CHARACTERIZING RESISTANCE IN FLUE-CURED TOBACCO TO *GLOBODERA TABACUM SOLANACEARUM*

by

Jie Wang

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APPROVED:

Charles S. Johnson

C. S. Johnson, Co-chairman

J. D. Eisenback, Co-chairman

G. J. Griffin

C. A. Wilkinson

T. D. Reed

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C. S Johnson, J. D. Eisenback, Co-chairmen

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(ABSTRACT)

Resistance to *Globodera tabacum solanacearum* in flue-cured tobacco was characterized *in vitro*, in the greenhouse, and field. The objectives of this study included evaluation of hatching of *G. t. solanacearum* as stimulated by root exudate, penetration and development of *G. t. solanacearum* on roots, and the effects of *G. t. solanacearum* on growth, yield, and quality of flue-cured tobacco.

Root exudate from resistant (NC 567) and susceptible (K 326) flue-cured tobacco cultivars had similar effects on hatching of *G. t. solanacearum*. Dilution of root exudate reduced hatching and hatching

appeared to increase with increased temperature. More swollen and flask-shaped nematodes developed in roots of K 326 than those of NC 567. Resistance reduced development of vermiform juveniles to swollen nematodes in NC 567. However, development of swollen nematodes to flask-shaped nematodes was similar for both cultivars. Resistance to *G. t. solanacearum* was effevtive at 17, 22, 27, and 31°C. Infection by *G. t. solanacearum* suppressed number of leaves, plant height, fresh weight of leaves, and feeder roots. Reduction in fresh leaf weight at 11 weeks after transplanting was greater for the susceptible cultivar, K 326, than for the resistant cultivar, NC 567. However reduction in fresh feeder root weight and increases in the ratio of leaf weight to feeder root weight at 11 weeks after transplanting between both cultivars. Yield and grade index of cured leaves were negatively correlated with area under curves of total nematode population densities per gram of feeder root. The rate parameters of regression models suggested that the two cultivars responded similarly to infection by *G. t. solanacearum* in yield and quality reduction. However, K 326 had a higher yield potential in the absence of nematodes.

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The tobacco cyst nematode complex comprises three sub-species, including *Globodera tabacum tabacum* (Lownsbery and Lownsbery, 1954) Behrens, 1975 (Behren, 1975), *Globodera tabacum virginiae* (Miller and Gray, 1968) Behrens, 1975 (Behren, 1975) and *Globodera tabacum solanacearum* (Miller and Gray, 1972) Behrens, 1975 (Behren, 1975) (Lownsbery and Lownsbery,1954; Miller and Gray, 1968, 1972). Tobacco cyst nematodes are distinguished from the closely related potato cyst nematodes by host range and other characters, including morphology and morphometrics. All tobacco cyst nematodes can reproduce on one or more cultivars of tobacco, *Nicotiana tabacum* (L.), whereas potato cyst nematodes can not (Miller and Gray, 1968, 1972)

G. t. tabacum is an important parasite of shade and broadleaf tobacco in the Connecticut River Valley of Connecticut and Massachusetts (LaMondia, 1990; Lownsbery, 1951; Lownsbery, 1955). The host range of *G. t. tabacum* also includes tomato (*Lycopersicon esculentum*), eggplant (*Solanum melongena*), and solanaceous weeds such as black nightshade (*Solanum nigrum*) (Harrison and Miller, 1969). Despite treatment with nematicides, losses are estimated at $50,000 annually, in addition to $60,000 spent for chemical control (Miller, 1986). *G. t. virginiae* was

originally found on horsenettle in Suffolk, Virginia (Miller and Gray, 1968). Although it is pathogenic to certain cultivars of tobacco, tomato, and eggplant, it is not known to infest soil where any of those crops are grown commercially; thus, it does not pose an imminent threat to agriculture. *G. t. solanacearum* is a serious pathogen of flue-cured tobacco (*Nicotiana tabacum*) in Virginia. Average yield reduction has been estimated at 15%, and complete crop failures have been recorded (Komm et al., 1983). An estimated one quarter of the total acreage of flue-cured tobacco in Virginia has been infested by *G. t. solanacearum* (Johnson unpubl.). Application of contact nematicides to control *G. t. solanacearum* costs an average of $139 per hectare (Johnson, 1989). *G. t. tabacum* was originally found and remains in the Connecticut River Valley. *G. t. virginiae* is only known in Virginia. *G. t. solanacearum* mainly occurs in Virginia, but several counties in North Carolina were recently reported to be infested (Melton et al., 1991; T. A. Melton, personal communication). Tobacco cyst nematodes have been reported from many parts of the world, but only in Morocco in Africa (Shepherd and Barker, 1990).

Taxonomy. Each subspecies was originally described as a separate species; however, the three forms can interbreed producing viable hybrids and are similar morphologically. They are, therefore considered as subspecies (Miller, 1983; Stone, 1983; Mugniery, et al., 1992). The

genetic distance between *G. t. virginiae* and *G. t. solanacearum* inferred from a comparison of patterns of total proteins generated by two-dimensional gel electrophoresis was very small (0.05), i.e. quite similar to that observed between populations of the same species (Bossis and Mugniery, 1993). The genetic distance between *G. t. tabacum* and the other two subspecies of *Globodera tabacum* was slightly greater (0.17), corresponding to intraspecific variability (Bossis and Mugniery, 1993). The status of these three forms as subspecies was recently confirmed by comparisons of the morphology and morphometrics of cysts, white females, males, and second-stage juveniles (Mota and Eisenback, 1993a-c).

Cyst shape is the most reliable character for distinguishing *G. t. tabacum* from *G. t. virginiae* and *G. t. solanacearum*. The globose shape of the cyst was very consistent among *G. t. tabacum* isolates from several locations (Mota and Eisenback, 1993c). Cysts of *G. t. solanacearum* and *G. t. virginiae* were elliptical. The ratio of length to width for *G. t. tabacum* was 1.0, compared to 1.1 to 1.2 for *G. t. virginiae* and *G. t. solanacearum* (Mota and Eisenback, 1993c). Shape of the fenestra was also used to distinguish the three subspecies. The fenestra is elliptical with obtuse ends in *G. t. tabacum*, circular to elliptical in *G. t. virginiae*, and barrel-shaped with convex ends for *G. t. solanacearum* (Mota and Eisenback, 1993b). Patterns of ridges and grooves in the terminal area

of females were also used to differentiate the three subspecies. In *G. t. tabacum*, the anus is closer to the fenestra than in *G. t. solanacearum* and *G. t. virginiae*. Ridges in the terminal area of females are typically parallel and perpendicular to the vulva-anus axis for *G. t. tabacum*. In *G. t. virginiae*, the ridges between the fenestra and anus are usually compacted and typically form a maze-like pattern. The anus is small and relatively inconspicuous in *G. t. virginiae*. In *G. t. solanacearum*, the anus is generally separated from the surrounding grooves in the center of a small anal basin. Large, wide grooves are normally found between the anus and fenestra. Mota and Eisenback (1993b) observed that the patterns of ridges and grooves show considerably more variability within the three subspecies than previously reported (Green, 1971).

The most useful character for separating white females of the three subspecies is the shape of the stylet knobs. The basal knobs in *G. t. tabacum* slope sharply posterior. In *G. t. virginiae*, the dorsal knob is curved anteriorly like a "Dutch shoe". The basal knobs in *G. t. solanacearum* are similar to those of *G. t. tabacum*, but are not as sharply sloped posteriorly.

The morphology and morphometrics of males and second-stage juveniles are similar among the three subspecies (Mota and Eisenback, 1993a,c). No useful characters have been found in males and second-stage

juveniles to distinguish the three subspecies.

Biology and Epidemiology. Research on the biology of tobacco cyst nematodes has focused on hatching and the effects of temperature on development. Hatching of *G. t. tabacum* and *G. t. solanacearum* increases after exposure to tobacco root exudate (Fox and Webber, 1970; LaMondia, 1993, 1995b). Root exudate from susceptible and resistant tobacco cultivars seems to stimulate hatching of *G. t. tabacum* similarly (LaMondia, 1988). In addition, some solanaceous crops (tomato) and weeds (nightshade) appear to stimulate hatching of *G. t. tabacum* (LaMondia, 1995b). Increased concentrations of root exudate and exudate from three week old plants stimulate greater hatching than lower concentrations and exudate from younger plants (LaMondia, 1995b). Natural hatching of *G. t. tabacum* has been estimated at 1 to 3% (Miller and Taylor, 1967).

The rate of development, size of cysts, and number of eggs and juveniles in cysts of *G. t. solanacearum* is affected by soil temperature (Adams et al., 1982). The life cycle of *G. t. solanacearum* is completed in 33 days at 27°C, 38 days at 32°C, 43 days at 21°C, and more than 48 days at 15°C. Cyst size and number of eggs and juveniles within cysts are greatest at 27°C. Both cyst size and numbers of eggs and juveniles decrease at temperatures above and below 27°C.

Seasonal changes in *G. t. solanacearum* population densities in the

field have also been investigated using two extraction techniques (Reilly and Grant, 1985). Numbers of cysts and juveniles of *G. t. solanacearum* were lowest in soil during June and July, following land tillage in May, with a peak in September-October. Freezing during early spring, however, could have greatly increased recovery of cysts from soil. Trends in population densities estimated as eggs of *G. t. solanacearum* in soil in July can be higher than those in May and October (Johnson, 1990).

Tobacco Cyst Nematode-Host Interaction. Very little research has been published concerning the mechanisms of compatibility and incompatibility in the interaction between tobacco cyst nematodes and tobacco. However, the nature of the interaction might be similar to that of other cyst nematode-host interactions. Second-stage juveniles penetrate host roots intracellularly, moving directly towards the central cylinder of vascular tissue (Jones, 1981). Characteristics of compatibility in the interaction are indicated by the development of invading juveniles and formation of syncytia (nurse cells) resulting from initial feeding. Incompatibility in the interaction is characterized by failure to remain in roots, death or delayed development of the invading juvenile. The syncytium may never develop, or its initial development may be restricted by necrosis of the surrounding xylem parenchyma cells (Rice et al., 1985). Another indication of incompatibility is an imbalance in

the proportion of males to females in the nematode population favoring the production of males (Trudgill, 1969).

Two flue-cured tobacco cultivars resistant to *G. t. solanacearum*, VA 81 and PD 4, also show resistance to *G. t. tabacum* (LaMondia, 1988). Second-stage juveniles penetrate the two cultivars, as well as the susceptible broadleaf cultivar CT86-4; however, juveniles invading VA 81 and PD 4 have little chance of developing into the adult stage but either leave roots or die (LaMondia, 1988). Classifying plants as resistant or susceptible on the basis of the presence of females, resistance to *G. t. tabacum* in VA 81 and PD 4 is reported to be conferred by a single dominant gene (LaMondia, 1991).

Resistance to *G. t. solanacearum* has been found in some wild *Nicotiana* species (Baalawy and Fox, 1971). Resistance in these species prevented development of second stage juveniles, rather than reducing their penetration. By counting the number of females recovered from plants nine weeks after inoculation, inheritance of resistance to *G. t. solanacearum* in a burley breeding line (BVA 523) and a dark-fired breeding line (DVA 606) suggested that resistance is multigenicly inherited (Miller et al., 1972; Spasoff et al., 1971). Different methods of classifying plants as resistant or susceptible were used in the above genetic studies. Resistance in two flue-cured tobacco cultivars VA 81 and

PD 4 was stable after three years of continuous cropping. Low indices of reproduction suggested that the resistance incorporated into the two cultivars may be multigenic, as in BVA 523 and DVA 606 (Elliott et al., 1986). Currently, flue-cured tobacco cultivars Coker 371-Gold, NC 567, and Speight G-80 are also reported to be resistant to *G. t. solanacearum* (Reed et al., 1995).

An association between inheritance of resistance to *G. t. solanacearum* and wildfire has been suggested (Spasoff et al., 1971; Komm and Terrill, 1982). All flue-cured tobacco cultivars with known or suspected *N. longiflora* parentage demonstrated a close linkage between wildfire and *G. t. solanacearum* resistance. However, the linkage appears to be broken in a fire-cured tobacco cultivar (KY 190) which possesses the *N. longiflora* source of wildfire resistance, but is susceptible to *G. t. solanacearum* (Hayes, 1995). Furthermore, the linkage may not exist for lines with resistance from a source other than *N. longiflora*. Several breeding lines derived from *N. repanda* were susceptible to wildfire, but resistant to *G. t. solanacearum* (Gwynn et al., 1986). *N. miersii* is susceptible to wildfire, and resistant to *G. t. solanacearum* (Hayes, 1995).

Resistant cultivars can also sustain considerable yield losses (Johnson et al., 1989; Johnson, 1990). Evidence suggests that resistance

and tolerance to *G. t. solanacearum* may be inherited independently (Fox and Spasoff, 1976).

Effects on Tobacco. High population densities of tobacco cyst nematodes can cause considerable reduction in tobacco yield and quality. *G. t. tabacum* can reduce leaf yields of shade tobacco by up to 45% compared with uninfected plants (LaMondia, 1995a). Average yield reductions caused by *G. t. solanacearum* have been estimated at 15%, but complete crop failure has also been recorded (Komm et al., 1983). Green yield of shade tobacco is negatively correlated with initial *G. t. tabacum* density (LaMondia, 1990, 1995a). At two months after inoculation, decreases of shoot dry weight of susceptible cultivars McNair 944 and Coker 319 were linearly correlated with initial populations of *G. t. solanacearum* (Grant et al., 1982). *G. t. solanacearum* increases alkaloid concentrations in cured leaf (Johnson et al, 1995). Increases in reducing sugar concentration are also seen in cured leaf from the first harvest when a nematicide has been applied to control *G. t. solanacearum.*

Interaction with Other Soil-borne Pathogens. Incidence of fusarium wilt, caused by *Fusarium oxysporum* (Schlecht.) Wr., has been reported to increase in the presence of *G. t. tabacum* (LaMondia and Taylor, 1987). Infection by *G. t. tabacum* predisposes broadleaf tobacco to Fusarium wilt (LaMondia, 1992). Presence of *G. t. solanacearum* and the Granville wilt

pathogen (*Pseudomonas solanacearum* (Smith) Smith) together reduces plant height more than either *G. t. solanacearum* or the bacterium alone (Elmer and Miller, 1980). *G. t. solanacearum* increases black shank disease development (Bower et al., 1980). The types of interaction between *Phytophthora parasitica* Dastur var. *nicotianae* (Breda de Haan) Tucker and *G. t. solanacearum* are dependent upon inoculum levels of the pathogens, resistance or susceptibility to one or both pathogens, soil temperature, and moisture (Grant et al., 1984). All combinations of these factors resulted in synergistic interactions between *P. p.* var. *nicotianae* and *G. t. solanacearum* on cultivar VA 81, highly resistant to *G. t. solanacearum* and susceptible to *P. p.* var. *nicotianae*. Interactions between the two pathogens were also synergistic at lower inoculum levels, but antagonistic at high inoculum levels on cultivars McNair 944 and Coker 319, which were both susceptible to *G. t. solanacearum* but possessed moderate or low level resistant to *P. p.* var. *nicotianae*. All interactions were synergistic at high inoculum levels under field conditions. In split-root experiments, *G. t. solanacearum* development accelerated on one half of the root system when the other half of the root system was infected by *P. p.* var. *nicotianae* (Reilly and Elliott, 1984). *G. t. solanacearum* and *P. p.* var. *nicotianae* both needed to be present on the same half of root system to cause severe root necrosis.

G. t. tabacum inhibits reproduction of *Pratylenchus penetrans* (Cobb) Filipjev Stekhoven 1917 on shade tobacco (Miller and Wihrheim, 1968). Although large populations of *P. penetrans* can also slow the increase of *G. t. tabacum*, moderate to high populations of *G. t. tabacum* suppress *P. penetrans* to the point that populations of *P. penetrans* become undetectable (Miller, 1970).

Control of Tobacco Cyst Nematodes. *G. t. tabacum* is managed intensively by fumigant and nonfumigant nematicides on shade tobacco, primarily because of limited choices of rotation crops (LaMondia, 1995a). Oxamyl, a systemic insecticide-nematicide, increased green leaf yield of shade tobacco by 10.7 to 21.0% when used at 2.2 to 6.7 kg a.i./ha, and where initial density of second-stage juveniles of *G. t. tabacum* ranged from 33 to 154/cm^3 soil (LaMondia, 1990). An integrated tactic is recommended for *G. t. solanacearum* management. Cultural practices such as crop rotation and early destruction of tobacco debris can help control *G. t. solanacearum*. Resistant cultivars can be grown for several years in order to reduce *G. t. solanacearum* population in heavily-infested fields (Johnson et al., 1989; Johnson, 1990). Fumigants such as methyl bromide, 1,3-dichloropropene, and chloropricrin are commonly used to control *G. t. solanacearum* (Reed et al., 1995). However, satisfactory control results from proper placement of appropriate rates when soil

conditions (porosity, moisture content, and temperature) allow fumigants to diffuse throughout the soil, partition between the water and air phases of the soil, and remain long enough to adequately kill the nematode (Lembright, 1990). Some non-fumigant nematicides are also recommended to control *G. t. solanacearum*. Fenamiphos is recommended at higher rates to control *G. t. solanacearum* than those used in root-knot nematode control. However, loss of efficacy has been reported for soil treated repeatedly with fenamiphos because of accelerated degradation (Davis et al., 1993). Therefore, continuous use of fenamiphos is not recommended when fields are not rotated. Aldicarb should not be applied more than one week before transplanting when it is used to control *G. t. solanacearum* (Reed et al., 1995).

LITERATURE CITED

Adams, H. S., W. W. Osborne, and A. J. Webber, Jr. 1982. Effect of temperature on development and reproduction of *Globodera solanacearum*. Nematropica 12:305-311.

Baalawy, H. A., and J. A. Fox. 1971. Resistance to Osborne cyst nematode in selected *Nicotiana* species. Journal of Nematology 3:395-398.

Behrens, E. 1975. *Globodera* Skarbilovich, 1959, eine selbstandige Gattung in der Unterfamililie Herteroderinae Skarbilovich, 1947 (Nematoda: Heteroderidae). Pp 12-26 in Vortragstagung (1) zu aktuellen problemen der Phytonematologie am 29.5. 1975 in Rostock, DDR., ed. Rostock.

Bossis, M., and D. Mugniery. 1993. Specific status of six *Globodera* parasites of solanaceous plants studied by means of two-dimensional gel electrophoresis with a comparison of gel patterns by a computed system. Fundamental and Applied Nematology 16:47-56.

Bower, L. A., J. A. Fox, and L. I. Miller. 1980. Influence of *Meloidogyne incognita* and *Globodera solanacearum* on development of black shank of tobacco. Phytopathology 70:688 (Abstr.).

Davis, R. F., A. W. Johnson, and R. D. Wauchope. 1993. Accelerated degradation of fenmiphos and its metabolites in soil previously treated with fenamiphos. Journal of Nematology 25:679-685.

Elliott, A. P., P. M. Phipps, and T. R. Terrill. 1986. Effects of continuous cropping of resistant and susceptible cultivars on reproduction potential of *Heterodera glycines* and *Globodera solanacearum*. Journal of Nematology 18:375-379.

Elmer, W. H., and L. I. Miller. 1980. Influence of *Psuedomonas solanacearum* and *Globodera solanacearum* on growth of tobacco. Virginia Journal of Science 31:78 (Abstr.).

Fox, J. A., and A. J. Webber. 1970. The hatching response of Osborne's cyst nematode to root leachates from germ-free tobacco plants. Journal of Parasitology 56:105 (Abstr.).

Fox, J. A., and L. Spasoff. 1976. Resistance and tolerance of tobacco to *Heterodera solanacearum*. Journal of Nematology 8:284-285.

Grant, C. E., J. J. Reilly, and A. P. Elliott. 1982. Reproduction of *Globodera solanacearum* and its effect on growth and development of three flue-cured tobacco cultivars under greenhouse conditions. Journal of Nematology 14:443 (Abstr.).

Grant, C. E., J. J. Reilly, and A. P. Elliott. 1984. Interaction of *Globodera solanacearum* (=*G. tabacum solanacearum*) and *Phytophthora parasitica* var. *nicotianae* with flue-cured tobacco. Phytopathology 74:756-757 (Abstr.).

Green, C. D. 1971. The morphology of the terminal area of the round-cyst nematodes S.G. *Heterodera rostochensis* and allied species. Nematologica 17:34-46.

Gwynn, G. R., K. R. Barker, J. J. Reilly, D. A. Komm, L. G. Burk, and S. M. Reed. 1986. Genetic resistance to tobacco mosaic virus, cyst nematode, root-knot nematodes, and wildfire from *Nicotiana repanda* incorporated into *N. tabacum*. Plant Disease 70:958-962.

Hayes, A. J. 1995. Identification and genetic characterization of tobacco accessions possessing resistance to tobacco cyst nematode. M.S. Thesis. Virginia Polytechnic Institute and State University. Pp 46.

Harrison, M. B. and Miller. L. I. 1969. Additional hosts of the tobacco cyst nematode. Plant Disease Report 53:949-951.

Johnson, C. S. 1989. Managing root-knot on tobacco in the southeastern United States. Supplement to Journal of Nematology 21,4s:604-608.

Johnson, C. S. 1990. Control of *Globodera tabacum solanacearum* by rotating susceptible and resistant flue-cured tobacco cultivars. Supplement to Journal of Nematology 22:700-706.

Johnson, C. S., D. A. Komm, and J. L. Jones. 1989. Control of *Globodera tabacum solanacearum* by alternating host resistance and nematicide. Journal of Nematology 21:16-23.

Johnson, C. S., J. Wang, and W. M. Tilson. 1995. Effects of tobacco cyst nematode control measures on tobacco leaf chemistry. Phytopathology 85:1141 (Abstr.).

Jones, M. G. K. 1981. Host cell responses to endoparasitic nematode attack: structure and function of giant cells and syncytia. Annala of Applied Biology 97:353-372.

Komm, D. A., and T. R. Terrill. 1982. A rapid screening technique for the determination of resistance in *Nicotiana tabacum* to a tobacco cyst nematode, *Globodera solanacearum*. CORESTA Information Bulletin. Pp 64-65.

Komm, D. A., J. J. Reilly, and A. P. Elliott. 1983. Epidemiology of a tobacco cyst nematode (*Globodera solanacearum*) in Virginia. Plant Disease 67:1249-1251.

LaMondia, J. A. 1988. Tobacco resistance to *Globodera tabacum*. Annals of Applied Nematology 2:77-80.

LaMondia, J. A. 1990. Effect of oxamyl on *Globodera tabacum* population dynamics and shade tobacco growth and yield. Journal of Nematology 22:654-657.

LaMondia, J. A. 1991. Genetics of tobacco resistance to *Globodera tabacum tabacum*. Plant Disease 75:453-454.

LaMondia, J. A. 1992. Predisposition of broadleaf tobacco to fusarium wilt by early infection with *Globodera tabacum solanacearum* or *Meloidogyne hapla*. Journal of Nematology 24:425-431.

LaMondia, J. A. 1993. Juvenile emergence and reproduction of *Globodera tabacum* in response to tobacco, tomato and black nightshade. Phytopathology 83:245 (Abstr.).

LaMondia, J. A. 1995a. Shade tobacco yield loss and *Globodera tabacum solanacearum* population changes in relation to initial nematode density. Journal of Nematology 27:114-119.

LaMondia, J. A. 1995b. Hatch and reproduction of *Globodera tabacum tabacum* in response to tobacco, tomato, or black nightshade. Journal of Nematology 27:382-386.

LaMondia, J. A., and G. S. Taylor. 1987. Influence of the tobacco cyst nematode (*Globodera tabacum*) on fusarium wilt of Connecticut broadleaf tobacco. Plant Disease 71:1129-1132.

Lembright, H. W. 1990. Soil fumigation: principles and application technology. Supplement to Journal of Nematology 22(4s):632-644.

Lownsbery, B. F. 1951. Tobacco attacked by a species of *Heterodera* in Connecticut. Cooperative Economic Insect Report. Vol. 1. Special Report No. 1:59-60.

Lownsbery, B. F., and Lownsbery, J. W. 1954. *Heterodera tabacum* new species, a parasite of solanaceous plants in Connecticut. Proceedings of the Helminthological Society of Washington 21:42-47.

Lownsbery, B. F., and B. G. Peters. 1955. The relation of the tobacco cyst nematode to tobacco growth. Phytopathology 45:163-167.

Melton, T. A., J. A. philips, J. L. Imbriani, and K. R. Barker. 1991. First report of *Globodera tabacum solanacearum* on flue-cured tobacco outside Virginia. Plant Disease 75:1074.

Miller, L. I. 1983. Diversity of selected taxa of *Globodera* and *Heterodera* and their interspecific and intergeneric hybrids. Pp. 207-220 in A. R. Stone, H. M. Platt, and L. F. Khalil, eds. Concepts in Nematode Systematics. London: Academic Press.

Miller, L. I. 1986. Economic importance of cyst nematodes in North America. Pp. 373-386 in F. Lambert and C. E. Taylor, eds. Cyst Nematodes. New York: Plenum Publishing.

Miller, L. I., and Gray, B. T. 1968. Horsenettle cyst nematode, *Heterodera virginiae* n. sp., a parasite of solanaceous plants. Nematologica 14:535-543.

Miller, L. I., and Gray, B. T. 1972. *Heterodera solanacearum* n. sp., a parasite of solanaceous plants. Nematologica 18:404-413.

Miller, L. I., J. A. Fox, and L. Spasoff. 1972. Genetic relationship of resistance to *Heterodera solanacearum* in dark-fired and burley tobacco. Phytopathology 62:778 (Abstr.).

Miller, P. M. 1970. Rate of increase of a low population of *Heterodera tabacum* reduced by *Pratylenchus penetrans* in the soil. Plant Disease Reporter 54:25-26.

Miller, P. M., and G. S. Taylor. 1967. Effects of fungicides on hatching, survival, and nematicidal killing of eggs of *Heterodera tabacum* in the field. Plant Disease 51:609-613.

Miller, P. M., and S. E. Wihreim. 1968. Mutual antagonism between *Heterodera tabacum* and some other parasitic nematodes. Plant Disease Reporter 52:57-58.

Mota, M. M., and Eisenback, J. D. 1993a. Morphology of second-stage juveniles and males of *Globodera tabacum tabacum*, *G. t. virginiae*, and *G. t. solanacearum* (Nemata: Heteroderinae). Journal of Nematology 25:27-33.

Mota, M. M., and Eisenback, J. D. 1993b. Morphology of females and cysts of *Globodera tabacum tabacum*, *G. t. virginiae*, and *G. t. solanacearum* (Nemata: Heteroderinae). Journal of Nematology 25:136-147.

Mota, M. M., and Eisenback, J. D. 1993c. Morphometrics of *Globodera tabacum tabacum*, *G. t. virginiae*, and *G. t. solanacearum* (Nemata: Heteroderinae). Journal of Nematology 25:148-160.

Mugniery, D., M. Bossis, and J. S. Pierre. 1992. Hybridation entre *Globodera rostochensis* (Wollenweber), *G. pallida* (Stone), *G. virginiae* (Miller & Gray), *G. solanacearum* (Miller & Gray) et *G. "mexican"* (Campos-Vela). Description et devenir des hybrides. Fundamental and Applied Nematology 15:375-382.

Reed, T. D., J. L. Jones, C. S. Johnson, P. J. Semtner, B. B. Ross, and C. A. Wilkinson. 1995. 1995 flue-cured tobacco production guide. Virginia Cooperative Extension. Publication 436-048. Pp.90.

Reilly, J. J., and A. P. Elliott. 1984. Disease development and cyst reproduction on tobacco split root system infected by *Globodera solanacearum* and *Phytophthora parasitica* var. *nicotianae*. Phytopathology 74:758 (Abstr.).

Reilly, J. J., and C. E. Grant. 1985. Seasonal fluctuation of *Globodera tabacum solanacearum* as estimated by two soil extraction techniques. Journal of Nematology 17:354-360.

Rice, S. L., B. S. C. Leadbeater, and A. R. Stone. 1985. Changes in cell

structure in roots of resistant potatoes parasitized by potato-nematode. I. Potatoes with resistance gene H1 derived from *solanum tuberosum* ssp. *andigena*. Physiological Plant Pathology 27:219-234.

Shepherd, J. A., and K. R. Barker. 1990. Nematode parasites of tobacco. Pp 493-517. In M. Luc, R. A. SiKora, and J. Bridge, eds. Plant parasitic nematodes in subtropical and tropical agriculture. CAB International Wallingford, UK.

Spasoff, L., L. A. Fox, and L. I. Miller. 1971. Multigenitic inheritance of resistance to Osborne's cyst nematode in tobacco. Journal of Nematology 3:329-330 (Abstr.).

Stone, A. R. 1983. Three approaches to the status of a species complex, with a revision of some species of *Globodera* (Nematoda: Heteroderidae). Pp. 221-233 in A. R. Stone, H. M. Platt, and L. F. Khalil, eds. Concepts in Nematode Systematics. London: Academic Press.

Trudgill, D. L., and D. M. Parrott. 1969. The behaviour of nine populations of the potato cyst-nematode *Heteroera rostochiensis* towards three resistant potato hybrids. Nematologica 15:381-388.

Hatching of *Globodera tabacum solanacearum* Juveniles by Root Exudate of Flue-cured Tobacco (*Nicotiana tabacum*) Cultivars

Abstract: Hatching of a tobacco cyst nematode (*Globodera tabacum solanacearum*) stimulated by root exudates from resistant 'NC 567' and susceptible 'K 326' flue-cured tobacco, *Nicotiana tabacum* (L.), was determined *in vitro*. Root exudate was collected by soaking seedlings in deionized water for 2 h at 22°C in the dark. Fifteen mature and uniformly-sized cysts were exposed to undiluted root exudate, to root exudate diluted 1:1 or 1:3 with deionized water, or to deionized water alone, at 15, 20, or 25°C. Hatched juveniles were counted and removed at weekly intervals for 42 and 53 days of exposure. Hatching appeared to increase with increased temperature. Root exudate from K 326 and NC 567 had similar effects on hatching. Root exudate from either cultivar induced significantly more hatching than deionized water at 25°C in experiment 1 and at all temperatures in experiment 2. Dilution of root exudate reduced hatching at 25°C in experiment 1 and at all temperatures in experiment 2.

Key words: tobacco cyst nematode, resistance, temperature.

Tobacco cyst nematode, *Globodera tabacum solanacearum* (Miller and Gray) Behrens, 1975, is one of the most serious pathogens of flue-cured tobacco, *Nicotiana tabacum* (L.) in Virginia (Miller and Gray, 1972). Average yield reductions caused by *G. t. solanacearum* have been estimated at 15% and complete crop failures have also been recorded (Komm et al., 1983). An estimated one quarter of the total acreage of flue-cured tobacco in Virginia is infested by *G. t. solanacearum* (Johnson, unpubl.). Tactics of *G. t. solanacearum* management include crop rotation, nematicides, and host resistance. The limited availability of effective nematicides, the relatively high survival rate of *G. t. solanacearum*, and the limited choices of rotation crops suggest host resistance could play an important role in improving management of *G. t. solanacearum*. Various degrees of resistance to *G. t. solanacearum* exist in wild *Nicotiana* species (Baalawy and Fox, 1971). Most widely used commercial cultivars are susceptible to *G. t. solanacearum*, but cultivars resistant to *G. t. solanacearum* have been identified (Johnson et al., 1989). However, significant yield reductions continue to occur with resistant cultivars in the presence of high *G. t. solanacearum* populations. Consequently, the main management tactic used is the application of contact nematicides to susceptible cultivars at an average cost of $139/ha (Johnson et al., 1989).

Hatching is a very important stage in the life cycle of cyst nematodes. Juveniles of *Globodera* have long been known to hatch in response to leachings from host roots (Triffitt, 1930). Althoug little has been confirmed about the chemical properties of various root exudate, their production of root exudate might be results of passive leaching of host tissue. Hatching of *G. t. solanacearum* increased following after exposure of cyst to tobacco root exudate in combination with soil microorganisms (Fox and Webber, 1970). Hatching of *G. t. tabacum* is also stimulated by some non-tobacco plants (LaMondia, 1995). Efficiency of hatching stimulation has been correlated with resistance to potato cyst nematodes in several investigations (Arntzen et al., 1993 and 1994; Farrer and Philips, 1983; Turner and Stone, 1981). Poor hatching activity has also been suggested as a component of host tolerance (Evans, 1983; Arntzen et al., 1994).

The mechanisms of resistance to *G. t. solanacearum* in cultivated flue-cured tobacco are unknown. This research was undertaken to compare hatching of *G. t. solanacearum* after exposure to root exudates from a resistant (NC 567) and a susceptible (K 326) flue-cured tobacco cultivar. The influence of root exudate concentration and temperature were also evaluated.

MATERIALS AND METHODS

Two hatching experiments were carried out with two different sources of cysts of *G. t. solanacearum*. However, hatching procedures in these experiments were similar.

Root exudate preparation: Plants of K 326 and NC 567 suitable for transplanting to the field (10-week-old) were collected from plant beds. Root systems of plants were carefully removed from soil, washed, and blotted dry on paper towels. Dried root systems were cut below ground level and weighed before transfer to beakers. Approximately 55 g root was used in this study. Beakers containing root systems were filled with deionized water of five times greater than the weight of the roots. Undiluted root exudate was collected by decanting beakers over a 325 mesh screen (45 μm) after 2 hours of soaking at 22°C in dark. Root exudate was diluted by mixing undiluted root exudate with equal (1:1) or triple (1:3) volumes of deionized water. All root exudate was kept at 4°C throughout these experiments. Experiments 1 and 2 were carried out in 1994 and 1995, respectively.

Hatching chamber: Hatching occurred in chambers made by cutting off the tapered end of a polyethelene BEEM capsule and severing the cap from the capsule. Individual caps were perforated with an 8 mm diameter

hole. A piece of 400 mm^2 nylon screen (150 μm) was put on one of the ends of each capsule before placing a punctured cap back on the end of the capsule. The other end of each capsule was left open. A capsule with a piece of nylon screen and a punctured cap was placed on the bottom of a 20 X 150 mm test tube with the open end on the top. Each test tube contained one capsule and served as a hatching chamber throughout the experiment.

Preparation of cysts: Cysts were propagated on flue-cured tobacco cultivar Coker 319 in the greenhouse. Soil containing cysts was thoroughly mixed with water in a plastic bucket and allowed to settle for 15 seconds. The supernatant was poured through 20 mesh (850 μm) and 60 mesh (250 μm) sieves with the 20 mesh sieve on the top. Cysts retained on the bottom 60 mesh sieve were collected for the hatching experiments. A single batch of cysts were used for each experiment. Cysts were washed in tap water twice before the start of each experiment. Cysts in experiment 1 were stored in tap water for 12 weeks at 4°C. Cysts used for experiment 2 were stored in tap water at 4°C for one week after harvesting. Cysts were crushed manually to determine average egg content. Eggs from crushed cysts were stained with acid-fuchsin (Barker, 1985). The egg solution was rinsed with tap water and the number of eggs stained was counted under low magnification. Each cyst contained an initial

average of 185 eggs in experiment 1. Each cyst had an average of 218.8 eggs and 25.6 well-developed second-stage juveniles at the beginning of experiment 2.

Exposure of cysts to root exudate: Fifteen uniformly-sized cysts from the stored cysts were placed in each hatching chamber at the start of each experiment and immersed into 1.5 ml of root exudate solution in each hatching chamber. Root exudate was renewed weekly in each hatching chamber throughout the period of the experiments. Deionized water was used as a control.

Treatments and experimental design: Root exudate treatments were organized using a complete factorial design of cultivars and dilutions with four replications. Root exudate from two cultivars (K 326 and NC 567) and 3 dilutions (undiluted, 1:1, and 1:3,) were included in the experiments. A deionized water treatment was used as a control for the two cultivars. Three temperatures (15, 20, and 25°C) were imposed as a main plot factor on all treatments and treatments were randomized within each temperature. Hatching chambers were maintained in dark at tested temperature provided by incubators. Number of hatched juveniles were recorded on a weekly basis and removed from hatching chambers after counting. Experiment 1 lasted for 42 days and experiment 2 lasted for 53 days.

Statistical analysis: Analysis of variance was conducted on the number of hatched juveniles at each of the individual sampling dates (SAS, 1985). Duncan's multiple range test was performed in all analyses.

RESULTS

The general pattern of hatching differed between experiment 1 and 2 (Figs. 2.1 and 2.2). Few juveniles hatched during the first two weeks in experiment 1. More juveniles were counted at 20 and 25°C at each sampling date toward the end of the experiment. In experiment 2, more juveniles had hatched at the first sampling date than at any of the other sampling dates. No interaction between cultivar and dilution was detected except on sampling date 27 at 20°C in experiment 2. In that case, the 1:3 dilution of root exudate from NC 567 reduced hatching, whereas similar dilution of root exudate from K 326 had no effect.

Very few hatched juveniles were found at 15° in experiment 1 (Fig. 2.1). Root exudate from the two cultivars had similar effects on hatching. Root exudate from K 326 induced higher ($P \leq 0.05$) hatching in experiment 1 than in deionized water from 21 to 42 days of exposure at 25°C. Root exudate from NC 567 induced higher ($P \leq 0.05$) hatching than that in deionized water at 28 and 35 days of exposure at 25°C. However, no

differences in hatching were observed between either cultivar and deionized water at 15 or 20°C (Fig. 2.1).

In experiment 2, root exudate from K 326 induced significantly higher ($P \leq 0.05$) hatching than in deionized water at 25°C (Fig. 2.1). Higher ($P \leq 0.05$) hatching was observed after exposure to root exudate from K 326 compared to deionized water during the first four weeks at 20 and 15°C. More ($P \leq 0.05$) hatched juveniles were recorded after 7, 36 , 44, and 53 days of exposure to root exudate from NC 567 than in deionized water at 25°C (Fig. 2.1). Root exudates from NC 567 stimulated higher ($P \leq 0.05$) hatching for the first 27 and 21 days after exposure at 20° and 15°C, respectively. Hatching stimulation was similar between root exudates from both cultivars in experiment 2, except that root exudate from K 326 induced greater hatching ($P \leq 0.05$) than from NC 567 after 21 days of exposure at 25°C (Fig. 2.1).

In experiment 1, the 1:3 dilution of root exudates significantly ($P \leq 0.05$) reduced hatching compared to the 1:1 dilution and undiluted root exudate after 28 and 42 days of exposure at 25°C, respectively (Fig. 2.2). Diluted root exudate stimulated hatching, although hatching at the 1:3 dilution was higher ($P \leq 0.05$) than in deionized water only at 35 days of exposure at 25°C. No differences in hatching were detected among the three dilutions and deionized water at 20 or 15°C, except that hatching

in the 1:3 dilution was higher ($P \leq 0.05$) than in deionized water at 35 and 42 days at 20°C.

In experiment 2, 1:1 dilution of root exudate did not significantly reduced hatching compared to the undiluted root exudate at 25° except at 36 and 44 days of exposure (Fig. 2.2). Hatching in the 1:3 dilution was lower than in the undiluted root exudate after 36 days of exposure at 25°C. Dilutions of root exudate stimulated hatching, although hatching in 1:3 dilution was only higher ($P \leq 0.05$) than in deionized water at 14 days of exposure at 25°C. Hatching in 1:1 dilution of root exudate was lower ($P \leq 0.05$) than in undiluted root exudate after 21 and 36 days of exposure at 20°C. The 1:3 dilution reduced hatching significantly at 7, 14, and 27 days of exposure. Significant differences in hatching between 1:3 dilution and deionized water were found at 7, 14, and 27 days of exposure at 20°C. The 1:1 dilution had lower ($P \leq 0.05$) hatching than undiluted root exudate at dates 27 and 36 of exposure at 15°C. More ($P \leq 0.05$) hatching was found in undiluted root exudate than in the 1:3 dilution from 14 to 44 days of exposure at 15°C. Differences ($P \leq 0.05$) in hatching between 1:3 dilution and deionized water were found at 14 and 44 days of exposure.

Very few juveniles hatched at 15°C in experiment 1 despite exposure to root exudate from the two cultivars (Fig. 2.3). Higher temperatures stimulated greater hatching in the presence of root exudate. In

experiment 2, hatching in root exudate from K 326 was always higher at 25°C than at 20 or 15°C except at 7 days of exposure (Fig. 2.3). On the other hand, differences in hatching between 15 and 20°C did not persist beyond the first two weeks. Differences in hatching among the three temperatures were smaller for NC 567 than for susceptible cultivar K 326.

DISCUSSION

Although the influence of root exudates on hatching was similar across experiments, percentage of hatched juveniles from eggs counted in cysts was much smaller in experiment 1 than experiment 2. Variation in hatching was reported for *Globodera tabacum tabacum* (LaMondia, 1995). Hatching of cysts of *G. rostochensis* is affected by diapause (Shepherd and Cox, 1967; Hominick et al. 1985). Day length and light intensity during cyst formation influence hatching of *G. rostochensis* and *G. pallida*. Cysts of *G. rostochensis* and *G. pallida* that develop during seasons of the year with increasing day-lengths conditions have higher hatching rates than those developing under short-day conditions (Salazar and Ritter, 1993). Diapause has also been suggested to contribute to variation in hatching of *G. t. tabacum* (LaMondia, 1995). Cysts used in experiment 1 were harvested in March, while cysts in experiment 2 were

harvested in June. Short-day conditions experienced by cysts in experiment 1 may have imposed diapause on cysts, resulting in lower hatching. Low storage temperature (5°C) reduced hatching of *G. rostochensis* (Muhammad, 1994). Cysts used in experiment 1 had been stored at 4°C for 12 weeks before the experiment. The long period of low temperature may have had a detrimental effect on eggs in cysts, resulting in a low percentage of hatchable eggs. Hyphae of unidentified fungi were also observed in storage vials during the period of storage. Lower hatching has been attributed to fungal disintegration of cyst walls under cold and wet conditions (Oostenbrink, 1967). Hatching was higher in the first two weeks than thereafter in experiment 2. The initial active hatching in this experiment may have resulted from hatching of well-developed second-stage juveniles within cysts. The cumulative percent hatching in our experiments is relatively small compared to those of potato cyst nematodes (Arntzen et al., 1993). The highest cumulative percent hatching was 13 and 18% after 53 days at 20 and 25°C, respectively. Different procedures collecting root exudate such as growing palnts in solution or leaching roots in media with water and diluting root exudate from different initial solution prevented a direct comparison between studies. A similarly small hatching response by *G. t. tabacum* to tobacco root exudate has also been reported (Lamondia, 1995).

An overall hatching rate of *G. t. tabacum* has also been reported at 1 to 3% (Miller and Taylor, 1967). Generally lower hatching by tobacco cyst nematodes compared to potato cysts could be an advantage, preventing over-crowding of juveniles when infection sites are limited.

Although root exudate stimulated greater hatching than deionized water, root exudate from both cultivars stimulated hatching similarly. Study of hatching by *G. t. tabacum* also indicated no differences in hatching among susceptible and resistant cultivars (LaMondia, 1988). Hatching of *G. t. solanacearum* decreased similarly with increasing dilution of root exudates from both cultivars. Influence of dilution on hatching was also observed on *G. t. tabacum* (LaMondia, 1995). Our results suggest that hatching stimulation by root exudate probably plays no role in resistance to *G. t. solanacearum*. However, the concentrations of root exudate in these experiments were related to the blot dry weight of root systems. Vigorous root growth probably generates greater amounts and higher concentration of root exudate. Hatching of *G. rostochensis* was positively correlated with increased root weight during the first three weeks after emergence (Rawsthorne and Brodie, 1986). Concentrations of root exudate also likely decline with distance away from active root tissues. Differences in root growth should, therefore, not be overlooked when evaluating host effects on hatching. Age of host has been reported

to influence hatching. Root exudate from 3-week-old tobacco does not stimulate as much hatching of *G. t. tabacum* as older plants (Lamondia, 1995). Further research is needed to evaluate the influence of root growth and distribution on hatching stimulation. Persistence of hatching factor has been reported as less than two months in soil in the absence of potato (Perry et al., 1981). Root exudate is a good source of nutrients for various soil organisms. As a result, effectiveness of root exudate may also be influenced by microorganisms associated with roots as well as by the surrounding soil environment.

Raw root exudate was used in this study. The uncertain identity of the hatching factor in tobacco root exudate complicates comparisons between different studies and increases the difficulty in further understanding the mechanism of hatching. Purifying the substances responsible for hatching stimulation, or at least partially purifying the hatching factor(s) may allow more accurate quantification of the hatching response, evaluation of their relative stability in the soil environment, and their usefulness in cyst nematode management. Chemically identified hatching factors would also offer the opportunity to develop faster screening methods to identify hatching differences among host genotypes in a commercial plant breeding program.

In summary, hatching appeared to increase with increased

temperature and dilutions of root exudate reduced hatching. Root exudate from K 326 and NC 567 had similar effects on hatching. Research of *G. t. tabacum* has also concluded that hatching stimulation was not different between susceptible and resistant cultivars. Hatching stimulation may not play an important role in tobacco and tobacco cyst nematode interaction. Resistance evaluation, therefore, may focus on other stages of nematode development.

Fig. 2.1. Number of juveniles of *Globodera tabacum solanacearum* hatched in root exudate from K 326, NC 567, and deionized water at 15, 20, and 25°C.

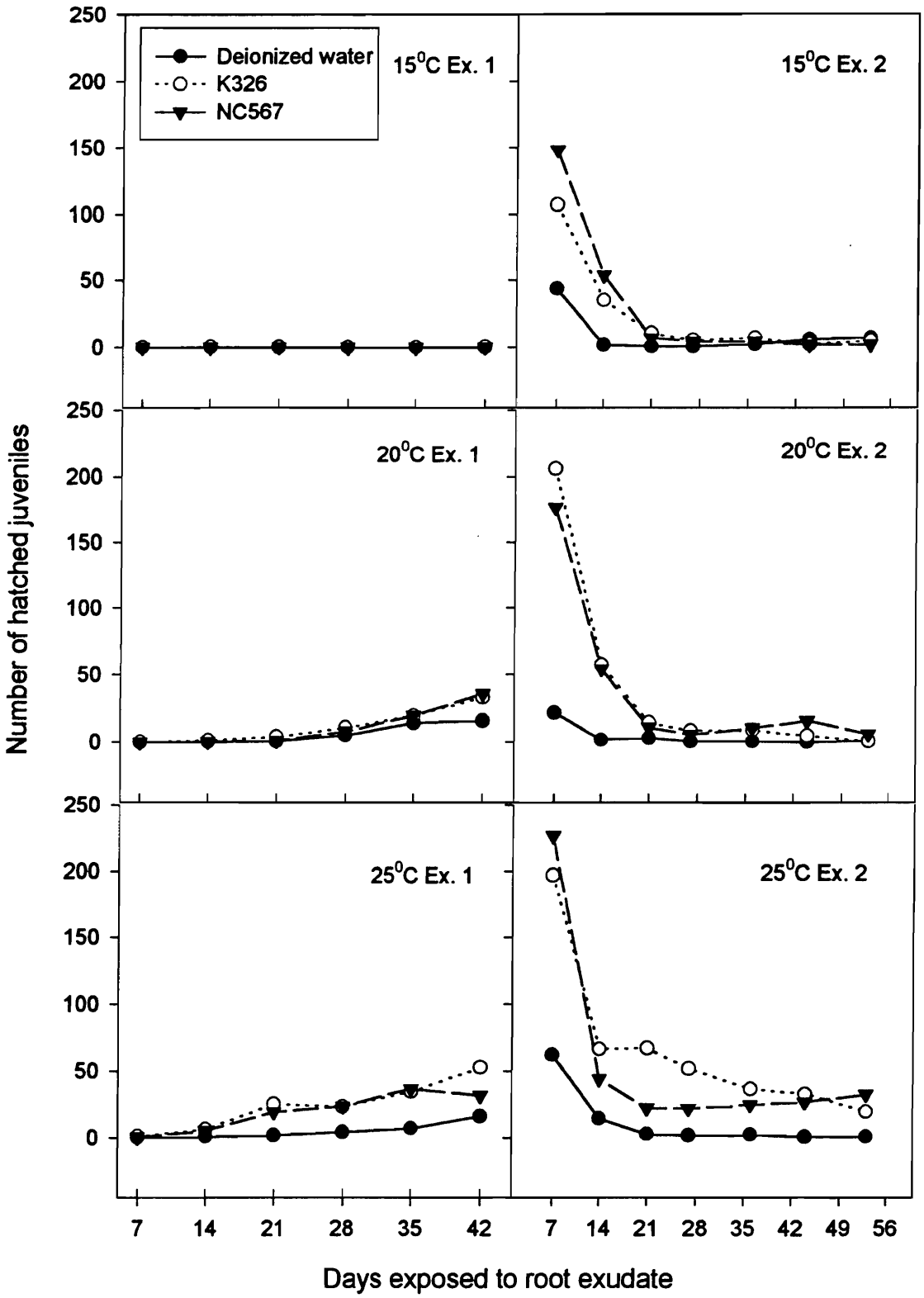
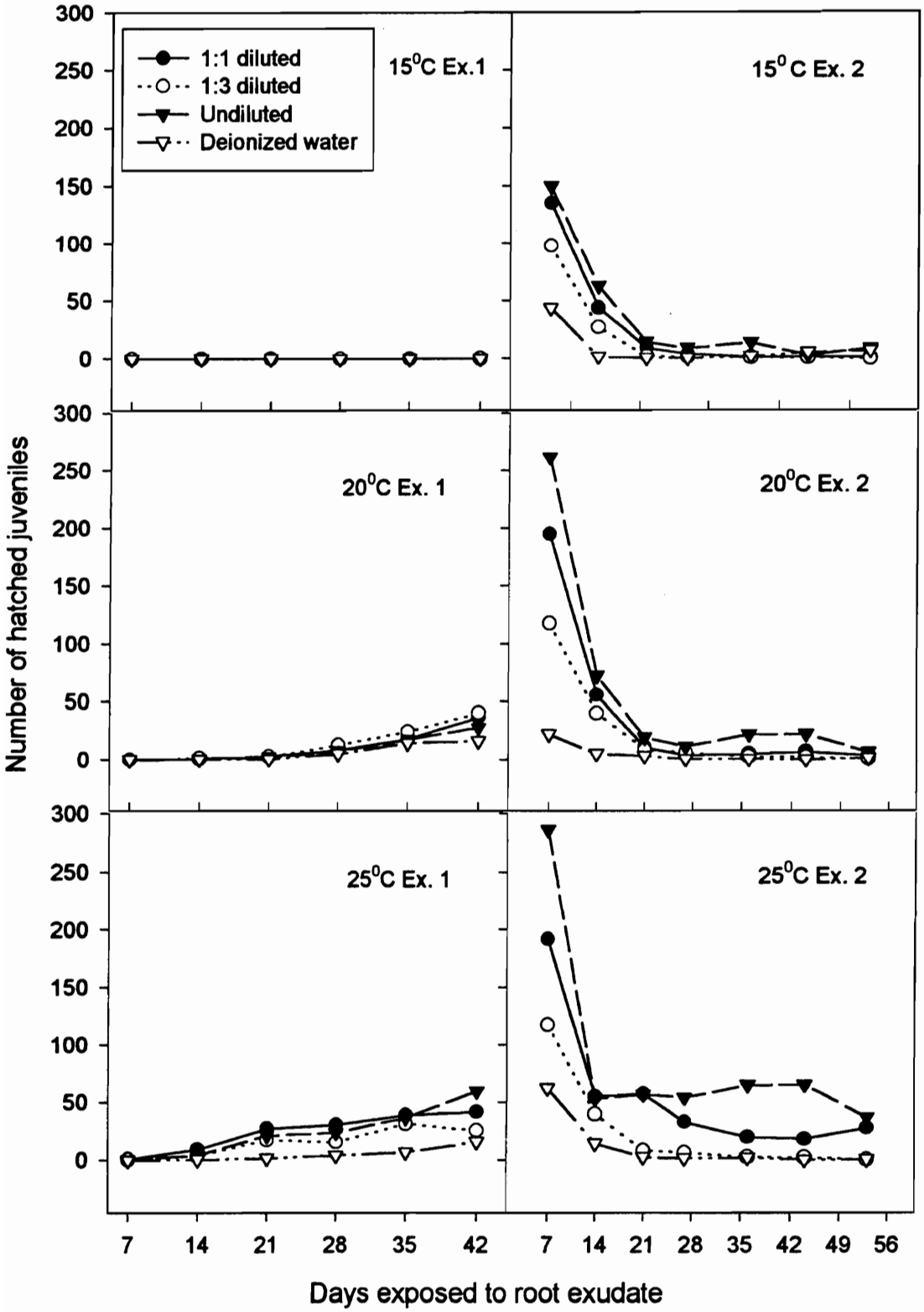

Deionized water
K326
NC567
15°C Ex. 1
15°C Ex. 2
20°C Ex. 1
20°C Ex. 2
25°C Ex. 1
25°C Ex. 2
Number of hatched juveniles
Days exposed to root exudate
0
50
100
150
200
250
7
14
21
28
35
42
49
56

Fig. 2.2. Number of juveniles of *Globodera tabacum solanacearum* hatched in 1:1 diluted, 1:3 diluted, undiluted root exudate, and deionized water at 15, 20, and 25°C.


1:1 diluted
1:3 diluted
Undiluted
Deionized water
15°C Ex.1
15°C Ex. 2
20°C Ex. 1
20°C Ex. 2
25°C Ex. 1
25°C Ex. 2
Number of hatched juveniles
Days exposed to root exudate
0
50
100
150
200
250
300
7
14
21
28
35
42
49
56

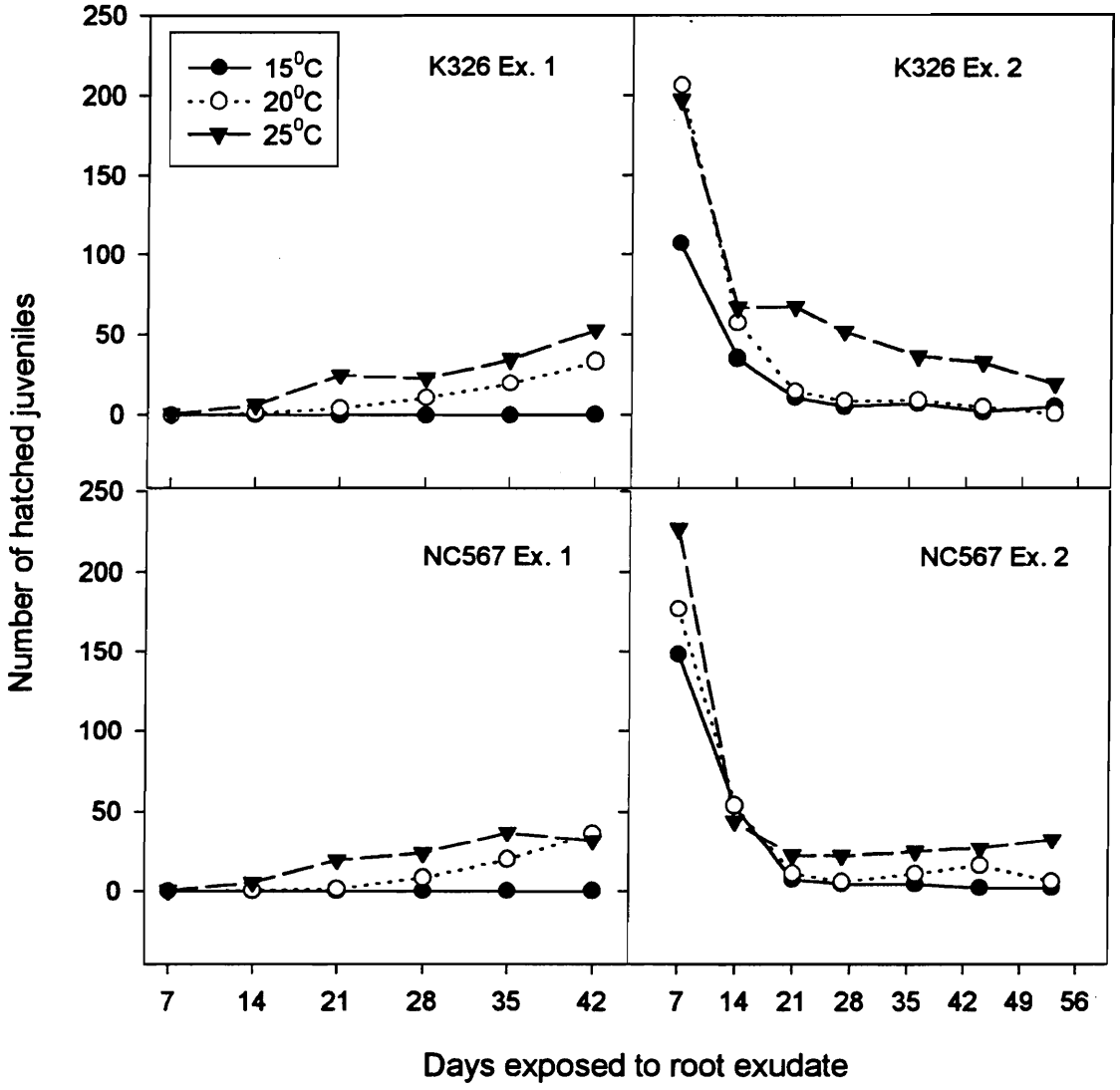


Fig. 2.3. Number of juveniles of *Globodera tabacum solanacearum* hatched in root exudate from K 326 and NC 567 at 15, 20, and 25°C.

LITERATURE CITED

Arntzen, F. K., J. H. M. Visser, and J. Hoogendoorn. 1994. The effect of the potato cyst nematode *Globodera pallida* on in vitro root growth of potato genotypes, differing in tolerance. Annals of Applied of Biology 124:59-64.

Arntzen, F. K., J. H. M. Visser, and J. Hoogendoorn. 1993. Hatching of *Globodera pallida* juveniles by diffusate of potato genotypes, differing in tolerance to *G. pallida*. Annals of Applied of Biology 123:83-91.

Baalawy, H. A., and J. A. Fox. 1971. Resistance to Osborne's cyst nematode in selected *Nicotiana* species. Journal of Nematology 3:395-398.

Barker, K. R. 1985. Nematode extraction and bioassays. Pp 19-35 in K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An Advanced Treatise on Meloidogyne Volume II Methodology. Raleigh: North Carolina State University.

Evans, K. 1983. Hatching of potato cyst nematodes in root diffusates collected from twenty-five potato cultivars. Crop Protection 2:97-103.

Farrer, L. A., and M. S. Philips. 1983. In vitro hatching of *Globodera pallida* in response to *Solanum vernei* and *S. tuberosum* X *S. vernei* hybrids. Revue de Nematology 6:165-169.

Fox, J. A., and A. J. Weber. 1970. The hatching response of Osborne's cyst nematode to root leachates from germ-free tobacco plants. Journal of Parasitology 56:105 (Abstr.)

Hominick, W. M., J. M. Forrest, and A. A. F. Evans. 1985. Diapause in *Globodera rostochiensis* and variability in hatching trials. Nematologica 31:159-170

Johnson, C. S., D. A. Komm, and J. L. Jones. 1989. Control of *Globodera tabacum solanacearum* by alternating host resistance and nematicide. Journal of Nematology 21:16-23.

Komm, D. A., J. J. Reilly, and A. P. Elliott. 1983. Epidemiology of a tobacco cyst nematode (*Globodera solanacearum*) in Virginia. Plant Disease 67:1249-1251.

LaMondia, J. A. 1988. Tobacco resistance to *Globodera tabacum*. Annals of Applied Nematology 2:77-80.

LaMondia, J. A. 1995. Hatch and reproduction of *Globodera tabacum tabacum* in response to tobacco, tomato, or black nightshade. Journal of Nematology 27: 382-386

Miller, L. I., and B. J. Gray. 1972. *Heterodera solanacearum* n. sp., a parasite of solanaceous plants. Nematologica 18:404-413.

Miller, P. M., and G. S. Taylor. 1967. Effects of fungicides on hatching, survival, and nematicidal killing of eggs of *Heterodera tabacum* in the field. Plant Disease 51:609-613.

Muhammad, Z. 1994. The effects of storage conditions on subsequent hatching of *Globodera rostochiensis*. Nematologica Mediterranea. 22:163-169.

Oostenbrink, M. 1967. Studiies on the emergence of encysted *Heterodera* larvae. Mededelingen Rijksfaculteit Landbouw-Wetenschappen Gent 32:503-539.

Perry, R. N., J. A. Hodges and J. Beane. 1981 Hatching of *Globodera rostochiensis* in response to potato root diffusate persisting in soil. Nematologica 27:349-352.

Rawsthorne, D., and B. B. Brodie. 1986. Relationship between root growth of potato, root diffusate production, and hatching of *Globodera rostochiensis*. Journal of Nematology 18:379-384.

Salazar, A., and E. Ritter. 1993. Effects of daylenth during cyst formation, storage time and temperature of cysts on the in vitro hatching of *Globodera rostochiensis* and *G. pallida*. Fundamental of Applied Nematology 16:567-572.

SAS Institute Inc. 1985. User's Guide: Statistics, Version 5 Edition. Statistical Anaylysis System Institute, Inc., Cary, NC.

Shepherd A. M., and P. M. Cox. 1967. Observations on periodicity of hatching of eggs of the potato cyst nematode, *Heterodera rostochensis* Woll. Annals of Applied of Biology 60:143-150.

Triffitt, M. J. 1930. On the bionomics of *Heterodera schachtii* on potatoes with special reference to the influence of mustard on the escape of the juveniles from the cyst. Journal of Helminthology 8:19-48.

Turner, S. J., and A. R. Stone. 1981. Hatching of potato cyst-nematodes (*Globodera rostochiensis*, *G. pallida*) in root exudate of *Solanum vernei* hybrids. Nematologica 27:315-318.

Development of *Globodera tabacum solanacearum* on Resistant and Susceptible Flue-cured Tobacco (*Nicotiana tabacum*)

Abstract: Penetration and development of a tobacco cyst nematode (*Globodera tabacum solanacearum*) on a resistant (NC 567) and a susceptible (K 326) cultivar of flue-cured tobacco (*Nicotiana tabacum* L.) were determined in growth chamber experiments. Development of vermiform juveniles into swollen and flask-shaped nematodes appeared to be similar across tested temperatures (17, 22, 27, and 31°C). More vermiform, swollen, and flask-shaped nematodes were found in roots of K 326 than those of NC 567. Although more vermiform juveniles developed into swollen nematodes on K 326 than on NC 567, development from swollen to flask-shaped nematodes appeared to be similar between the two cultivars. Differences in resistance between the two cultivars remained stable across tested temperatures.

Key words: tobacco cyst nematode, temperature, penetration

Tobacco cyst nematode, *Globodera tabacum solanacearum* (Miller and Gray) Behrens, 1975, is one of the most serious pathogens of flue-cured tobacco, *Nicotiana tabacum* (L.), in Virginia (Miller and Gray, 1972). Average yield reductions caused by *G. t. solanacearum* have been estimated at 15% and complete crop failures have also been recorded (Komm et al., 1983). An estimated one quarter of the total flue-cured tobacco acreage in Virginia is infested by *G. t. solanacearum* (C. S. Johnson, unpub.). Several counties in North Carolina are reported to have *G. t. solanacearum* (Melton et al., 1991; T. A. Melton personal communication). Tactics of *G. t. solanacearum* management include crop rotation, nematicides, and host resistance. The limited availability of effective nematicides, the relatively high survival rate of *G. t. solanacearum*, and limited choices of rotation crops suggest host resistance could play a more important role in successful management of *G. t. solanacearum*. Various degrees of resistance to *G. t. solanacearum* exist in wild *Nicotiana* species (Baalawy and Fox, 1971). Most widely used commercial cultivars, however, lack resistance. Cultivars with resistance to *G. t. solanacearum* have been identified, but significant yield reductions occur in the presence of high *G. t. solanacearum* populations (Komm et al., 1983; Johnson, 1990). Consequently, contact nematicides are routinely applied to susceptible cultivars at an average cost of $139/ha (Johnson

et al, 1989).

Failure of juveniles to penetrate roots and retardation or failure of juveniles to develop, have been noted as attributes of host resistance to *Meloidogyne* species (Sasser and Taylor, 1952; Griffin and Waite, 1972; Jatala and Russell, 1972; Huang, 1986). Resistance to cyst nematodes can involve failure to remain in roots, death, slowing of development, and imbalance of adult sex ratios towards males (Rice et al., 1985). Resistance to soybean cyst nematode, *Heterodera glycines* Ichinohe, 1952, varies depending on the source of resistance and appears to be developmental stage-related (Acedo et al., 1984; Halbrendt et al., 1992). Although resistance to potato cyst nematodes can involve loss of hatching-factor activity, tobaco cyst nematodes hatch similarly in response to resistant and susceptible tobacco cultivars (LaMondia, 1985; Trudgill, 1991; Wang, 1996). Resistance to *G. t. solanacearum* in some wild species and hybrids appears to be related to juvenile development rather than penetration (Baalawy and Fox, 1971). Increased knowledge about penetration and early development of *G. t. solanacearum* in flue-cured tobacco could facilitate improving resistance by identifying more appropriate screening criteria.

Temperature is considered to be of major importance among the environmental factors that affect nematode development (Alston and

Schmitt, 1988; Ferris, 1957). Adams et al. (1982) reported that the rate of development of *G. t. solanacearum* is affected by soil temperature, with a minimum generation time of 33 days at 27°C. Partial loss of resistance at high temperature has been well documented in various crops (Ammati et al., 1986; Griffin, 1969; Omwega et al., 1990). Stability at various temperatures is an important characteristic of resistance. Our objectives in this research were to investigate the effects of host resistance and susceptibility on *G. t. solanacearum* penetration and development at varying root zone temperatures.

MATERIALS AND METHODS

Two experiments were carried out with the same source of inoculum (*G. t. solanacearum* cysts).

Seedling preparation: Seeds of K 326 (susceptible to *G. t. solanacearum*) and NC 567 (resistant to *G. t. solanacearum*) were sown on the surface of vermiculite media held in a 22 X 16 X 7 cm^3 plastic container. Vermiculite media was watered through holes punched in the bottom of the container. Care was taken to maintain suitable moisture and nutrients for germination and seedling growth. Seedlings with one true leaf and vigorous growth were transferred to 11 cm diameter clay pots

pre-filled with 300 cm^3 of steam sterilized loamy sand:fine quartz sand (1:1). Pots with transferred seedlings were placed in a growth chamber for one week at 24°C to allow seedlings to establish themselves before inoculation.

Inoculum preparation: Cysts were propagated on flue-cured tobacco cultivar Coker 319 in the greenhouse. Soil containing cysts was thoroughly mixed with water in a plastic bucket. The supernatant was poured through a 20 mesh (850 μm) sieve on top of a 60 mesh (250 μm) sieve after the mixture had settled for 15 seconds. Cysts were collected from those retained on the bottom sieve and stored in a refrigerator at 4°C for 2 to 4 months. Inoculum preparation began by crushing cysts in a blender on "frappe" for one minute. Contents of the blender were rinsed through a 100 mesh (150 μm) sieve on top of a 500 mesh (25 μm) sieve. Eggs caught on the 500 mesh sieve were transferred to a beaker and standardized to a concentration of 1,750 eggs/ml by adding tap water.

Inoculation of eggs: Inoculum was applied by inserting a 10 ml syringe 5 cm below the surface of the soil. Twenty ml of egg suspension was applied around the seedling in each pot to deliver 35,000 eggs per pot. The trench made by inoculation was immediately covered with soil.

Experimental design and sampling: Two cultivars were arranged in a randomized complete block design with four replications within each

growth chamber. Temperatures were randomly assigned to each of the four growth chambers (Root Zone Cabinet Model R-1, Environmental Growth Chambers, Chagrin, Ohio). Immediately after inoculation, growth chambers were programmed for day-night temperatures of 31-29, 27-25, 22-20, and 17-15°C at 12 h cycles. Individual plants were sampled on a weekly basis, starting one week after inoculation for five weeks. At sampling time, one plant of each cultivar was removed from each replication at each temperature. A total of 4 pots were sampled of each variety at each temperature. Each plant was carefully washed free of soil. The fresh weight of entire plants, total roots, and feeder roots (excluding tap roots) were recorded after blotting plants dry. Feeder roots were cut into segments, mixed, and a 1 g sample was randomly removed for staining. If total feeder root weight was less than 1 g, all feeder roots were stained.

Penetration and development: Lateral and feeder roots were stained with acid fuchsin (Byrd et al., 1983). Nematodes were counted and their stages of development were assigned to one of four classes based on overall body shape under low magnification (4 X 10): a) vermiform (successfully penetrated roots without obvious feeding); b) swollen (a distinct sausage shape); c) flask-shaped; d)saccate adults bearing eggs. Nematode penetration was indicated by the presence of vermiform juveniles

in roots. The number of swollen, flask-shaped, and saccate nematodes in roots indicated nematode development. Cumulative percentages of swollen or flask-shaped nematodes were calculated by adding the percentage of swollen or flask-shaped nematodes (the number of swollen or flask-shaped nematodes divided by total vermiform juveniles) across all sample dates. Percentages were based upon penetrated vermiform juveniles in order to remove the potential influence of temperature on hatching. Effects of temperature on development were estimated based on cumulative percentages.

Stage-related development: Ratios of the number of nematodes at one stage of development to those in the earlier stage were used to estimate stage-related development. All nematodes in an advanced stage on a particular sampling date were assumed to have developed from their immediately lower stage since the previous sampling date. The values of $R_{S/V}$ and $R_{F/S}$ (where $R_{S/V}$ is the proportion of vermiform juveniles which developed into swollen stage and $R_{F/S}$ is the proportion of swollen juveniles which developed into flask-shaped stage) were square root transformed prior to statistical analysis.

Statistical analysis: The two experiments were combined and analyzed using a split-plot design, with temperature as the main plot factor. Analyses of variance were conducted on total nematodes,

cumulative percentages of swollen or flask-shaped nematodes, and the ratios of developmet in lateral and feeder roots. In all analyses, Duncan's multiple range test was performed on means at a level of $P \leq 0.05$.

RESULTS

Penetration and development of *G. t. solanacearum* were greater in experiment 2 than experiment 1. Very few egg-bearing adults were detected in either experiment. Egg-bearing adults were excluded, therefore, from the following analyses. No consistent interactions were found between temperature and cultivars. Consequently, the effects of temperature and cultivar are presented separately.

Very few vermiform juveniles were detected in roots 7 days after inoculation (Fig. 3.1). Number of vermiform juveniles in roots peaked 14 days after inoculation at 22 and 27°C. At 31°C, number of vermiform juveniles increased during the first 21 days after inoculation, followed by a decrease. Very few juveniles were detected at 17°C. At 14 and 21 days after inoculation, more ($P \leq 0.05$) juveniles were found at 22 and 27°C than 17 or 31°C.

More ($P \leq 0.05$) swollen nematodes developed at 22, 27, and 31° than at 17°C at 35 days after inoculation (Fig. 3.2). Development of swollen

nematodes was similar at 22, 27, and 31°C. No significant ($P \leq 0.05$) differences in flask-shaped nematodes developing from vermiform juveniles were found among temperatures (Fig. 3.2). Effects of temperature on ratio of development were not significantly different during the period of the experiment.

Penetration by vermiform juveniles of *G. t. solanacearum* followed similar patterns in both susceptible (K 326) and resistant (NC 567) cultivars (Fig. 3.3). Little penetration occurred during the first 7 days after inoculation. Penetration apparently peaked at 14 days after inoculation in both cultivars. More vermiform juveniles ($P \leq 0.05$) were found in roots of susceptible K 326 at 14, 21, and 28 days after inoculation than in roots of resistant NC 567. More ($P \leq 0.05$) swollen nematodes developed on K 326 than on NC 567 (Fig. 3.3). More ($P \leq 0.05$) flask-shaped nematodes had developed on K 326 than on NC 567 at 28 days and 35 days after inoculation (Fig. 3.3). More vermiform juveniles ($P \leq 0.05$) developed into the swollen stage on K 326 than NC 567, particularly at 21 and 28 days after inoculation (Fig. 3.4). No consistent differences in development from the swollen to flask-shaped stage were observed between both cultivars, although the ratio for K 326 is higher than NC 567 ($P=0.08$) at 28 days after inoculation (Fig. 3.4).

DISCUSSION

The inoculum used in these experiments consisted of nematode eggs that were apparently viable when observed under lower magnification (4 X 10). Nematode juveniles observed in roots result from both hatching and penetration. Because resistant and susceptible cultivars stimulate hatching of *G. t. solanacearum* similarly, the effects of resistance on penetration and/or development reported here should be comparable to those based on inoculation of juveniles directly on roots (Wang, 1996). Previous studies have also indicated no difference in hatching and penetration between resistant and susceptible tobacco genotypes (Baalaway and Fox, 1971; LaMondia, 1988). The higher number of vermiform juveniles in roots of the susceptible cultivar K 326 could have resulted from juveniles leaving the roots of the resistant cultivar NC 567 after unsuccessfully attempts to establish a feeding site (Lamondia, 1988; Schneider, 1991).

Resistance to *G. t. solanacearum* in NC 567 was clearly expressed by lower number of swollen and flask-shaped nematodes, compared to the susceptible cultivar, as early as 14 days after inoculation (Fig. 3.2). These differences were consistent throughout the period of the investigation. However, resistance may not be equally effective on all

stages of development before become fully mature adults. More vermiform juveniles developed into swollen nematodes in roots of K 326 than NC 567 indicating greater success in initiating feeding. Inconsistent differences in the ratio of flask-shaped to swollen nematodes ($R_{F/S}$) between cultivars suggests that resistance in NC 567 does not inhibit nematode development once juveniles have begun feeding. Stage-related resistance has been reported in soybean and soybean cyst (*Heterodera glycines*) interactions (Halbrendt, 1992; Endo, 1965). The reduction in number of flask-shaped nematodes at 35 days after inoculation was partially due to further development of flask-shaped nematodes and losses of mature nematodes in sample processing. Flask-shaped nematodes and egg-bearing adults are less firmly attached to host tissue as they develop into cysts. Small females and reduced fecundity were associated with *H. glycines* resistance in some soybean cultivars (Noel, 1983). Very few adults were observed at the end of this study which prevented estimation of influence of the resistance on fecundity.

Effects of temperature on penetration or development were confounded with its effects on hatching. However, cumulative percent swollen or flask-shaped nematodes and the stage-related ratios of development were based upon penetrated juveniles rather than the number of eggs added as initial inoculum. Therefore the influence of temperature

on hatching should not have influenced the estimation of temperature effects on development. No significant differences were found among temperatures, except a lower percent of swollen nematodes at 17°C. The maintenance of similar developmental rates over the range of temperatures tested indicated that *G. t. solanacearum* is acclimated to the temperatures prevailing throughout the flue-cured tobacco growing area in Virginia. Effects of cultivars on nematode development were not influenced by temperature, suggesting that resistance to *G. t. solanacearum* in NC 567 is stable across the temperatures typically occurring in Virginia.

Growth chamber research provides a fast and cost-efficient way to identify and evaluate resistance. However, plant growth is inevitably modified to some extent by conditions in greenhouse pots. Numerous fine feeder roots were observed in plants in this study. Easy access to tender roots may lessen competition among nematodes for entry, thus artificially increasing penetration and reducing differences in number of juveniles in roots of cultivars compared to what might have occurred in the field. Fine roots have also been reported to support production of fewer syncytia required for development (Ross and Trudgill, 1969). Therefore, differences observed in pot experiments may need to be confirmed in more natural growing environments. The presence of flower buds towards the end

of the experiments also indicated that plants had undergone physiological stress during the relatively short (5 weeks) period of growth. Some characters of plant growth that may play an important role in reducing damage from nematode infection (such as vigorous growth of root systems or mechanisms for compensation) could not be evaluated in relatively short-term experiments or in a growth chamber. The results of this experiments may account, therefore, for only a part of the resistance in NC 567.

Various cyst-host interactions indicated that both host genotypes and composition of population play an important role in success of interactions. Difference in parasitism among different populations of *G. t. solanacearum* have been suggested, but not demonstrated (Miller and Gray, 1972). The results reported here are based on a single population.

In summary, the results of this experiment indicated that the resistance in NC 567 prevented the establishment of feeding sites, thereby fewer swollen nematodes developed in the roots. The resistance does not appear to affect nematode development beyond the swollen stage. The response of both cultivars to infection by *G. t. solanacearum* was similar at various temperatures.

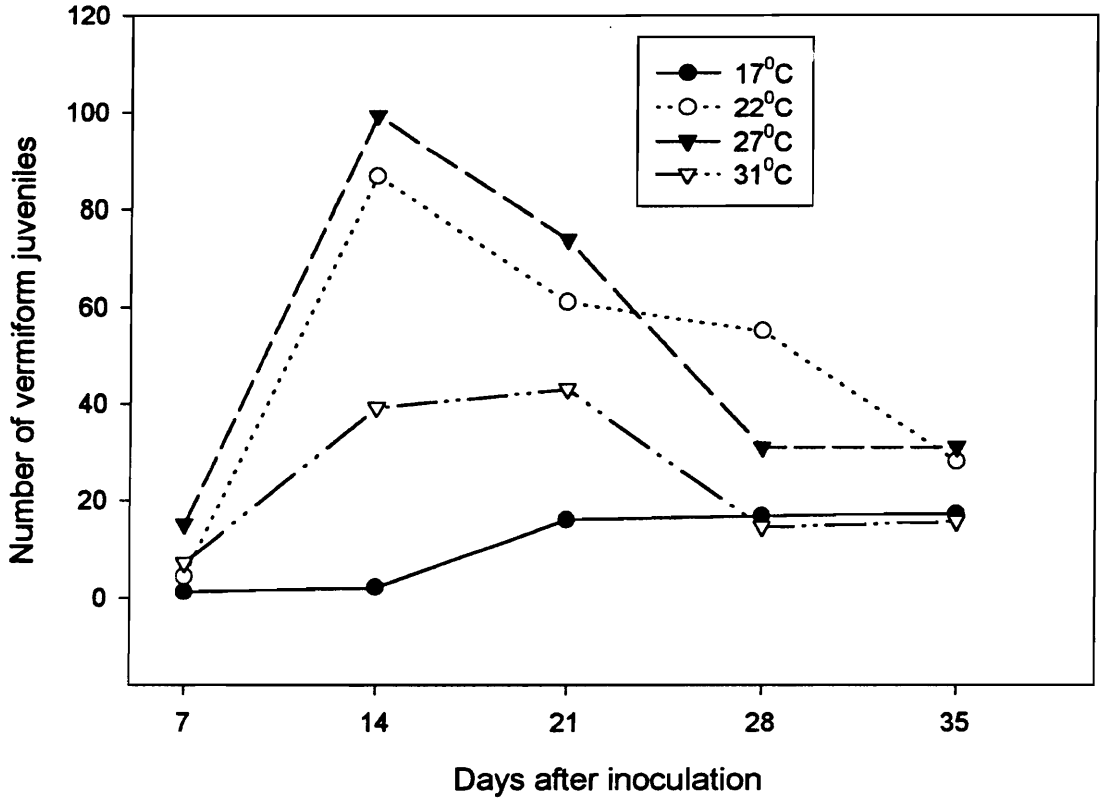


Fig. 3.1. Number of vermiform juveniles of *Globodera tabacum solanacearum* in lateral and feeder roots at four root zone temperatures. Data are means of 16 observations.

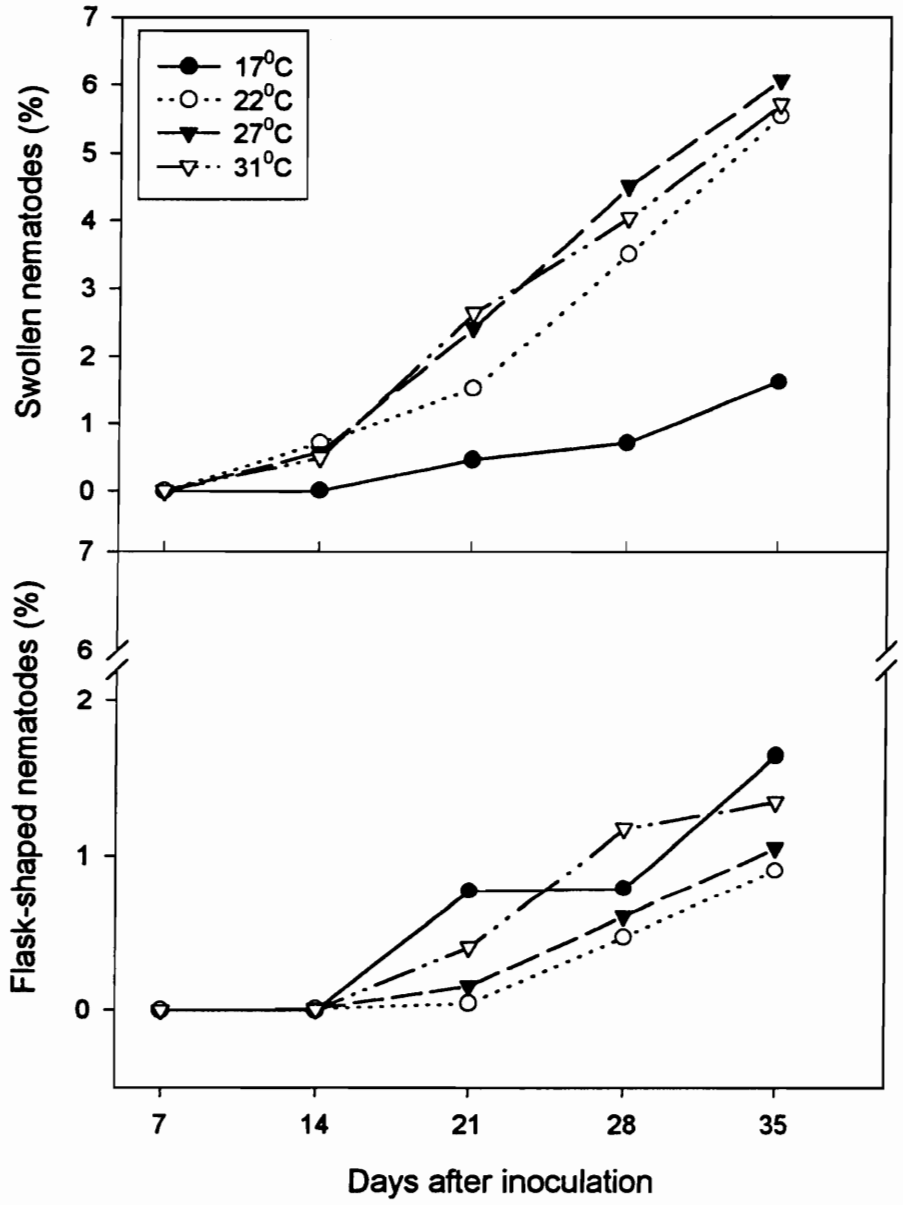
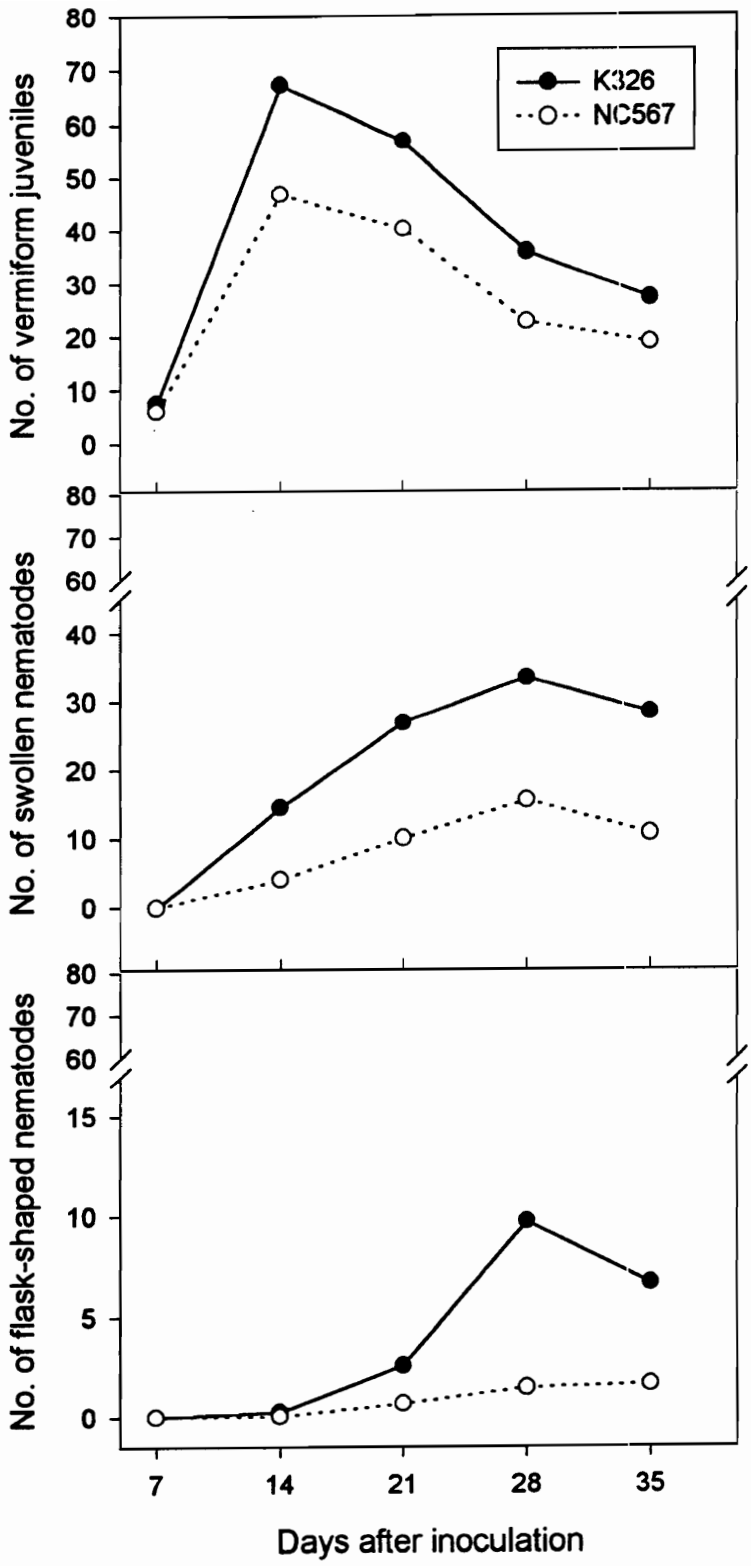


Fig. 3.2. Cumulative percent swollen and flask-shaped nematodes of *Globodera tabaum solanacearum* developed in lateral and feeder roots at four root zone temperatures.

Fig. 3.3. Number of vermiform juveniles, swollen and flask-shaped nematodes of *Globodera tabacum solanacearum* in lateral and feeder roots of cultivar K 326 and NC 567.


No. of vermiform juveniles
No. of swollen nematodes
No. of flask-shaped nematodes
K326
NC567
Days after inoculation
7
14
21
28
35

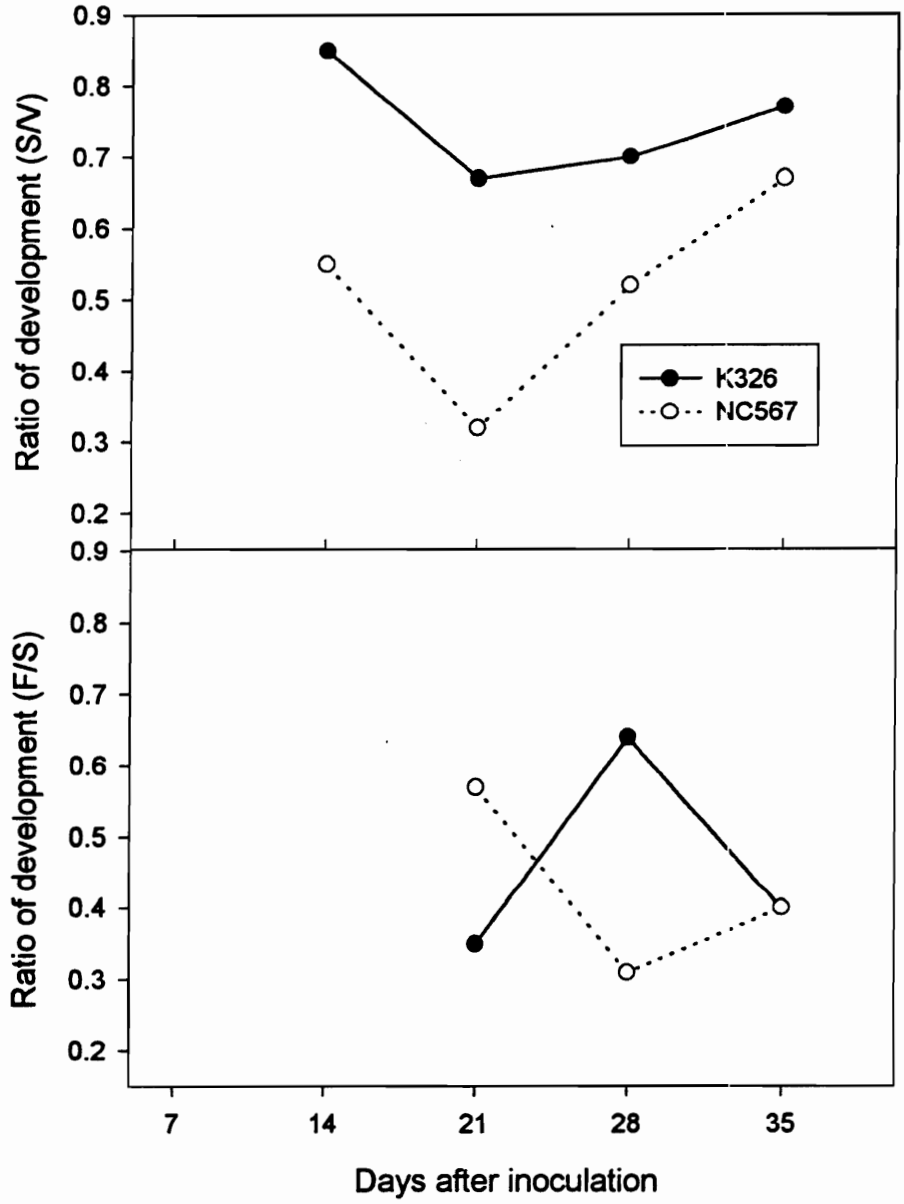


Fig. 3.4. Ratio of vermiform juveniles of *Globodera tabacum solanacearum* developing into swollen nematodes (S/V) and swollen nematodes developing into flask-shaped nematodes (F/S) in lateral and feeder roots of cultivar K 326 and NC 567.

LITERATURE CITED

Acedo, J. R., V. H. Dropkin, and V. D. Luedders. 1984. Nematode population attrition and histopathology of *Heterodera Glycines*-soybean association. Journal of Nematology 16:48-57.

Adams, H. S., W. W. Osborne, and A. J. Webber, Jr. 1982. Effects of temperature on development and reproduction of *Globodera solanacearum*. Nematropica 12:305-311.

Alston, D. G., and D. P. Schmitt. 1988. Development of *Heterodera glycines* life stage as influenced by temperature. Journal of Nematology 20:366-372.

Ammati, M., I. J. Thomason, and H. E. McKiney. 1986. Retention of resistance to *Meloidogyne incognita* in Lycopersicon genotypes at high soil temperature. Journal of Nematology 18:491-495.

Byrd, D. W., T. Kirkpatrick, and K. R. Barker. 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. Journal of Nematology 15:142-143.

Baalawy, H. A., and J. A. Fox. 1971. Resistance to Osborne's cyst nematode in selected Nicotiana species. Journal of Nematology 3:395-398.

Endo, B. Y. 1965. Histological responses of resistance and susceptible soybean varieties and back-cross progeny to entry and development of *Heterodera glycines*. Phytopathology 55:375-381.

Ferris, J. M. 1957. Effect of soil temperature on the life cycle of the golden nematode in host and nonhost species. Phytopathology 47:221-230.

Ferris, H., H. S. DuVernay, and R. H. Small 1978. Development of soil-temperature data base on *Meloidogyne arenaria* for a simulation model. Journal of nematology 10:39-42.

Grffin, G. D., and W. W. Waite. 1972. Attraction of *Ditylenchus dipsaci* and *Meloidogyne hapla* by resistant and susceptible Alfalfa seedlings. Journal of Nematology 3:215-219.

Griffin, G. D. 1969. Effects of temperature on *Meloidogyne hapla* in

alfalfa. Phytopathology 59:599-609.

Halbrendt, J. M., S. A. Lewis, and E. R. Shipe. 1992. A technique for evaluating *Heterodera glycines* development in susceptible and resistant soybean. Journal of Nematology 24:84-91.

Huang, S. P. 1986. Penetration, development, reproduction, and sex ratio of *Meloidogyne javanica* in three carrot cultivars. Journal of Nematology 18:408-412.

Jatala, P., and C. C. Russell. 1972. Nature of sweet potato resistance to *Meloidogyne incognita* and the effects of temperature on parasitism. Journal of Nematology 4:1-6.

Johnson, C. S. 1990. Control of *Globodera tabacum solanacearum* by rotating susceptible and resistant flue-cured tobacco cultivars. Supplement to Journal of Nematology 22:700-706.

Johnson, C. S., D. A. Komm, and J. L. Jones. 1989. Control of *Globodera tabacum solanacearum* by Alternating host resistance and nematicide. Journal of Nematology 21:16-23.

Komm, D. A., J. J. Reilly, and A. P. Elliott. 1983. Epidemiology of a tobacco cyst nematode (*Globodera solanacearum*) in Virginia. Plant Disease 67:1249-1251.

LaMondia, J. A. 1988. Tobacco resistance to *Globodera tabacum*. Annals of Applied Nematology 2:77-80.

Melton, T. A., J. A. Philips, J. L. Imbriani, and K. R. Barker. 1991. First report of *Globodera tabacum solanacearum* on flue-cured tobacco outside Virginia. Plant Disease 75:1074.

Miller, L. I., and B. J. Gray. 1972. *Heterodera solanacearum* n. sp., a parasite of solanaceous plants. Nematologica 18:404-413.

Noel, G. R., B. A. Stanger, and P. V. Bloor. 1983. Population development, reproductive behavior, and morphology of race 4 of soybean cyst nematode, *Heterodera glycines* on resistance soybeans. Plant Disease 67:179-182.

Omwega, C. O., I. J. Thomason, and P. A. Roberts. 1990. Effect of

temperature on expression of resistance to *Meloidogyne spp.* in common Bean (*Phaseolus vulgaris*). Journal of Nematology 22:446-451.

Rice, S. L., S. C. Leadbeater, and A. R. Stone. 1985. Changes of cell structure in roots of resistant potatoes parasitized by potato cyst-nematodes. I. Potatoes with resistance gene H_1 derived from *Solanum tuberosum ssp. andigena*. Physiological Plant Pathology 27:219-234.

Ross, G. J. S., and D. L. Trudgill. 1969. The effect of population density on the sex ratio of *Heterodera rostochiensis*: A two-dimensional model. Nematologica 15:601-607.

Sasser, J. N., and A. L. Taylor. 1952. Studies on the entry of larvae of root-knot nematodes into roots of susceptible and resistant plants. Phytopathology 42:474 (Abstract).

Schneider, S. M. 1991. Penetration of susceptible and resistant tobacco cultivars by *Meloidogyne* juveniles. Journal of Nematology 23:225-228.

Trudgill, D. L. 1991. Resistance to and tolerance of plant parasitic nematodes in plants. Annual Review of Phytopathology 29:167-192.

Wang, J. 1996. Hatching of *Globodera tabacum solanacerum* juveniles by root exudate of flue-cured tobacco (*Nicotiana tabacum*). Pp 19-40 in Ph.D dissertation. Characterizing Resistance in Flue-cured Tobacco to *Globodera tabacum solanacearum*. Virginia Polytechnic Institute and State University.

Effects of *Globodera tabacum solanacearum* on Growth of Flue-cured Tobacco (*Nicotiana tabacum*)

Abstract: The effects of infection by *Globodera tabacum solanacearum* on root and shoot growth of flue-cured tobacco cultivars NC 567 (resistant) and K 326 (susceptible) were evaluated in 1993 and 1994. Different infection levels of *G. t. solanacearum* were achieved by applying an experimental nematicide (fosthiazate) to half of the experiment approximately one week before transplanting. Infection by *G. t. solanacearum* was determined by sampling plants weekly and counting nematodes in the feeder roots of sampled plants. Infection by *G. t. solanacearum* suppressed number of leaves, plant height, fresh weight of leaves, and feeder roots. Correlation coefficients between weekly egg densities of *G. t. solanacearum* collected from soil around sampled plants and plant height, fresh leaf weight, number of leaves, fresh feeder root weight, and a ratio of fresh leaf weight to feeder root weight at 11 weeks after transplanting were small and varied between cultivars and years. Fresh leaf weight and feeder root weight were significantly correlated with nematode days of total nematode (NDT), calculated as the area under the curve for total number of nematodes per gram of feeder root, for both cultivars in 1994 and for K 326 in 1993. The ratio of leaf

weight to feeder root weight was significantly correlated with ND for both cultivars in 1994 and for NC 567 in 1993. Reduction in fresh leaf weight by *G. t. solanacearum* was greater ($P \leq 0.0748$) for K 326 than NC 567 in 1994. Reduction in the fresh weight of feeder roots and increases in the ratio of leaf weight to feeder root weight induced by infection by *G. t. solanacearum* were similar for the resistant and susceptible cultivars.

Key words: cyst nematode, resistance, tolerance, multiple point model, damage function.

Tobacco cyst nematode, *Globodera tabacum solanacearum* (Miller and Gray) Behrens, 1975, is one of the most serious pathogens of flue-cured tobacco, *Nicotiana tabacum* (L.) in Virginia (Miller and Gray, 1972). Average yield reductions caused by *G. t. solanacearum* have been estimated at 15% and complete crop failures have also been recorded (Komm et al., 1983). An estimated one quarter of the total flue-cured tobacco acreage in Virginia is infested with *G. t. solanacearum* (Johnson, unpub.). Tactics of *G. t. solanacearum* management include crop rotation, nematicides, and host resistance. The limited availability of effective nematicides, the relatively high survival rate of *G. t. solanacearum*, and limited choices of rotation crops suggest host resistance could play a more important role in successful management of *G. t. solanacearum*. Various degrees of resistance to *G. t. solanacearum* exist in wild *Nicotiana* species (Baalawy and Fox, 1971). Most widely used commercial cultivars, however, lack resistance. Cultivars with resistance to *G. t. solanacearum* have been identified, but significant yield reductions still occur in the presence of high populations of these nematodes. Mechanisms of resistance to *G. t. solanacearum* in flue-cured tobacco are unknown. Consequently, the main management tactic used is the application of contact nematicides to susceptible cultivars at an average cost of $139/ha (Johnson et al., 1989).

Many studies have related yield loss to initial population density of cyst nematodes (Seinhorst, 1965; Schmitt et al., 1987; LaMondia, 1995). Various factors affect crop growth and cyst population development, causing variation in the relationships between yield losses and nematode population densities (Trudgill, 1986). Understanding changes in crop growth resulting from infection by cyst nematodes may provide useful information to help understand relationships between nematode population densities and yield losses. *Globodera* species are reported to impair plant growth by inhibiting new root development and causing degeneration of existing roots, thereby suppressing shoot growth (Trudgill and Cotes, 1983). Differences in growth of the tops of plants can be used to separate genotypes of potato that are tolerant and intolerant to potato cyst nematodes (Dale and Brown, 1989). Tolerance in wheat to *Heterodera avenae* is also closely correlated with early growth (Stanton and Fisher, 1987). Early season damage to wheat roots by *Heterodera avenae* and subsequent effects on shoot growth have been related to yield losses (Simon and Rovira, 1982). The effects of infection by *G. t. solanacearum* on early growth of flue-cured tobacco have not been well characterized (Grant et al., 1982). Our objectives were to investigate the influence of infection by *G. t. solanacearum* on growth of a resistant (NC 567) and susceptible (K 326) cultivar of flue-

cured tobacco during the first 11 weeks of the growing season.

MATERIALS AND METHODS

Field preparation: Experimental plots were located at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, Virginia. The experimental plots had been in a two year tobacco-fescue rotation for several cycles. However, flue-cured tobacco was grown in each field the year before each experiment. The soil type of experimental plots was a Chesterfield-Mayodan-Bourne sandy loam complex in 1993 and a Dothan-Norfolk sandy loam in 1994. Each plot was 4.88 m wide and 12.12 m long and contained four rows 1.2 m apart. The first and fourth rows served as border rows. Plants in the second row were destructively sampled. The third row was used for evaluation of yield and quality. All plots were maintained similarly to commercial tobacco fields except that only foliar sprays were used for insect control (Jones et al., 1993). Irrigation was used only as necessary to minimize leaf scalding and to maintain plant stand.

Treatments and experimental design: Four treatments were imposed using a complete factorial design with 12 replications. Cultivars included in the experiments were NC 567 (resistant) and K 326

(susceptible). An experimental organophosphate nematicide, fosthiazate (ISK Biotech), was used because of a reported high level of nematicide activity and a lack of insecticidal effect. Rates of the nematicide were 0 and 6.84 L/ha in 1993 and 0 and 6.78 L/ha in 1994. Nematicides were applied as experimental treatments using a tractor mounted, CO_2-powered, hydraulic sprayer. Plots were disked immediately after nematicide application. Tobacco seedlings were transplanted approximately one week after nematicide application.

Measurements of plants: One plant was randomly sampled from each plot every week for the first 11 weeks after transplanting. Plants were washed free of soil and blotted dry before measurements were taken. Plant height, number of leaves, fresh weight of leaves, and fresh weight of feeder roots were recorded.

Determination of nematode infection: G. t. solanacearum infection was determined by counting the number and characterizing the stage of nematodes in feeder roots. Feeder roots from each plant were separated, cut into segments, and mixed. One gram of feeder roots was randomly sampled from the mixture for each plant. If the weight of feeder roots was less than one gram, all feeder roots were used for counting nematodes. Roots were stained with acid fuchsin (Byrd et al., 1983). Stained roots were mounted on a transparent plastic petri dish. Nematodes

in stained roots were counted and assigned to one of four classes based on overall shape under low magnification: a) vermiform nematodes (those juveniles that had successfully penetrated roots without obvious feeding); b) swollen (nematodes with a distinct sausage shape); c) flask-shaped; and d) saccate adult nematodes bearing eggs.

Determination of soil population of G. t. solanacearum: Soil samples (1000 cm^3) were collected from around the roots of one randomly selected plant per plot every week for the first 11 weeks after transplanting. Soil samples were air dried and *G. t. solanacearum* cysts were extracted from a 250 cm^3 subsample using a modified Fenwick can. After crushing cysts in a blender on "frappe" for one minute, two aliquots of egg suspension were counted to determine the number of eggs. Egg counts were expressed per 500 cm^3 of soil.

Populations of *G. t. solanacearum* and infection by nematodes were quantified as the area under the curve for several parameters describing nematode population dynamics. Populations of *G. t. solanacearum* in soil were described by the area under the curve for nematode egg density in 500 cm^3 soil over the first 11 weeks after transplanting (NDE). NDS was the area under the curve for vermiform and swollen nematodes per gram of feeder root over the first 11 weeks after transplanting. NDT was the area under the curve for total number of nematodes per gram of feeder

root over the first 11 weeks after transplanting. The three methods of quantification used a similar mathematical formula as follows:

$$\text{NDE, NDS, or NDT} = [PGTS_{i+1}+PGTS_i)/2](X_{i+1}-X_i)$$

where $PGTS_i$=number of eggs in 500 cm^3 soil for NDE, number of vermiform and swollen nematodes per gram of feeder root for NDS, and number of total nematodes per gram of feeder root for NDT at the i'th sampling date. X_i=the julian date of the i'th sampling date.

Statistical Analysis: Plant height, fresh weight of leaves, number of leaves, fresh weight of feeder roots, a ratio of fresh weight of leaves to fresh weight of feeder roots, and total nematodes per gram of feeder root were subjected to analysis of variance (SAS, 1985). The responses of the host to infection by *G. t. solanacearum* were compared over the first 11 weeks after transplanting. Where appropriate, data were subjected to regression analysis.

RESULTS

1993 and 1994 experienced very different growing seasons. The early part of 1993 was dry, and experimental plots were irrigated four times. Host responses to nematode were, therefore, analyzed separately for the two years.

Number of nematodes per gram of feeder root decreased with time in all treatments in both years (Fig. 4.1). K 326 had higher ($P \leq 0.05$) number of nematodes than NC 567 from 2 to 4 and from 6 to 11 weeks after transplanting (WAT) in 1993 (Fig. 4.1). Number of nematodes were higher ($P \leq 0.05$) for K 326 than NC 567 at 4, 5, 7, 8, 9, 10, and 11 WAT in 1994. Fosthiazate did not significantly reduce nematode parasitism for NC 567 at 5, 10 and 11 WAT in 1993. However nematicide treatment ($P \leq 0.05$) suppressed nematode infection on both cultivars in 1994. Infection levels on both cultivars were higher in 1994 than in 1993 when not treated with fosthiazate, especially for the earlier part of the sampling periods.

No significant increases in feeder root weight were observed during the first 5 WAT in either cultivar in either year (Fig. 4.2). No differences in feeder root weight were found between the two cultivars in either year. Application of fosthiazate increased ($P \leq 0.05$) feeder root weight from 3 to 8 WAT in 1993 and at 5 WAT and from 7 to 11 WAT in 1994.

Feeder root weight was heavier towards the end of the sampling period in 1993 than in 1994.

There were generally no differences in number of leaves or leaf weight between the two cultivars in either year (Figs. 4.3,4). However, leaves of NC 567 were heavier ($P \leq 0.05$) than those of K 326 at 1 and 4 WAT in 1993 and at 2 and 6 WAT in 1994 (Fig. 4.3). Application of fosthiazate increased ($P \leq 0.05$) leaf weight from 2 to 8 WAT in 1993 and from 4 to 11 WAT in 1994. Reduction in *G. t. solanacearum* infection resulting from use of fosthiazate increased the number of leaves from 2 WAT until topping (9 WAT) in 1993 and 4 WAT and thereafter in 1994 (Fig. 4.4).

Plant height of NC 567 was greater ($P \leq 0.05$) than that of K 326 from 1 to 3 and at 10 WAT in 1993 from 2 to 5 WAT and at 7 WAT in 1994 (Fig. 4.5). Plants treated with fosthiazate were taller ($P \leq 0.05$) from 4 WAT until topping (9 WAT) in 1993 and after 6 WAT in 1994. However, fosthiazate did not increase plant height for K 326 at 4 WAT and for NC 567 at 9 WAT in 1993. NC 567 was taller than K 326 when fosthiazate was used, but height of both cultivars was similar in untreated soil at 6, 8, and 9 WAT 1994.

No differences in the ratio of leaf fresh weight to root fresh weight were found between the two cultivars in 1993. The ratio of fresh leaf weight to feeder root weight was higher ($P \leq 0.05$) for NC 567 than for

K 326 at 1, 3, and 6 WAT in 1994 (Fig. 4.6). Fosthiazate increased ($P \leq 0.05$) the leaf to root weight ratio at 4 WAT in 1993 and from 3 to 6 WAT in 1994, but decreased the ratio at 10 and 11 WAT for both cultivars in both years. At 6 WAT, fosthiazate only increased the ratio for K 326 in 1993.

Egg densities of *G. t. solanacearum* were not correlated ($P \leq 0.05$) with plant height, number of leaves, or the ratio of leaf weight to feeder root weight for K 326 in either year (Tables 4.1,2). Significant correlations were detected between plant height of NC 567 and nematode egg densities 11 WAT in 1993 and from 3 to 6 WAT in 1994. Significant correlations were found between nematode egg densities and number of leaves at 7 and 11 WAT and the ratio of leaf to feeder root weight at 3 and 6 WAT for NC 567 in 1993 (Table 4.1). Egg densities of *G. t. solanacearum* at 6 WAT were correlated ($P \leq 0.05$) with leaf weight of K 326 in both years. Significant correlations between egg densities and feeder root weight were found 5 and 9 WAT for K 326 in both years. Nematode egg densities 9 WAT were correlated ($P \leq 0.05$) with feeder root weight of NC 567 in both years. Correlations between leaf weight and egg densities were inconsistent between years for NC 567.

Leaf weight and feeder root weight of K 326 in 1993 and all plant growth measurements of K 326 in 1994 were significantly correlated with

nematode days of total nematodes per gram of feeder root (NDT) and nematode days of vermiform and swollen nematodes per gram of feeder root (NDS) (Table 4.3). No significant correlations were found between leaf and feeder root weight of K 326 and nematode days of eggs (NDE), except root weight in 1994. All plant growth measurements except the ratio of leaf weight to feeder root weight (RSR) of NC 567 were not correlated with NDT and NDS in 1993. Feeder root weight of NC 567 was significantly correlated with NDE in 1993. All plant growth measurements except number of leaves were correlated ($P \leq 0.05$) with NDT and NDS in 1994 (Table 4.3). However, NDE was only correlated ($P \leq 0.05$) with Plant height and leaf weight in 1994.

The largest correlation coefficients were obtained between NDT and plant growth parameters. It was, therefore, used in regression analysis to quantify the effects of infection by *G. t. solanacearum* on tobacco growth. NDT correlated consistently with leaf weight, feeder root weight, and the ratio of leaf weight to feeder root weight (RSR) of both cultivars, so these three plant growth measurements were selected for regression analysis. Regression analysis was performed only for three measurements in 1994 since significant correlations were not found between plant growth parameters and NDT for NC 567 in 1993. The rate of reduction in fresh leaf weight was greater ($P \leq 0.0748$) for susceptible K

326 than for the resistant cultivar NC 567 (Fig. 4.7). Rates of decline in fresh weight of feeder roots with increasing nematode pressure were not significantly different between the two cultivars (Fig. 4.8). However, K 326 also produced a higher ($P \leq 0.05$) leaf weight in the absence of nematode infection. Rates of increase in the ratios of leaf weight to feeder root weight were similar for the two cultivars (Fig. 4.9).

DISCUSSION

No other significant diseases or insect pests were observed in experimental plots throughout these experiments. Differences in growth in these experiments probably resulted, therefore, from control of the tobacco cyst nematode. Fosthiazate has not been reported to directly influence tobacco growth. Leaf and root weight were negatively correlated with nematode infection in both years. However, environmental conditions were quite different between the two growing seasons. Feeder roots were much heavier at the end of the sampling period in 1993 than in 1994. Soil moisture stress has been reported to stimulate root growth (Osmond and Raper, 1982). Differences in root growth would inevitably affect nematode development and their damage to the host.

The usual time period between transplanting and topping in flue-

cured tobacco is 8 to 12 weeks (Shew and Lucas, 1990). Number of leaves available for harvest are determined by removal of the terminal inflorescence (topping). Much of the final root system may be established by the time plants are topped (Osmond and Raper, 1982). Therefore, the first 11 to 12 weeks of the growing season provide a good time window to investigate interactions between *G. t. solanacearum* and flue-cured tobacco.

Nematode egg densities from soil samples are one of the most frequently used methods to estimate populations of cyst nematodes (LaMondia, 1990). At least two generations of *G. t. solanacearum* occur during the first 11 weeks after transplanting, with the peaks of development of mature adults at 5 and 10 WAT (Wang and Johnson, 1994). In our experiments, higher correlations between leaf and root weight and egg densities of *G. t. solanacearum* from 4 to 9 WAT may reflect population development of *G. t. solanacearum* and damage by the first two generations of nematodes to plant growth. However, correlations between leaf weight and the individual weekly nematode egg densities were relatively low and often inconsistent between years. Accumulating nematode egg densities during the sampling period provided no better correlations with plant growth compared to egg densities in individual WAT during the growing season. Application of a nematicide such as

fosthiazate may have contributed to the poor correlation between nematode egg densities in soil and growth of tobacco. Nonfumigants impair neuromuscular activity, interfering with movement and invasion, and probably also with hatching and feeding (Hague and Growen, 1987). Fosthiazate may have prevented juveniles from penetrating roots without obviously affecting survival of eggs in cysts. Some of the eggs counted from soil samples may have been apparently viable, but may have lost their ability to hatch. Eggs in cysts extracted from soil samples may not, therefore, have accurately indicated the number of juveniles potentially capable of attacking host roots. Cysts resulting from infection by the first generations of nematodes may be counted in samples taken later in the sampling period (6 to 11 WAT). However, egg densities in the later period of sampling did not greatly improve correlations with plant growth compared to egg densities early in the sampling period (1 to 5 WAT).

Infection levels estimated by penetrated nematodes excluded juveniles that might be counted in egg extraction but failed to penetrate host roots due to nematicide activity and other factors. During the first five weeks after transplanting, root systems are relatively small. Infection by *G. t. solanacearum* further delayed the establishment of root systems, reducing plant growth. The importance of early invasion by *G. t. solanacearum* and delay in establishment of root systems was somewhat

reflected by the nematode days. Because root systems were small, nematodes per gram of feeder root early in the sampling period were higher than those in the later period, although the number of nematodes counted during the first few weeks may not have been very high (Fig. 4.1). The quantification based on area under the curves gives the same weight to each of the densities taken during the sampling period. Sampling plants to determine population development at various points during growth of tobacco is time consuming. Its usage may, therefore, be limited in nematode management.

Research has related tolerance to various characteristics of plant growth (Trudgill and Cotes, 1983). A common feature of tolerance is a high capacity for enhanced root growth after an initial period of nematode attack (Wallace, 1988; Davy de Virvill and Person-Dedryver, 1989). Intolerant potato cultivars to potato cyst nematode, *G. rostochiensis* (Woll.) Behrens, 1975, suffer a reduction in the weight and length of root systems when grown in heavily infested soil (Trudgill and Cotes, 1983). The most sensitive plant growth characteristics to infection by *G. t. solanacearum* in this study appeared to be leaf weight and feeder root weight. The greater ($P \leq 0.078$) rate of reduction in leaf weight for K 326 under infection by *G. t. solanacearum* indicated that it is less tolerant to infection by *G. t. solanacearum* than NC567.

However, reductions in feeder root weight and increases in the ratio of leaf to feeder root weight were similar for both cultivars in the presence of *G. t. solanacearum*. Feeder root weight of K 326 was comparable to that for NC 567 at 11 WAT in spite of heavy infection by *G. t. solanacearum*. The similar ratio of leaf weight to feeder root weight between both cultivars indicated that the relative effectiveness of root systems in supporting plant growth and accumulating yield was similar between the two cultivars. Producing a significantly higher leaf weight in the absence of nematode infection and maintaining similar amounts of feeder root under infection by *G. t. solanacearum* may compensate to some extent for early losses at the end of growing season. Early growth responses may not, therefore, reflect the final yield response. Yield and quality of cured leaves need to be evaluated at the end of growing season to fully evaluate responses of flue-cured tobacco to *G. t. solanacearum*.

In conclusion, infection by *G. t. solanacearum* reduced plant height, number of leaves, fresh weight of leaves, and fresh weight of feeder roots of a resistant and a susceptible flue-cured tobacco cultivar during the first 11 weeks after transplanting. Although both cultivars had comparable feeder root system and leaf weight at end of the first 11 weeks, the susceptible cultivar K 326 was more likely to have lower leaf

weight than NC 567 as indicated by the steeper rate parameter in regression models.

Table 4.1. Correlations between plant height (Ht.), fresh weight of leaves (Lwt.), number of leaves (No.), fresh weight of feeder roots (Rwt.), and the ratio of fresh leaf weight to feeder root weight (RSR) at 11 weeks after transplanting (WAT) and *Globodera tabacum solanacearum* egg densities in the first 11 weeks in 1993[a].

		Correlation coefficients[b]				
Cultivar	WAT	Ht.	Lwt.	No.	Rwt.	RSR
K 326	1	.132	-.336	.081	-.374	.193
	2	.063	-.286	.084	-.297	.246
	3	-.081	-.541**	-.088	-.377	.220
	4	-.225	-.473*	-.234	-.274	.065
	5	-.196	-.596**	-.141	-.478*	.347
	6	-.191	-.440*	-.268	-.334	.308
	7	.269	-.156	.068	-.307	.170
	8	-.050	-.626**	-.103	-.463	.363
	9	.268	.128	.168	-.014*	-.165
	10	.323	-.236	.334	-.193	.040
	11	.268	-.373	.141	-.258	.098
NC 567	1	.220	.003	-.008	-.332	.199
	2	.113	-.204	-.035	-.390	.257
	3	.177	-.073	.224	-.429*	.571**
	4	.249	.168	.037	-.218	.206
	5	.078	-.237	.051	-.308	.217
	6	.303	-.308	.039	-.373	.568**
	7	-.334	-.189	-.481*	-.343	.083
	8	.326	.071	.113	-.408*	.403
	9	.205	-.163	-.110	-.435*	.278
	10	-.102	-.044	-.144	.204	-.188
	11	-.430*	-.534**	-.605**	-.341	.018

a. Egg densities estimated at eggs/500 cm^3 soil.
b. Pearson correlation coefficients.
*, **. Correlations significant at $P \leq 0.05$, $P \leq 0.01$, respectively.

Table 4.2. Correlations between plant height (Ht.), fresh weight of leaves (Lwt.), number of leaves (No.), fresh weight of feeder roots (Rwt.), and the ratio of fresh leaf weight to feeder root weight (RSR) at 11 weeks after transplanting (WAT) and *Globodera tabacum solanacearum* egg densities in the first 11 weeks in 1994[a].

		Correlation coefficients[b]				
Cultivar	WAT	Ht.	Lwt.	No.	Rwt.	RSR
K 326	1	-.220	-.230	-.051	-.347	.143
	2	-.275	-.339	-.147	-.395	.015
	3	-.242	-.248	-.364	.027	-.104
	4	-.034	-.194	.080	-.315	.298
	5	-.287	-.327	-.002	-.457*	.374
	6	-.403	-.472*	-.366	-.492*	.408
	7	-.331	-.394	-.235	-.406	.228
	8	-.017	-.102	.257	-.281	.327
	9	-.030	-.198	-.006	-.419*	.497
	10	-.217	-.269	-.116	-.360	.378
	11	.104	-.111	.029	-.334	.509
NC 567	1	.037	-.078	.120	-.253	.202
	2	-.173	-.025	.116	-.042	.029
	3	-.683*	-.438*	-.380	-.297	.306
	4	-.589*	-.483*	-.168	-.470*	.502
	5	-.711*	-.561*	-.329	-.377	.177
	6	-.430*	-.327	-.056	-.308	.199
	7	-.275	-.404*	.057	-.369	.307
	8	-.121	-.196	.212	-.213	.151
	9	-.720	-.571*	-.321	-.486*	.331
	10	-.005	-.074	.207	-.314	.245
	11	.015	-.196	.173	-.326	.499

a. Egg densities estimated at eggs/500 cm^3 soil.
b. Pearson correlation coefficients.
*. Correlations significant at $P \leq 0.05$.

Table 4.3. Correlations between plant height (Ht.), fresh weight of leaves (Lwt.), number of leaves (No.), fresh weight of feeder roots (Rwt.), and the ratio of fresh leaf weight to feeder root weight (RSR) at 11 weeks after transplanting (WAT) and nematode days for total number of nematodes per gram of feeder roots (NDT), vermiform and swollen nematodes per gram of feeder roots (NDS), and eggs in 500 cm^3 soil (NDE) of *Globodera tabacum solanacearum* over the first 11 weeks after transplanting in 1994 and 1993.

		Correlation coefficients[a]				
Cultivar	ND	Ht.	Lwt.	No.	Rwt	RSR
			1993			
K 326	NDT	.018	-.520**	-.023	-.480*	.341
	NDS	.050	-.510*	.007	-.477*	.348
	NDE	.269	-.360	.171	-.368	.113
NC 567	NDT	.313	.024	.192	-.260	.550**
	NDS	.317	.021	.196	-.261	.550**
	NDE	.142	-.247	-.146	-.490*	.377
			1994			
K 326	NDT	-.689**	-.803**	-.641**	-.777**	.694**
	NDS	-.686**	-.797**	-.645**	-.758**	.647**
	NDE	-.275	-.397	-.137	-.508*	.372
NC 567	NDT	-.470*	-.481*	.083	-.556**	.637**
	NDS	-.469*	-.481*	.085	-.554**	.638**
	NDE	-.538**	-.415*	-.094	-.397	.325

a. Pearson correlation coefficients.
*, **. Correlations significant at $P \leq 0.05$, $P \leq 0.01$, respectively.

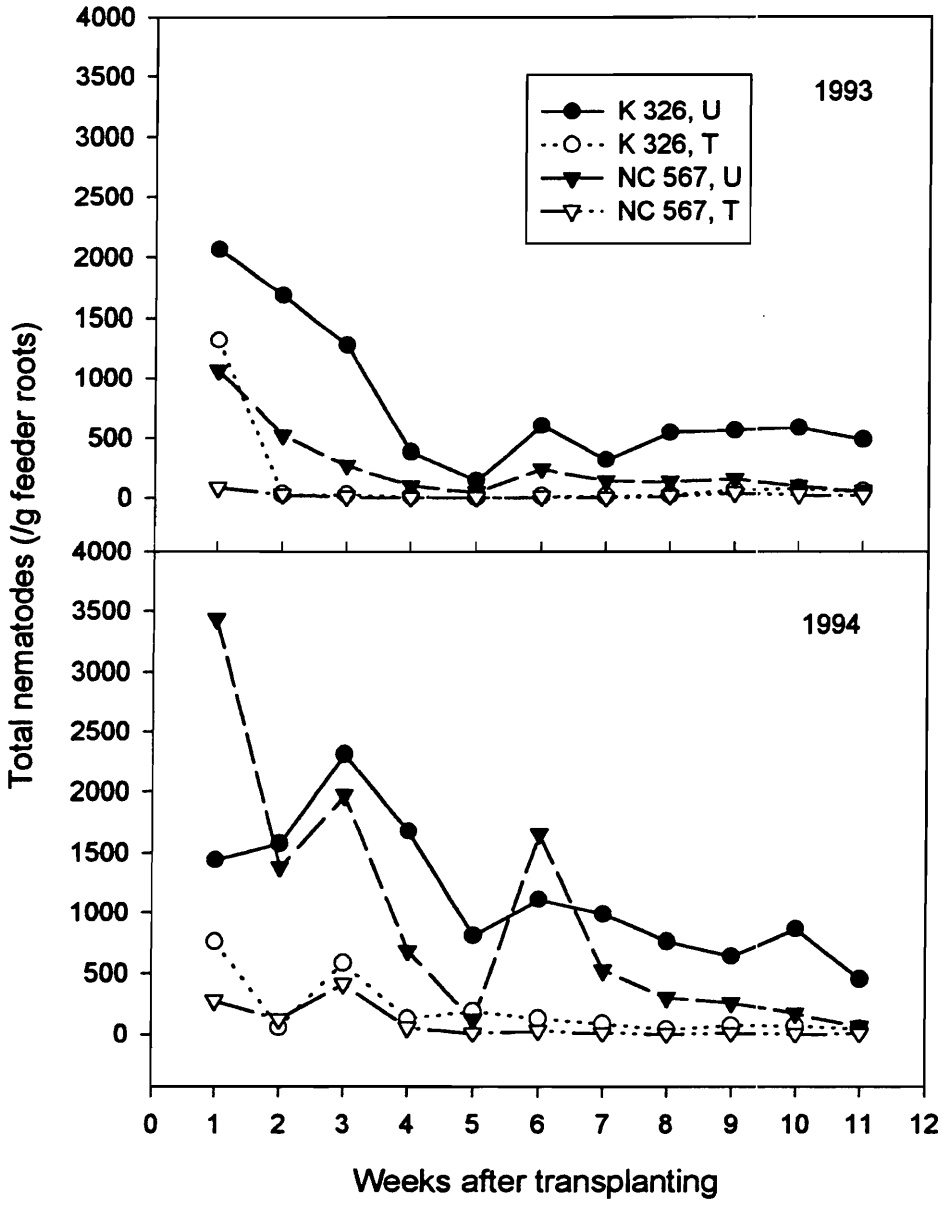


Fig. 4.1. Total nematodes of *Globodera tabacum solanacearum* per gram of feeder roots in the first 11 weeks after transplanting. Cultivar K 326 and NC 567 treated (T) or untreated (U) with fosthiazate. Data are means of 12 replicates.

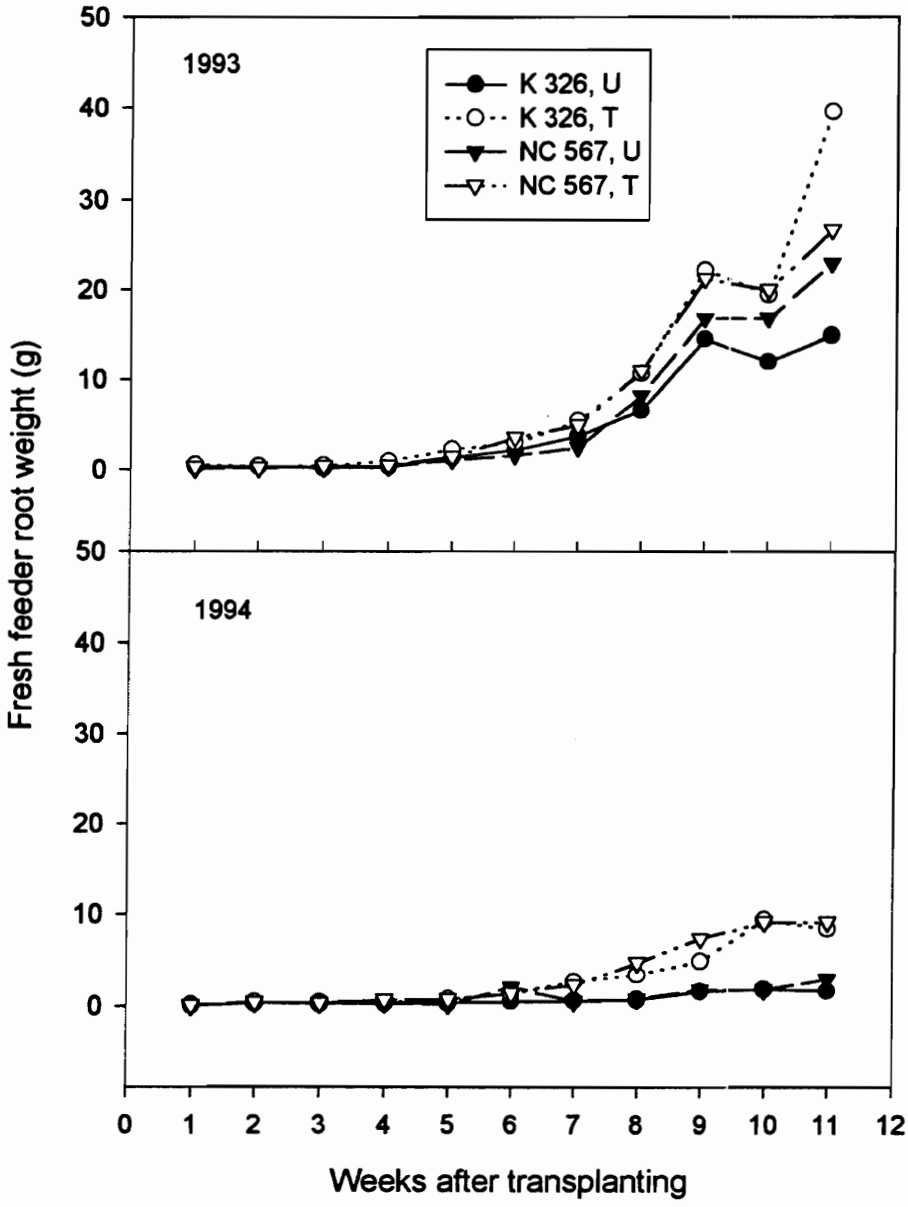


Fig. 4.2. Effects of infection by *Globodera tabacum solanacearum* on fresh feeder root weight in the first 11 weeks after transplanting. Cultivar K 326 and NC 567 treated (T) or untreated (U) with fosthiazate. Data are means of 12 replicates.

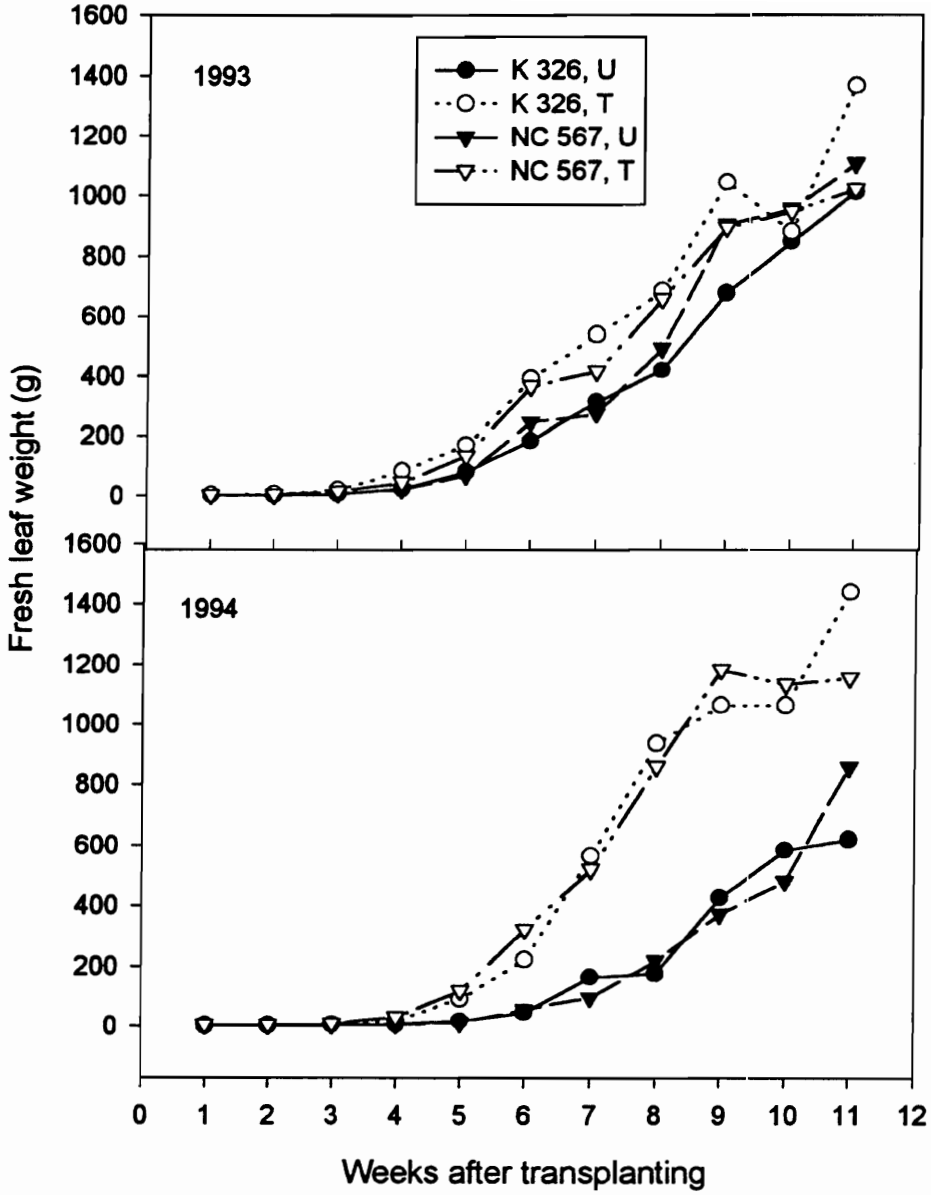


Fig. 4.3. Effects of infection by *Globodera tabacum solanacearum* on fresh leaf weight in the first 11 weeks after transplanting. Cultivar K 326 and NC 567 treated (T) or untreated (U) with fosthiazate. Data are means of 12 replicates.

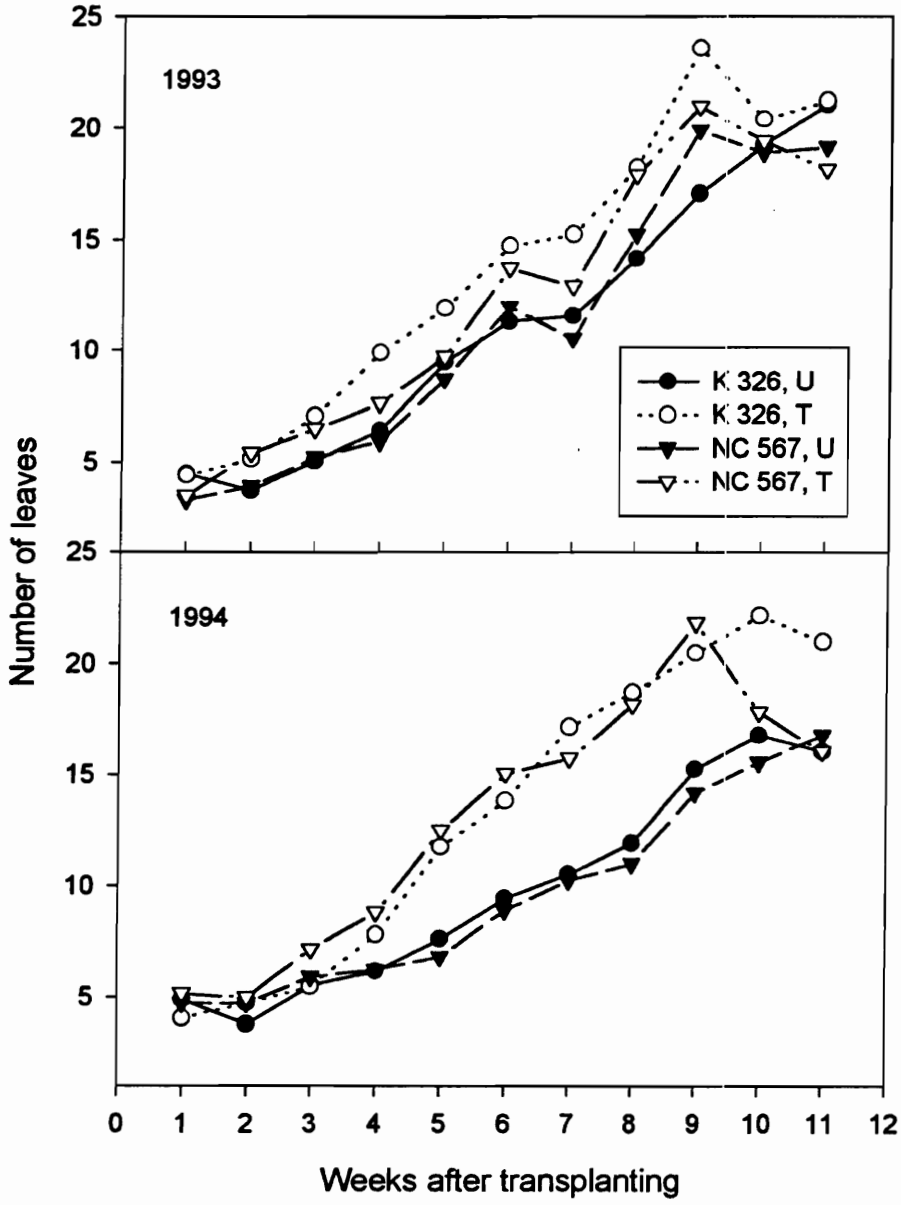


Fig. 4.4 Effects of infection by *Globodera tabacum solanacearum* on numbers of leaves in the first 11 weeks after transplanting. Cultivar K 326 and NC 567 treated (T) or untreated (U) with fosthiazate. Data are means of 12 replicates.

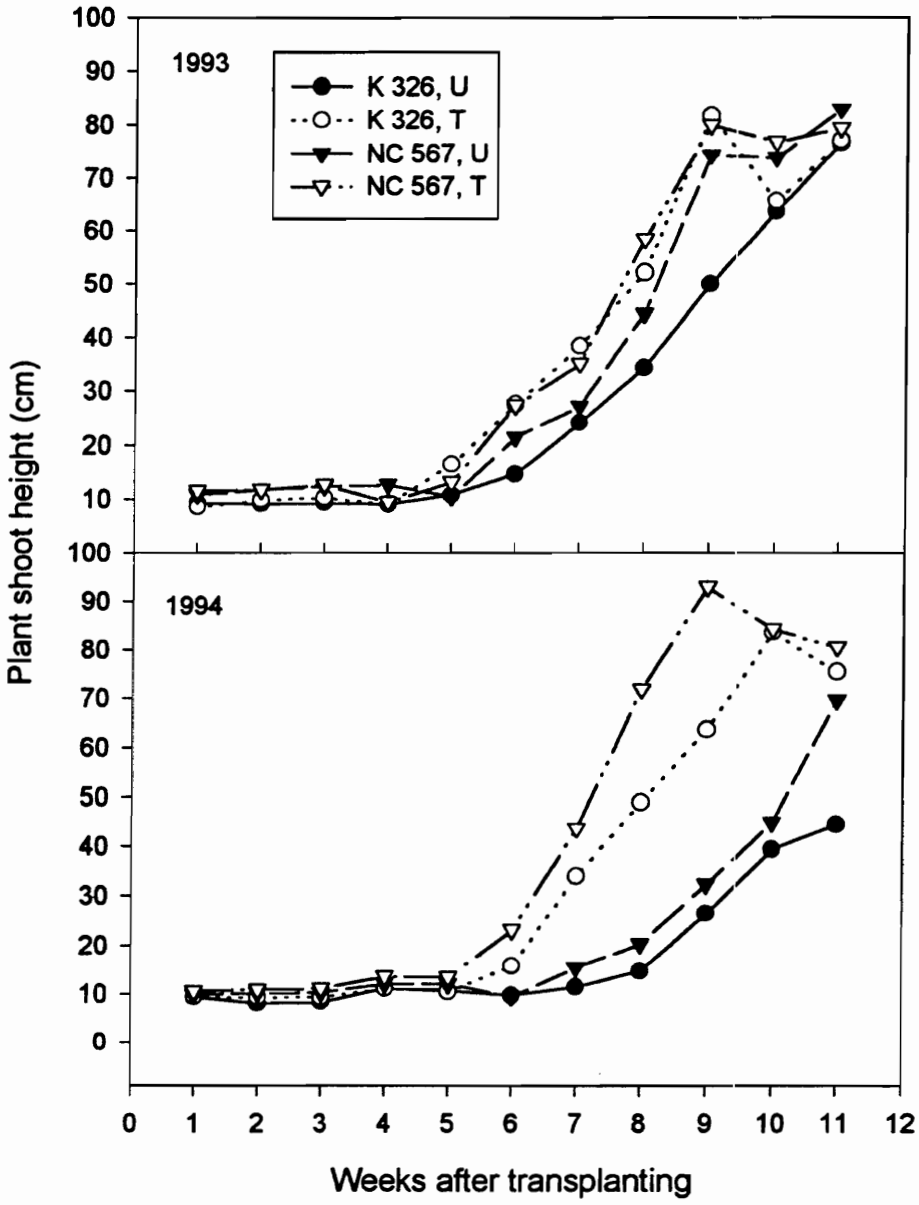


Fig. 4.5. Effects of infection by *Globodera tabacum solanacearum* on shoot height in the first 11 weeks after transplanting. Cultivar K 326 and NC 567 treated (T) or untreated (U) with fosthiazate. Data are means of 12 replicates.

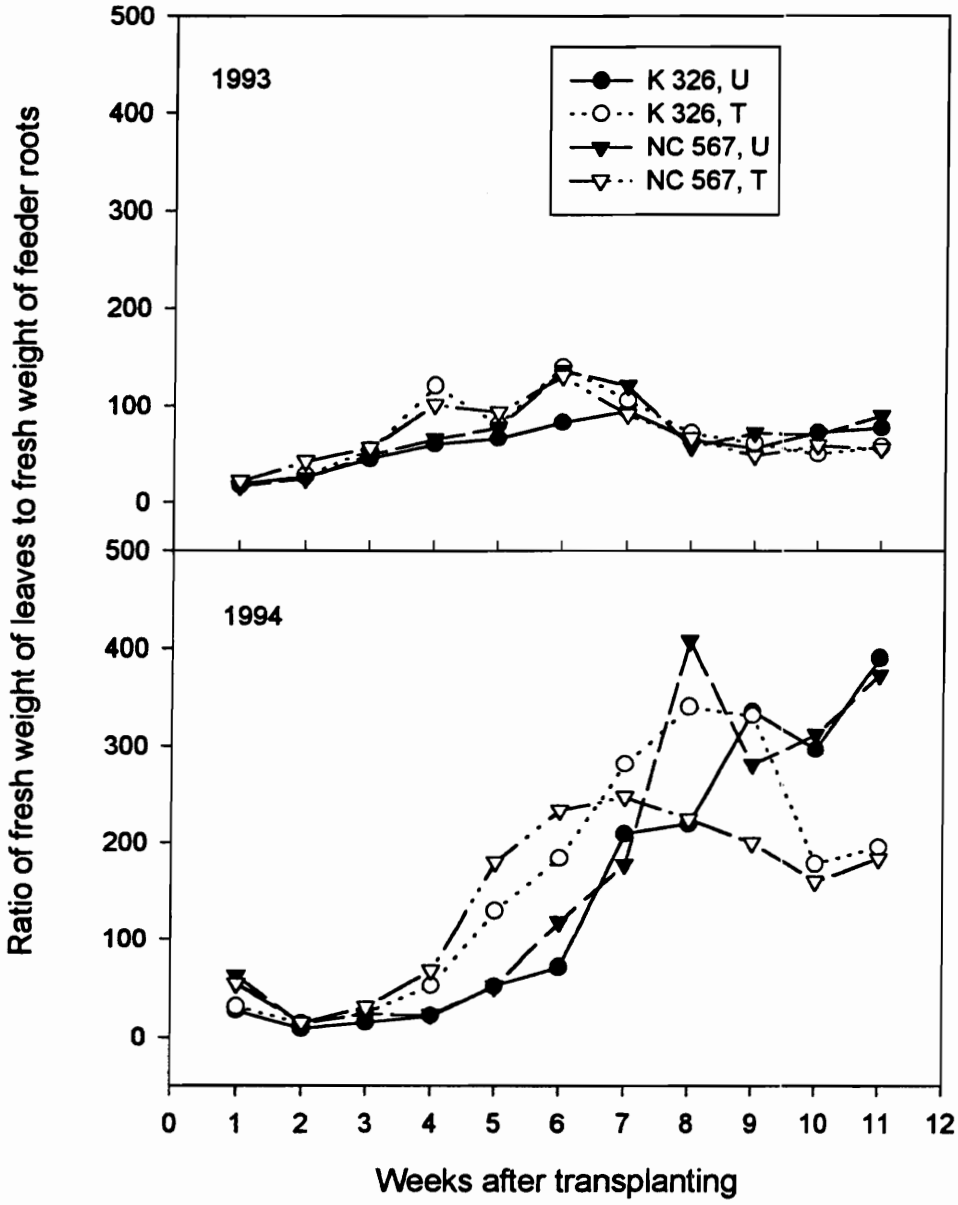


Fig. 4.6. Effects of infection by *Globodera tabacum solanacearum* on ratio of fresh leaf weight to feeder root weight in the first 11 weeks after transplanting. Cultivar K 326 and NC 567 treated (T) or untreated (U) with fosthiazate. Data are means of 12 replicates.

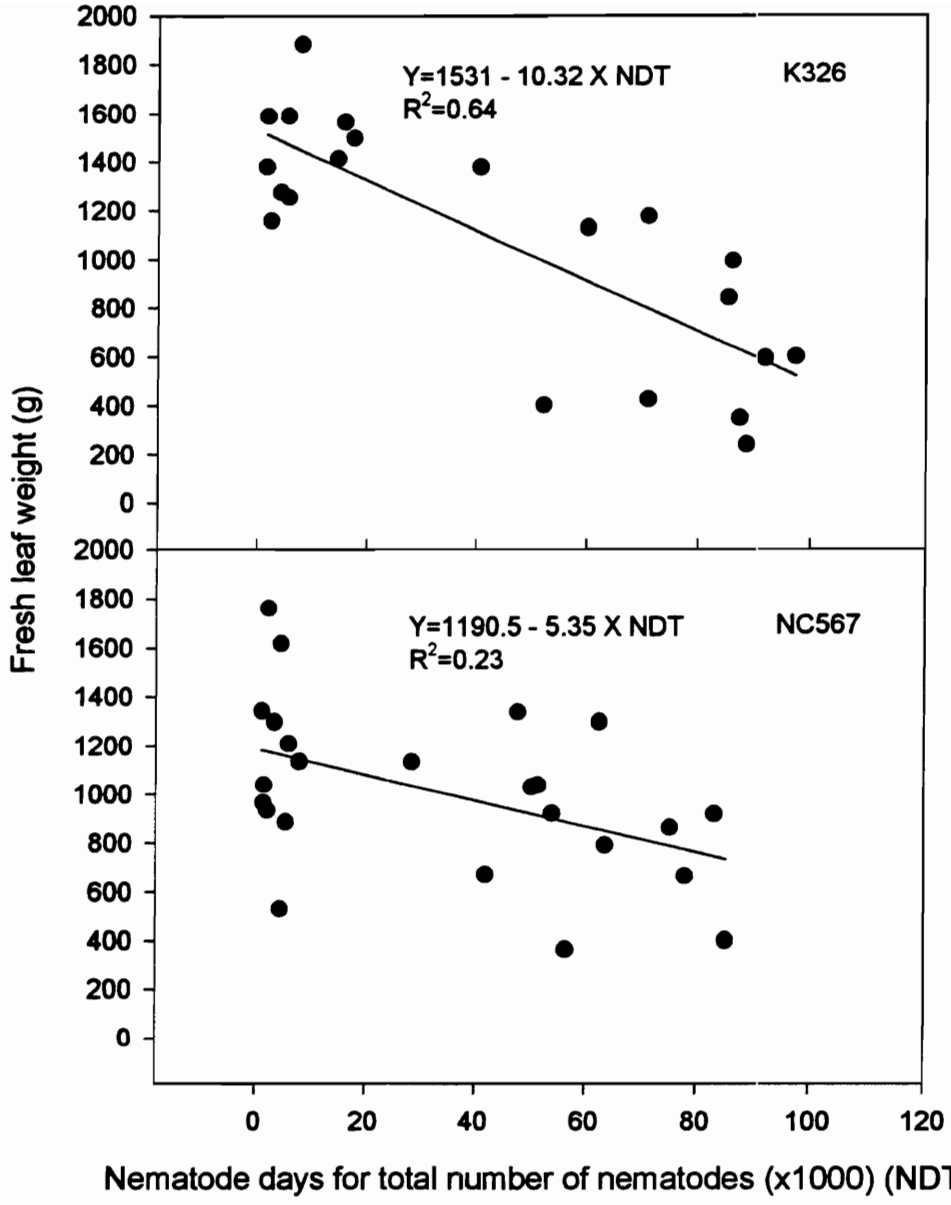


Fig. 4.7. Relationships between fresh weight of leaves of cultivar K 326, NC 567 and nematode days for total number of nematodes of *Globodera tabacum solanacearum* per gram of feeder roots (NDT) for the first 11 weeks after tansplanting in 1994.

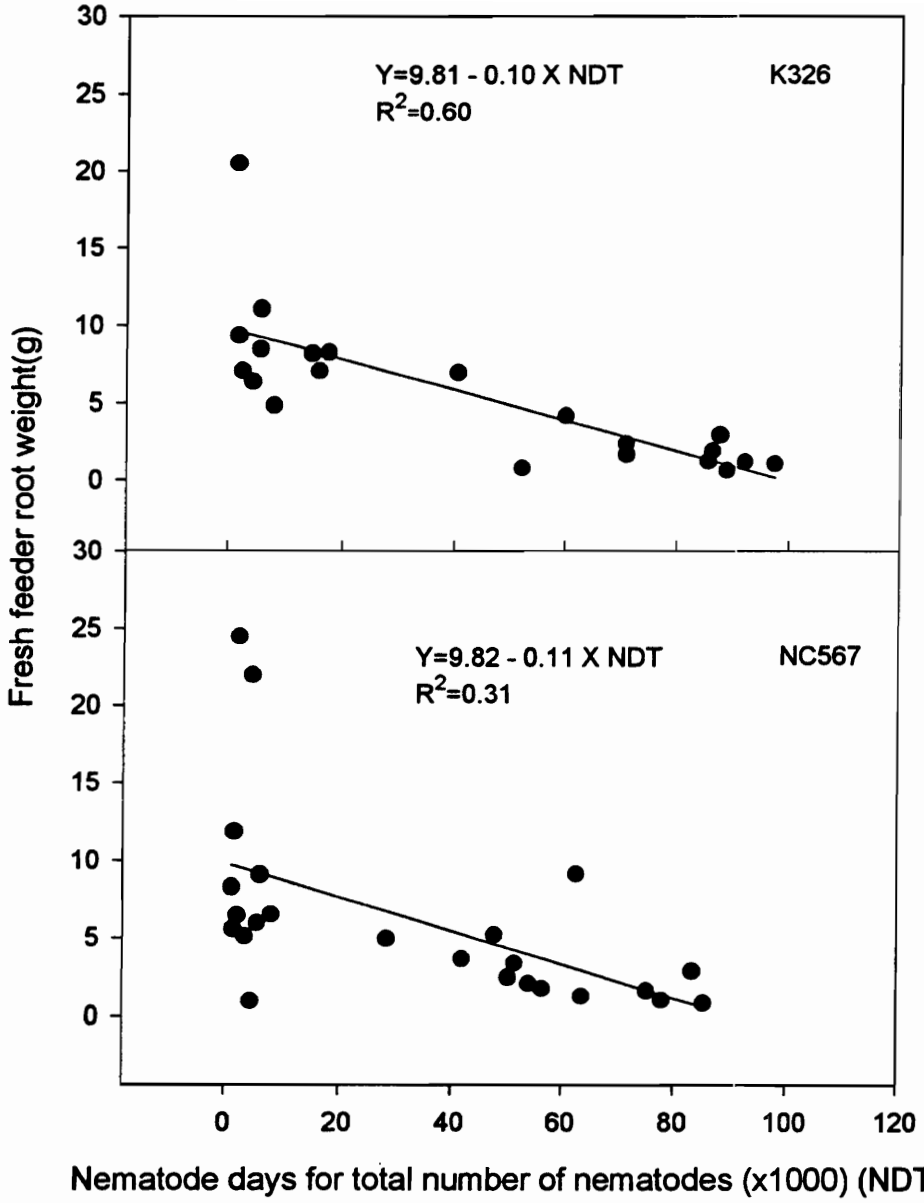


Fig. 4.8. Relationships between fresh weight of feeder roots of cultivar K 326, NC 567 and nematode days for total number of nematodes of *Globodera tabacum solanacearum* per gram of feeder roots (NDT) for the first 11 weeks after tansplanting in 1994.

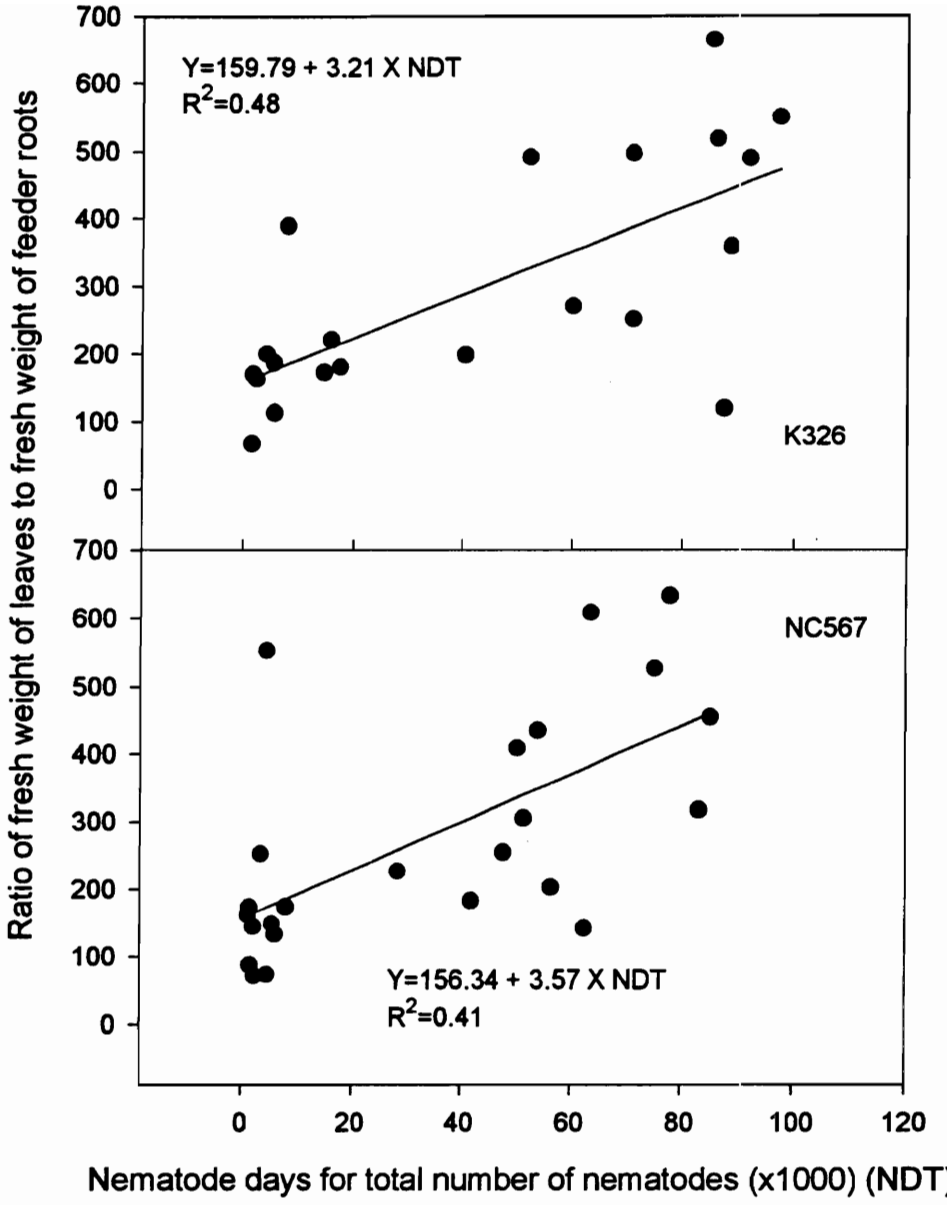


Fig. 4.9. Relationships between ratio of fresh weight of leaves to fresh weight of feeder roots of cultivar K 326, NC 567 and nematode days for total number of nematodes of *Globodera tabacum solanacearum* per gram feeder roots (NDT) for the first 11 weeks after transplanting in 1994.

LITERATURE CITED

Baalawy, H. A., and J. A. Fox. 1971. Resistance to Osborne's cyst nematode in selected *Nicotiana* species. Journal of Nematology 3:395-398.

Byrd, D. W., T. Kirkpatrick, and K. R. Barker. 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. Journal of Nematology 15:142-143.

Dale, M. F. B., and J. Brown. 1989. The use of foliage assessment to improve the identification of tolerance to damage by nematodes (*Globodera pallida*) in potatoes. Annals of Applied of Biology 115:313-319.

Davy de Virville, J., and J. Person-Dedryver. 1989. Growth and respiratory activity of roots of various Triticeae tolerant or resistant to *Heterodera aveneae* Woll. with or without infection by the nematode. Revue de Nematologie 12:379-386.

Grant, C. E., J. J. Reilly, and A. P. Elliot. 1982. Reproduction of *Globodera solanacearum* and its effect on growth and development of three flue-cured tobacco cultivars under greenhouse conditions. Journal of Nematology 14:443 (Abstr.)

Hague, N. G. M., and S. R. Gowen. 1987. Chemical control of nematodes. Pp. 131-178 in R. H. Brown, and B. R. Kerry. eds. Principles and Practice of Nematode Control in Crops. Australia: Academic Press.

Johnson, C. S., D. A. Komm, and J. L. Jones. 1989. Control of *Globodera tabacum solanacearum* by alternating host resistance and nematicide. Journal of Nematology 21:16-23.

Jones, J. L., C. S. Johnson, P. J. Semtner, T. D. Reed, B. B. Ross, and C. A. Wilkinson. 1993. 1993 flue-cured tobacco production guide. Virginia Cooperative Extension. Publication 436-420. Pp.84.

Komm, D. A., J. J. Reilly, and A. P. Elliott. 1983. Epidemiology of a tobacco cyst nematode (*Globodera solanacearum*) in Virginia. Plant Disease 67:1249-1251.

LaMondia, J. A. 1990. Effect of oxamyl on *Globodera tabacum* population dynamics and shade tobacco growth and yield. Journal of Nematology

22:654-657.

LaMondia, J. A. 1995. Shade tobacco yield loss and *Globodera tabacum tabacum* population changes in relation to initial nematode density. Journal of Nematology 27:114-119.

Miller, L. I., and B. J. Gray. 1972. *Heterodera solanacearum* n. sp., a parasite of solanaceous plants. Nematologica 18:404-413.

Osmond, D. L., and C. D. Raper, Jr. 1982. Root development of field-grown flue-cured tobacco. Agronomy Journal 74:541-546.

SAS Institute Inc. 1985. User's Guide: Statistics, Version 5 Edition. Statistical Anaylysis System Institute, Inc., Cary, NC.

Schmitt, D. P., H. Ferris, and K. R. Barker. 1987. Response of soybean to *Heterodera glycines* races 1 and 2 in different soil types. Journal of Nematology 19:240-250.

Seinhorst, J. M. 1965. The relation between nematode density and damage to plants. Nematogica 11:137-154.

Shew, H. D., and G. B. Lucas. 1990. Compendium of tobacco diseases. American Phytopathology Society Press.

Simon, A., and A. D. Rovira. 1982. The relation between wheat yield and early damage of roots by cereal cyst nematode. Australian Journal of Experimental Agriculture and Animal Husbandry 22:201-208.

Stanton, J. M., and J. M. Fisher. 1987. Field assessment of factors associated with tolerance of wheat to *Heterodera avenae*. Nematologica 33:357-360.

Trudgill, D. L. 1986. Yield losses caused by potato cyst nematodes: a review of the current position in Britain and prospects for improvements. Annals of Applied Biology. 108:181-198.

Trudgill, D. L., and L. M. Cotes. 1983. Tolerance of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) in relation to the growth and efficiency of the root system. Annals of Applied Biology 102:385-397.

Wang, J., and C. S. Johnson. 1994. Development of *Globodera tabacum solanacearum* population on a resistant and susceptible cultivar of flue-cured tobacco in Virginia. Proceedings of 36th Tobacco Workers' Conference. (Abstr.) In Press.

Wallace, H. R. 1988. A perception of tolerance. Nematologica 33:419-432.

Influence of Infection by *Globodera tabacum solanacearum* on Flue-cured Tobacco (*Nicotiana tabacum*) Yield and Quality

Abstract: The influence of infection by *Globodera tabacum solanacearum* on the yield and quality of flue-cured tobacco cultivars K 326 (susceptible) and NC 567 (resistant) were investigated in 1993 and 1994. Different infection levels of *G. t. solanacearum* were achieved by applying an experimental nematicide (fosthiazate) approximately one week before transplanting. Infection by *G. t. solanacearum* was determined by sampling plants weekly and counting nematodes in feeder roots. Infection suppressed yield and reduced grade index of cured leaves of both cultivars in both years. Linear regression models described relationships between yield and quality and nematode days for total number of nematodes, calculated by integrating areas under curves for total nematode population densities per gram of feeder root in 1994. Rate parameters in the regression models suggested that the yield and quality of leaf from resistant and susceptible cultivars decreased similarly with increasing infection although NC 567 generally supported lower populations of *G. t. solanacearum*. Results suggest that placing resistance gene in genetic background with higher yield and quality potential might result in improved resistant cultivars.

Key words: cyst nematode, resistance, tobacco grade index, damage function, multiple point model.

Tobacco cyst nematode, *Globodera tabacum solanacearum* (Miller and Gray) Behrens, 1975, is one of the most serious pathogens of flue-cured tobacco, *Nicotiana tabacum* (L.), in Virginia (Miller and Gray, 1972). An estimated one quarter of the total acreage of flue-cured tobacco in Virginia is infested by *G. t. solanacearum* (Johnson, unpub.). *G. t. solanacearum* also occurs in several counties in North Carolina (Melton et al., 1991; T. A. Melton, personal communication). Average yield reductions caused by *G. t. solanacearum* have been estimated at 15% (Komm et al., 1983). Nonfumigant nematicides are routinely applied to control *G. t. solanacearum*, at an average cost of $139/ha (Johnson et al., 1989). Nematicides that provide effective control of *G. t. solanacearum* are limited in number and subject to withdrawal due to environmental concerns (Johnson et al., 1989). Choices of economically feasible rotation crops are also restricted. Host resistance may, therefore, present the best opportunity for improving management of *G. t. solanacearum*.

Several flue-cured tobacco cultivars have been identified that suppress populations of *G. t. solanacearum*, but significant yield losses

still occur when these cultivars are planted in nematode-infested soil that has not been treated with a nematicide (Komm et al., 1983; Johnson et al., 1989; Johnson, 1990). These yield losses have prevented widespread use of resistant cultivars to manage *G. t. solanacearum*, and have been cited as evidence that resistance to *G. t. solanacearum* is linked with a lack of tolerance to these nematodes (Fox and Spasoff, 1976; Komm et al., 1983). Accurate characterization of the interactions between populations of *G. t. solanacearum* and resistant and susceptible cultivars of flue-cured tobacco would enable more appropriate use of resistance genes in plant breeding programs. An improved quantitative understanding of the influence of *G. t. solanacearum* on flue-cured tobacco yield and quality would also prove extremely useful in optimizing use of currently available nematode management options. Information regarding tolerance to *G. t. solanacearum* is not available for most currently available cultivars of flue-cured tobacco.

The field research reported here was conducted in order to investigate the relationships between infection by *G. t. solanacearum* and yield and quality of resistant and susceptible flue-cured tobacco.

MATERIALS AND METHODS

Field preparation: Experimental plots were located at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, Virginia. Each plot was 4.88 m wide and 12.12 m long and contained four rows 1.2 m apart. The first and fourth rows served as border rows. Plants in the second row were destructively sampled. The third row was used for evaluation of yield and quality. All plots were maintained similarly to commercial tobacco fields (Jones et al., 1993). Only foliar sprays were used for insect control. Irrigation was used only as necessary to minimize leaf burn and to prevent losses in plant stand. scalding and to maintain plant stand. Further details about the fields used and experimental methods employed are reported in the previous chapter (Wang, 1996).

Treatments and experimental design: Four treatments were imposed using a complete factorial design with 12 replications. Flue-cured cultivars K 326 (susceptible to *G. t. solanacearum*) and NC 567 (resistant to *G. t. solanacearum* were planted in untreated soil or in plots treated with 6.84 L/ha or 6.78 L/ha in 1993 and 1994, respectively. Fosthiazate is an experimental organophosphate nematicide under development by ISK Biosciences Corporation reported to possess a high level of nematicidal

activity without significant insecticidal effect on tobacco (Johnson, 1995). Fosthiazate was applied using a tractor-mounted, CO_2-powered, hydraulic sprayer, and was incorporated by disking immediately after application. Tobacco seedlings were transplanted approximately one week after nematicide application.

Determination of soil population and infection by G. t. solanacearum: One plant was randomly sampled from each plot every week for the first 11 weeks after transplanting. A 1000 cm^3 soil sample was also collected from around the roots of each plant. Soil populations of *G. t. solanacearum* were estimated by extracting cysts from a 250 cm^3 subsample, crushing cysts and counting eggs. Infection by *G. t. solanacearum* was determined by counting the number and characterizing the stage of nematodes within roots stained with acid fuchsin (Byrd et al., 1983). Nematodes in roots were counted and assigned to one of four classes based on overall shape under low magnification (40X): a) vermiform nematodes (those juveniles that had successfully penetrated roots without obvious feeding); b) swollen (nematodes with a distinct sausage shape); c) flask-shaped; and d) saccate adult nematodes bearing eggs. Further details about methods used are reported in the previous chapter (Wang, 1996).

Yield and quality of cured leaves: Plots were harvested or 'primed' four times, based upon the ripeness of leaves, and cured according to standard practices. Primings from all plots were weighed after harvesting had been completed and were graded by USDA marketing service inspectors. A 0-100 grade index that groups federal flue-cured tobacco grades according to equivalent quality characteristics was used to estimated the quality of each harvest or priming (Bowman et al., 1988).

Population of *G. t. solanacearum* and infection by nematodes were quantified as areas under curves for the first 11 weeks after transplanting. Areas under curves were calculated as "nematode days" for the number of nematodes of each class or the total number of nematodes per gram of feeder roots during the first 11 weeks after transplanting. The formula used to calculated ND was as follows:

$$\text{Nematode days} = [PGTS_{i+1}+PGTS_i)/2](X_{i+1}-X_i)$$

where $PGTS_i$=number of nematodes per gram of feeder roots at i'th sampling date and X_i=the julian date of the i'th sampling date.

Statistical Analysis: The influence of experimental treatments on yield and grade index were subjected to analysis of variance. Regression analyses were used to describe relationships between yield and quality

of flue-cured tobacco and with infection levels of *G. t. solanacearum*.

RESULTS

Application of fosthiazate successfully created different infection levels as indicated by total nematodes per gram of feeder root (Fig. 5.1). Application of fosthiazate also significantly increased yields of the first three harvests in 1993, but yields in the fourth harvest were similar between nematicide treatments (Table 5.1). As a result, the total yield of plots treated with fosthiazate was higher ($P \leq 0.05$) than that from those leaf untreated. Significant increases in yield were recorded in all harvests of plots treated with nematicide in 1994. Yields of all harvests of susceptible K 326 were higher ($P \leq 0.05$) than those for resistant NC 567 in 1993 (Table 5.2). Yields of the first two harvests of K 326 were also higher ($P \leq 0.05$) than those for NC 567 in 1994, but NC 567 produced more ($P \leq 0.05$) yield in the third harvest than K 326. Both cultivars yielded similarly in the fourth harvest. The total yield for both cultivars was similar in 1994.

Significant negative correlations were detected between yield of K 326 and densities of *G. t. solanacearum* in both years (Tables 5.3,4). However, total yield of NC 567 was only significantly correlated with

densities of vermiform juveniles and swollen nematodes of *G. t. solanacearum* at 11 WAT in 1993. Correlations ($P \leq 0.05$) between total yield of NC 567 and population densities of *G. t. solanacearum* were usually found in 1994 (Table 5.4). Among the correlations between yield and egg densities for both year, egg densities at the sixth week after transplanting tended to have higher correlation coefficients. Significant linear regressions were found for both cultivars in 1994 and for K 326 in 1993 (Fig. 5.2).

Total yields were also negatively correlated with nematode days for total nematodes within feeder roots (NDT) (Table 5.5). Correlations between yield and (NDT) were higher and more consistent for both cultivars in 1994 than in 1993. Correlations between yield and NDT were also relatively higher than nematode days for specific nematode classes or eggs in soil for both cultivars in both years. The yield response of both cultivars to infection by *G. t. solanacearum* was, therefore, evaluated using NDT during the first 11 WAT.

No significant regression relationships were found between total yield and NDT for either cultivar in 1993 (Fig. 5.3). However, yield reductions of the third harvest for K 326 and the second harvest for NC 567 in 1993 were significantly linearly correlated with NDT with $R^2=0.20$ and 0.30, respectively. The total yield of both cultivars declined

linearly with increasing NDT in 1994. Rate parameters in regression models associating total yield with NDT were similar for both cultivars (Fig. 5.3). Yields of individual harvests were also reduced linearly in 1994 with increasing NDT (Fig. 5.4). The rates of decline in yield with increasing nematode pressure were lower for the first harvest than for all subsequent harvests for both cultivars. However no differences in rates of decline were found between cultivars for yields at individual harvests. Yield potential was higher for K 326 than for NC 567 at the first and second harvest.

Application of fosthiazate increased ($P \leq 0.05$) cured grade index for the first harvest only in 1993 (Table 5.6). As a result, average grade index was similar between nematicide treatments. However, treatment with fosthiazate increased ($P \leq 0.05$) cured grade index for all harvests in 1994. Grade index for K 326 was higher ($P \leq 0.05$) in both years than that for NC 567, except in the first harvest (Table 5.7).

Average grade index and the index of the first two harvests for NC 567 was significantly linearly correlated with NDT in 1993 although the coefficients of determination were small (Fig. 5.5). However, no significantly linear regression was found between average grade index for K 326 and grade index of individual harvests and NDT in 1993. Average grade index was highly significantly correlated with NDT for both

cultivars in 1994. Significant linear relationships were also found between grade index of cured leaves from individual harvests and NDT for both cultivars in 1994, except for the third harvest of K 326 (Fig. 5.6). Reduction in grade index of the first harvest was greater ($P \leq 0.05$) for NC 567 than for K 326, but nematode effects on grade index were similar on the resistant (NC 567) and susceptible (K 326) cultivars at all other harvests.

DISCUSSION

No significant damage from other diseases or insect pests were observed throughout these experiments. Fosthiazate has not been reported to directly influence tobacco growth. Differences in yield and quality recorded in these experiments, therefore, probably resulted from control of the tobacco cyst nematode. Application of fosthiazate reduced populations of *G. t. solanacearum* significantly for both cultivars. Yield of cured leaf was correlated with densities of *G. t. solanacearum*, further indicating that control of *G. t. solanacearum* was responsible for the yield increases and quality improvements in these experiments.

The different growing conditions in 1993 and 1994 greatly influenced host response to infection by *G. t. solanacearum*. This was

clearly demonstrated by the differences in yield and grade index between plots treated with fosthiazate in the two years. The average yield of plots treated with fosthiazate was 445.7 kg/ha lower in 1993 than in 1994. Reducing nematode populations using fosthiazate increased yield 8.9% in 1993 and 46.9% in 1994, compared to the previously estimated average of 15% (Komm et al., 1983). The average grade index was 28.9 lower in 1993 than in 1994. Because other factors lessened the impact of infection by *G. t. solanacearum* on yield in 1993, no significant correlations were found between total yield and nematode population densities. However, differences in yield response to infection by *G. t. solanacearum* between cultivars appeared to be similar in both years (Table 5.8). Similar responses to infection by the resistant and susceptible cultivars were clearly indicated by regression models in 1994.

Several models have been proposed to relate yield loss to nematode infection (Seinhorst, 1965; Brown, 1969; Noe et al., 1991). Asymptotic models provide estimates of minimum yield and tolerance level which are important parameters related to nematode management. These models are based on the observation that damage per nematode decreases at high nematode infection levels, when nutrients for full development of individual nematodes may become limiting. The extensively high nematode

population densities required for this phenomenon may be not common in naturally infested soil, largely due to various mechanisms of population regulation. There was no evidence that leaf yields had reached minimum levels in this study. Correlating yields with population densities of *G. t. solanacearum* using Seinhorst's equation did not appear to significantly improve regression results compared to simple linear models (Wang and Johnson, 1995). Nematode days based on changes of nematode population over time reflects the continuous association of nematodes and hosts and has been used to quantify parasitism of *Meloidogyne hapla* (Noling and Ferris). Simple linear regression models were used to describe the relationships between the reduction in yield and grade index of flue-cured tobacco and the infection by *G. t. solanacearum*. Relatively higher correlations between yield and nematode populations at 5 or 6 weeks after transplanting suggest that populations during these period may be used in a critical point model as an alternative to describe host response to infection by *G. t. solanacearum*. Significant correlations between nematode days for eggs (NDE) and nematode days for total number of nematodes (NDT) may suggest substituting NDE for NDT in evaluating host responses to infection to reduce the amount of work associated to collet NDT (Fig. 5.7).

The focus of this study was on evaluating the response of resistant

and susceptible cultivars of flue-cured tobacco to infection by *G. t. solanacearum*. Proportional decreases in yield and quality with increasing nematode population provide a measure of host tolerance to nematode infection. The resistant and susceptible cultivars tolerated infection by *G. t. solanacearum* similarly, as indicated by the rate parameters in the regression models. However, the higher yield potential and grade index demonstrated for K 326 make it much more acceptable in tobacco production. These results strongly suggest that a backcrossing program to combine resistance to *G. t. solanacearum* with genes for desired agronomic traits should result in resistant cultivars satisfactory to flue-cured tobacco.

Table 5.1. Influence of infection by *Globodera tabacum solanacearum* on cured tobacco yield in 1993 and 1994*.

Year	Rate of fosthiazate (Liters/ha)	Yield (kg/ha)**				
		Total	1st harvest	2nd harvest	3rd harvest	4th harvest
1993	6.84	3438.1[a]	451.2[a]	674.1[a]	1248.7[a]	1066.0[a]
	0	3156.7[b]	402.2[b]	608.8[b]	1071.7[b]	1071.6[a]
1994	6.78	3883.8[a]	575.3[a]	874.8[a]	1103.0[a]	1333.6[a]
	0	2644.6[b]	373.0[b]	571.1[b]	889.9[b]	812.7[b]

* Yields are means of 12 replications.
** Means with the same letter within a column for each year are not significantly different ($P \leq 0.05$) according to Duncan multiple-range test.

Table 5.2. Influence of infection by *Globodera tabacum solanacearum* on cured tobacco yield in 1993 and 1994*.

Year	Cultivar	Yield (kg/ha)**				
		Total	1st harvest	2nd harvest	3rd harvest	4th harvest
1993	K 326	3561.1[a]	462.2[a]	688.7[a]	1220.8[a]	1188.5[a]
	NC 567	3033.7[b]	391.1[b]	594.2[b]	1099.5[b]	949.1[b]
1994	K 326	3257.8[a]	525.5[a]	804.8[a]	915.3[b]	1016.3[a]
	NC 567	3270.6[a]	422.7[b]	641.1[b]	1077.7[a]	1130.1[a]

* Yields are means of 12 replications.

** Means with the same letter within a column for each year are not significantly different ($P \leq 0.05$) according to Duncan multiple-range test.

Table 5.3. Correlations between yield and population densities of vermiform juveniles, swollen nematodes, flask-shaped nematodes, egg-bearing adults per gram feeder roots, and eggs in 500 cm^3 soil of *Globodera tabacum solanacearum* in the first 11 weeks after transplanting (WAT) in 1993.

		Correlation coefficients[a]				
Cultivar	WAT	Vermform	Swollen	Flask-shaped	E.Adult	Egg in soil
K 326	1	-.32	-.46*	.	.	-.37
	2	-.31	-.33	-.31	.	-.31
	3	-.48*	-.46*	-.41*	-.04	-.51*
	4	-.60**	-.52**	-.54**	-.46	-.18
	5	-.53**	-.58**	-.57**	-.55**	-.55
	6	-.24	-.25	-.26	-.48*	-.57*
	7	-.30	-.15	-.25	-.12	-.23
	8	-.20	-.35	-.32	-.37	-.44*
	9	-.26	-.17	-.13	-.15	-.00
	10	-.37	-.32	-.36	-.42*	-.03
	11	-.38	-.31	-.27	-.41*	-.22
NC 567	1	-.15	-.23	.	.	.12
	2	-.34	-.30	-.17	.	-.07
	3	-.38	-.16	-.14	-.32	-.20
	4	-.33	-.37	-.27	-.37	-.11
	5	-.22	-.02	-.10	-.01	-.08
	6	-.25	-.33	-.04	-.24	-.28
	7	-.34	-.24	-.11	-.22	-.10
	8	-.35	-.40	-.23	-.04	-.36
	9	-.19	-.18	.09	.26	-.25
	10	-.25	-.02	-.13	-.08	-.06
	11	-.56**	-.53**	-.13	-.09	-.16

a. Pearson correlation coefficients. *, **. Correlations significant at $P \leq 0.05$, $P \leq 0.01$, respectively.

Table 5. 4. Correlations between yield and population densities of vermiform juveniles, swollen nematodes, flask-shaped nematodes, egg-bearing adults per gram feeder roots, and eggs in 500 cm^3 soil of *Globodera tabacum solanacearum* in the first 11 weeks after transplanting (WAT) in 1994.

		Correlation coefficients[a]				
Cultivar	WAT	Vermform	Swollen	Flask-shaped	E.Adult	Egg in soil
K 326	1	-.53*	-.79**	.	.	-.35
	2	-.79**	-.85**	-.65**	.	-.48*
	3	-.58**	-.68**	-.78**	-.20	-.20
	4	-.85**	-.74**	-.86**	-.78**	-.57**
	5	-.70**	-.69**	-.73**	-.85**	-.62**
	6	-.74**	-.72**	-.65**	-.38	-.79**
	7	-.75**	-.71**	-.69**	-.60**	-.50*
	8	-.84**	-.58**	-.70**	-.72**	-.45*
	9	-.74**	-.87**	-.70**	-.71**	-.62**
	10	-.68**	-.78**	-.59**	-.56**	-.50*
	11	-.76**	-.73**	-.75**	-.58**	-.61**
NC 567	1	-.59**	.	.	.	-.20
	2	-.74**	-.61**	-.40	.	-.44*
	3	-.61**	-.63**	-.25	.	-.33
	4	-.74**	-.81**	-.58**	-.08	-.64**
	5	-.55**	-.54**	-.47*	-.13	-.62**
	6	-.85**	-.59**	-.33	-.10	-.55**
	7	-.71**	-.51*	-.19	-.17	-.74**
	8	-.89**	-.67**	-.48*	-.07	-.45*
	9	-.73**	-.73**	-.61**	-.27	-.64**
	10	-.89**	-.85**	-.75**	-.44*	-.49*
	11	-.49*	-.65**	-.57**	-.27	-.51**

a. Pearson correlation coefficients.
*, **. Correlations significant at $P \leq 0.05$, $P \leq 0.01$, respectively.

Table 5.5. Correlations between yield and nematode days of vermiform juveniles (NDV), swollen nematodes (NDS), flask-shaped nematodes (NDF), egg-bearing adults (NDA), total nematodes (NDT) per gram feeder roots, and eggs in 500 cm^3 soil (NDE) of *Globodera tabacum solanacearum* in the first 11 weeks after transplanting (WAT) in 1993 and 1994.

		Correlation coefficients[a]					
Year	Cultivar	NDV	NDS	NDF	NDA	NDT	NDE
1993	K 326	-.44*	-.38	-.40	-.56**	-.45*	-.47*
	NC 567	-.27	-.30	-.18	-.08	-.30	-.12
1994	K 326	-.80**	-.82**	-.85**	-.81**	-.82**	-.70**
	NC 567	-.87**	-.73**	-.60**	-.28	-.86**	-.67**

a. Pearson correlation coefficients.
*, **. Correlations significant at $P \leq 0.05$, $P \leq 0.01$, respectively.

Table 5.6. Influence of infection by *Globodera tabacum solanacearum* on cured tobacco quality in 1993 and 1994*.

Year	Rate of fosthiazate (Liters/ha)	Grade index**				
		Average	1st harvest	2nd harvest	3rd harvest	4th harvest
1993	6.84	47.0[a]	48.3[a]	53.8[a]	43.3[a]	45.6[a]
	0	42.3[a]	43.8[b]	46.7[a]	43.8[a]	38.5[a]
1994	6.78	75.9[a]	60.4[a]	79.6[a]	79.6[a]	76.9[a]
	0	63.5[b]	32.5[b]	66.5[b]	73.5[b]	64.8[b]

* Yields are means of 12 replications.
** Means with the same letter within a column for each year are not significantly different ($P \leq 0.05$) according to Duncan multiple-range test.

Table 5.7. Influence of infection by *Globodera tabacum solanacearum* on cured tobacco quality in 1993 and 1994*.

Year	Cultivar	Grade index**				
		Average	1st harvest	2nd harvest	3rd harvest	4th harvest
1993	K 326	51.4[a]	46.7[a]	59.8[a]	53.1[a]	47.1[a]
	NC 567	37.9[b]	45.4[a]	40.6[b]	34.0[b]	37.1[b]
1994	K 326	72.7[a]	49.6[a]	76.3[a]	79.0[a]	75.0[a]
	NC 567	66.7[b]	43.3[a]	69.8[b]	74.2[b]	66.7[b]

* Yields are means of 12 replications.
** Means with the same letter within a column for each year are not significantly different ($P \leq 0.05$) according to Duncan multiple-range test.

Table 5.8. Yield response to infection by *Globodera tabacum solanacearum* measured as percent yield differences between cultivars NC 567 and K 326 with and without fosthiazate treatment in 1993 and 1994*.

	Yield change (%)**				
Year	Total	1st harvest	2nd harvest	3rd harvest	4th harvest
1993	-4.62[a]	9.45[a]	15.45[a]	-8.70[a]	-21.28[a]
1994	-12.83[a]	-3.81[a]	22.90[a]	-15.71[a]	-42.08[a]

*. Yield change (%) is differeces in percent yield incrseases for NC567 between treated and untreated with fosthiazte minus percent yield increases for K326 between treated and untreated with fosthiazte.
**. yield are means of 12 replications. Means with the same letter within a column are not significant different (P≤0.05) according to Duncan multiple-range test.

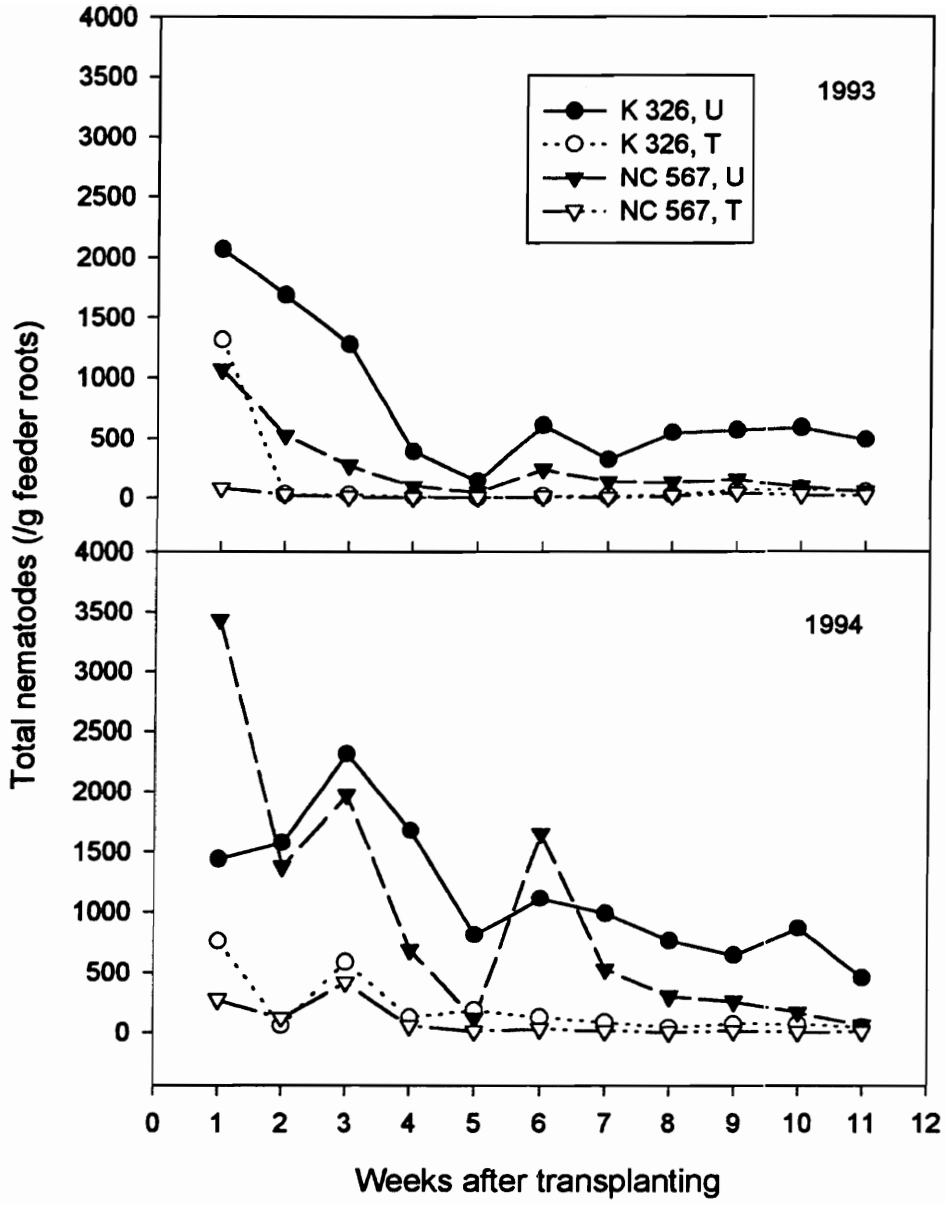


Fig. 5.1. Total nematodes of *Globodera tabacum solanacearum* per gram of feeder roots in the first 11 weeks after transplanting. Cultivar K 326 and NC 567 treated (T) or untreated (U) with fosthiazate. Data are means of 12 replicates.

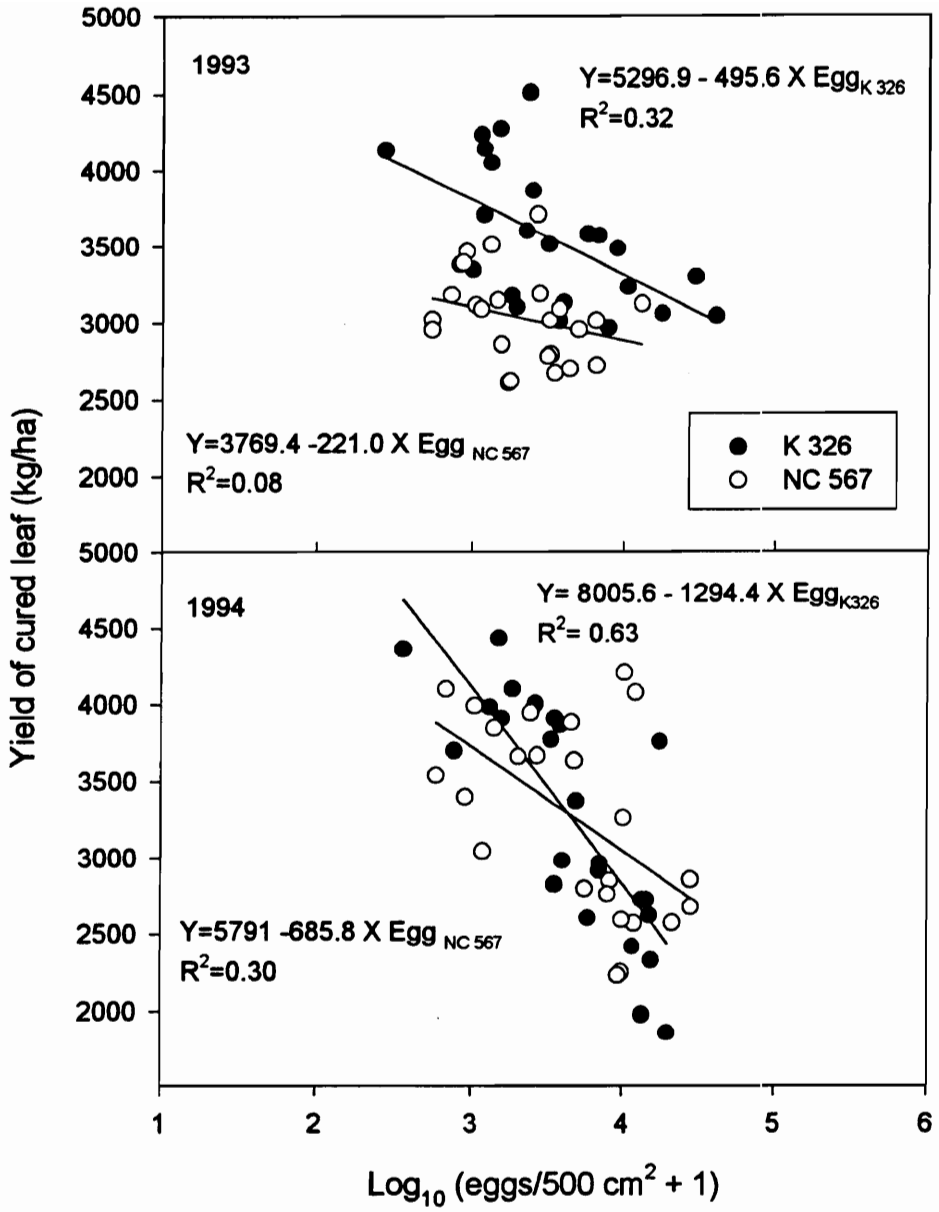


Fig. 5.2 Relationships between yield of cured leaf (kg/ha) of cultiva K 326, NC 567 and egg densities of the sixth week after transplanting in 1993 and 1994.

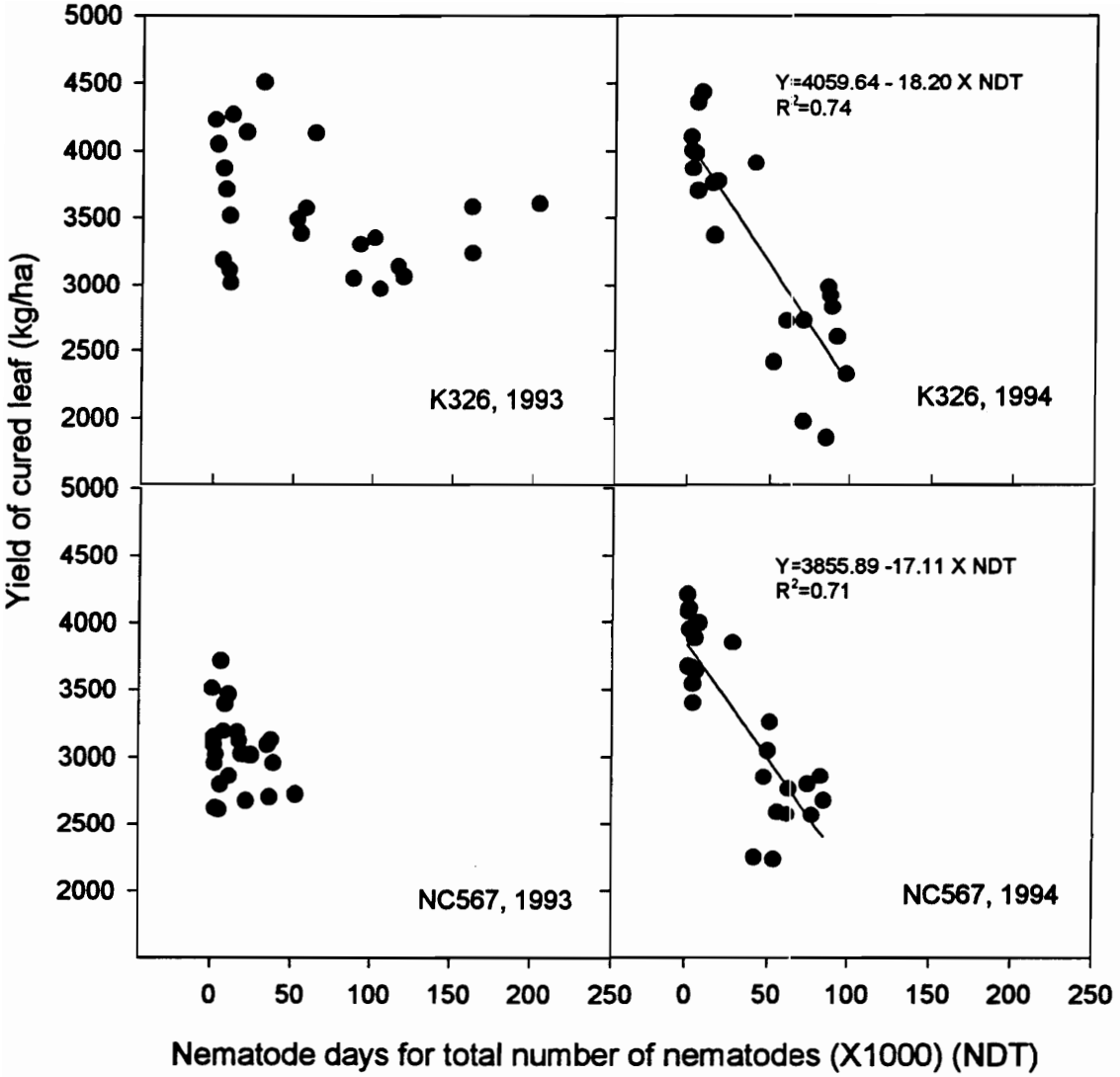
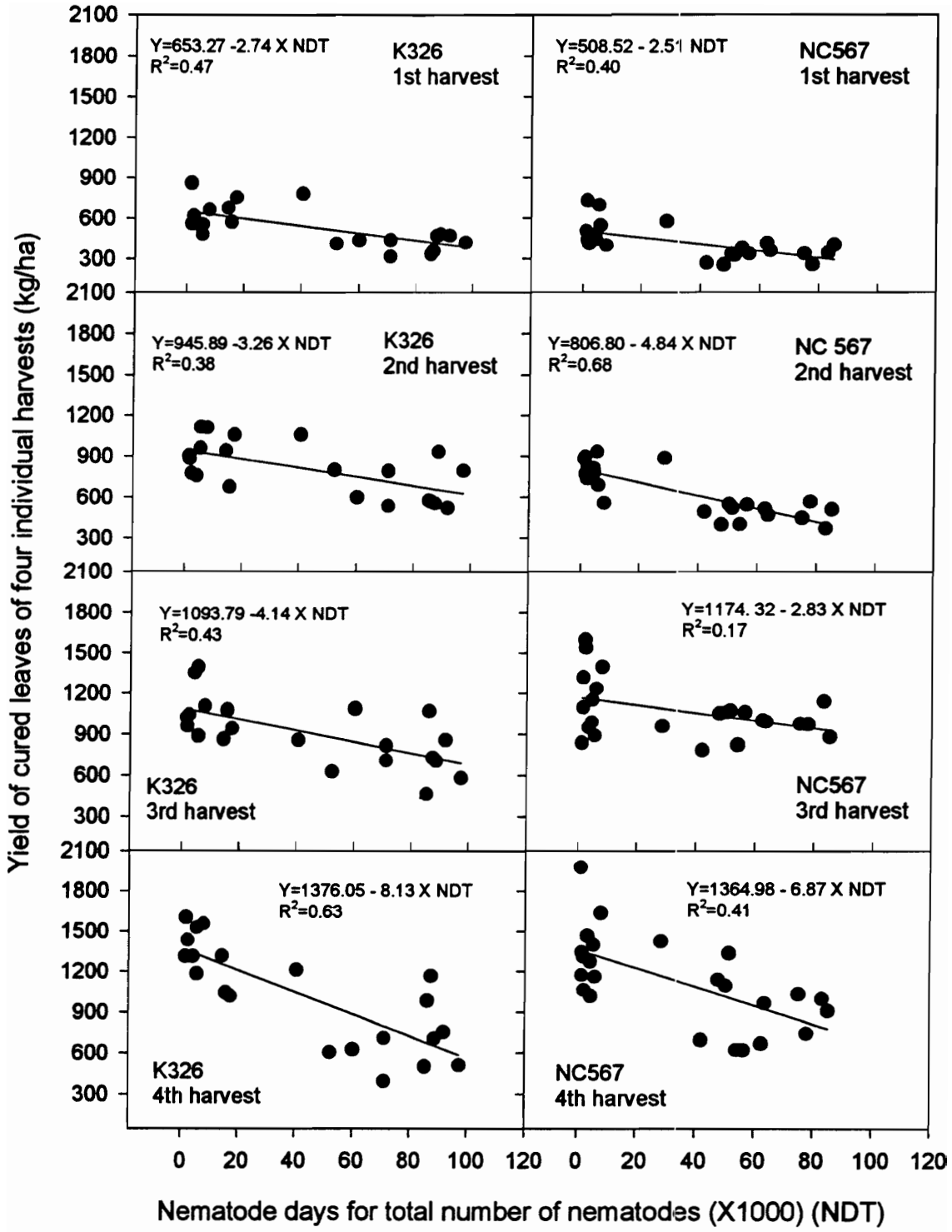


Fig. 5.3. Relationships between yield of cured leaf (kg/ha) of cultivar K 326, NC 567 and nematode days for total number of nematodes of *Globodera tabacum solanacearum* per gram of feeder roots (NDT) in the first 11 weeks after transplanting in 1993 and 1994.

Fig. 5.4. Relationships between yield of cured leaf from individual harvests of cultivars K 326, NC 567 and nematode days for total number of nematodes of *Globodera tabacum solanacearum* per gram of feeder roots (NDT) in the first 11 weeks after transplanting in 1994.


Y=653.27 -2.74 X NDT
R²=0.47
K326
1st harvest
Y=508.52 - 2.51 NDT
R²=0.40
NC567
1st harvest
Y=945.89 -3.26 X NDT
R²=0.38
K326
2nd harvest
Y=806.80 - 4.84 X NDT
R²=0.68
NC 567
2nd harvest
Y=1093.79 -4.14 X NDT
R²=0.43
K326
3rd harvest
Y=1174. 32 - 2.83 X NDT
R²=0.17
NC567
3rd harvest
Y=1376.05 - 8.13 X NDT
R²=0.63
K326
4th harvest
Y=1364.98 - 6.87 X NDT
R²=0.41
NC567
4th harvest
Yield of cured leaves of four individual harvests (kg/ha)
Nematode days for total number of nematodes (X1000) (NDT)

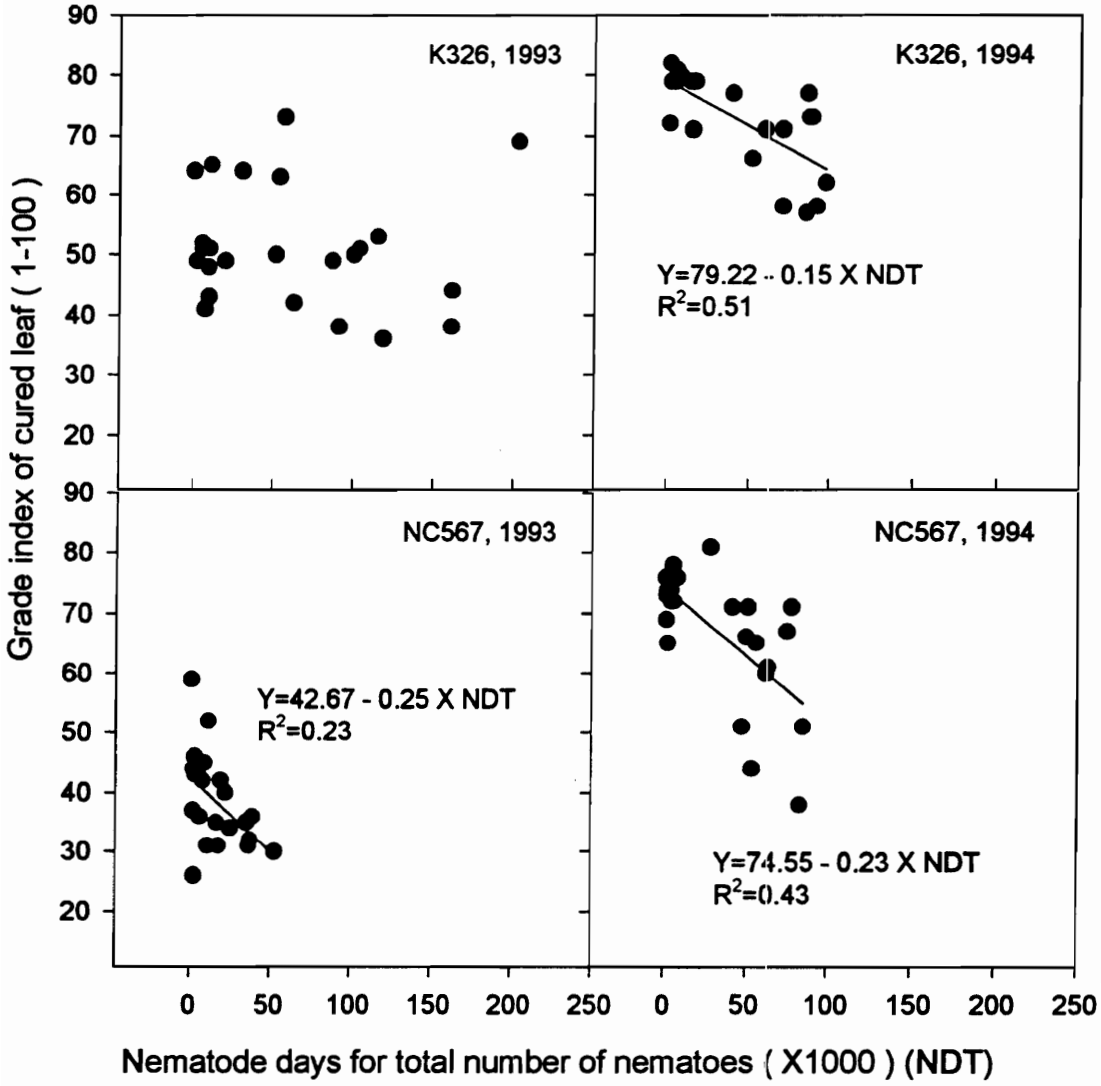
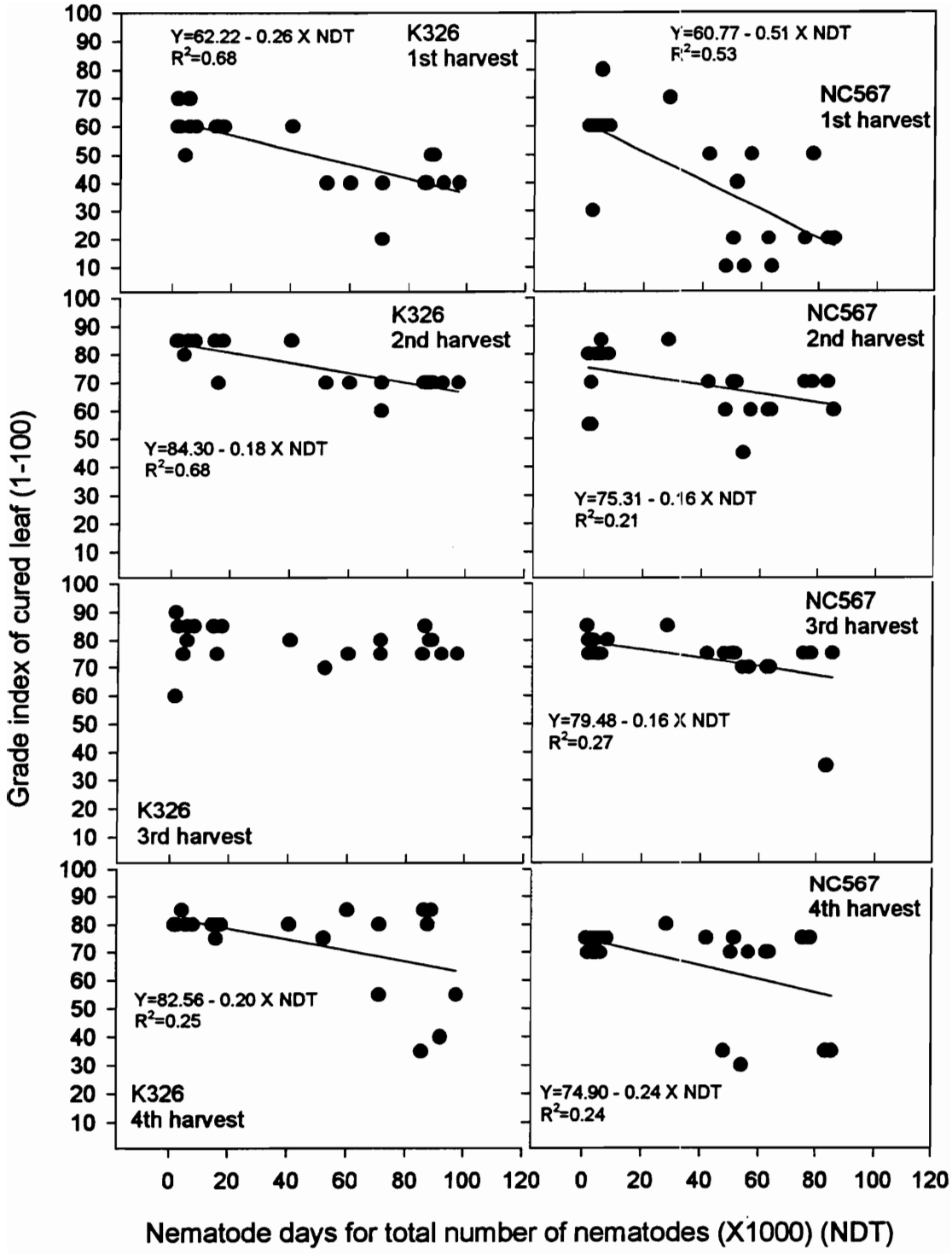


Fig. 5.5. Relationships between average grade index of cured leaf of cultivar K 326, NC 567 and nematode days for number of total nematodes of *Globodera tabacum solanacearum* per gram of feeder roots (NDT) in the first 11 weeks after transplanting in 1993 and 1994.

Fig. 5.6. Relationships between grade index of cured leaf from individual harvests of cultivars K 326, NC 567 and nematode days for total number of nematodes *Globodera tabacum solanacearum* per gram of feeder roots (NDT) in the first 11 weeks after transplanting in 1994.


Y=62.22 - 0.26 X NDT
R2=0.68
K326
1st harvest
Y=60.77 - 0.51 X NDT
R2=0.53
NC567
1st harvest
K326
2nd harvest
Y=84.30 - 0.18 X NDT
R2=0.68
NC567
2nd harvest
Y=75.31 - 0.16 X NDT
R2=0.21
NC567
3rd harvest
Y=79.48 - 0.16 X NDT
R2=0.27
K326
3rd harvest
NC567
4th harvest
Y=82.56 - 0.20 X NDT
R2=0.25
K326
4th harvest
Y=74.90 - 0.24 X NDT
R2=0.24
Grade index of cured leaf (1-100)
Nematode days for total number of nematodes (X1000) (NDT)

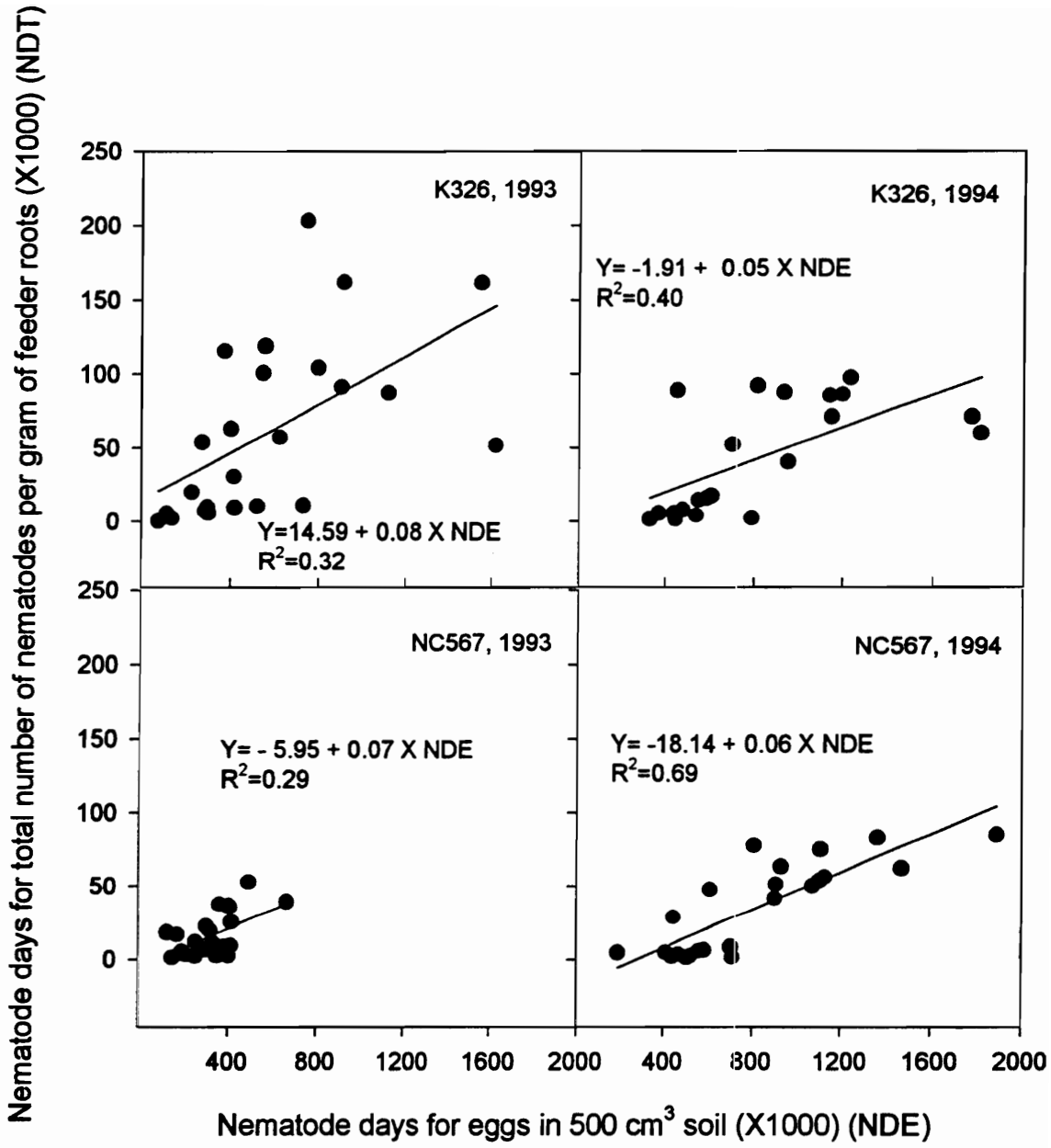


Fig. 5.7. Relationships between nematode days for total number of nematodes of *Globodera tabacum solanacearum* per gram of feeder roots (NDT) of cultivar K 326, NC 567 and nematode days of eggs in 500 cm^3 soil (NDE) in the first 11 weeks after transplanting in 1993 and 1994.

LITERATURE CITED

Bowman, D. T., A. G. Tart, E. A. Wernsman, and T. C. Corbin. 1988 Revised North Carolina grade index for flue-cured tobacco. Tobacco Science 32:39-40.

Brown, E. B. 1969. Assessment of damage caused to potato by potato cyst eelworm, *Heterodera rostochiensis* Woll. Annals of Applied Biology 63:493-502.

Byrd, D. W., T. Kirkpatrick, and K. R. Barker. 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. Journal of Nematology 15:142-143.

Fox, J. A., and L. J. Spasoff. 1976. Resistance and tolerance of tobacco to *Heterodera solanacearum*. Journal of Nematology 8:284-285 (Abstr.)

Johnson, C. S. 1990. Control of *Globodera tabacum solanacearum* by rotating susceptible and resistant flue-cured tobacco cultivars. Supplement to Journal of Nematology 22:700-706.

Johnson, C. S. 1995. Use of fosthiazate to control tobacco cyst nematodes in flue-cured tobacco. Tobacco Science 69-76.

Johnson, C. S., D. A. Komm, and J. L. Jones. 1989. Control of *Globodera tabacum solanacearum* by alternating host resistance and nematicide. Journal of Nematology 21:16-23.

Jones, J. L., C. S. Johnson, P. J. Semtner, T. D. Reed, B. B. Ross, and C. A. Wilkinson. 1993. 1993 flue-cured tobacco production guide. Virginia Cooperative Extension. Publication 436-420. Pp.84.

Komm, D. A., J. J. Reilly, and A. P. Elliott. 1983. Epidemiology of a tobacco cyst nematode (*Globodera solanacearum*) in Virginia. Plant Disease 67:1249-1251.

Melton, T. A., J. A. Philips, J. L. Imbriani, and K. R. Barker. 1991. First report of *Globodera tabacum solanacearum* on flue-cured tobacco outside Virginia. Plant Disease 75: 1074.

LaMondia, J. A. 1995. Shade tobacco yield loss and *Globodera tabacum tabacum* population change in relation to initial nematode density.

Journal of Nematology 27:114-119.

Miller, L. I., and B. J. Gray. 1972. *Heterodera solanacearum* n. sp., a parasite of solanaceous plants. Nematologica 18:404-413.

Noe, J. P., J. A. Sasser, and J. L. Imbriani 1991. Maximizing the potential of cropping systems for nematode management. Journal of Nematology 23:353-361.

Noling, J. W., and H. Ferris. 1987. Nematode-degree days, a density-time model for relating epidemiology and crop losses in perennials. Journal of Nematology 19:108-118.

Seinhorst, J. W. 1965. The relation between population density and damage to plants. Nematologica 11:137-154.

Wang, J. 1996. Effects of *Globodera tabacum solanacerum* on Growth of flue-cured tobacco (*Nicotiana tabacum*). Pp 62-94 in Ph.D dissertation. Characterizing Resistance in Flue-cured Tobacco to *Globodera tabacum solanacearum*. Virginia Polytechnic Institute and State University.

Wang, J., and C. S. Johnson. 1995. Using various damage functions to associate tobacco cyst nematode population with yield of flue-cured tobacco. Journal of nematology 27:524.

VITA

Jie Wang was born to Xueru Wang and Shuqin Liu on June 23, 1964 in Weiyuan, Sichuan, China. He attended public school and graduated from Guanzhuan high school in Daxian, Sichuan in 1981. He enrolled in Southwestern Agricultural University in 1981 and received a B.S. with a major in Plant Protection in 1985.

In August of 1985, he was employed by Ministry of Agriculture of China as a project officer. In January of 1987, he transferred to Cotton Research Institute, Chinese Academy of Agricultural Sciences. He was a research assistant in Plant Pathology. He held a position of research pathologist before he left the institute for his graduate education.

From January of 1990 to January of 1991, he was a visiting scientist in Department of Plant Pathology, Physiology and Weed Science at Virginia Polytechnic Institute and State University. In January of 1993, he returned to the University for his graduate education.

Jie Wang is married to Jie Fang. They have two children. Mrs. Fang received her B.S. in Chemistry in May 1989.