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Effects of Tobacco Cyst Nematode on Growth of Flue-cured Tobacco[1]

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Abstract: The effects of infection by tobacco cyst nematode (*Globodera tabacum solanacearum*) on growth of flue-cured tobacco cultivars NC 567 (resistant) and K 326 (susceptible) were evaluated in the field in 1993 and 1994. Infection by *G. t. solanacearum* suppressed number of leaves, plant height, and fresh weight of leaves and feeder roots. Correlations between weekly egg densities of *G. t. solanacearum* collected from soil and host growth during 11 weeks after transplanting (WAT) were often inconsistent between cultivars and years. However, consistent correlations were obtained between root weight and egg densities collected 9 WAT, as well as between leaf weight from susceptible K 326 and nematode egg densities 6 WAT. Leaf and feeder root weights were significantly correlated with the area under the curve for all nematodes per gram of feeder root for K 326 in 1993 and for both cultivars in 1994. Reduction in feeder root weight by *G. t. solanacearum* was similar for the resistant and susceptible cultivars. Reduction in fresh leaf weight by *G. t. solanacearum* was twice as great ($P \leq 0.07$) for K 326 as for NC 567 in 1994. Incorporating nematode resistance into germplasm possessing improved yield and quality traits should produce cultivars more acceptable to growers.

Key words: cyst nematode, damage function, flue-cured tobacco, fosthiazate, *Globodera tabacum solanacearum,* multiple-point model, nematode, *Nicotiana tabacum,* plant disease loss, plant growth, resistance, tolerance.

One fourth of the total flue-cured tobacco acreage in Virginia is infested with the tobacco cyst nematode *Globodera tabacum solanacearum* (Miller and Gray) Behrens, 1975 (Miller and Gray, 1972). This nematode has also been found in Maryland and is spreading in the flue-cured tobacco-producing area of North Carolina (Johnson, 1998). *Globodera tabacum tabacum,* a closely related nematode, can reduce tobacco yield by more than 40% (LaMondia, 1995). Average yield reductions caused by *G. t. solanacearum* have been estimated at 15%, and complete crop failures also have been recorded (Komm et al., 1983).

Understanding changes in crop growth resulting from infection by cyst nematodes may provide 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). Yield losses caused by *Heterodera avenae* have been related to early-season damage to wheat roots and subsequent effects on shoot growth (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). Resistance to *G. t. solanacearum* has been associated with host intolerance to the nematode (Komm et al., 1983). Our objectives were to investigate the influence of infection by *G. t. solanacearum* on growth of resistant (NC 567) and susceptible (K 326) cultivars 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

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been in a 2-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, and 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 as necessary to minimize leaf scalding and to maintain plant stand.

Treatments and experimental design: Four treatments were imposed in a complete factorial design with 12 replications. Flue-cured tobacco cultivars included in the experiments were NC 567 (resistant to *G. t. solanacearum*) 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 (Johnson, 1995). Rates of the nematicide were 0 and 6.84 liters/ha in 1993 and 0 and 6.78 liters/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 1 week after nematicide application.

Measurement of plants: One plant was randomly sampled from each plot every week for the first 11 weeks after transplanting (WAT). Plants were washed free of soil and blotted dry before plant height, number of leaves, fresh weight of leaves, and fresh weight of feeder roots were measured.

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 of roots for each plant. If the weight of feeder roots was less than 1 gram, all feeder roots were used for counting nematodes. Roots were stained with acid fuchsin (Byrd et al., 1983). Nematodes in stained roots were counted under low magnification and assigned to one of four classes based on overall shape: (i) vermiform (those juveniles that had successfully penetrated roots without obvious feeding), (ii) swollen (nematodes with a distinct sausage shape), (iii) pyriform (flask-shaped nematodes), and (iv) adult (saccate nematodes bearing eggs).

Determination of soil population: Soil samples (1,000 cm^3) were collected from around the roots of one randomly selected plant per plot every week for the first 11 WAT. Soil samples were air-dried, and *G. t. solanacearum* cysts were extracted from a 250-cm^3 subsample with a modified Fenwick can (Caswell et al., 1985). After crushing cysts in a blender on ''frappe'' for 1 minute, two aliquots of egg suspension were counted to determine the number of eggs. Egg counts were expressed per 500 cm^3 of soil.

Infection pressure by *G. t. solanacearum* was quantitatively described by the area under the curve for each of several parameters describing nematode population dynamics over the first 11 WAT. Populations of *G. t. solanacearum* eggs within cysts in soil were described by the area under the curve for nematode egg densities (AUCNED). Nematode parasitism was described by the area under the curve for vermiform and swollen nematodes (AUCVSN) and by AUCAN, the area under the curve for all nematodes (vermiform, swollen, pyriform, and adult), in each gram of feeder root. The three methods of quantification used a similar mathematical formula, as follows:

$$\text{AUCNED, AUCVSN, or AUCAN} = \Sigma[\text{NUMNEMAS}_{i+1} + \text{NUMNEMAS}_i)/2](X_{i+1} - X_i) \quad (1)$$

where NUMNEMAS_i = number of eggs in 500 cm^3 soil for AUCNED, number of vermiform and swollen nematodes per gram of feeder root for AUCVSN, and number of

nematodes at all life stages per gram of feeder root for AUCAN at the i'th sampling date. X_i = the julian date of the i'th sampling date.

Statistical analysis: Differences among experimental treatments in plant height, fresh weight of leaves, number of leaves, fresh weight of feeder roots, 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 for each sample date. Host responses to infection by *G. t. solanacearum* also were evaluated by correlation and regression analysis of relationships between host growth 11 WAT and nematode population densities and infection during the first 11 weeks of the growing season.

Results

Initial population densities of *G. t. solanacearum* eggs in 500 cm^3 of soil ranged from 1,277 to 20,292 in 1993 and from 16,918 to 70,862 in 1994. Initial nematode population densities varied significantly ($P \leq 0.01$) among replications but were similar across experimental treatments, with coefficients of variability of 42% and 31% in 1993 and 1994, respectively.

Although host resistance and use of fosthiazate generally reduced nematode parasitism and sometimes increased host growth, these effects were not statistically significant at every sample date. Fewer nematodes were found per gram of root ($P \leq 0.05$) in the resistant cultivar NC 567 than in susceptible cultivar K 326 from 2 to 4 and from 6 to 11 weeks after transplanting (WAT) in 1993 and at 4, 5, and 7 to 11 WAT in 1994 (Fig. 1). Fosthiazate did not retard nematode parasitism for NC 567 at 5, 10, and 11 WAT in 1993, but suppressed nematode infection on both cultivars in 1994 ($P \leq 0.05$). Number of nematodes per gram of feeder root decreased with time in all treatments in both years. No differences ($P \leq 0.05$) were observed in feeder root weight among cultivars in either year of the study (Fig. 2). Application of fosthiazate increased ($P \leq 0.05$) feeder root weight from 3 to 8 WAT in 1993

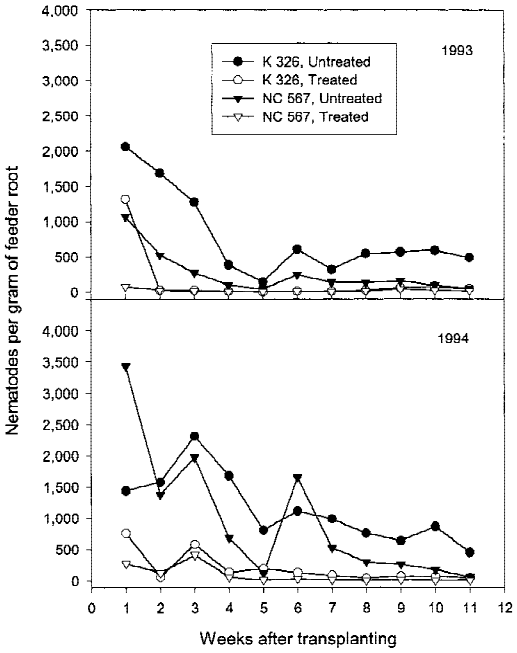

Fig. 1. Numbers of *Globodera tabacum solanacearum* on resistant (NC 567) and susceptible (K 326) cultivars of flue-cured tobacco in untreated soil or soil treated before planting with fosthiazate.

and at 5 WAT and from 7 to 11 WAT in 1994. Fresh leaf weight for NC 567 exceeded ($P \leq 0.05$) that of K 326 at 1 and 4 WAT in 1993 and at 2 and 6 WAT in 1994 (Fig. 2). Significant increases ($P \leq 0.05$) in leaf weight were observed from use of fosthiazate at all but the first sample date in 1993 and after the first 3 WAT in 1994.

In 1993 and 1994, feeder root weight of K 326 at 11 WAT correlated significantly ($P \leq 0.05$) with egg densities of *G. t. solanacearum* from soil samples collected 5 and 8 WAT (r = –0.478 and –0.463, respectively). Feeder root weight of NC 567 at 11 WAT also correlated ($P \leq 0.05$) with egg densities from soil samples collected 9 WAT (r = –0.435 and –0.486 in 1993 and 1994, respectively). Plant height and number of leaves of K 326 did not correlate with weekly egg densities in either year. Height of NC 567 correlated ($P \leq 0.05$) with nematode egg densities 11 WAT in 1993 (r = –0.430) and from 3 to 6 WAT (r = –0.683, –0.589, –0.711, and –0.430, respectively) and 9 WAT (r = –0.720) in 1994. Number of NC 567 leaves corre-

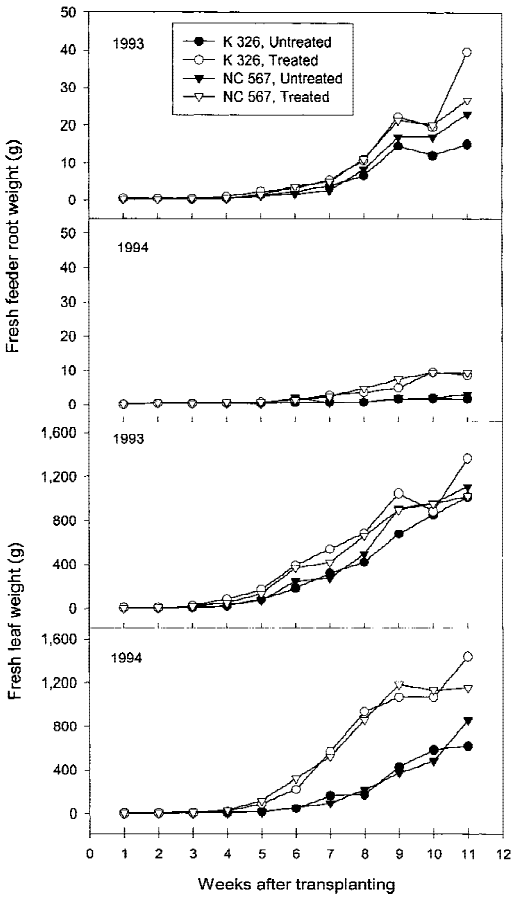

FIG. 2. Accumulation of fresh leaf weight and fresh root weight by resistant (NC 567) and susceptible (K 326) cultivars of flue-cured tobacco in soil infested by *Globodera tabacum solanacearum* and either untreated or treated before planting with fosthiazate.

lated ($P \leq 0.05$) with nematode egg densities at 7 and 11 WAT (r = –0.481 and –0.605, respectively) in 1993. Leaf weight of K 326 correlated ($P \leq 0.05$) with egg densities of *G. t. solanacearum* at 6 WAT (r = –0.440 and –0.472 in 1993 and 1994, respectively) and also with nematode egg densities 3 to 5 and 8 WAT (r = –0.541, –0.473, –0.596, and –0.626, respectively) in 1993. Leaf weight for NC 567 correlated ($P \leq 0.05$) with nematode egg densities for soil samples collected 11 WAT (r = –0.534) in 1993 and 3 to 5, 7, and 9 WAT (r = –0.438, –0.483, –0.561, –0.404, and –0.571, respectively) in 1994. Correlations between the ratio of leaf weight to feeder root weight 11 WAT and weekly nematode egg densities were never significant for K 326 but were significant ($P \leq 0.05$) for NC 567 in 1993, when nematode egg densities had been sampled 3 or 6 WAT (r = 0.571 or 0.568, respectively).

Leaf weight and feeder root weight of K 326 in 1993 and all plant-growth measurements of K 326 in 1994 correlated significantly with the area under the curves for all nematodes per gram of feeder root (AUCAN) and for vermiform and swollen nematodes per gram of feeder root (AUCVSN) (Table 1). The area under the curve for eggs in soil (AUCNED) did not correlate with leaf weight for K 326 in either year but was associated with root weight in 1994. For NC 567, only the ratio of leaf weight to feeder root weight correlated significantly with AUCAN and AUCVSN in 1993. All plant-growth measurements for NC 567, except number of leaves, correlated ($P \leq 0.05$) with AUCAN and AUCVSN in 1994. AUCNED correlated significantly with feeder root weight of NC 567 in 1993 and with plant height and leaf weight in 1994 ($P \leq 0.05$).

Plant-growth parameters correlated best with AUCAN (Table 1). It was, therefore, used in regression analysis to quantify the effects of infection by *G. t. solanacearum* on tobacco growth. AUCAN correlated consistently with leaf weight, feeder root weight, and the ratio of leaf weight to feeder root weight for both cultivars, so these three plant-growth measurements were selected for regression analysis. Regression analysis was performed only on 1994 data because significant correlations did not occur between AUCAN and most plant-growth parameters for NC 567 in 1993. In 1994, susceptible cultivar K 326 produced a higher leaf weight than resistant NC 567 in the absence of nematode infection. However, the rate of reduction in fresh leaf weight was somewhat greater ($P \leq 0.07$) for susceptible K 326 than for resistant NC 567 (Figs. 2,3). Rates of decline in fresh weight of feeder roots with increasing nematode pressure were not significantly different between the two cultivars (Figs. 2,3). Rates of increase in the ratios of leaf weight to feeder root

TABLE 1. Pearson correlation coefficients between characteristics of flue-cured tobacco growth and three measures of *Globodera tabacum solanacearum* population development over the first 11 weeks after transplanting in 1993 and 1994.

Year, cultivar	Area under nematode curve[a]	Plant height	Leaf number	Fresh leaf weight	Fresh root weight	Ratio of leaf weight to root weight
1993						
K 326	AUCAN	0.018	–0.230	–0.520**	–0.480**	0.341
	AUCVSN	0.050	0.007	–0.510*	–0.477**	0.348
	AUCNED	0.269	0.171	–0.360	–0.368	0.113
NC 567	AUCAN	0.313	0.192	0.024	–0.260	0.550**
	AUCVSN	0.317	0.196	0.021	–0.261	0.550**
	AUCNED	0.142	–0.146	–0.247	–0.490*	0.377
1994						
K 326	AUCAN	–0.689**	–0.641**	–0.803**	–0.777**	0.694**
	AUCVSN	–0.686**	–0.645**	–0.797**	–0.758**	0.647**
	AUCNED	–0.275	–0.137	–0.397	–0.508*	0.372
NC 567	AUCAN	–0.470*	0.083	–0.481*	–0.556**	0.637**
	AUCVSN	–0.469*	0.085	–0.481*	–0.554**	0.638**
	AUCNED	–0.538**	–0.094	–0.415*	–0.397	0.325

[a] AUCAN = area under the population development curve for all nematodes per gram of feeder root; AUCVSN = area under the curve for vermiform and swollen nematodes per gram of feeder root; AUCNED = area under the curve for *G. t. solanacearum* eggs per 500 cm^3 of soil.

weight were similar for the two cultivars ($P \leq 0.05$) (Fig. 4).

DISCUSSION

Resistance to *G. t. solanacearum* has been linked with severe root necrosis and stunting, as well as reduced nematode reproduction (Baalawy and Fox, 1971; Komm et al., 1983). In this study, resistance to *G. t. solanacearum* in NC 567 was not associated with increased damage caused by the nematode, at least in comparison with the susceptible cultivar K 326. Negative correlations between population densities of *G. t. solanacearum* and host growth were smaller and less consistent for the resistant cultivar NC 567 than for the susceptible cultivar K 326. The rate of reduction in leaf weight with increasing nematode population pressure was also smaller for NC 567 compared to K 326. Incorporating this resistance into germplasm possessing improved yield and quality traits should produce cultivars more acceptable to growers.

Differences between the resistant and susceptible cultivars in the effect of *G. t. solanacearum* on leaf weight may have resulted from the influence of nematode feeding on root system efficiency. Nematode penetration is similar among tobacco genotypes resistant or susceptible to *G. t. solanacearum* (Baalawy and Fox, 1971). Root size (as measured by fresh weight), nematode suppression of root growth, and the ratio of fresh leaf weight to feeder root weight were all similar for both the resistant and susceptible cultivars. The higher relative reduction in leaf weight by nematode parasitism of susceptible K 326 may, therefore, be related to the feeding by the increased number of *G. t. solanacearum* in the roots of the susceptible cultivar.

Resistance to *G. t. solanacearum* did not completely prevent nematode reproduction and damage by the nematode. Although fewer nematodes developed on the resistant cultivar NC 567, *G. t. solanacearum* suppressed root growth similarly on the resistant and susceptible cultivars. Improvement in the agronomic performance of cultivars resistant to this nematode may require greater inhibition of nematode feeding. However, cyst nematodes may also damage host root systems by their intercellular (vs. intracellular) movement through the root prior to establishing a successful feeding

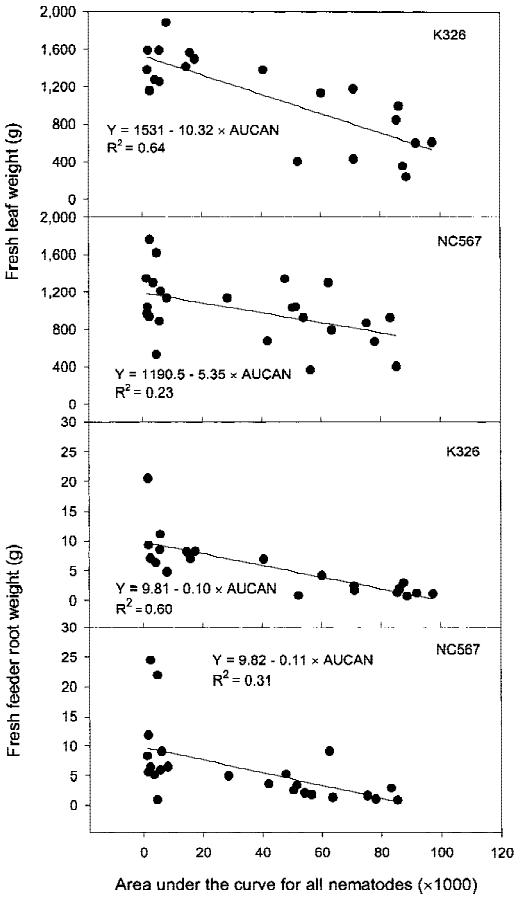

FIG. 3. Influence of parasitism by *Globodera tabacum solanacearum* (AUCAN) on fresh weight of roots and leaves of resistant (NC 567) and susceptible (K 326) cultivars of flue-cured tobacco in 1994.

site. Inhibition of such root injury from use of an effective nematicide might help explain the early onset (2 to 4 WAT) of significant increases in leaf number and weight that were associated with use of fosthiazate in these experiments. Furthermore, no significant differences in early host growth were recorded when juvenile penetration was similar between the resistant and susceptible cultivars. Development of cultivars that produce satisfactory yield in *G. t. solanacearum*-infested soil in the absence of a nematicide may require incorporation of mechanisms that reduce cyst nematode penetration as well as subsequent development.

Nematode egg densities from soil samples are frequently used to estimate population densities of cyst nematodes (LaMondia, 1990). Correlations between egg densities in soil and measures of host growth were often inconsistent across the 2 years of this study. At least some of this variability can be attributed to environmental effects on the response of tobacco to nematode parasitism (Barker, 1989). Use of fosthiazate (a contact nematicide) to adjust initial nematode population densities also may have contributed to the poor correlation between nematode egg densities in soil and tobacco growth. Eggs rendered incapable of penetrating roots by the nematicide may have appeared viable under microscopic examination. The hatching rate of *G. t. solanacearum* is also fairly low on flue-cured tobacco, probably due to effects such as diapause (Wang et al., 1998). Similar initial population densities of *G. t. tabacum* were estimated to reduce shade tobacco yield by less than 5% (LaMondia, 1995). However, the initial nematode egg densities in our experiments were also similar to those at which fosthiazate significantly increased flue-cured tobacco yield and value (Johnson, 1995).

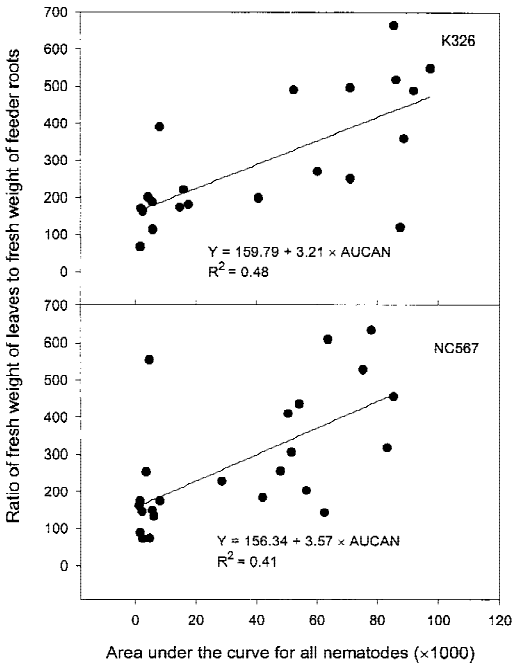

FIG. 4. Effect of parasitism by *Globodera tabacum solanacearum* (AUCAN) on partitioning between roots and leaves of resistant (NC 567) and susceptible (K 326) cultivars of flue-cured tobacco in 1994.

The initial nematode population densities in this study are also generally representative of those found in soil samples from commercial flue-cured tobacco fields in Virginia. Fresh feeder root weight of both cultivars correlated consistently with egg densities collected 9 WAT. Fresh leaf weight correlated consistently with nematode egg densities 6 WAT for susceptible K 326, but not for resistant NC 567. At least two generations of *G. t. solanacearum* occur during the first 11 weeks after transplanting, with peaks of development of mature adults at 5 and 10 WAT (Wang and Johnson, 1994). The timing of these correlations may reflect the generation time of the nematode.

Yield loss due to foliar plant disease is often described using an ''area under the disease progress curve (AUDPC)'' approach (Campbell and Madden, 1990). Integration of changes in disease pressure over the course of the growing season provides a single, weighted summation of disease pressure over time. Accumulating nematode egg densities over time into an ''area under the curve for nematode egg densities'' (AUCNED) did not improve correlations with plant growth. However, significant correlations with host growth were found when the number of vermiform and swollen nematodes and the number of nematodes at all life stages were integrated into AUCVSN and AUCAN, respectively. Our results suggest that nematode eggs in soil may not provide a sufficiently accurate estimate of the number of juveniles attacking host roots for crop modeling purposes. Monitoring nematode populations within host roots is usually impractical for routine use in most crop management scenarios. However, such detailed monitoring may be necessary to accurately describe the relationships between population dynamics of plant-parasitic nematodes and host growth, yield, and quality. Use of an AUDPC approach may also be beneficial when host response to nematode parasitism cannot be adequately explained by single or critical-point models of the nematode-host relationship.

Our examination of the growth response of flue-cured tobacco to *G. t. solanacearum* was limited to the first 11 WAT (up to the topping stage). Flue-cured tobacco leaf is harvested sequentially (Collins and Hawks, 1993; Shew and Lucas, 1990). Harvest commenced approximately 12 WAT in our experiments and was completed 19 to 20 WAT. Although the number of harvested leaves is fixed at topping, significant weight and quality attributes develop between topping and final harvest (Marshall and Seltmann, 1964). Consequently, growth responses during the first 11 WAT may not always accurately reflect the ultimate yield response.

In conclusion, infection by *G. t. solanacearum* reduced the growth of both a susceptible and a resistant cultivar of flue-cured tobacco during the first 11 weeks after transplanting. However, stunting was somewhat less severe on the resistant host compared to a standard susceptible cultivar. Incorporating nematode resistance into germplasm possessing improved yield and quality traits should produce cultivars more acceptable to growers. Further research will be necessary to demonstrate the mechanisms by which *G. t. solanacearum* reduces yield of flue-cured tobacco. Other sources of resistance to *G. t. solanacearum* are also available within *Nicotiana* species (Hayes et al., 1997; Herrero et al., 1996). These other sources of resistance may involve different mechanisms of suppressing nematode reproduction that may limit damage to flue-cured tobacco more effectively.

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