Effects of Host Resistance and Temperature on Development of *Globodera tabacum solanacearum*[1]

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Abstract: Penetration and development of juveniles of tobacco cyst nematode (*Globodera tabacum solanacearum*) on a resistant (NC567) and a susceptible (K326) cultivar of flue-cured tobacco (*Nicotiana tabacum* L.) were determined in root zone chamber experiments. More vermiform juveniles developed into a swollen shape at 22, 27, and 31 °C than at 17 °C. Development of flask-shaped nematodes appeared to be similar across tested temperatures (17, 22, 27, and 31 °C). General patterns of penetration and development of juveniles in both resistant and susceptible cultivars were similar under all temperatures tested. More vermiform, swollen, and flask-shaped nematodes were found in roots of K326 than in those of NC567. Development from swollen to flask-shaped nematodes appeared to be similar between the two cultivars, although more vermiform juveniles developed into swollen nematodes on K326 than on NC567. Differences in resistance between the two cultivars remained stable across tested temperatures.

Key words: development, flue-cured tobacco, *Globodera tabacum solanacearum, Nicotiana tabacum,* parasitism, resistance, screening, temperature, tobacco cyst nematode.

The tobacco cyst nematode, *Globodera tabacum solanacearum* (Miller and Gray) Behrens, 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 flue-cured tobacco hectarage in Virginia is infested with *G. t. solanacearum* (Johnson, unpubl.). The nematode causes estimated average yield reductions of 15%, and complete crop failures have been recorded (Komm et al., 1983). The nematode is also found in several counties in North Carolina (Melton et al., 1991; Melton, pers. comm.). 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 the nematode, and limited choices of rotation crops suggest that host resistance could play a more important role in successful management of *G. t. solanacearum.* Various levels of resistance to *G. t. solanacearum* exist in wild *Nicotiana* species, but widely used commercial cultivars lack resistance (Hayes et al., 1997). Cultivars with resistance to *G. t. solanacearum* suffer significant yield suppression in the presence of high population densities of the nematode (Johnson, 1990; Johnson et al., 1989; Komm et al., 1983). Consequently, fumigants are widely used to control the nematode in Virginia.

Plant resistance to nematodes may be expressed in several different ways. Failure of juveniles to penetrate roots and retardation or failure of juveniles to develop have been noted as a consequence of host resistance to *Meloidogyne* species (Griffin and Waite, 1972; Huang, 1986; Jatala and Russell, 1972). Resistance to potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*) can result in failure of juveniles to remain in roots, death, slowing of development, and shift of adult sex ratios toward males (Rice et al., 1985). Resistance to soybean cyst nematode, *Heterodera glycines,* 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 a loss of nematode hatching factor activity, tobacco cyst nematodes hatch at similar rates on resistant and susceptible tobacco cultivars (LaMondia, 1988; Wang et al., 1997). Resistance to *G. t. solanacearum* in some wild *Nicotiana* species and tobacco hybrids appears to be related to juvenile development rather than reduced penetration (Baalawy and Fox, 1971). *Globodera t. tabacum* was reported to penetrate both susceptible and resistant shade tobacco in the field, with more juveniles developing in the susceptible host (LaMondia, 1996). Increased knowledge about penetration and early development of *G. t. solanacearum* in flue-cured tobacco could help identify more appropriate screening criteria for resistance breeding programs.

Temperature is considered to be an important environmental factor affecting nematode development (Alston and Schmitt, 1988; Ferris, 1957; Ferris et al., 1978; Melton et al., 1986). 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 root-knot nematode resistance at high temperature has been documented in various crops (Ammati et al., 1986; Griffin, 1969; Omwega et al., 1990). Stability of resistance at various temperatures is a desirable characteristic. Our objectives were to investigate the effects of host resistance and temperature on penetration and development of *G. t. solanacearum* in flue-cured tobacco.

MATERIALS AND METHODS

Seedling preparation: Seeds of flue-cured tobacco cultivars K326 (susceptible to *G. t. solanacearum*) and

NC567 (resistant) were sown on the surface of vermiculite in $22 \times 16 \times 7\text{-cm}^3$ plastic containers. Care was taken to maintain suitable moisture and nutrients for germination and seedling growth by watering through holes punched in the bottom. Seedlings with one true leaf, displaying vigorous growth, were transferred to 11-cm-diam. clay pots containing 300 cm^3 steam-sterilized loamy sand:fine quartz sand (1:1). Pots with transferred seedlings were placed in a constant soil temperature apparatus (Root Zone Cabinet Model R-1, Environmental Growth Chambers, Chagrin, OH) for 1 week at 24 °C to allow seedlings to become established before inoculation.

Inoculum preparation and inoculation: Cysts used in the experiments originated from a single field population and were propagated on flue-cured tobacco cultivar Coker 319 in a greenhouse. Soil, containing cysts, was thoroughly mixed with water in a plastic bucket. The supernatant was poured through a 850-µm-pore sieve nested over a 250-µm-pore sieve after the mixture had settled for 15 seconds. Cysts captured on the 250-µ-pore sieve were rinsed into vials with tap water and stored in a refrigerator at 4 °C for 2 to 4 months. Inoculum was prepared from cysts crushed in a kitchen blender (10-speed Osterizer) on ''frappe'' for 1 minute. Contents of the blender were rinsed through a 150-µm-pore sieve nested over a 25-µm-pore sieve. Eggs caught on the 25-µm-pore sieve were transferred to a beaker and standardized to a concentration of 1,750 eggs/ml by adding tap water.

Plants were inoculated by infesting the soil in which each plant was growing. Inoculum was applied with a 10-ml syringe inserted 5 cm below the surface of the soil around each seedling. Twenty ml of egg suspension (35,000 eggs/pot) was applied to each seedling. The holes made by the syringe were immediately covered with soil.

Experimental design and sampling: The experiments were performed twice. Cultivars were arranged in randomized complete blocks with four replications within each root zone chamber. Soil temperatures were randomly assigned to each of four root zone chambers. Immediately after infestation, chambers were programmed for day-night temperatures of 31-29, 27-25, 22-20, and 17-15 °C in 12-hour cycles. Beginning 1 week after infestation, and continuing for 5 weeks, one randomly chosen plant of each cultivar was removed from each replication at each temperature for a total of 32 plants per sample date (2 cultivars × 4 temperatures × 4 replications). Each plant was carefully washed free of soil. The fresh weights of entire plants, total roots, and lateral and feeder roots (excluding tap roots) were recorded after plants were blotted dry. Lateral and feeder roots were cut into segments, mixed, and a 1-g sample was randomly chosen for staining. When total lateral and feeder root weight was less than 1 g, all lateral and feeder roots were stained and counts converted to number per gram root.

The nematodes within root samples 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 40× magnification as follows: (a) vermiform; (b) swollen; (c) flask-shaped; (d) saccate females bearing eggs. Cumulative percentages of swollen or flask-shaped nematodes were calculated from 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 on penetrated vermiform juveniles to remove the potential influence of temperature on hatching and penetration. Effects of temperature on development were estimated based on cumulative percentages.

Ratios 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 that developed into swollen stage and $R_{F/S}$ is the proportion of swollen juveniles that developed into flask-shaped stage) were transformed by square root before statistical analysis.

Data analysis: Saccate females bearing eggs were excluded from the analysis because the sampling technique used in the experiments may have resulted in a disproportionate loss of saccate females compared with juveniles. Data from the two experiments conducted were combined and analyzed as a split-plot design, with temperature as the main plot factor and cultivar as the sub-plot factor. Analyses of variance (SAS Institute, Cary, NC) were conducted on total nematodes, cumulative percentages of swollen or flask-shaped nematodes, and the ratios of development in lateral and feeder roots. Means were separated with Duncan's multiple range test at ($P \leq 0.05$).

Results and Discussion

Soil temperature influenced nematode infection. Temperature effects were greatest 14 and 21 days after infestation, when more vermiform juveniles were found at constant temperatures of 22 and 27 °C than at 17 or 31 °C ($P \leq 0.05$) (Fig. 1). Very few vermiform juveniles were detected in roots 7 days after infestation at any temperature. The number of vermiform juveniles in roots peaked 14 days after infestation at 22 and 27 °C. At 31 °C, the number of vermiform juveniles increased during the first 21 days after infestation and then decreased. Very few juveniles were detected at 17 °C at any sample date. Differences in the cumulative percentage
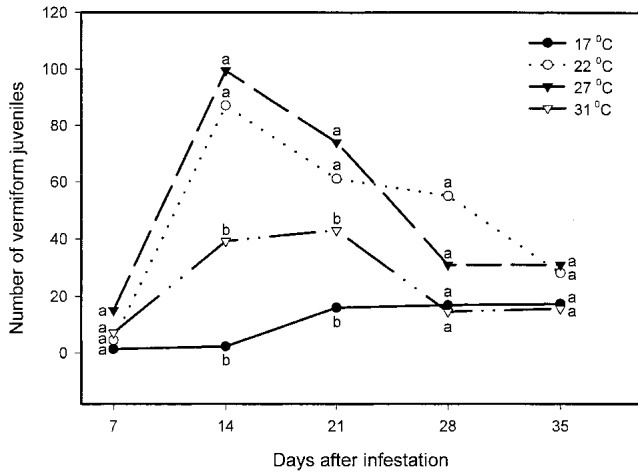
Fig. 1. Influence of soil temperature on numbers of *Globodera tabacum solanacearum* juveniles in 1 g of lateral and feeder roots of flue-cured tobacco.

of swollen nematodes were not statistically significant until 35 days after infestation, when more swollen nematodes had developed at 22, 27, and 31 °C than at 17 °C ($P \leq 0.05$) (Fig. 2A). Development of swollen
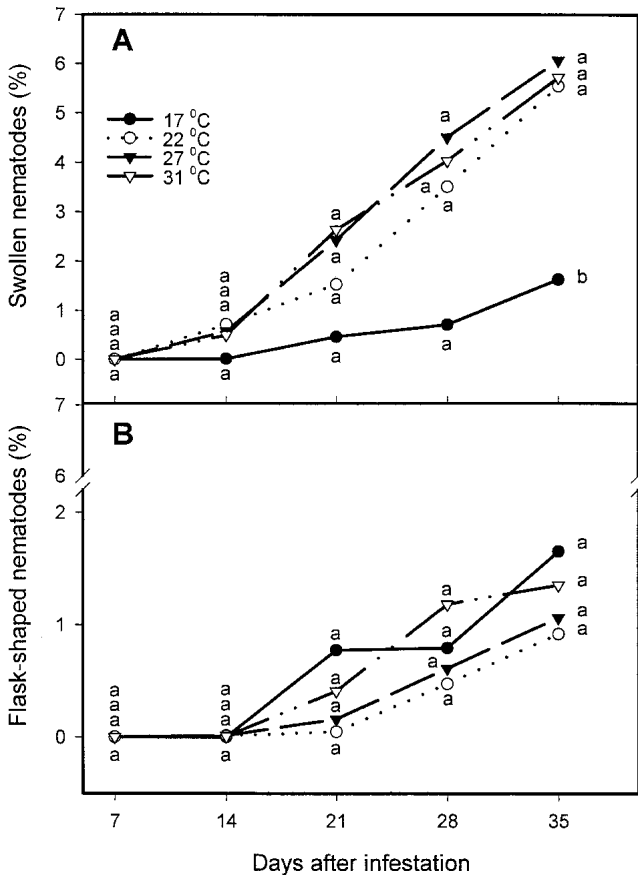

Fig. 2. Influence of temperature on cumulative percentage of swollen (a) and flask-shaped (b) juveniles of *Globodera tabacum solanacearum* in 1 g of lateral and feeder roots of flue-cured tobacco. Data points are means of 16 observations. Data points within a figure section associated with the same letter were not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

nematodes was similar at 22, 27, and 31 °C at all sample dates. Temperature had no effect on the development of flask-shaped nematodes from vermiform juveniles ($P \leq 0.05$) (Fig. 2B).

Adams et al. (1982) noted a delayed appearance of white females of *G. t. solanacearum* on a susceptible cultivar at 15 and 21 °C, and lower fecundity at 15 and 32 °C. Our results suggest that this temperature-mediated delay may have resulted from influences on the infection stage in the life cycle of the nematode, as we observed temperature effects on infection but not on development. Wang et al. (1997) found little or no hatching by *G. t. solanacearum* at 15 °C compared to 20 and 25 °C. Our results in this study also showed fewer vermiform juveniles in host roots at 17 °C. Trends in the cumulative percentage of swollen vs. vermiform nematodes in the roots suggested that feeding site establishment was also inhibited when temperatures were below 20 °C (Fig. 2A). The lack of differences in the cumulative percentage of flask-shaped vs. vermiform nematodes among the temperatures tested indicated few if any temperature effects on development of *G. t. solanacearum* once a juvenile successfully established a feeding site (Fig. 2B).

Penetration by *G. t. solanacearum* followed a similar pattern in roots of resistant cultivar NC567 to that in susceptible cultivar K326 (Fig. 3A). Little penetration occurred during the first 7 days after infestation of the pots but peaked at 14 days after infestation. More vermiform juveniles were found in roots of K326 compared to NC567 at 14, 21, and 28 days after infestation ($P \leq 0.05$). More swollen nematodes developed on K326 than on NC567 at 21, 28, and 35 days after infestation ($P \leq 0.05$) (Fig. 3B). The ratios for juvenile development from the vermiform to the swollen stage ($R_{S/V}$) also indicated slower development on NC567 compared to K326, particularly 21 and 28 days after infestation (Fig. 4A). More flask-shaped nematodes had developed on susceptible K326 than on NC567 at 28 and 35 days after infestation ($P \leq 0.05$) (Fig. 3C). However, differences between the resistant and susceptible cultivars in the ratios for development from swollen to flask-shaped nematodes ($R_{F/S}$) were inconsistent (Fig. 4B).

Stage-related resistance also has been reported in soybean to the soybean cyst nematode (*H. glycines*) (Endo, 1965; Halbrendt et al., 1992). Previous tobacco cyst nematode studies have shown no differences in hatching and penetration between tobacco genotypes resistant or susceptible to *Globodera* spp. (Baalawy and Fox, 1971; LaMondia, 1988; Wang et al., 1997). The reduced number of vermiform juveniles in roots of resistant cultivar NC567 compared to susceptible cultivar K326 may have resulted from juveniles leaving resistant roots after unsuccessful attempts to establish a feeding site (Fig. 3A) (LaMondia, 1988; Schneider, 1991). The inconsistent differences in $R_{F/S}$ between the resistant
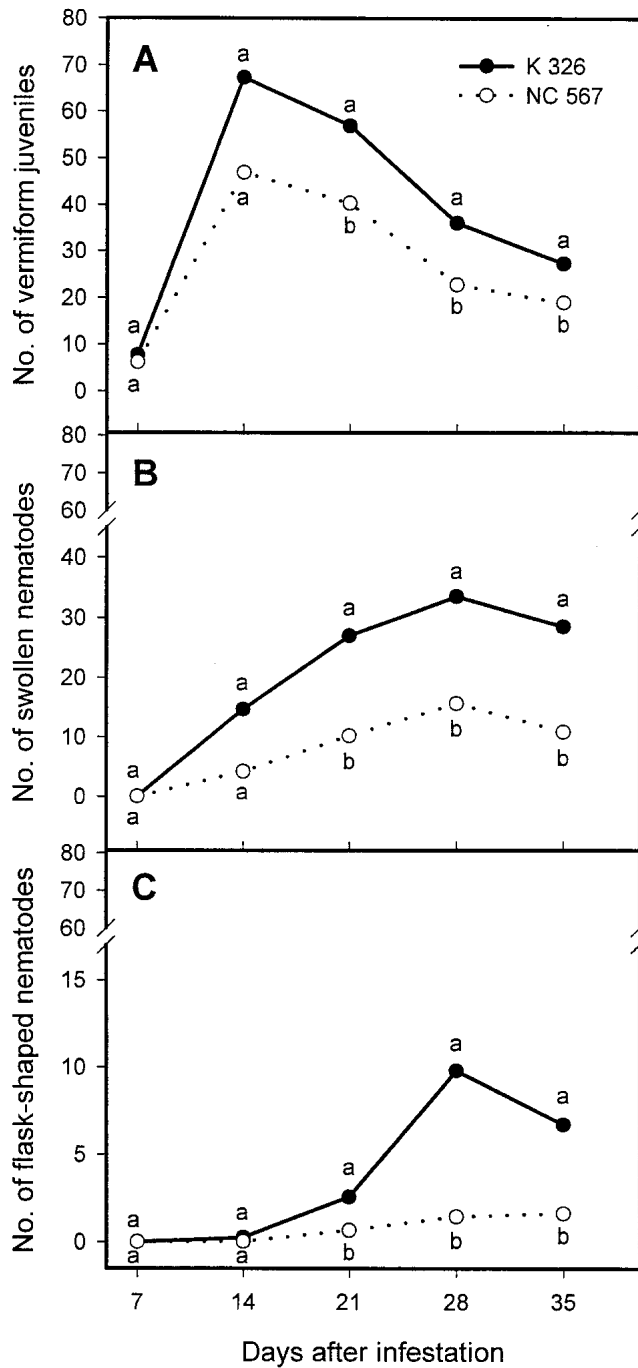
FIG. 3. Development of vermiform (a), swollen (b), and flask-shaped (c) *Globodera tabacum solanacearum* juveniles in 1 g of lateral and feeder roots of flue-cured tobacco-susceptible cultivar K326 and resistant NC567. Data points are means of 32 observations. Data points within a sample date and figure section associated with the same letter were not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).
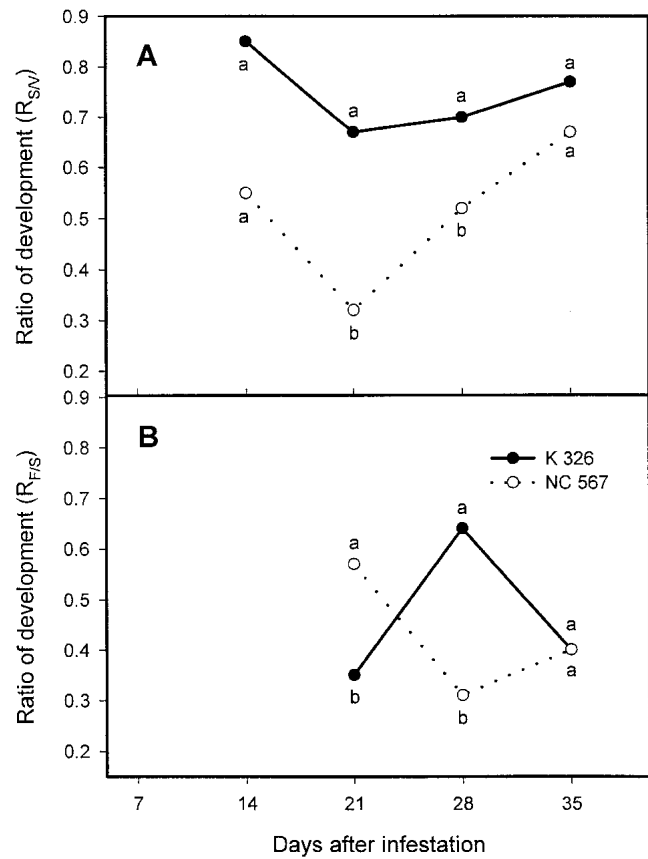

FIG. 4. Ratios of vermiform *Globodera tabacum solanacearum* developing into swollen (S/V) (a) and swollen developing into flask-shaped (F/S) (b) in 1 g of lateral and feeder roots of flue-cured tobacco-susceptible cultivar K326 and resistant NC567. Data points are means of 32 observations. Data points within a sample date and figure section associated with the same letter were not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

and susceptible cultivars in this study, when compared to the consistently lower $R_{S/V}$ observed in NC567 than in K326, suggest that the resistance in NC567 does not inhibit nematode development once juveniles have established feeding sites. No attempt to differentiate male and female nematodes was made, so the development observed on NC567 may have been of either sex. How-

ever, the reduction in the number of flask-shaped nematodes for the susceptible cultivar at 35 days after infestation could have been partially due to increased development of flask-shaped juveniles into mature male and female nematodes that may have been lost in sample processing or from male migration from roots. Therefore, nematode development after feeding could have been delayed on the resistant cultivar without the trend being observed in our data.

Smaller females with reduced fecundity have been associated with resistance to *H. glycines* in some soybean cultivars (Melton et al., 1986; Noel et al., 1983). However, very few egg-bearing females were observed at the end of our study, preventing estimation of the possible influence of resistance to *G. t. solanacearum* on nematode fecundity. Contrary to other reports, we also observed few, if any, males in roots of either cultivar (Baalawy and Fox, 1971; LaMondia, 1996). It is possible that some unknown proportion of the flask-shaped nematodes we counted were males. However, our focus was on the impact of resistance within a single generation of the parasite. Differentiation of male from female adults might have enabled us to identify an addi-

tional mechanism of resistance and gain information on the impact of resistance over subsequent generations of *G. t. solanacearum.*

High temperatures have been associated with partial loss of resistance to *Meloidogyne* species (Ammati et al., 1986; Griffin, 1969; Omwega et al., 1990) but not to *H. glycines* (Melton et al., 1986). Our results did not contain consistent interactions between temperature and resistance to *G. t. solanacearum.* Gradual breakdown in root-knot resistance at high temperatures has been attributed, at least in part, to differences in the optimal temperature range for the parasite, vs. the host, that favor the nematode (Canto-Saenz, 1985; Cook and Evans, 1987). Temperatures between 25 and 30 °C may be optimum for root-knot nematode activity but suboptimal for host growth. Inhibition of protein activity thought to be important in gene-for-gene interactions may also decrease resistance at higher temperatures (Canto-Saenz, 1985; Cook and Evans, 1987). However, our results, along with those of others, suggest that the optimal temperature range is similar for both *G. t. solanacearum* and tobacco (Adams et al., 1982; Collins and Hawks, 1993; Tso, 1990). Consequently, the biochemical processes responsible for inhibiting nematode development in *G. t. solanacearum* -resistant cultivars should remain effective over the range of relevant temperatures. These soil temperatures also represent those typical for soils in the flue-cured tobacco-producing areas of Virginia and North Carolina over the course of a growing season. Flue-cured tobacco cultivars with the resistance to *G. t. solanacearum* found in NC567 should be expected to restrict nematode development throughout the growing season, regardless of normal variation in temperature.

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