley 21, 'Burley 49', Burley 64, MD 40, Pennbell 69, 'Pennlan', TI 1597, and TI 1625. Tobacco introduction 551 and 'KY 190' were found to be resistant to wildfire, but susceptible to TCN, and *N. miersii* to be resistant to TCN, but susceptible to wildfire. Wildfire resistance was found to be highly correlated with TCN resistance, but the genes responsible for resistance to these two pathogens may not be as closely linked as previously thought. Some accessions evaluated did not possess a linkage between the two pathogens. Therefore, wildfire resistance may not be a reliable indicator of TCN resistance (Hayes et al., 1997).

For many years, tobacco cultivar PD 4 has been used as the conventional source of TCN resistance in flue-cured tobacco breeding programs (C. A. Wilkinson, personal communication, 1999). However, PD 4 has inferior agronomic quality compared to commercially available susceptible cultivars. Only within the past five years has a new source of resistance been used in tobacco breeding programs (C. A. Wilkinson, personal communication, 1999). This source of resistance comes from Kutsaga 110, which is not commercially grown in the United States. Kutsaga 110 tends to sucker more than the typical cultivars grown in the US, which is an undesirable trait. Recently, Coker 371 Gold, which is grown in the US, has been shown to be resistant to TCN. Therefore, the potential use of Coker 371 Gold and Kutsaga 110 as possible sources of resistance in future breeding programs was examined. The objective of this study was to determine the mode of inheritance of resistance to TCN in the flue-cured tobacco cultivars Coker 371 Gold and Kutsaga 110.

MATERIALS AND METHODS

Greenhouse experiments were conducted at the Southern Piedmont Agricultural Research and Extension Center in Blackstone, Virginia, in 1999 and 2000. Crosses were made between the TCN susceptible commercial flue-cured tobacco cultivar K 326 and two resistant cultivars, Coker 371 Gold and Kutsaga 110, to produce F_1, F_2, BC_1P_s (susceptible parent X F_1), and BC_1P_r (resistant parent X F_1) seed. Coker 371 Gold (PI 552524) was developed in the USA and released in 1988. It has a high level of resistance to black shank [*Phytophthora parasitica* Dastur var. *nicotianae* (Breda de Haan) Tucker syn. *P. nicotianae* Breda de Haan var. *nicotianae* G. M. Waterhouse] and has been shown to possess resistance to TCN. Planting Coker 371 Gold is one of several cultivars currently recommended to growers as a method of reducing TCN population densities (Reed et al., 1999). Resistance to TCN has also been shown in Kutsaga 110, which was developed in Zimbabwe. In a diallel study, Kutsaga 110 was found to significantly contribute to increased resistance to TCN based on general combining ability (GCA) effects (Hayes et al., 1995). Each test was conducted twice as a randomized complete block design with 15 replications. One replication consisted of one P_s, one P_r, one F_1, seven F_2, seven BC_1P_s, and seven BC_1P_r for a total of 15 plants for non segregating generations and 105 plants for segregating generations evaluated in each test.

Inheritance of TCN Resistance in Coker 371 Gold and Kutsaga 110

Four-week-old seedlings were transplanted into 11-cm clay pots containing 300 cm^3 of 2:1 sterilized sand:soil mix. A piece of number two Whatman filter paper was placed in the bottom of each pot to retain the soil in the pot. Transplants were grown for two weeks prior to inoculation. TCN cysts for inoculum were extracted, using a modified Fenwick can (Caswell et al., 1985), from infested field soil collected from the B.J. Coffee farm, Kenbridge, VA and the Southern Piedmont Agricultural Research and Extension Center, Blackstone, VA. Cysts were dried on filter paper and stored in glass vials at room temperature prior to inoculation. TCN eggs were obtained by crushing cysts in a blender at high speed for 60 s. Each test pot was inoculated with 6000 TCN eggs and juveniles to ensure a high level of infection. Inoculations were performed by pipetting

the egg suspension into a trench around the root zone of a single tobacco plant. An additional 100 cm^3 of soil was added to each pot after inoculation to cover exposed eggs and encourage additional root growth (Hayes et al., 1997).

The first tests to evaluate Coker 371 Gold and Kutsaga 110 were watered daily using an automatic watering system. Fertilizer was applied once a week using a Dosatron® Injector (Dosatron® International Inc., 2090 Sunnydale Blvd., Clearwater, FL 33765 USA) at a rate of 125 mg/kg N of 20-10-20 (Miller Greenhouse Special Soluble Fertilizer, Miller Chemical & Fertilizer Corporation, Hanover, PA 17331, USA). The Dosatron® Injector was set to deliver 1 L of nutrient per 64 L of water. The automatic watering system was set up to deliver water/nutrient for 1 min/d. The watering time was increased to 2 min/d as the plants matured. The repeated tests were subirrigated and fertilized as described above. Greenhouse temperatures were maintained at approximately 24°C during the day and 18°C during the night.

Plants were scored for TCN resistance approximately 8 wk after inoculation. The shoots of each plant were removed and discarded. The root ball was removed from each pot and rinsed under a stream of tap water over a 400 mesh sieve. The contents of the sieve were rinsed into an erlenmeyer flask. The root ball of each plant was weighed and a one gram sample of fibrous root was randomly collected. The feeder root subsample and root wash materials were combined and stained with acid fuchsin (Byrd et al., 1983). The number of vermiform, swollen, pyriform, and adult nematodes were counted using a dissecting microscope. Cysts were extracted from the remaining soil in pots using a modified Fenwick can (Caswell, 1985). The number of cysts per pot were counted and crushed in a blender for 60 s to release the eggs. Eggs were suspended in tap water and stained with acid fuchsin. The number of TCN eggs in two 10 ml aliquots were counted for each sample and averaged to estimate the total number of eggs per pot. The number of cysts per pot and eggs per pot were standardized and expressed as number of cysts per 400 cm^3 of soil and number of eggs per 400 cm^3 of soil for statistical analysis.

Data Analysis

Standard deviations were proportional to the means, so the data were log transformed [$\log_{10}(x+1)$] prior to statistical analysis (SAS Institute, Inc., 1998). The GLM procedure of SAS was used to perform an analysis of variance and treatment means were compared using Duncan's multiple range test (Gomez and Gomez, 1984). Error variances were homogeneous between the Coker 371 Gold tests for all parameters measured except for the number of cysts per 400 cm^3 of soil. Therefore, a combined analysis was performed. Error variances between the Kutsaga 110 tests were heterogeneous for most parameters. However, genotype ranks were similar between tests, allowing for combined analysis (Herrero et al., 1996). Significant test by treatment interactions were observed, but were due to changes in magnitude, not to changes in genotype rank.

Genetic Analysis

Generation means analysis was performed using the SAS procedure IML (SAS Institute, Inc., 1998; Mather and Jinks, 1977) to assess the inheritance of resistance to TCN. A three-parameter model (*m, a,* and *d*) was fitted and tested for goodness of fit by a chi-square test with three degrees of freedom. The three genetic parameters were defined as follows: *m* = the midparent value, *a* = the amount of variation among the means resulting from the additive effect of the genes, and *d* = the amount of variation among the means resulting from the dominance effect of the genes. A six-parameter model (*m, a, d, aa, ad,* and *dd*) was fitted if a significant chi-square value (poor fit) was obtained for the three-parameter model. The six genetic parameters were defined as follows: *m* = the mean of the inbred population, *a* and *d* as defined for the three parameter model, *aa* = the amount of variation among the means attributed to additive X additive epistasis, *ad* = the amount of variation among the means resulting from additive X dominance epistasis, and *dd* = the amount of variation among the means resulting from dominance X dominance epistasis. Standard errors of genetic estimates were compared with the estimated genetic values to determine significance. If the absolute value of an

estimate was greater than twice its standard error, the estimate was considered significantly different from zero.

Estimating Gene Number

Quantitative estimates of the number of genes (n) segregating in the F_2 generation for TCN resistance based on the sum of swollen, pyriform, and adult nematodes, the number of cysts per 400 cm^3 of soil, and the number of eggs per 400 cm^3 of soil were obtained using the following formula: $n = \{(P_s - P_r)^2[1.5 - 2h(1-h)]\}/8[V_{F2} - (V_{Ps} + V_{Pr} + 2V_{F1})/4]$, where P_s = the mean of the susceptible parent (K 326), P_r = the mean of the resistant parent (Coker 371 Gold or Kutsaga 110), $h = (F_1 - P_r)/(P_s - P_r)$, F_1 = mean of the F_1 generation, V_{F2} = the variance of the F_2 generation, V_{Ps} = the variance of the susceptible parent, V_{Pr} = the variance of the resistant parent, and V_{F1} = the variance of the F_1 generation (Wright, 1968). This formula estimates the number of genes by dividing the square of the genotypic range by the genotypic variance. The genotypic range was estimated from the difference of the parental means, which provides a conservative estimate of the number of genes segregating for resistance. The genotypic variance was estimated by subtracting the environmental variance from the phenotypic variance of the segregating population. The environmental variance was estimated from the parental and F_1 populations, according to Mather and Jinks (1977), as follows: $V_E = (V_{Ps} + V_{Pr} + 2V_{F1})/4$, where V_E = the environmental variance, V_{Ps}, V_{Pr}, and V_{F1} are defined as described above.

Estimating Heritability

Broad-sense heritability (H) estimates were derived using population variance components in the following formula described by Wright (1968): $H = (V_{F2} - V_E)/V_{F2}$. Additive variance is a component of the total genetic variance and cannot be easily distinguished from the dominance and environmental components. However, an estimate of the additive variance can be obtained from backcross data to calculate narrow-sense heritability (Simmonds, 1979). Narrow-sense heritability (h^2) was estimated using F_2 and backcross generation variance components as described by Warner (1952): $h^2 = [2V_{F2} -$

$(V_{BC1Ps} + V_{BC1Pr})]/V_{F2}$, where V_{BC1Ps} and V_{BC1Pr} = the variance for the backcross of the F_1 to the susceptible parent and to the resistant parent, respectively. Genetic gains per cycle for selection at the 10% level (Gs) were calculated as $Gs = 1.76(h^2)(V_{F2})$. The degree of dominance was calculated as the deviation of the F_1 from the midparent divided by the departure of the resistant parent from the midparent (Mather and Jinks, 1982). Heterosis was calculated as the difference between the F_1 mean and the resistant parent mean according to Mather and Jinks (1982).

RESULTS

The number of vermiform, swollen, pyriform, and adult nematodes observed in the roots, the number of cysts per 400 cm^3 of soil, and the number of eggs per 400 cm^3 of soil were analyzed by an analysis of variance for each test. Since vermiform nematodes invade both resistant and susceptible roots, resistance is evaluated by the ability of the nematode to establish a feeding site and thereby develop into an adult cyst nematode (Baalawy and Fox, 1971). Therefore, the sum of swollen, pyriform, and adult nematodes observed in the roots was also analyzed.

Significant differences were observed among generation means for each stage of development for each cross in each test and in the combined analyses (Appendix Tables 1, 2, 3, 4, 5, and 6). Significant differences were detected between tests for the K 326 X Coker 371 Gold cross based on adult nematode and egg counts only. Differences between tests were not detected for other measured parameters. Significant test by generation mean interactions were detected for vermiform, adult, cyst, and egg counts. No significant test by generation mean interactions were shown for swollen, pyriform, and sum of swollen, pyriform, and adult counts. When a combined analysis was performed for the K 326 X Kutsaga 110 cross, significant differences were detected between tests for all measured parameters except vermiform counts. Significant test by generation mean interactions were detected for all parameters measured except cyst and egg counts. A graphical presentation of the data showed this interaction to be the result of changes in magnitude between the generation means and not changes in rank; therefore, a combined analysis could be justified (Herrero et al., 1996).

Generation means for the parameters measured are shown in Table 1. The sum of swollen, pyriform, and adult nematodes was used as a measure of resistance because summing these life stages eliminates some variability associated with nematode development and provides a more useful indicator of resistance.

The sum of swollen, pyriform, and adult nematodes for the resistant parent was significantly different from the susceptible parent for the K 326 X Coker 371 Gold cross. Although the F_1 generation mean was in between the two parents, it was not significantly different from the resistant parent in either test. The F_2 generation mean was not significantly different from the F_1 generation mean in either test. The BC_1P_s generation mean was intermediate between the two parents and was significantly different from both parent generation means in both tests. The BC_1P_r generation mean was also intermediate between the two parents and was not significantly different from the resistant parent generation mean in either test. The BC_1P_s generation mean was more susceptible than the BC_1P_r generation mean. Similar trends were observed for the individual means comprising the sum of swollen, pyriform, and adult nematodes for each test (Appendix Tables 1, 2, and 3).

Similar trends were observed for cysts per 400 cm^3 of soil generation means for both tests. However, the F_2 generation mean in test 1 was significantly different from the F_1 generation mean. Egg count means per 400 cm^3 of soil also showed similar generation mean ranks in the first test. However, the F_1 generation mean was not significantly different from the susceptible parent mean in the second test and the F_2 generation mean was not significantly different from any other generation mean in test 2.

Generation means for each parent for the sum of swollen, pyriform, and adult nematodes in the K 326 X Kutsaga 110 cross were significantly different from each other in both tests. The F_1 generation mean was not significantly different from the resistant parent generation mean in either test. The F_2 generation mean was not significantly different from the F_1 generation mean in either test. The BC_1P_s generation mean was significantly different from both parent generation means in both tests, but was not significantly different from the F_2 generation mean in test 1. The BC_1P_r generation mean was not significantly different from the resistant parent generation mean in either test. Similar relationships among generation means were observed for the individual swollen, pyriform, and adult nematode count means (Appendix Tables 4, 5, and 6).

Parent generation means were significantly different from each other based on cyst counts per 400 cm^3 of soil for both tests. The F_1 generation mean was significantly different from the resistant parent in test 1, but not in test 2. The F_2 generation mean was significantly different from the F_1 generation mean in test 2, but not in test 1. The BC_1P_s generation mean was significantly different from both parents in test 1, but not significantly different from the susceptible parent generation mean in test 2. The BC_1P_r generation mean was significantly different from both parents in test 1, but not significantly different from the resistant parent generation mean in test 2. The BC_1P_r generation mean was not significantly different from the F_2 generation mean in either test. Egg counts per 400 cm^3 of soil showed similar generation mean ranks between tests.

Fit to a 3:1 Segregation Ratio

Graphical representation of the F_2 data (Figure 1) led to testing the data for fit to a 3:1 (resistant:susceptible) segregation ratio (Table 2). The F_2 data were skewed toward the resistant parent with a large portion of the progeny being resistant and a smaller portion exhibiting a range of susceptibility. Progeny were classified as resistant when the number of nematodes for the measured parameter was below a threshold of 10% of the susceptible parent mean. Chi-square values below 3.84 were obtained for the sum of swollen, pyriform, and adult nematodes, the number of cysts per 400 cm^3 of soil, and the number of eggs per 400 cm^3 of soil, indicating acceptable fits to the 3:1 ratio in the first test for the K 326 X Coker 371 Gold cross. The sum of swollen, pyriform, and adult nematodes in the second test also fit a 3:1 ratio, but the number of cysts and eggs did not. Poor fit to the 3:1 ratio for cysts and eggs is probably related to poor nematode development in the second test. BC_1P_s data (Figure 2) fit a 1:1 segregation ratio and support these findings when F_2 data fit the 3:1 ratio.

Fit to a 3:1 ratio, based on 10% of the susceptible parent mean threshold values, was rejected for the K 326 X Kutsaga 110 cross. Graphical representation of the F_2 data (Figure 3) appeared to be more continuous than F_2 data for the K 326 X Coker 371 Gold cross, however data were skewed toward the resistant parent. To obtain a good fit to the

3:1 ratio, threshold values would have to be larger and would include genotypes not considered to be resistant.

Generation Mean Analyses

The three-parameter model was sufficient for explaining the inheritance of resistance to TCN for the K 326 X Coker 371 Gold cross. Results from analyses of individual life stages were inconsistent (Appendix Tables 7, 8, 9, and 10). Mean, additive, and dominance effect estimates were significant in both tests and the combined analyses for sum of swollen, pyriform, and adult nematodes and the number of cysts per 400 cm^3 of soil (Tables 3 and 4). Additive effects were positive and similar in magnitude to dominance effect estimates which were all negative. Mean effect estimates were similar to additive effects estimates. Additive effect estimates seemed to be important in the K 326 X Coker 371 Gold cross. Significant chi-square values were obtained based on egg counts per 400 cm^3 of soil, indicating a poor fit to the three-parameter model.

The three-parameter model was sufficient for explaining the inheritance of resistance to TCN for the K 326 X Kutsaga 110 cross based on all parameters measured except for the sum of swollen, pyriform, and adult nematodes in the second test. The poor fit to the three-parameter model in test 2 for the cross can be attributed to the poor reproduction in the test. Mean effect estimates were similar to or larger than additive effect estimates for all parameters (Tables 3, 4, and 5). Additive effect estimates were all positive and significant. Dominance effect estimates were all negative and were significant in both tests for all parameters except in the second test based on egg counts per 400 cm^3 of soil. Additive effect estimates were larger in magnitude than dominance effect estimates for all parameters. Additive genetic effects account for a greater portion of variation in the K 326 X Kutsaga 110 cross as compared to the K 326 X Coker 371 Gold cross.

A six-parameter model was tested when the three-parameter model was insufficient in explaining the inheritance of resistance to TCN (Appendix Tables 11, 12,

13, 14, 15, 16, and 17). Therefore, resistance based on egg counts per 400 cm^3 of soil for the K 326 X Coker 371 Gold cross and resistance based on the sum of swollen, pyriform, and adult nematodes for the K 326 X Kutsaga 110 cross were tested for fit to a six-parameter model. Chi-square values indicate fits to the six-parameter model for both tests and the combined analysis for both crosses (Table 6).

For the K 326 X Coker 371 Gold cross, additive gene effect estimates were positive and significant for both tests and the combined analysis. Dominance effect estimates were larger in magnitude than additive effect estimates but were not significant. Significant positive additive X dominance and negative dominance X dominance epistatic effect estimates were obtained for the second test. Both estimates were larger in magnitude than the additive effect estimate. Additive, additive X dominance, and dominance X dominance effect estimates are important in the second test for the K 326 X Coker 371 Gold cross based on egg counts.

For the K 326 X Kutsaga 110 cross, additive effect estimates were positive and significant for both tests. Dominance effect estimates were negative, smaller in magnitude than additive effect estimates, and non significant. A significant positive additive X additive effect estimate was obtained in the second test. However, no other significant epistatic effects were obtained.

Variance Components, Heritability Estimates, and Gene Number

Variance components were estimated and used to calculate broad- and narrow-sense heritability for both crosses based on the sum of swollen, pyriform, and adult nematodes, the number of cysts per 400 cm^3 of soil, and the number of eggs per 400 cm^3 of soil. For the K 326 X Coker 371 Gold cross, additive variance was negative for both tests based on the sum of swollen, pyriform, and adult nematodes (Table 7). Dominance variance was positive in both tests for this parameter, but was smaller in magnitude than the additive variance in the first test. Environmental variance was smaller in magnitude than additive and dominance variances for all parameters in both tests. Negative broad-

and narrow-sense heritabilities were obtained in the first test based on the sum of swollen, pyriform, and adult nematodes. Negative narrow-sense heritability was obtained in the second test for this parameter. Negative heritability estimates are due to the negative additive variance component in the two tests and were set to zero. The broad-sense heritability estimate in the second test was 0.72.

Broad-sense heritability estimates based on cyst counts were 0.65 and 0.48 for the first and second test respectively. Negative narrow-sense heritability was obtained in the second test based on cyst counts due to negative additive variance. Results were similar for narrow-sense heritability estimates based on egg counts. Broad-sense heritability estimates were 0.81 for the first test and 0.37 for the second test based on egg counts.

Gene number estimates based on Wright's formula (1968) were less than one for both tests for each parameter and was negative for the first test based on the sum of swollen, pyriform, and adult nematodes. Heterosis was significant for both tests based on all parameters. Due to the zero estimate of narrow-sense heritability, the genetic gain per cycle for selection at the 10% level (Gs) was estimated to be zero. Positive genetic gain estimates were evident in the first test based on cyst counts and in the first test based on egg counts.

For the K 326 X Kutsaga 110 cross, heritability estimates were considered zero due to negative values for both tests based on the sum of swollen, pyriform, and adult nematodes (Table 8). Additive variance estimates were negative in both tests for this parameter. Negative estimates for gene number were also obtained for both tests for this parameter. Additive variance based on cyst counts was negative in the first test and positive in the second test. Broad-sense heritabilities were low in both tests (0.19 and 0.33). Wright's formula estimated 2.41 genes in the first test based on cyst counts. However, estimates of gene number were less than one for the second test and for both tests based on egg counts. Broad-sense heritability estimates were moderate to large based on eggs counts (0.84 and 0.61). Heterosis was significant for both tests based on all parameters. Due to the zero estimate of narrow-sense heritability, the genetic gain per

cycle for selection at the 10% level (Gs) was estimated to be zero. Positive genetic gain was evident in the second test based on cyst counts.

DISCUSSION

Inheritance of resistance to TCN in Coker 371 Gold and Kutsaga 110 appears to be different. Both cultivars possess partial dominance for resistance based on F_1 generation means (Table 1). The F_1 generation means were intermediate to the parental means, but deviate from the midparent value toward the resistant parent mean. Graphical representation of the F_2 data show the distribution of data to be skewed toward the resistant parent mean for both cultivars (Figures 1 and 3). The F_2 distribution for the K 326 X Coker 371 Gold cross fit a 3:1 segregation ratio when the threshold for resistance was 10% of the susceptible parent mean (K 326) (Table 2). The BC_1P_s data (Figure 2) support the F_2 data by fitting a 1:1 segregation ratio. These findings suggest the presence of a single dominant gene in Coker 371 Gold conditioning resistance to TCN. LaMondia (1991) found similar results for the cultivar PD 4 and the breeding line VA 81 when evaluating resistance against *G. t. tabacum*. *Globodera tabacum tabacum* and *G. t. solanacearum* are part of a species complex and are morphologically very similar to one another, differing slightly in host preference. Therefore, it is reasonable to conclude that the inheritance of resistance to TCN for the two subspecies would be similar.

Coker 371 Gold has high resistance to black shank [*Phytophthora parasitica* Dastur var. *nicotianae* (Breda de Haan) Tucker syn. *P. nicotianae* Breda de Haan var. *nicotianae* G. M. Waterhouse] race 0, which was probably derived from an interspecific source. The black shank resistance in Coker 371 Gold closely resembles the resistance to race 0 obtained from *N. longiflora* or *N. plumbaginifolia* (Carlson et al., 1997). Baalawy and Fox (1971) found *N. longiflora* and *N. plumbaginifolia* to be resistant to tobacco cyst nematode. However, it is not known if *N. longiflora* or *N. plumbaginifolia* are in the parentage of Coker 371 Gold. If one of these species is in the background of Coker 371 Gold, resistance to tobacco cyst nematode is most likely derived from that source.

Resistance to TCN in Kutsaga 110 appears to be quantitative in nature. The F_2 generation data was more continuous, although a large proportion of the plants were similar to the resistant parent (Figure 3). Using 10% of the susceptible parent mean as a

threshold value for resistance was not sufficient to achieve a fit to the 3:1 segregation ratio (Table 2). The threshold limit would have to be larger than 10% of the susceptible parent mean to achieve a fit to the 3:1 ratio. Increasing this threshold would include susceptible phenotypes and would not accurately portray the inheritance of resistance in Kutsaga 110.

Kutsaga 110 is an anther-derived dihaploid from the cross TW 438 X Kutsaga 51E (Anne Jack, personal communication, 2000). TW 438 is a breeding line with resistance to wildfire and tobacco mosaic virus and has Burley 21 in its parentage. Resistance to TCN may be derived from *N. longiflora*, incorporated via Burley 21. However, there are also quantitative genes in Kutsaga 110 for tolerance to wildfire derived from Meadows Giant and Kutsaga 51 which may or may not be associated with TCN resistance.

Hayes et al. (1995) performed a diallel analysis involving Kutsaga 110 and found general combining ability to be significant. In the study, Kutsaga 110 contributed significantly to increased resistance to TCN based on general combining ability effects. Since general combining ability is a function of additive genetic effects, additive gene action plays a significant role in the inheritance of resistance to TCN in Kutsaga 110 (Hayes et al., 1995).

The findings in the study by Hayes et al. (1995) support the findings from the generation means analysis conducted in this study. Generation means analysis showed that additive gene action plays a role in the resistance to TCN in Coker 371 Gold and Kutsaga 110 (Tables 3, 4, 5, and 6). Additive gene action accounted for a larger proportion of the variation in Kutsaga 110 than in Coker 371 Gold. Dominance gene effects accounted for a larger proportion of the variation in Coker 371 Gold when compared to Kutsaga 110. Dominance gene effects were negative in both the K 326 X Coker 371 Gold cross and the K 326 X Kutsaga 110 cross because resistance values are obtained by measuring in the negative direction or corresponding smaller values.

It is possible that TCN resistance in Coker 371 Gold may have been derived from a source similar to the resistance in the breeding line VA 81 and the cultivar PD 4. Resistance to TCN in the breeding line VA 81 was obtained from the breeding line Burley 523. All commercial burley cultivars grown today have high resistance to wildfire which can be traced back to TL 106. Spasoff et al. (1971) evaluated resistance to TCN in Burley 523 and concluded that resistance to TCN is multigenic in this breeding line. This contrasts with LaMondia's (1991) results which suggest resistance to *G. t. tabacum* is controlled by a single dominant gene.

Resistance to TCN in the cultivar PD 4 is probably derived from Burley 21. Burley 21 was the first burley tobacco to be developed with resistance to wildfire [*Pseudomonas syringae* pv. *tabaci* (Wolf & Foster) Young et al.] (Heggestad, 1966). The breeding line TL 106 is included in the parentage of Burley 21. A single dominant gene for resistance to wildfire was transferred from *N. longiflora* to TL 106. The gene for wildfire resistance may be linked, although not tightly, to the gene(s) for tobacco cyst nematode resistance (Hayes et al., 1997; Komm and Terrill, 1982). Therefore, resistance to TCN in PD 4 is most likely derived from *N. longiflora*. LaMondia (1991) concluded that a single dominant gene confers resistance to *G. t. tabacum* in VA 81 and PD 4, which is consistent with the findings in this study for Coker 371 Gold.

Gene number estimates based on Wright's formula (1968) are conservative, expressing the minimum number of genes controlling a character. Gene number estimates obtained in this study were less than one for all parameters measured except in the first test based on cyst counts per 400 cm^3 of soil for the K 326 X Kutsaga 110 cross where the estimate was greater than two (Tables 7 and 8). Several assumptions are made when using Wright's (1968) gene number (n) formula. The parent genotypes for the trait of interest, resistance to TCN, should be at opposite extremes. The loci are segregating independently of each other and their effects are additive. All loci make equal contributions and there is semi-dominance at all loci (Wright, 1968). Breaking any of these assumptions will lead to an underestimate of gene number.

Broad-sense heritabilities ranged from low to high depending on the parameter evaluated (Tables 7 and 8). Narrow-sense heritabilities were estimated to be zero for many parameters due to negative additive variance, but were otherwise low. Negative additive variances were most likely obtained because the variability in the data was large enough to produce negative estimates, even though the true value of the variance component was positive. Environmental variation and variation in nematode development made it difficult to obtain reliable estimates of heritability and genetic gain.

Heterogeneous error variances between tests can be attributed to the different irrigation methods used for each test. Drip irrigation was used in the first test for each cross while subirrigation was used in the second test. The drip irrigated test had greater nematode reproduction as is reflected in the mean nematode counts for each life stage. Therefore, drip irrigation is recommended in further cyst nematode studies.

Reports on mode of inheritance of TCN resistance have been contradictory. Discrepancies can be due to source of resistance, variation in environmental conditions under which plants were evaluated, and criteria used for assessing resistance. Nematode reproduction is highly influenced by environmental factors, complicating the study of resistance. Reproducing results from potato cyst nematode experiments is very difficult because of the variability in nematode reproduction due to environmental influences (Phillips et al., 1989). Cyst nematodes are greatly influenced by temperature (Adams et al., 1982). The rate of development, size of cysts, and number of eggs and larvae in cysts of *G. t. solanacearum* vary with different soil temperatures (Adams et al., 1982). Egg hatching depends on the concentration of root exudates and temperature (Wang, 1996). Root exudates stimulate hatching and hatching seems to increase with increasing temperature (Wang, 1996). LaMondia (1995) observed hatching of *G. t. tabacum* on tobacco, tomato, and black nightshade roots and showed that hatching was stimulated by root exudates. The relationship between dilution of host exudates or plant age and hatch was similar, but the magnitude of hatch was greatly reduced when the experiments were repeated (LaMondia, 1995). These kinds of variability can be reflected in the range of susceptibility observed among plants.

The tests in this study were inoculated with eggs. The sum of swollen, pyriform, and adult nematodes was counted and used as an indicator of resistance to account for differential hatching among pots. LaMondia (1991) inoculated with juveniles, eliminating the variability associated with hatching and rated resistance according to the number of white females observed on the roots. While methods for scoring resistance differed between LaMondia's (1991) study and this study, results for Coker 371 Gold were consistent with those for VA 81 and PD 4. It is reasonable that inheritance of resistance to TCN in Kutsaga 110 be different from that in Coker 371 Gold. While Kutsaga 110 has *N. longiflora* in its parentage, quantitative genes from other sources could contribute to resistance to TCN. Inoculating with juveniles would help reduce the variability associated with hatching and ensure more uniform infection of plants. Inoculating with juveniles would also reduce the amount of time required for the nematode to complete its lifecycle, thus allowing for more rapid screening of plants for resistance.

The choice of breeding strategy for incorporating TCN resistance into agronomically acceptable cultivars would depend on the type and magnitude of gene effects. Additive and dominance gene effects were equally important in the K 326 X Coker 371 Gold cross, indicating that selection would be efficient. However, developing hybrids with Coker 371 Gold may be appropriate. Resistance to TCN appears to be inherited as a single dominant gene in Coker 371 Gold. This would allow for quick incorporation of resistance into agronomically superior lines. Additive gene effects were more important in the inheritance of resistance to TCN in the K 326 X Kutsaga 110 cross than in the K 326 X Coker 371 Gold cross. Therefore, a breeding program that leads to development of desirable homozygous cultivars would be suggested. It may be beneficial to test for allelism between Coker 371 Gold and Kutsaga 110 for TCN resistance. The gene for resistance in Coker 371 Gold may have been part of a quantitative trait loci that was responsible for a major portion of the genetic variation. This would also provide evidence as to whether or not resistance to TCN in Coker 371 Gold was derived from the interspecific source *N. longiflora*.

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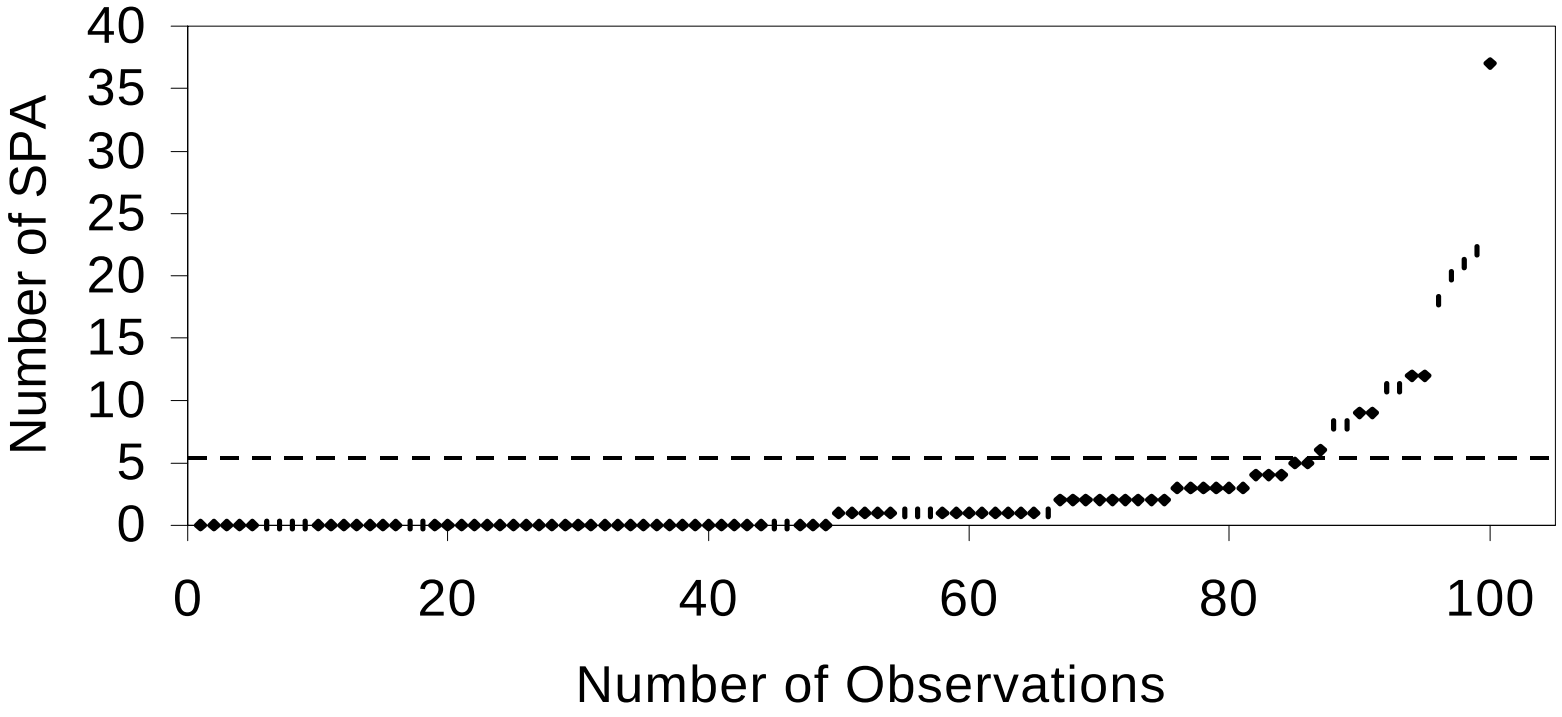

Figure 1. Distribution of the sum of swollen, pyriform, and adult nematodes (SPA) in the F_2 progeny from test one of the tobacco cross K 326 X Coker 371 Gold. The dashed line represents the resistance threshold calculated as 10% of the susceptible parent (K 326) mean.

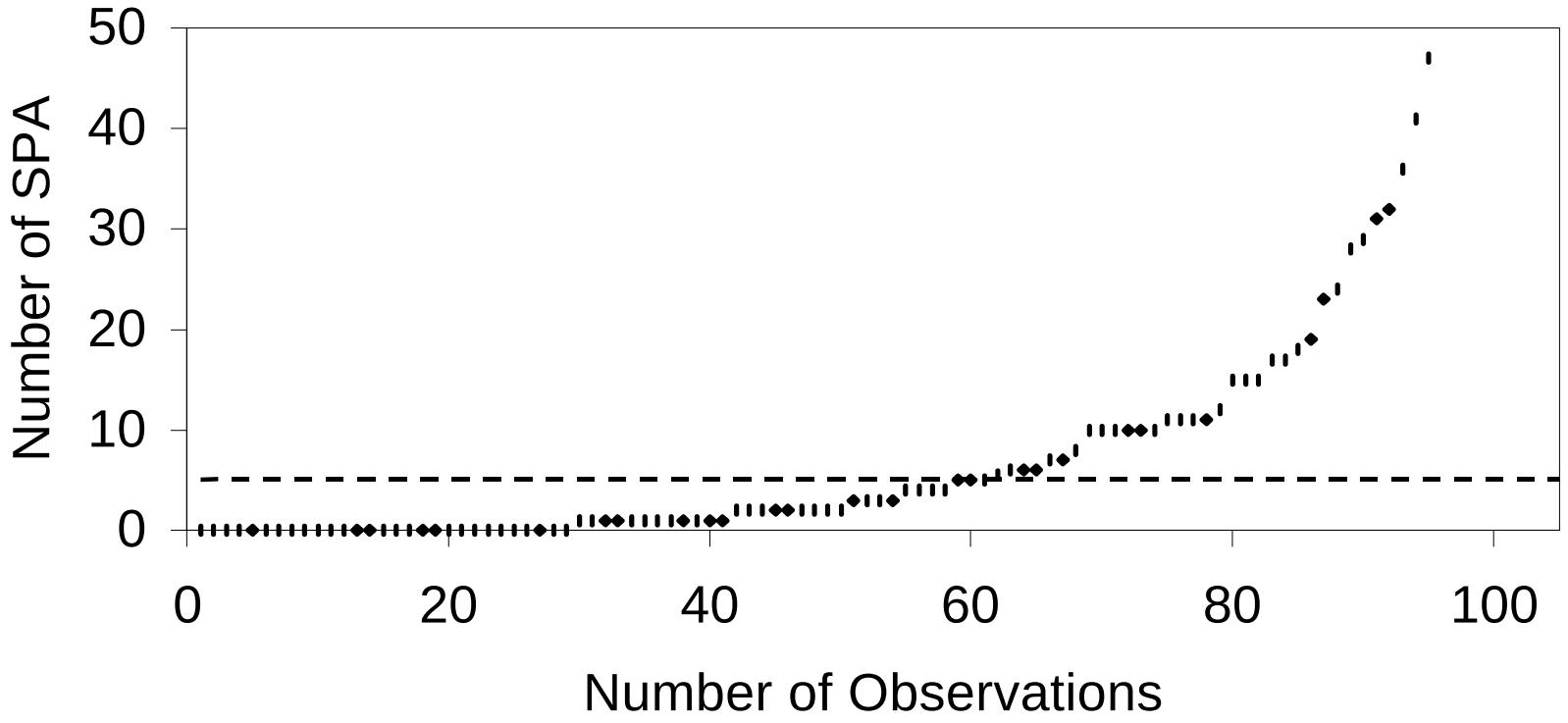

Figure 2. Distribution of the sum of swollen, pyriform, and adult nematodes (SPA) in the BC_1P_s progeny from test one of the tobacco cross K 326 X Coker 371 Gold. The dashed line represents the resistance threshold calculated as 10% of the susceptible parent (K 326) mean.

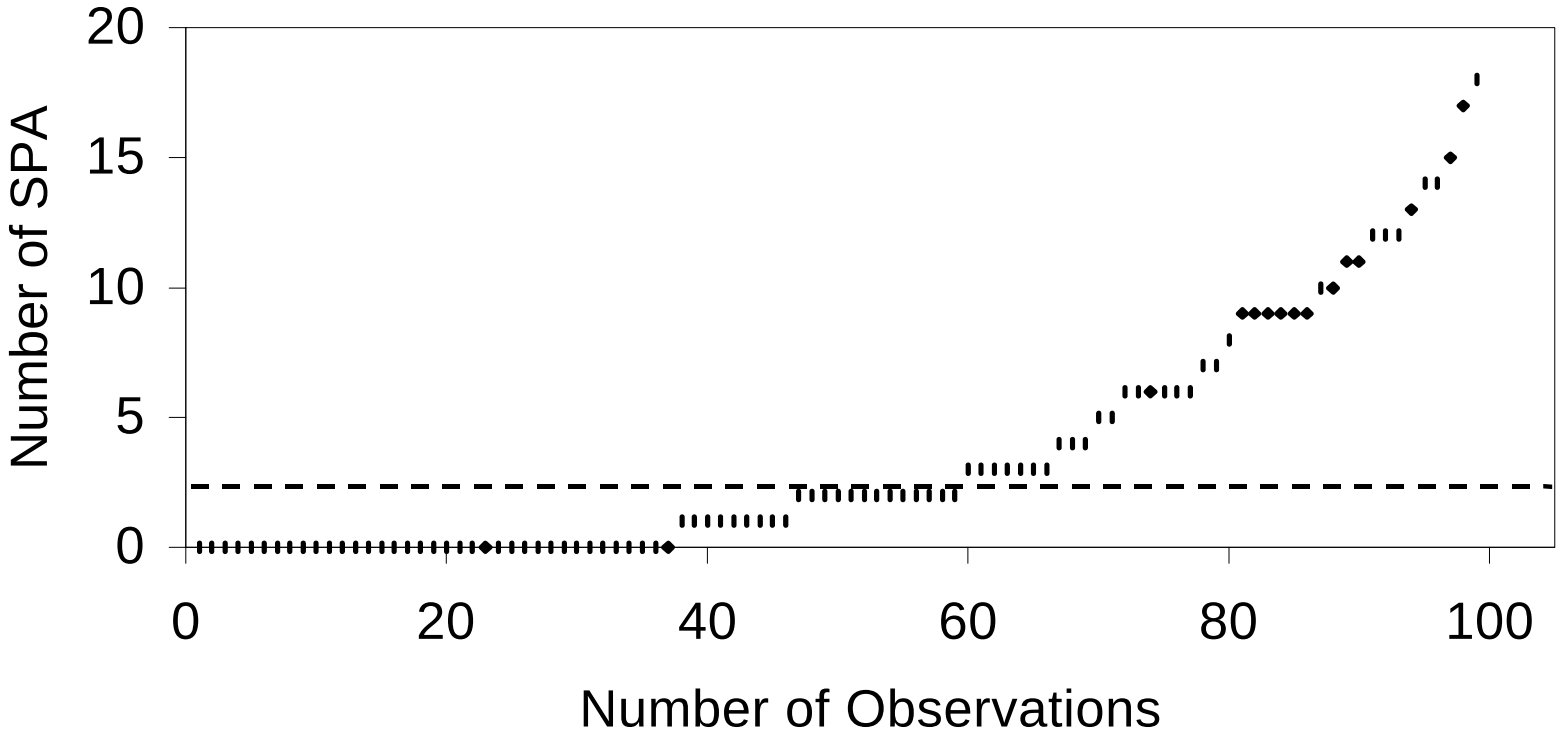

Figure 3. Distribution of the sum of swollen, pyriform, and adult nematodes (SPA) in the F_2 progeny from test one of the tobacco cross K 326 X Kutsaga 110. The dashed line represents the resistance threshold calculated as 10% of the susceptible parent (K 326) mean.

Table 1. Means for tobacco cyst nematode (TCN) resistance based on the sum of swollen, pyriform, and adult nematodes per 1-g root sample, the number of cysts per 400 cm^3 of soil, and the number of eggs per 400 cm^3 of soil for the two tobacco crosses K 326 X Coker 371 Gold and K 326 X Kutsaga 110 for two tests.

	Sum of Swollen, Pyriform, and Adult		Cysts per 400 cm^3 of Soil		Eggs per 400 cm^3 of Soil	
Cross	Test 1†	Test 2	Test 1	Test 2	Test 1	Test 2
K 326 X Coker 371 Gold						
P_s	54.1 a	9.1 a	107.2 a	16.5 a	11265 a	253 ab
P_r	0.5 d	0.3 c	0.7 d	1.3 d	258 cd	76 bc
F_1	1.2 cd	1.2 c	2.5 d	2.6 cd	436 cd	154 a
F_2	7.3 c	3.3 c	45.6 c	5.8 c	5270 c	183 abc
BC_1P_s	26.6 b	8.6 b	60.0 b	13.1 b	7538 b	530 ab
BC_1P_r	0.9 cd	0.1 c	1.1 d	1.7 cd	153 d	76 c
K 326 X Kutsaga 110						
P_s	32.9 a	6.1 a	223.8 a	15.8 a	7210 a	1156 a
P_r	0.9 d	0.0 c	34.7 e	2.0 c	261 c	70 c
F_1	2.7 cd	0.3 c	79.6 c	2.2 c	1800 b	397 bc
F_2	5.6 bc	0.3 c	91.9 cd	6.3 b	3495 b	700 bc
BC_1P_s	13.2 b	1.4 b	153.2 b	7.8 a	7529 b	572 b
BC_1P_r	2.2 d	0.1 c	59.7 d	2.6 bc	935 c	207 bc

† Generation means represent the sum of swollen, pyriform, and adult nematodes per 1-g root sample, the number of cysts per 400 cm^3 of soil, and the number of eggs per 400 cm^3 of soil from non-transformed data. Letters represent significance of log base 10 transformed data. Means followed by the same letter within a test for a given parameter are not significantly different at the 0.05 level based on Duncan's multiple range test.

Table 2. Phenotypic ratios of resistant to susceptible (R:S) plants for two tobacco crosses, K 326 X Coker 371 Gold and K 326 X Kutsaga 110, based on the sum of swollen, pyriform, and adult nematodes, the number of cysts per 400 cm^3 of soil, and the number of eggs per 400 cm^3 of soil.

Cross	No. of F_2 Plants R:S	χ^2 3:1	P-value	No. of BC_1P_s Plants R:S	χ^2 1:1	P-value
K 326 X Coker 371 Gold						
Test 1						
SPA†	86:19	2.67	0.05-0.20	61:44	2.75	0.05-0.20
Cyst	74:31	1.15	0.20-0.30	44:60	2.46	0.05-0.20
Egg	80:25	0.08	0.70-0.80	50:54	0.15	0.50-0.70
Test 2						
SPA	78:27	0.03	0.80-0.95	60:45	2.14	0.05-0.20
Cyst	49:56	44.96	<0.01	24:81	30.94	<0.01
Egg	37:68	88.54	<0.01	25:80	28.81	<0.01
K 326 X Kutsaga 110						
Test 1						
SPA	66:39	8.26	<0.01	57:48	0.77	0.30-0.50
Cyst	20:85	175.32	<0.01	12:91	60.59	<0.01
Egg	59:46	19.81	<0.01	45:58	1.64	0.20-0.30
Test 2						
SPA	90:15	6.43	0.01-0.05	58:45	1.64	0.20-0.30
Cyst	39:66	80.26	<0.01	14:91	56.47	<0.01
Egg	57:48	24.03	<0.01	38:67	8.01	<0.01

† SPA = the sum of swollen, pyriform, and adult nematodes, Cyst = the number of cysts per 400 cm^3 of soil, Egg = the number of eggs per 400 cm^3 of soil.

Table 3. Estimates of genetic effects for tobacco cyst nematode resistance based on the non-transformed sum of swollen, pyriform, and adult counts on two flue-cured tobacco crosses fitted to a three-parameter model.

Cross and test	Parameter†			
	m	*a*	*d*	χ^2‡
K 326 X Coker 371 Gold				
Test 1	16.69**§	16.17**	-15.47**	2.36
Test 2	6.17**	5.95**	-6.15**	6.56
Average	11.74**	11.46**	-10.83**	1.94
K 326 X Kutsaga 110				
Test 1	10.54**	9.65**	-7.69**	4.25
Test 2	0.77**	0.77**	-0.63**	11.22
Average	5.74**	5.30**	-4.24**	3.24

† Mean, additive, and dominance genetic effects for the model $y = m + a + d$, where y equals the generation mean.

‡ For values larger than 7.81, the probability of a fit is less than 0.05.

§ * And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

Table 4. Estimates of genetic effects for tobacco cyst nematode resistance based on non-transformed cyst counts per 400 cm^3 of soil on two flue-cured tobacco crosses fitted to a three-parameter model.

Cross and test	Parameter†			χ^2‡
	m	*a*	*d*	
K 326 X Coker 371 Gold				
Test 1	62.17**§	61.54**	-60.41**	4.37
Test 2	10.20**	9.06**	-7.90**	1.85
Average	36.79**	35.92**	-34.74**	2.13
K 326 X Kutsaga 110				
Test 1	123.13**	87.78**	-42.44*	1.46
Test 2	8.02**	5.74**	-5.34**	2.13
Average	65.82**	47.05**	-24.07*	0.66

† Mean, additive, and dominance genetic effects for the model $y = m + a + d$, where y equals the generation mean.

‡ For values larger than 7.81, the probability of a fit is less than 0.05.

§ * And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

Table 5. Estimates of genetic effects for tobacco cyst nematode resistance based on non-transformed egg counts per 400 cm^3 of soil on two flue-cured tobacco crosses fitted to a three-parameter model.

Cross and test	Parameter†			χ^2‡
	m	*a*	*d*	
K 326 X Coker 371 Gold				
Test 1	7204**§	7074**	-6997**	11.51
Test 2	206**	144**	-82	9.20
Average	3782**	3684**	-3626**	6.61
K 326 X Kutsaga 110				
Test 1	4427**	4169**	-2734**	2.18
Test 2	500**	431**	-149	2.42
Average	2558**	2395**	-1533*	1.10

† Mean, additive, and dominance genetic effects for the model $y = m + a + d$, where y equals the generation mean.

‡ For values larger than 7.81, the probability of a fit is less than 0.05.

§ * And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

Table 6. Estimates of genetic effects for tobacco cyst nematode resistance based on non-transformed egg counts per 400 cm^3 of soil on the flue-cured tobacco cross K 326 X Coker 371 Gold and the sum of swollen, pyriform, and adult nematodes on the flue-cured tobacco cross K 326 X Kutsaga 110 fitted to a six-parameter model.

	Parameter†						
Cross and test	*m*	*a*	*d*	*aa*	*ad*	*dd*	χ^2
K 326 X Coker 371 Gold	Egg Counts per 400 cm^3 of Soil						
Test 1	11460	5503**‡	-13735	-5699	3762	2711	1×10^{-20}
Test 2	-314	88*	1521	478	732*	-1053*	5×10^{-25}
Average	5573	2796*	-6107	-2610	2247	829	3×10^{-21}
K 326 X Kutsaga 110	Sum of Swollen, Pyriform, and Adult Nematodes						
Test 1	8.60	16.00**	-6.06	8.27	-10.06	0.19	1×10^{-24}
Test 2	1.24	3.07*	-2.84	1.83*	-3.44	1.86	3×10^{-18}
Average	4.92	9.53**	-4.45	5.05	-6.75	1.03	2×10^{-25}

† Mean, additive, dominance, additive X additive, additive X dominance, and dominance X dominance genetic effects for the model $y = m + a + d + aa + ad + dd$, where y equals the generation mean.

‡ * And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

Table 7. Estimates of additive (V_A), dominance (V_D), and environmental (V_E) variances, broad-(H) and narrow-(h^2) sense heritabilities, genetic gain through selection (Gs), number of genes (n), dominance ratio (D_R), and heterosis for *Globodera tabacum solanacearum* resistance based on the sum of swollen, pyriform, and adult nematodes, the number of cysts per 400 cm^3 of soil, and the number of eggs per 400 cm^3 of soil in tobacco cross K 326 X Coker 371 Gold for two tests.

	Sum of Swollen, Pyriform, and Adult		Cysts per 400 cm^3 of Soil		Eggs per 400 cm^3 of Soil	
Cross	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
K 326 X Coker 371 Gold						
V_A†	-7234.86	-159.60	9777.74	-270.51	241721643.19	-1588859.52
V_D	4502.24	245.28	-4639.48	349.83	-73804800.64	1615010.58
V_E	3226.79	33.94	2817.72	84.51	39485468.74	45188.22
H‡	0.00	0.72	0.65	0.48	0.81	0.37
h^2	0.00	0.00	1.23	0.00	1.17	0.00
Gs§	0.00	0.00	17208.82	0.00	425430092.01	0.00
n¶	0.00	0.15	0.40	0.49	0.13	0.15
D_R#	-1.12	-1.75	-0.97	-1.61	-0.78	-1.45
Heterosis††	0.73	0.93	1.81	1.29	178.43	77.66

† Variance components calculated as follows: $V_A = 2V_{F2} – (V_{BC1Ps} + V_{BC1Pr})$, $V_E = (V_{Ps} + V_{Pr} + 2V_{F1})/4$, $V_D = V_{BC1Ps} + V_{BC1Pr} - V_{F2} – V_E$.

‡ Heritabilities calculated as follows: $H = (V_{F2} – V_E)/V_{F2}$, $h^2 = V_A/V_{F2}$.

§ Genetic gain for selection at 10% calculated as follows: $Gs = 1.76(h^2)(V_{F2})$.

¶ Number of genes (n) calculated as follows: $n = \{(P_s – P_r)^2[1.5 – 2h(1-h)]\}/8[V_{F2} – (V_{Ps} + V_{Pr} + 2V_{F1})/4]$, $h = (F_1 – P_r)/(P_s – P_r)$.

Dominance ratio is the degree of dominance.

†† Heterosis calculated as the difference between the F_1 mean and the resistant parent mean (P_r).

‡‡ Estimate assumed to be zero due to negative estimate.

Table 8. Estimates of additive (V_A), dominance (V_D), and environmental (V_E) variances, broad-(H) and narrow-(h^2) sense heritabilities, genetic gain through selection (Gs), number of genes (n), dominance ratio (D_R), and heterosis for *Globodera tabacum solanacearum* resistance based on the sum of swollen, pyriform, and adult nematodes, the number of cysts per 400 cm^3 of soil, and the number of eggs per 400 cm^3 of soil in tobacco cross K 326 X Kutsaga 110 for two tests.

	Sum of Swollen, Pyriform, and Adult		Cysts per 400 cm^3 of Soil		Eggs per 400 cm^3 of Soil	
Cross	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
K 326 X Kutsaga 110						
V_A†	-625.10	-6.40	-2250.53	188.88	-237433895.60	6915388.55
V_D	447.89	-16.05	4359.67	-144.49	321958700.67	-4336850.56
V_E	275.81	23.56	8751.26	91.03	16127531.20	1661142.80
H‡	0.00	0.00	0.19	0.33	0.84	0.61
h^2	0.00	0.00	0.00	1.39	0.00	1.63
Gs§	0.00	0.00	0.00	332.43	0.00	12171083.85
n¶	0.00	0.00	2.41	0.80	0.08	0.06
D_R#	-1.26	2.27	1.84	-1.18	-1.67	-1.10
Heterosis††	1.87	0.27	44.83	0.18	1539.30	326.97

† Variance components calculated as follows: $V_A = 2V_{F2} – (V_{BC1Ps} + V_{BC1Pr})$, $V_E = (V_{Ps} + V_{Pr} + 2V_{F1})/4$, $V_D = V_{BC1Ps} + V_{BC1Pr} - V_{F2} – V_E$.

‡ Heritabilities calculated as follows: $H = (V_{F2} – V_E)/V_{F2}$, $h^2 = V_A/V_{F2}$.

§ Genetic gain for selection at 10% calculated as follows: $Gs = 1.76(h^2)(V_{F2})$.

¶ Number of genes (n) calculated as follows: $n = \{(P_s – P_r)^2[1.5 – 2h(1-h)]\}/8[V_{F2} – (V_{Ps} + V_{Pr} + 2V_{F1})/4]$, $h = (F_1 – P_r)/(P_s – P_r)$.

Dominance ratio is the degree of dominance.

†† Heterosis calculated as the difference between the F_1 mean and the resistant parent mean (P_r).

‡‡ Estimate assumed to be zero due to negative estimate.

APPENDIX A

Table 1. Evaluation of tobacco cyst nematode resistance in Coker 371 Gold: test 1 means table of non-transformed data.

	Vermiform	Swollen	Pyriform	Adult	SPA†	Cyst	Egg
K 326	282.3 a	34.5 a	5.1 a	14.5 a	54.1 a	107.2 a	11265 a
C 371 G	23.7 c	0.3 b	0.0 c	0.1 b	0.5 b	0.7 c	258 b
F_1	20.0 c	0.4 b	0.3 bc	0.5 b	1.2 b	2.5 c	436 b
F_2	42.9 c	2.7 b	0.8 bc	3.8 b	7.3 b	45.6 b	5270 ab
BC_1P_1	169.1 b	20.1 ab	2.6 b	3.9 b	26.6 ab	60.0 b	7538 a
BC_1P_2	16.4 c	0.4 b	0.3 bc	0.3 b	0.9 b	1.1 c	153 b

† Generation means represent the sum of swollen, pyriform, and adult nematodes per 1-g root sample, the number of cysts per 400 cm^3 of soil, and the number of eggs per 400 cm^3 of soil from non-transformed data. Means followed by the same letter within a test for a given parameter are not significantly different at the 0.05 level based on Duncan's multiple range test.

Table 2. Evaluation of tobacco cyst nematode resistance in Coker 371 Gold: test 2 means table of non-transformed data.

	Vermiform	Swollen	Pyriform	Adult	SPA†	Cyst	Egg
K 326	73.8 a	3.1 a	4.2 a	1.8 a	9.1 a	16.5 a	253 ab
C 371 G	35.6 ab	0.3 a	0.0 b	0.0 b	0.3 b	1.3 c	76 b
F_1	61.3 ab	1.1 a	0.1 b	0.1 b	1.2 b	2.6 c	154 ab
F_2	36.0 ab	2.2 a	0.9 b	0.2 b	3.3 ab	5.8 bc	183 ab
BC_1P_1	53.4 ab	6.0 a	1.9 b	0.7 b	8.6 a	13.1 ab	530 a
BC_1P_2	25.3 b	0.1 a	0.03 b	0.01 b	0.1 b	1.7 c	76 b

† Generation means represent the sum of swollen, pyriform, and adult nematodes per 1-g root sample, the number of cysts per 400 cm^3 of soil, and the number of eggs per 400 cm^3 of soil from non-transformed data. Means followed by the same letter within a test for a given parameter are not significantly different at the 0.05 level based on Duncan's multiple range test.

Table 3. Evaluation of tobacco cyst nematode resistance in Coker 371 Gold: combined means table of non-transformed data.

	Vermiform	Swollen	Pyriform	Adult	SPA†	Cyst	Egg
K 326	178.1 a	18.8 a	4.7 a	8.1 a	31.6 a	61.9 a	5759 a
C 371 G	29.6 c	0.3 b	0.0 c	0.1 b	0.4 c	1.0 c	167 b
F_1	40.7 c	0.7 b	0.2 c	0.3 b	1.2 c	2.6 c	290 b
F_2	39.4 c	2.4 b	0.9 bc	2.0 b	5.3 bc	25.7 b	2727 ab
BC_1P_1	111.2 b	13.1 ab	2.2 b	2.3 b	17.6 ab	36.5 b	4017 a
BC_1P_2	20.8 c	0.2 b	0.1 c	0.2 b	0.5 c	1.4 c	115 b

† Generation means represent the sum of swollen, pyriform, and adult nematodes per 1-g root sample, the number of cysts per 400 cm^3 of soil, and the number of eggs per 400 cm^3 of soil from non-transformed data. Means followed by the same letter within a test for a given parameter are not significantly different at the 0.05 level based on Duncan's multiple range test.

Table 4. Evaluation of tobacco cyst nematode resistance in Kutsaga 110: test 1 means table of non-transformed data.

	Vermiform	Swollen	Pyriform	Adult	SPA†	Cyst	Egg
K 326	234.7 a	11.7 a	7.5 a	13.7 a	32.9 a	223.8 a	7210 ab
K 110	30.1 c	0.5 c	0.2 c	0.2 b	0.9 c	34.7 d	261 b
F_1	128.7 abc	0.8 c	0.3 bc	1.6 b	2.7 c	79.6 cd	1800 ab
F_2	131.3 abc	2.3 bc	1.2 bc	2.1 b	5.6 bc	91.9 c	3495 ab
BC_1P_1	204.6 ab	7.6 ab	2.4 b	3.1 b	13.2 b	153.2 b	7529 a
BC_1P_2	64.6 bc	0.7 c	0.3 bc	1.2 b	2.2 c	59.7 cd	935 ab

† Generation means represent the sum of swollen, pyriform, and adult nematodes per 1-g root sample, the number of cysts per 400 cm^3 of soil, and the number of eggs per 400 cm^3 of soil from non-transformed data. Means followed by the same letter within a test for a given parameter are not significantly different at the 0.05 level based on Duncan's multiple range test.

Table 5. Evaluation of tobacco cyst nematode resistance in Kutsaga 110: test 2 means table of non-transformed data.

	Vermiform	Swollen	Pyriform	Adult	SPA†	Cyst	Egg
K 326	110.0 ab	2.7 a	2.7 a	0.7 a	6.1 a	15.8 a	1156 a
K 110	96.7 abc	0.0 b	0.0 b	0.0 c	0.0 b	2.0 c	70 b
F_1	114.5 a	0.1 b	0.1 b	0.1 bc	0.3 b	2.2 c	397 b
F_2	54.1 c	0.1 b	0.1 b	0.1 c	0.3 b	6.3 bc	700 ab
BC_1P_1	86.0 abc	0.5 b	0.5 b	0.4 ab	1.4 b	7.8 b	572 ab
BC_1P_2	64.0 bc	0.0 b	0.05 b	0.02 c	0.1 b	2.6 c	207 b

† Generation means represent the sum of swollen, pyriform, and adult nematodes per 1-g root sample, the number of cysts per 400 cm^3 of soil, and the number of eggs per 400 cm^3 of soil from non-transformed data. Means followed by the same letter within a test for a given parameter are not significantly different at the 0.05 level based on Duncan's multiple range test.

Table 6. Evaluation of tobacco cyst nematode resistance in Kutsaga 110: combined means table of non-transformed data.

	Vermiform	Swollen	Pyriform	Adult	SPA†	Cyst	Egg
K 326	172.4 a	7.2 a	5.1 a	7.2 a	19.5 a	119.8 a	4183 a
K 110	63.4 c	0.2 c	0.1 c	0.1 c	0.4 c	18.4 d	165 b
F_1	121.6 abc	0.4 c	0.2 c	0.9 bc	1.5 c	40.9 cd	1098 ab
F_2	92.7 bc	1.2 bc	0.7 bc	1.1 bc	3.0 bc	49.1 c	2098 ab
BC_1P_1	145.9 ab	4.1 b	1.5 b	1.8 b	7.4 b	79.8 b	4017 a
BC_1P_2	64.3 c	0.4 c	0.2 c	0.6 bc	1.2 c	31.2 cd	571 b

† Generation means represent the sum of swollen, pyriform, and adult nematodes per 1-g root sample, the number of cysts per 400 cm^3 of soil, and the number of eggs per 400 cm^3 of soil from non-transformed data. Means followed by the same letter within a test for a given parameter are not significantly different at the 0.05 level based on Duncan's multiple range test.

Table 7. Estimates of genetic effects for tobacco cyst nematode resistance based on non-transformed vermiform counts on two flue-cured tobacco crosses fitted to a three-parameter model.

Cross and test	Parameter†			χ^2‡
	m	*a*	*d*	
K 326 X Coker 371 Gold				
Test 1	93.87**§	78.13**	-76.38**	10.68
Test 2	50.46**	24.26**	-24.06	2.48
Average	77.10**	54.05**	-58.40*	6.08
K 326 X Kutsaga 110				
Test 1	147.17**	119.10**	-39.08	0.76
Test 2	90.20**	16.99	-42.96	11.12
Average	120.29**	63.70**	-40.94	1.52

† Mean, additive, and dominance genetic effects for the model $y = m + a + d$, where y equals the generation mean.

‡ For values larger than 7.81, the probability of a fit is less than 0.05.

§ * And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

Table 8. Estimates of genetic effects for tobacco cyst nematode resistance based on non-transformed swollen counts on two flue-cured tobacco crosses fitted to a three-parameter model.

Cross and test	Parameter†			χ^2‡
	m	*a*	*d*	
K 326 X Coker 371 Gold				
Test 1	6.09**§	5.76**	-5.69**	4.06
Test 2	2.08**	1.87**	-2.13**	12.14
Average	5.50**	5.33**	-5.20*	3.32
K 326 X Kutsaga 110				
Test 1	5.15**	4.67**	-4.34**	3.11
Test 2	0.32**	0.32**	-0.32**	6.57
Average	2.83**	2.59**	-2.40**	2.21

† Mean, additive, and dominance genetic effects for the model $y = m + a + d$, where y equals the generation mean.

‡ For values larger than 7.81, the probability of a fit is less than 0.05.

§ * And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

Table 9. Estimates of genetic effects for tobacco cyst nematode resistance based on non-transformed pyriform counts on two flue-cured tobacco crosses fitted to a three-parameter model.

Cross and test	Parameter†			
	m	*a*	*d*	χ^2‡
K 326 X Coker 371 Gold				
Test 1	1.58**§	1.58**	-1.24**	3.25
Test 2	1.87**	1.87**	-1.81**	0.11
Average	1.79**	1.79**	-1.58**	1.11
K 326 X Kutsaga 110				
Test 1	2.52**	2.29**	-2.14**	3.33
Test 2	0.33**	0.33**	-0.25*	6.34
Average	1.45**	1.33**	-1.22**	3.27

† Mean, additive, and dominance genetic effects for the model $y = m + a + d$, where y equals the generation mean.

‡ For values larger than 7.81, the probability of a fit is less than 0.05.

§ * And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

Table 10. Estimates of genetic effects for tobacco cyst nematode resistance based on non-transformed adult counts on two flue-cured tobacco crosses fitted to a three-parameter model.

Cross and test	Parameter†			
	m	*a*	*d*	χ^2‡
K 326 X Coker 371 Gold				
Test 1	4.12**§	3.98**	-3.65**	2.97
Test 2	0.60**	0.60**	-0.58**	2.16
Average	2.39**	2.33**	-2.13**	1.54
K 326 X Kutsaga 110				
Test 1	2.56**	2.35**	-0.84	4.10
Test 2	0.19**	0.19**	-0.16*	11.32
Average	1.43**	1.33**	-0.54	2.06

† Mean, additive, and dominance genetic effects for the model $y = m + a + d$, where y equals the generation mean.

‡ For values larger than 7.81, the probability of a fit is less than 0.05.

§ * And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

Table 11. Estimates of genetic effects for tobacco cyst nematode resistance based on non-transformed vermiform counts on two flue-cured tobacco crosses fitted to a six-parameter model.

Cross and test	Parameter†						
	m	*a*	*d*	*aa*	*ad*	*dd*	χ^2
K 326 X Coker 371 Gold							
Test 1	-46.42	129.33*‡	290.72	199.42*	46.79	-224.30	8×10^{-23}
Test 2	41.33	19.10	-41.47	13.37	17.99	61.48	3×10^{-27}
Average	-2.54	74.22*	124.62	106.39*	32.39	-81.41	1×10^{-25}
K 326 X Kutsaga 110							
Test 1	119.30	102.33**	38.66	13.10	75.45	-29.29	2×10^{-26}
Test 2	19.71	6.63	42.75	83.66*	30.73	52.01	3×10^{-26}
Average	69.50	54.48*	40.71	48.38	53.09	11.36	3×10^{-25}

† Mean, additive, dominance, additive X additive, additive X dominance, and dominance X dominance genetic effects for the model $y = m + a + d + aa + ad + dd$, where y equals the generation mean.

‡ * And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

Table 12. Estimates of genetic effects for tobacco cyst nematode resistance based on non-transformed swollen counts on two flue-cured tobacco crosses fitted to a six-parameter model.

Cross and test	Parameter†						
	m	*a*	*d*	*aa*	*ad*	*dd*	χ^2
K 326 X Coker 371 Gold							
Test 1	-12.63	17.10	48.43	30.06	5.34	-35.40	3×10^{-21}
Test 2	-1.86	1.40**‡	13.11	3.52	9.03*	-10.19	3×10^{-23}
Average	-7.24	9.25	30.77	16.79	7.18	-22.79	7×10^{-24}
K 326 X Kutsaga 110							
Test 1	-1.32	5.60**	12.39	7.39	2.63	-10.27	1×10^{-24}
Test 2	0.72	1.37*	-1.80	0.65	-1.67	1.15	2×10^{-18}
Average	-0.30	3.48*	5.29	4.02	0.48	-4.56	5×10^{-26}

† Mean, additive, dominance, additive X additive, additive X dominance, and dominance X dominance genetic effects for the model $y = m + a + d + aa + ad + dd$, where y equals the generation mean.

‡ * And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

Table 13. Estimates of genetic effects for tobacco cyst nematode resistance based on non-transformed pyriform counts on two flue-cured tobacco crosses fitted to a six-parameter model.

Cross and test	Parameter†						
	m	*a*	*d*	*aa*	*ad*	*dd*	χ^2
K 326 X Coker 371 Gold							
Test 1	0.14	2.57	2.48	2.42	-0.51	-2.29	9×10^{-19}
Test 2	1.83	2.10*‡	-1.91	0.27	-0.39	0.14	2×10^{-19}
Average	0.99	2.33	0.28	1.35	-0.45	-1.07	4×10^{-19}
K 326 X Kutsaga 110							
Test 1	3.20	3.63**	-5.00	0.63	-3.06	2.12	2×10^{-25}
Test 2	0.79	1.37*	-1.95	0.57	-1.86	1.22	1×10^{-20}
Average	2.00	2.50**	-3.47	0.60	-2.46	1.67	9×10^{-26}

† Mean, additive, dominance, additive X additive, additive X dominance, and dominance X dominance genetic effects for the model $y = m + a + d + aa + ad + dd$, where y equals the generation mean.

‡ * And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

Table 14. Estimates of genetic effects for tobacco cyst nematode resistance based on non-transformed adult counts on two flue-cured tobacco crosses fitted to a six-parameter model.

Cross and test	Parameter†						
	m	*a*	*d*	*aa*	*ad*	*dd*	χ^2
K 326 X Coker 371 Gold							
Test 1	13.98*‡	7.17**	-27.24	-6.68	-7.08	13.73	8×10^{-23}
Test 2	0.40	0.90*	-0.40	0.50	-0.47	0.07	2×10^{-22}
Average	7.19	4.03*	-13.82	-3.09	-3.77	6.90	4×10^{-23}
K 326 X Kutsaga 110							
Test 1	6.72*	6.77**	-13.45	0.25	-9.63*	8.33	8×10^{-26}
Test 2	-0.27	0.33*	0.92	0.61*	0.09	-0.51	1×10^{-22}
Average	3.22	3.55*	-6.27	0.43	-4.77	3.91	3×10^{-25}

† Mean, additive, dominance, additive X additive, additive X dominance, and dominance X dominance genetic effects for the model $y = m + a + d + aa + ad + dd$, where y equals the generation mean.

‡ * And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

Table 15. Estimates of genetic effects for tobacco cyst nematode resistance based on the non-transformed sum of swollen, pyriform, and adult counts on two flue-cured tobacco crosses fitted to a six-parameter model.

Cross and test	Parameter†						
	m	*a*	*d*	*aa*	*ad*	*dd*	χ^2
K 326 X Coker 371 Gold							
Test 1	1.47	26.83	23.72	25.83	-2.24	-23.99	2×10^{-21}
Test 2	0.36	4.40**‡	10.82	4.30	8.15	-9.98	2×10^{-22}
Average	0.92	15.62	17.27	15.07	2.96	-16.98	1×10^{-23}
K 326 X Kutsaga 110							
Test 1	8.60	16.00**	-6.06	8.27	-10.06	0.19	1×10^{-24}
Test 2	1.24	3.07*	-2.84	1.83*	-3.44	1.86	3×10^{-18}
Average	4.92	9.53**	-4.45	5.05	-6.75	1.03	2×10^{-25}

† Mean, additive, dominance, additive X additive, additive X dominance, and dominance X dominance genetic effects for the model $y = m + a + d + aa + ad + dd$, where y equals the generation mean.

‡ * And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

Table 16. Estimates of genetic effects for tobacco cyst nematode resistance based on non-transformed cyst counts per 400 cm^3 of soil on two flue-cured tobacco crosses fitted to a six-parameter model.

	Parameter†						
Cross and test	*m*	*a*	*d*	*aa*	*ad*	*dd*	χ^2
K 326 X Coker 371 Gold							
Test 1	114.02*‡	53.25**	-162.24	-60.07	11.35	50.74	3×10^{-21}
Test 2	2.27	7.62**	13.60	6.64	7.68	-13.28	4×10^{-23}
Average	58.15*	30.43**	-74.32	-26.71	9.52	18.73	3×10^{-21}
K 326 X Kutsaga 110							
Test 1	70.96	94.55**	75.06	58.32	-2.28	-66.46	6×10^{-25}
Test 2	13.19*	6.92*	-16.53	-4.28	-3.42	5.50	6×10^{-25}
Average	42.07	50.74**	29.27	27.02	-2.85	-30.48	2×10^{-25}

† Mean, additive, dominance, additive X additive, additive X dominance, and dominance X dominance genetic effects for the model $y = m + a + d + aa + ad + dd$, where y equals the generation mean.

‡ * And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

Table 17. Estimates of genetic effects for tobacco cyst nematode resistance based on non-transformed egg counts per 400 cm^3 of soil on two flue-cured tobacco crosses fitted to a six-parameter model.

	Parameter†						
Cross and test	*m*	*a*	*d*	*aa*	*ad*	*dd*	χ^2
K 326 X Coker 371 Gold							
Test 1	11460	5503**‡	-13735	-5699	3762	2711	1×10^{-20}
Test 2	-314	88*	1521	478	732*	-1053*	5×10^{-25}
Average	5573	2796*	-6107	-2610	2247	829	3×10^{-21}
K 326 X Kutsaga 110							
Test 1	788	3474**	9818	2948	6239	-8805	1×10^{-24}
Test 2	1854*	543*	-3160	-1242	-348	1702	1×10^{-24}
Average	1321	2009**	3329	853	2940	-3551	4×10^{-23}

† Mean, additive, dominance, additive X additive, additive X dominance, and dominance X dominance genetic effects for the model $y = m + a + d + aa + ad + dd$, where y equals the generation mean.

‡ * And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

VITA

Barbara Jean Crowder

Barbara Jean Crowder is the daughter of William E. and Sharon R. Crowder of Dinwiddie, VA. She was born on September 19, 1976 in Petersburg, VA and grew up in the small town of Sutherland in northern Dinwiddie County. Barbara was graduated third in her class from Dinwiddie County High School in June 1994. She attended James Madison University in Harrisonburg, VA where she was graduated Summa Cum Laude in May 1998. Barbara received her Bachelor of Science degree in Integrated Science and Technology with a concentration in Biotechnology and a minor in Chemistry. She is a member of Golden Key National Honor Society and the National Honor Society of Phi Kappa Phi. While at James Madison University, Barbara worked the summer of 1995-96 at the Virginia Tech Southern Piedmont Agricultural Research and Extension Center under the direction of Dr. Carol A. Wilkinson. During her remaining two years at James Madison University, Barbara interned with Merck & Co., Inc. where she completed a senior thesis entitled Analysis and Optimization of Sterilization Procedures at Merck & Co., Inc. Stonewall Plant. In 1998, Barbara returned to Southern Piedmont AREC and enrolled in Virginia Polytechnic Institute and State University to study plant breeding and genetics and to participate in the Molecular Cell Biology and Biotechnology option. She received her Master of Science degree in Crop and Soil Environmental Science under the direction of Dr. Carol A. Wilkinson in December 2000.