Chapter 1: Literature Review

In 1954, Lownsbery and Lownsbery reported the discovery of a cyst nematode parasitizing shade tobacco (*Nicotiana tabacum* L.) in Connecticut. The morphology of the newly discovered nematode resembled that of the potato cyst nematode, *Heterodera rostochiensis*. It was described as a new species and was subsequently named, *Heterodera tabacum*, the tobacco cyst nematode (Lownsbery and Lownsbery, 1954). Miller and Gray described a second cyst nematode parasitizing tobacco in 1968, and named it *Heterodera virginiae*. This nematode was found parasitizing horsenettle (*Solanum carolinense* L.) in Tidewater Virginia. Cysts were shaped distinctly different from those of *H. tabacum* (Miller and Gray, 1968). A third cyst nematode was found on tobacco in 1961 by W. W. Osborne on a flue-cured tobacco farm in Amelia Co., Virginia and was called Osborne's Cyst Nematode (Osborne, 1961). Miller and Gray described it in 1972 as *Heterodera solanacearum* (Miller and Gray, 1972). In 1975, Behrens placed the three cyst nematodes into the genus *Globodera* due to their globular cyst shape (Behrens, 1975). However, in 1983, Stone considered the three species as a tobacco cyst nematode (TCN) species complex, and assigned all three nematodes subspecific status. Thus, the name for the tobacco cyst nematode from Connecticut became *Globodera tabacum tabacum* (Lownsbery and Lownsbery, 1954) Behrens, 1975; the horsenettle cyst nematode was named *Globodera tabacum virginiae* (Miller and Gray, 1968) Behrens, 1975; and Osborne's cyst nematode became *Globodera tabacum solanacearum* (Miller

and Gray, 1972) Behrens, 1975 (Stone, 1983). This classification is the currently accepted scheme.

Starting with the original discovery of *G. t. tabacum*, morphological and genetic similarities were noted between TCN and the species of the potato cyst nematode (PCN), *Globodera pallida* (Stone, 1973) Behrens, 1975 and *G. rostochiensis* (Wollen., 1923) Behrens, 1975. Lownsbery (1951) was able to differentiate *G. t. tabacum* from *G. rostochiensis* with host range studies. Potato cyst nematodes did not reproduce on tobacco varieties that *G. t. tabacum* successfully parasitized. Lownsbery also cited several morphological characteristics that varied between *G. t. tabacum* and *G. rostochiensis*, including: lip region annulation, length of juvenile tail, head shape, and distance of the dorsal esophageal gland opening to the base of the stylet in males. In the original description of *G. t. virginiae*, Miller and Gray (1968) distinguished it from *G. rostochiensis* by its ability to reproduce on several tobacco cultivars. Also instrumental in this differentiation were morphological differences in cyst shape, Granek's ratio, and pattern of the cyst wall. *Globodera tabacum solanacearum* was differentiated from PCN using similar methods and characteristics (Miller and Gray, 1972; Stone and Miller, 1974). Stone (1983) confirmed these morphological differences in his reevaluation of the genus. Recent phylogenetic studies have supported this evidence, indicating that PCN and TCN differ significantly enough to be classified as separate species. Bossis and Mugniéry (1993) ran two-dimensional electrophoresis on proteins from the two PCN species and the three TCN subspecies and found that the tobacco cyst nematode subspecies were indeed more similar to each other than to the potato cyst nematodes,

enough to differentiate between separate species. Similar results were found when the ribosomal DNA of the PCN species were compared with that of the TCN subspecies (Ferris *et al.*, 1995; Thiéry and Mugniéry, 1996).

Taxonomy within the *Globodera tabacum* subspecies complex is a little more difficult and controversial. The three tobacco cyst nematodes were originally classified as separate species because of host range preferences and slight morphological differences. Three plants, in particular, are useful in determining the identity of the three subspecies: *Nicotiana tabacum*, DVA 312, *N. acuminata* 2-G-58, and *N. X sanderae*, 50-B. *Globodera tabacum virginiae* successfully reproduces on all three. *Globodera tabacum solanacearum* reproduces on all but *N. X sanderae*, 50-B. *Globodera tabacum tabacum* reproduces on all but *N. acuminata* 2-G-58 (Miller and Gray, 1972). Slight variation in cyst shape and color, posterior wall patterns, and fenestra shape were also detected among the three subspecies (Miller and Gray, 1968; Miller and Gray, 1972). Mulvey (1973) separated the three subspecies into distinct species using cyst characteristics such as vulval aperture length and fenestral length. However, this work was based on only one isolate of each subspecies, with the cysts being produced on different hosts under different conditions (Mota and Eisenback, 1993c). Green (1971) conducted similar work, but also only used one isolate of each of the three subspecies. The insufficient sample sizes in the older studies has prompted recent morphological and phylogenetic work on subspecies differientiation within the complex. Mota and Eisenback (1993c) studied variation in morphometrics among the three subspecies, and searched for more reliable distinguishing characteristics. They found that the morphometrics of eggs, males, and

second-stage juveniles were much less variable than those of females and cysts (Mota and Eisenback, 1993c). Additionally, it was found that the morphology of *Globodera tabacum virginiae* was more similar to that of *G. t. solanacearum* than to that of *G. t. tabacum* (Mota and Eisenback, 1993c). *Globodera tabacum tabacum* could be differentiated from the other two members of the complex by stylet knob width, tail length of males, and body length/width ratios of females and cysts (Mota and Eisenback, 1993c). Subsequent research was conducted involving males and second-stage juveniles to determine if morphological characteristics could be found that would ease subspecies identification. Unfortunately, no additional useful traits were found to be present (Mota and Eisenback, 1993a). Mota and Eisenback (1993b) also looked at the morphology of females and cysts of the three subspecies to determine additional characteristics that would be useful in identification. They found that the most useful characteristics in identification were female body shape, shape of stylet knobs, cyst shape, tail region shape and length, and clumping of perineal tubercles. Females and cysts of *G. t. tabacum* were found to be more rounded than those of the other subspecies in the tobacco cyst nematode complex. The tubercle regions of *G. t. tabacum* were also observed to be more clumped than those of the other tobacco cyst nematode subspecies. *Globodera tabacum virginiae* could be differentiated from the other subspecies due to a “dutch-shoe” shape of the dorsal stylet knob, whereas the dorsal stylet knobs of the other subspecies were more sloped. *Globodera tabacum solanacearum* was distinguished from the other subspecies due to the female nematodes’ distinct anus and crescent shaped tail region (Mota and Eisenback, 1993b).

Results from molecular studies have provided supporting evidence regarding the relationship between the three subspecies. Protein gel electrophoresis estimated genetic differences of 0.05 between *G. t. virginiae* and *G. t. solanacearum* and 0.17 between these two subspecies and *G. t. tabacum* (Bossis and Mugniéry, 1993). The genetic distance (0.05) between *G. t. virginiae* and *G. t. solanacearum* was similar to that observed within many species. The genetic distance between *G. t. tabacum* and the two other subspecies, though larger, was also low enough to correspond to intra-specific variation. Thus, Bossis and Mugniéry (1993) proposed clumping the three subspecies into one species. Ribosomal DNA restriction fragment length polymorphism data similarly found *G. t. tabacum* to be more distantly related to *G. t. virginiae* and *G. t. solanacearum*. However, all three subspecies were found to be highly related (Thiéry and Mugniéry, 1996).

Biological and Epidemiological Information: There have been a number of studies regarding the biology of TCN that have focused on juvenile hatching. Initial work on this subject examined the effects of fungicides on hatching by *G. t. tabacum* (Miller, 1967). Maneb and nabam both stimulated hatch in laboratory studies. Subsequent field experiments were conducted, hoping that fall application of these fungicides would induce hatching and cause nematode death due to lack of a host. However, this result did not materialize, perhaps due to insufficient incorporation of the fungicide or destruction of nematode parasitizing soil fungi (Miller and Taylor, 1967). Fox and Webber (1970) studied hatching by *G. t. solanacearum*. They found that hatching by TCN increased after exposing eggs to tobacco root leachates, compared to plain water or soil

microorganisms. More recent hatching experiments have compared the influences of resistant and susceptible tobacco cultivars and other hosts on the hatching of TCN. Work with *G. t. tabacum* indicated that hatching was slightly stimulated by exposure to exudates from resistant tobacco cultivar PD-4 compared to exudates from susceptible cultivar CT86-4 (LaMondia, 1988). However, no differences were found in number of hatched juveniles of *G. t. solanacearum* between the susceptible cultivar K326 and the resistant NC567 (Wang, 1996). LaMondia (1988) and Wang *et al.* (1997) both found that TCN hatching increased with root exudate concentration; however, Wang *et al.* (1997) concluded that temperature influenced hatching by *G. t. solanacearum* more than exudates from tobacco roots. LaMondia (1995b) found that variability in hatching of eggs from the field is greater than that of eggs from greenhouse-produced cysts. Three week old tobacco plants also induced more hatching than younger or older seedlings. TCN hatching trials have also examined hosts other than tobacco. LaMondia (1995b) found that black nightshade (*Solanum nigrum* L.) and tomato (*Lycopersicon esculentum* L.) root exudates stimulate greater hatching of *G. t. tabacum* than does tobacco. Along similar lines, exudates from horsenettle roots induced hatching by *G. t. solanacearum* significantly more than exudates from tobacco roots (Rideout and Johnson, unpublished data).

Reilly and Grant (1985) studied seasonal fluctuations in TCN population densities in soil over a two year period, using both centrifugal sugar floatation and sugar floatation extraction methods. They found that cyst populations in soil were fairly stable outside the growing season. However, cyst recovery dropped off significantly in June, after layby

cultivation. Numbers of cysts recovered rebounded around July and continued to increase to a maximum level in September. The number of cysts recovered after September was reduced by the destruction of plant material and burying it in the soil. Some annual variation was present, however, as in the second year of the study a larger number of cysts were found in February and March than in the preceding year. The explanation offered for this variation was that the freezing and thawing of the ground in an extremely cold January which could serve to push more cysts upwards in the soil concentrating them in the sampling region (Reilly and Grant, 1985). LaMondia (1995a) found that increases in population densities of *G. t. tabacum* could be fit to a log/log plot over the course of a growing season. LaMondia's (1995a) study was conducted in microplots that were reinoculated every year prior to transplanting and, therefore, no overwintering data was taken. Rideout and Johnson (1996) also found seasonal variation in cyst recovery from the fall to the spring over three years. However, their data did not show any consistent trend.

Adams *et al.* (1982) investigated the effect of temperature on the development of *G. t. solanacearum*. They found that the optimum temperature for TCN development was 27°C. At this temperature, the life cycle of *G. t. solanacearum* was completed in the shortest period of time (33 days), and the largest cysts and the most eggs per cyst were produced. When temperature was increased or decreased above or below this optimal amount, days to complete life cycle increased, and cyst size and eggs per cyst decreased. The optimal temperature for TCN development corresponds to Wang's 1996 study, which found 25°C to be the optimal hatching temperature for TCN, thus suggesting hatching is

an important part in determining the amount of nematodes present. Despite the fact that cooler temperatures reduce hatching, resulting in fewer juveniles, Wang (1996) found that cooler temperatures did not inhibit subsequent juvenile development and feeding. Signifying that the nematodes could reproduce successfully at the lower temperatures.

Effects of TCN on host: The host range of TCN is considered to be limited to a few species within the plant family *Solanaceae,* particularly the genera *Solanum* and *Nicotiana* (Baldwin and Mundo-Ocampo, 1991). *Globodera tabacum solanacearum* and *G. t. tabacum* were found to be parasitic on most cultivars of shade and flue-cured tobacco, while only being parasitic on some cultivars of dark-fired, sun-cured, and burley tobacco (Miller and Duke, 1969; Miller and Gray, 1972). Tobacco is a poorer host for *G. t. virginiae* than for the other subspecies (L.I. Miller, 1970). All of the subspecies have also been found to be parasitic on some cultivars of tomato (*Lycopersicon* spp. L.), pepper (*Capsicum* spp. L.) and eggplant (*Solanum melongena* L.) (Harrison and Miller, 1969; Miller and Gray, 1968,1972). Tobacco cyst nematodes also reproduce on many wild *Nicotiana* and *Solanum* weeds, such as black nightshade and horsenettle (Harrison and Miller, 1969; Miller and Gray, 1968, 1972). There is very little difference in the host range of the three subspecies. However, they may be differentiated according to reproduction on specific *Nicotiana* species, as mentioned earlier.

Very little research has been conducted on the etiology of parasitism by the tobacco cyst nematode complex. Research of this nature has been conducted on other cyst nematodes and is presumed to be applicable to TCN. As already mentioned, exposure to host root exudates increases hatching by TCN species (Fox and Webber,

1970; LaMondia, 1988; Wang *et al.*, 1997). Freshly hatched juveniles of the soybean cyst nematode, *Heterodera glycines* Ichinohe, 1952, were found to be chemically attracted to the host by root exudates (Huettel, 1986). Endo (1987) found that juveniles of *H. glycines* usually enter host roots near sites of root elongation. Once juveniles of the sugarbeet cyst nematode, *Heterodera schachtii* Schmidt, 1871 enter roots, they move intracellularly, causing extensive cell damage along their path to the vascular cylinder (Wyss and Zunke, 1986). The sugarbeet cyst nematode juvenile then inserts its hypodermic needle-like stylet into a selected root cell in the endodermis, cortex, pericycle, or vascular parenchyma, injecting unknown esophageal gland secretions into the host cell (Jones, 1981; Wyss, 1992). Cyst nematode secretions are thought to be responsible for the initiation of syncytia through increased metabolic activity by the host (Burrows, 1992). Additionally, these secretions polymerize into a feeding tube, through which the nematode may withdraw nutrients from the host (Hussey and Williamson, 1998). Nutrient uptake by *Heterodera schachtii* is accomplished through the actions of the metacorpal pump chamber, which contracts continuously for a duration of two hours to withdraw desired nutrients (Wyss, 1992). After feeding, the stylet is withdrawn from the host cell and a new feeding site is located by the nematode within the same syncytial cell (Wyss and Zunke, 1986). Within the next few days, the cell walls of cyst nematode infected cells begin to degrade, fusing cells to form a multinucleate syncytium (Endo, 1987). The cytoplasm within this cell becomes extremely dense; cytoplasmic streaming increases to a rate of 10 to 15 times that normal for root cells (Jones and Northcote, 1972). Also, the number of plastids, mitochondria, dictyosomes, rough endoplasmic

reticulum, and vacuoles increased within syncytia, signifying a high metabolism rate for soybean (*Glycine max* L.) cells infected by *H. glycines* (Gipson *et al*., 1971). The plant may be able to check syncytial expansion by lignifying surrounding xylem cells, about eight days after syncytial initiation (Burrows, 1992). At this point, it is too late for the plant to avoid primary damage, because the nematode has already extracted sufficient nutrients. The female may be fertilized soon, then the life cycle will be completed, and the syncytium may disintegrate (Burrows, 1992). Syncytial degradation could be the cause for the reduction of root systems, root pruning, and decay that was described on TCN-infected plants by Lucas and Shew (1991) and Reich (1991).

Numerous syncytia present in the root can have substantial impact on above ground plant parts. Trudgill (1992) considered that root-invading nematodes can effect top growth in three ways: 1) withdrawal of nutrients, 2) reduced uptake of nutrients and water and, 3) physiologically abnormal growth induced by plant responses to the nematode or nematode products. According to Lucas and Shew (1991), TCN-infected plants wilt during the day when soil moisture is not limiting and exhibit stunting and delayed flowering. The symptoms exhibited by TCN infected plants seem to closely follow the physiological effects described by Trudgill.

The earliest work investigating the effects of TCN on tobacco was performed by Lownsbery and Peters (1955) with *G. t. tabacum*. In their pot experiments, the height and weight of Connecticut shade tobacco was inversely proportional to the logarithm of the initial density of TCN eggs. In early field experiments, it was found that plants grown in nematicide-treated plots were 15 to 22% taller than those in untreated plots. In a later

study, initial nematode density was negatively correlated with total shoot weight and plant height as well (LaMondia, 1995a). However, the use of dichloropropene may have exerted effects other than on TCN. LaMondia (1990) used oxamyl to more effectively isolate the effects of *G. t. tabacum* on shade tobacco and was able to correlate a decrease in green leaf yield with increases in initial nematode density. LaMondia (1995a) also revealed a nonlinear damage function that related initial *G. t. tabacum* densities to yield loss. He reported that yield losses over 40% can occur where initial population densities are over 500 J2/cm^3.

Elmer and Miller (1980) found that potted plants inoculated with *G. t. solanacearum* were three to four times shorter than non-inoculated plants after two months of growth. Although control plants bloomed during this period, inoculated plants did not. Grant *et al.* (1982) observed differences in TCN effects among three different cultivars of flue-cured tobacco. The cultivar VA 81 seemed to be tolerant to TCN when compared to McNair 944 and Coker 319 and showed little damage caused by TCN. Additionally, nematode populations did not increase on this cultivar. The remaining two cultivars, McNair 944 and Coker 319, both exhibited a reduction in plant height, leaf number, and shoot and root dry weight, with the worst damage occurring on McNair 944. Nematode populations were also found to increase linearly on the two cultivars.

Most studies on the effects of *G. t. solanacearum* on tobacco have been conducted in the field. Osborne (1971) reported a 54% reduction in yield of untreated plots compared to the best nematicide treatment. Komm *et al.* (1983) reported an average 15% yield loss in infested fields (137 ha) in 1982, although several complete crop losses were

recorded. Detectable differences in yield due to nematicidal treatment varies with initial nematode density, year, and location (Komm and Elliott, 1983; Reilly and Komm, 1985). Wang (1996) investigated correlations between weekly egg densities of *G. t. solanacearum* during the first 11 weeks of the growing season and plant height, fresh leaf weight, leaf number, feeder root weight, and ratio of root to leaf weight. He found that *G. t. solanacearum* suppressed leaf number, plant height, and fresh weight of leaves and roots. Correlations varied between year and cultivar, but consistent negative correlations were found between egg densities in soil and fresh feeder root weight at 9 weeks after transplanting for both a resistant (NC567) and a susceptible (K326) cultivar. Additionally, it was found that fresh leaf weight correlated negatively with egg densities 6 weeks after transplanting of the susceptible cultivar K326. Negative correlations were consistently significant between fresh leaf and feeder root weights and the area under a curve for the total number of nematodes per gram of feeder root. Number of nematodes per gram of feeder root was determined using the method described by Byrd *et al.* (1983). Evaluation of nematode amounts in roots, versus soil, could provide more useful data for diagnostic lab work involving TCN, since damage was more highly correlated with nematodes per gram of feeder root than with TCN egg counts from soil.

Disease Complexes: A number of soil-borne pathogens have been associated with TCN in disease complexes. In most cases, disease severity is greater when TCN is present, most likely due to predisposition. Tobacco cyst nematodes can increase the number of infection sites for the secondary pathogen by leaving entry points in roots for the secondary pathogen to successfully penetrate the root system. Disease complex work

with *G. t. tabacum* has focused primarily on Fusarium wilt. Miller (1975) found that levels of Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici* (Schlecht.) Wr.) decreased in tomato pots that contained both pathogens, compared to pots containing only the fungal pathogen. However, Fusarium wilt of tobacco increased in the presence of *G. t. tabacum* (LaMondia and Taylor, 1987). Subsequent studies supported the theory that *G. t. tabacum* inoculated onto plants prior to exposure to *Fusarium oxysporum* f. sp. *nicotianae* increased wilt severity (LaMondia, 1992,1995c). However, the presence of *G. t. tabacum* did not increase wilt incidence on Fusarium wilt resistant cultivars (LaMondia, 1995c). Also, *G. t. tabacum* was shown to be more important in predisposition than *Meloidogyne hapla* Chitwood, 1949 (LaMondia, 1992). Miller (1975) found that the presence of *G. t. tabacum* increased the rate and severity of Verticillium wilt (*Verticillium albo-atrum*) on tomato.

Most disease interaction work with *G. t. solanacearum* has involved the tobacco black shank fungus, *Phytophthora parasitica* Dastur var. *nicotianae* (Breda de Haan) Tucker. One of the first studies found that *G. t. solanacearum* stimulated black shank occurrence, but not as much as *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 (Bowers *et al*., 1980). A split pot experiment conducted by Reilly *et al.* (1984) showed that *G. t. solanacearum* reproduction could be accelerated by the presence of *P. p. nicotianae*, even when the two pathogens were placed in separate pots containing roots of the same plant. Severe root necrosis occurred only when both pathogens were present on the same half of the root (Reilly *et al.*, 1984). Grant *et al*. (1984) found that the extent to which *G. t. solanacearum* and *P. p. nicotianae* were synergistic to each other

was dependent upon a number of factors. These factors included inoculum level, cultivars, and soil conditions. All field experiments showed a synergistic response on all cultivars. However, the TCN-resistant cultivar VA 81 showed a synergistic response in pot experiments, while the TCN-susceptible/black shank resistant cultivars McNair 944 and Coker 319 showed synergistic responses at low levels of inoculum and an antagonistic relationship at higher inoculum levels. In field experiments, higher yields were realized when a nematicide and a fungicide were both used to control both pathogens (Reilly *et al*., 1985).

An interaction between *G. t. solanacearum* and *Pseudomonas solanacearum* Smith (Granville wilt) has been hypothesized. Elmer and Miller (1980) found that plants treated with both pathogens were significantly shorter than the control pots or plants that were treated with just one pathogen. Unfortunately, no Granville wilt data was reported. Antagonism between *G. t. tabacum* and *Tylenchorhynchus claytoni* Steiner, 1937 and *Pratylenchus penetrans* (Cobb, 1917) Chitwoodi and Oteifa, 1952 has been reported (Miller and Wihrheim, 1968). *Globodera tabacum tabacum* reduced infection by and reproduction of these two nematodes. *Globodera tabacum tabacum* infection and population levels were reduced as well. A subsequent study found that even low populations of TCN reduced populations of *P. penetrans*. This could explain why few lesion nematodes are usually found in fields containing *G. t. tabacum* (Miller, 1970).

Economic Importance: *Globodera tabacum virginiae* is found only in the Tidewater section of Virginia. This pathogen parasitizes horsenettle and other solanaceous weeds in that area. No commercial problems or infestations have been

reported. *Globodera tabacum tabacum* is distributed throughout the Connecticut River Valley and parasitizes Connecticut shade tobacco grown in Connecticut and Massachusetts (Miller, 1986). Miller (1986) estimated that annual losses average about $50,000 a year, with an additional $60,000 a year going to chemical treatment. *Globodera tabacum solanacearum* is the most serious pest on flue-cured tobacco in Virginia. Komm *et al*. (1983) reported that *G. t. solanacearum* infested 10% of Virginia's flue-cured producing acreage in 1983. *Globodera tabacum solanacearum* currently infests one-third of the flue-cured tobacco acreage in Virginia and is spreading (C.S. Johnson, 1998, personal communication). *G. t. solanacearum* was first reported outside of Virginia in Warren County, North Carolina in 1991 (Melton and Phillips, 1991). Since that time, TCN has been reported in six additional counties in North Carolina (T.A. Melton, 1998, personal communication). TCN also infests one farm in Charles County, Maryland (Johnson, 1998). The recent spread of this pathogen threatens the long term profitability of tobacco production in Virginia and the Carolinas. Virginia flue-cured farmers lost approximately 3 million dollars in 1997 in damage and pesticide expenditures (C. S. Johnson, 1998, personal communication).

Control: Tobacco cyst nematodes are difficult to control due to their high reproductive potential, ability to survive for long periods in the absence of a host, and the protective effect of the 'cyst' covering. In order to successfully manage TCN, a grower has to implement multiple control techniques. Three main control strategies that can be adopted are cultural practices, resistance, and chemical control. The use of only one of

these strategies can result in heavy damages. Multiple management tools must be used to avoid TCN-related problems.

Cultural Practices: While cultural practices alone cannot control TCN, they may be very useful in suppressing existing populations and slowing spread to non-infested fields. The most useful cultural practice is crop rotation. Crop rotations including small grains, fescue, sorghum, and corn have been most useful in reducing TCN populations (Reed *et al*., 1997). Long rotation is most efficient because cysts remain viable in the soil without a host for at least seven years (Osborne, 1971). However, many tobacco farms lack the acreage to implement long rotation schemes involving less profitable crops. Johnson (1990) found that rotating TCN-resistant cultivars with TCN-susceptible cultivars reduced TCN populations compared to continuously planting susceptible cultivars. This would enable the farmer to continue planting tobacco, but at the same time suppress TCN reproduction. Johnson *et al*. (1989) also found that combining rotation with a resistant cultivar and application of a nematicide further reduced TCN population densities. However, resistant cultivar rotation did not produce yields as high as continuous use of a nematicide with a TCN-susceptible cultivar. Elliott *et al*. (1986) demonstrated that a three year rotation using TCN-resistant cultivars effectively reduced TCN reproduction for the course of the study. However, their study was conducted only on one isolate of the pathogen.

Sanitation is also an important aspect of TCN management. One important practice that can be implemented is early root and stalk destruction (Reed *et al*., 1997). This practice can reduce the number of generations TCN can complete in a year by

removing host tissue necessary for reproduction. Another important practice for limiting the spread of TCN is cleaning farm machinery and implements. Machinery that has been used in infested soil should be cleaned thoroughly before entering non-infested fields (Osborne, 1971). Of equal importance is acquiring transplants from a location that is not infested with TCN (Reed *et al*., 1997). LaMondia (1996) found that the use of trap crops could significantly reduce *G. t. tabacum* populations if allowed to grow 3 to 6 weeks after transplanting. Tomato or resistant tobacco cultivars were found to be the most efficient trap crops, reducing TCN populations by 96%. Nightshade and susceptible tobacco cultivars reduced TCN populations by 80%.

Resistance: Unfortunately, there are currently no agronomically desirable TCN-resistant cultivars. Flue-cured tobacco cultivars Coker 371-Gold, NC567, and Speight-80 inhibit TCN reproduction, but suffer significant yield loss from TCN parasitism. Additionally, these cultivars yield less than some susceptible cultivars with the application of a nematicide (Johnson, 1990; Johnson *et al.*, 1989).

Some of the earliest work on resistance to *G. t. solanacearum* was performed by evaluating wild *Nicotiana* species. Baalawy and Fox (1971) found that *N. longiflora* L., *N. glutinosa* L., *N. plumbaginiflora* L., and *N. paniculata* L. exhibited varying forms of resistance to TCN. The resistant plants allowed juvenile penetration, eliminating the possibility of a mechanical barrier to penetration. In all four *Nicotiana* species, the majority of penetrating nematodes remained in the root as a second stage juvenile and did not develop into adult female cysts. Spasoff *et al*. (1971) found TCN resistance was linked to wildfire (*Pseudomonas syringae* pv. *tabaci*) resistance through crosses of the

burley wildfire-resistant breeding line ‘BVA523’ and the TCN susceptible cultivar ‘NC2326’. These crosses revealed four levels of TCN resistance, indicating that TCN resistance is theoretically of a multigenic nature. Subsequent tests using the dark-fired tobacco breeding line DVA 606 also supported the conclusion that TCN resistance may be multigenic (Miller *et al*., 1972). Elliott *et al*. (1986) supported this theory by evaluating the performance of the TCN resistant VA81 and PD4 over a four year rotation period. Resistance remained stable over this time period further suggesting that TCN resistance is of a multigenic nature. Such a conclusion can be dangerous, as stability of resistance over any period does not necessarily equate to a multigenic nature of resistance. A good example of such a case is the *Mi* gene in tomatoes conferring resistance to *Meloidogyne incognita*. This single, dominant gene has been used extensively and effectively for over 40 years in tomato breeding programs (Roberts *et al.*, 1998). Additionally, Elliott’s study involved only one isolate of the pathogen. Other resistant breaking biotypes could be present in nature.

Resistance in tobacco to *G. t. tabacum* has been very similar to *G. t. solanacearum*. LaMondia (1988) found that flue-cured tobacco lines PD4 and VA 81, resistant to *G. t. solanacearum*, were also resistant to *G. t. tabacum*. *Globodera tabacum tabacum* penetrated the root but was unable to successfully develop into adults when compared to the TCN susceptible CT86-4. The total amount of reproduction by *G. t. tabacum* was somewhat less than observed for *G. t. solanacearum* (LaMondia, 1991). A study looking at the nature of resistance to *G. t. tabacum* was performed by crossing resistant and susceptible lines. Resistance to *G. t. tabacum* was attributed to a single,

dominant gene, contrary to previous conclusions for *G. t. solanacearum* (LaMondia, 1991). This discretion between LaMondia's study and the studies conducted on *G. t. solanacearum* could be due to a number of factors, including experimental techniques, different expression of resistance in separate cultivars, or a true difference in the subspecies (LaMondia, 1991).

Several recent studies have examined the relationship between resistance to wildfire and TCN. Gwynn *et al*. (1986) concluded that the relation between wildfire and TCN resistant may not be as strong as originally suggested because 9 out of 21 lines tested were resistant to TCN but were susceptible to wildfire. A subsequent study (Hayes *et al.*, 1997) found that wildfire resistance could not be relied upon as a screening technique for TCN resistance. Wildfire results were variable in tobacco lines that exhibited TCN-resistance, with many TCN-resistant lines showing susceptibility to wildfire (Hayes *et al*., 1997). Perhaps a more appropriate screening technique for TCN resistance is the use of nematode reproductive data such as cysts per pot and eggs per cyst. Herrero *et al*. (1996) found that this technique was extremely useful in correlating reduced nematode counts with known TCN resistance. The collection of nematode data as a screening technique is very time consuming, but it seems necessary to effectively characterize the true nature and degree of resistance to TCN.

Chemical: The last tool for TCN control is use of pesticides. Early studies involving TCN control through pesticides included oxamyl, 1,2 dichloropropane (D-D), and 1,3 dichloropropene (1,3 D) (Miller, 1966; Osborne, 1971). Early testing also evaluated fungicides to determine their ability to suppress TCN damage. The fungicides

benomyl and thiabendazole were found to slightly reduce root invasion, but not at levels high enough to provide satisfactory control (Miller, 1969). Two soil incorporated herbicides (trifluralin and pebulate) increased reproductive rates of *G. t. solanacearum* on tomato (Pirkey and Miller, 1980).

Ethroprop and fenamiphos were used as the primary chemicals for TCN control in the 1970s and 1980s, respectively; however, current control using these products alone has been inadequate. Oxamyl is currently used in Connecticut to control *G. t. tabacum* and is still a recommended choice as a non-fumigant nematicide choice (LaMondia, 1990). Fenamiphos recently dropped TCN from its label due to decreased control performance. This may be due to the enhanced biodegradation associated with continuous use as reported by Davis *et al.* (1993). The loss of fenamiphos has sent farmers and researchers searching for alternative chemicals. Another pesticide commonly used for TCN control is a mixture of methyl bromide and chloropicrin. With the imminent cancellation of methyl bromide, this option will not be available to farmers in the future. Other currently used pesticides for TCN control include 1,3 dichloropropene alone, 1,3 dichloropropene mixed with chloropicrin, and aldicarb (Reed *et al*., 1997). Another potential nematicide that has been tested for a number of years is fosthiazate. Fosthiazate has been found to significantly increase yield and quality of treated tobacco as well as decrease nematode population densities (Johnson, 1995). Additionally, Wang (1996) supported the efficacy of fosthiazate by finding that the combination of fosthiazate and a TCN resistant cultivar (NC567) significantly decreased TCN population densities. However, fosthiazate is currently not registered for use on flue-cured tobacco.

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