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Quantifying Potential Tolerance of Selected Cotton Cultivars to *Belonolaimus longicaudatus*

S. R. Koenning,[1] D. T. Bowman,[2] R. H. Morris[3]

Abstract: Glyphosate-tolerant cotton cultivars were evaluated for tolerance to *Belonolaimus longicaudatus* in field experiments conducted from 2004 to 2005. Field trials were arranged in a split-plot design that included treatment with four levels of 1, 3-dichloropropene (0.0, 13.9, 27.8, and 41.7 l a.i./ha) to establish a range of population densities of *B. longicaudatus.* Six cotton cultivars (early-to-mid maturity: DP444BG/RR SG501BR, ST5242BR; mid-to late maturity: DP451B/RR, ST5599BR, DP655BRR) were planted as whole plots. Fumigation was effective in suppressing *B. longicaudatus* population densities at mid-season, but not at cotton harvest, and increased cotton lint yield. The cultivar x fumigation interaction for cotton lint yield was not significant for the six cultivars evaluated, indicating that tolerance did not occur in this nematode-host combination. Early-to-mid maturity cultivars yielded significantly more than mid-to-late maturity cultivars in both years. Small but significant differences in nematode final population density were observed between cultivars that may be related to relative maturity.

Key words: *Belonolaimus longicaudatus*, cotton, crop loss, 1, 3-dichloropropene, fumigant nematicide, *Gossypium hirsutum*, glyphosate, herbicide-tolerant crops, host-plant tolerance, nematode, sting nematode, transgenic.

The sting nematode, *Belonolaimus longicaudatus* Sher, is found primarily in the southeastern US and is limited to very sandy soils with more than 80% sand content (Robbins and Barker, 1974). This nematode can be a destructive pathogen of cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), potato (*Solanum tuberosum* L.), and soybean (*Glycine max* L.) (Koenning et al., 1999; Crow et al., 2000a, 2000b; Koenning et al., 2004). Data on pathogenicity and cotton-crop losses due to *B. longicaudatus* is scarce as a result of its limited geographic distribution and its minor importance compared to other plant-parasitic nematodes that attack cotton, such as *Meloidogyne incognita* and *Rotylenchulus reniformis.* Still, increases in cotton hectarage in the southeastern US in the 1990s have resulted in the expansion of cotton production into sandy coastal-plain soils where sting nematode is relatively common.

Tactics for management of *B. longicaudatus* are limited (Crow et al., 2000a). Rotation is often not an option in fields infested with this nematode due to its wide host range (Robbins and Barker, 1973). Peanut and tobacco (*Nicotiana tabacum* L.) can be used in rotation with host crops, but the hectarage of and demand for these crops are limited, and the host status of different populations of *B. longicaudatus* on peanut varies (Robbins and Barker, 1973). Fumigant and nonfumigant nematicides are effective in preventing cotton yield suppression by this nematode, and cotton production in nematode-infested fields is highly dependent on nematicides (Crow et al., 2000c; Koenning et al., 2004).

Cotton cultivars vary in their response to parasitism by nematodes. Cultivars resistant to the southern root-knot nematode, *M. incognita*, are generally defined as limiting the reproduction of this species, whereas susceptible cultivars support large numbers of this nematode (Roberts, 2002; Davis and May, 2003). Although certain cotton cultivars were considered as poor hosts for some geographical isolates of sting nematode, it is difficult to evaluate experiments conducted under different conditions (Holdeman and Graham, 1953; Robbins and Barker, 1973). Population densities of *B. longicaudatus* increased on cotton cultivar DPL 5415 during the growing season, but the decrease of this nematode during the intercropping period in the absence of a host was such that it would result in population densities below the damage threshold after two to three years (Crow et al., 2000c). The impact of cotton cultivars on the population dynamics and relative cultivar tolerance to *B. longicaudatus* is lacking. Bermuda grass and St. Augustine grass accessions with less root suppression than sensitive types were identified and categorized as tolerant to *B. longicaudatus* (Busey et al., 1991, 1993; Giblin-Davis et al., 1992). Cultivars that support nematode reproduction but suffer less yield suppression compared to other cultivars are referred to as being tolerant (Cook and Evans, 1987; Barker, 1993; Roberts, 2002).

Cotton tolerance to *Hoplolaimus columbus* has been documented, but tolerance to *B. longicaudatus* and differences in reproduction among current cotton cultivars are unknown (Bowman and Schmitt, 1994; Koenning and Bowman, 2005). Information on cultivar resistance to root-knot nematodes may be obtained through greenhouse experiments, but tolerance must generally be evaluated through field experimentation (Boerma et al., 1986; Reese et al., 1988; Bowman and Schmitt, 1994; Roberts, 2002; Starr and Bendezu, 2002; Davis and May, 2003; Koenning and Bowman, 2005). The most common method of measuring tolerance to

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[1] Research Assistant Professor, Department of Plant Pathology, NC State University, Raleigh, NC 27695–7616.

[2] Professor, Crop Science Department, NC State University, Raleigh, NC 27695–7620.

[3] Regional Agronomist, Agronomic Division, North Carolina Department of Agriculture and Consumer Services, 3184 Old NC 41, Bladenboro, NC 28320.

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E-mail: stephen_koenning@ncsu.edu

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plant-parasitic nematodes is the comparison of plots treated with a nematicide vs. nontreated (Trudgill and Cotes, 1983; Cook and Evans, 1987; Bowman and Schmitt, 1994; Koenning and Bowman, 2005). Tolerance to plant-parasitic nematodes is generally considered to be density-dependent, so an alternative method for evaluating tolerance is the use of varying densities of the plant-parasitic nematode species in question and comparison of the slopes of the regression lines for different cultivars (Trudgill and Cotes, 1983; Boerma et al., 1986; Koenning et al., 1992). Although relatively simple in concept, this approach is difficult to achieve in the field. Natural variation in population densities may be the result of soil factors that may confound fertility effects with nematode population densities and also impact yield. A second approach is the imposition of different treatments on field plots than in previous years, such as the use of non- or poor hosts or the application of nematicides of varying efficacies (Koenning et al., 1992; Crow et al., 2000a, 2000b). In the current research, the population densities of *B. longicaudatus* were manipulated by the use of different rates of the fumigant nematicide 1,3-dichloropropene (1,3-D) (Telone II, Dow AgroSciences, Indianapolis, IN).

The widespread use of herbicide-tolerant and/or insect-resistant cotton cultivars has resulted in numerous cultivars becoming obsolete. Currently, over 90% of the cotton hectarage in North Carolina is planted with transgenic cultivars (USDA-AMS, 2004) tolerant to the herbicide glyphosate. Data on field tolerance and/or resistance of transgenic cultivars to pests and pathogens is generally lacking. Field research conducted from 2004 through 2005 evaluated the relative tolerance to *B. longicaudatus* of glyphosate-tolerant cotton cultivars with transgenic insect resistance. Cultivars were selected on the basis of perceived tolerance or lack thereof to *H. columbus* determined in previous research (Koenning and Bowman, 2005).

The objectives of this research were to evaluate the effect of *B. longicaudatus* on lint yield of selected cotton cultivars, quantify any differences in this nematode's reproductive capacity on these cultivars, and lastly to determine if measurable tolerance to this nematode was available in commonly used cotton cultivars.

Materials and Methods

Field experiments were conducted in 2004 and 2005 in growers' fields located in Robeson County, NC, infested with *B. longicaudatus*. The soil type in both years was a Wagram loamy sand (in 2004: 87% sand, 11% silt, 2% clay, < 1% organic matter; in 2005: 87% sand, 12% silt, 1% clay; < 1% organic matter). The mean Pi per 500 cm^3 soil was 129 ± 12.5 and 54 ± 5.8 for 2004 and 2005, respectively.

The experimental design was a split-plot with six replicates. Whole-plots contained six transgenic glyphosate-tolerant cotton cultivars with transgenic insect resistance, three of which were considered to be early-to-mid maturity and three mid-to-late maturity (early-to-mid: DP444BG/RR SG501BR, ST5242BR; mid-to late: DP451B/RR, ST5599BR, DP655BRR). Sub-plots were treated with 0.0, 13.9, 27.8, and 41.7 l a.i. /ha of 1,3-D. Standard management practices for cotton production were used throughout this research, and plots were not irrigated (Anonymous, 2005).

Selected plots were fumigated in early April, and all plots were planted in mid-May. All plots received an in-furrow application of aldicarb (Temik 15G, Bayer Crop Science Inc., Research Triangle Park, NC) at 0.5 kg a.i./ha at planting for early season insect control. Plots were two rows 7.62-m long with 1.01-m row spacing and 3.04-m alleys. Lint yield was determined by ginning sub-samples of seed-cotton taken from the first two replicates after picking with a commercial cotton picker.

Samples for nematode assays for each plot were collected prior to fumigation (Pi), mid-season (Pm), and at cotton harvest (Pf). Each composite soil sample consisted of eight to 10 cores (2.5-cm-diam.) taken to a depth of 15 cm. A 500-cm^3 sub-sample was processed by elutriation and centrifugation to extract adult and juvenile nematodes from soil (Jenkins, 1964; Byrd et al., 1976).

Data analysis consisted of General Linear Models for a split-plot design with $\log_{10}$ (Pi +1) as a covariate using PC/SAS software (SAS Institute, Cary, NC). Nematode numbers were transformed $\log_{10}(x + 1)$ prior to analysis to standardize the variance. Nontransformed numbers are presented in figures for clarity. Years for the field experiments were considered to be random effects, so the cultivar x year interaction was used to test differences for cultivar, fumigation, and cultivar x fumigation effects for combined analysis over years.

The Waller Duncan k-ratio t test (k-ratio = 100) was used for mean separation, and regression analysis was used to evaluate the influence of rate of 1,3-D on Pf, Pm and lint yield. Data on nematode Pf from nontreated plots were compared to results of all plots (fumigated and nonfumigated) and did not differ, so all data were subjected to regression analysis and used to determine if a relationship between nematode reproduction or cotton lint yield was present.

Results

Fumigation with 1, 3-D resulted in a linear decrease both years in the mid-season population levels of *B. longicaudatus* according to regression analysis Log_{10} $Y = 1.5 - 0.095\ x$ ($R^2 = 0.51$, $P = 0.0009$) and to the Waller-Duncan k-ratio t test (k-ratio = 100) (Fig. 1). Nematode numbers were not suppressed ($P \leq 0.10$) by fumigation at cotton harvest either year. Cotton cultivars did not differ ($P \leq 0.10$) in their ability to support

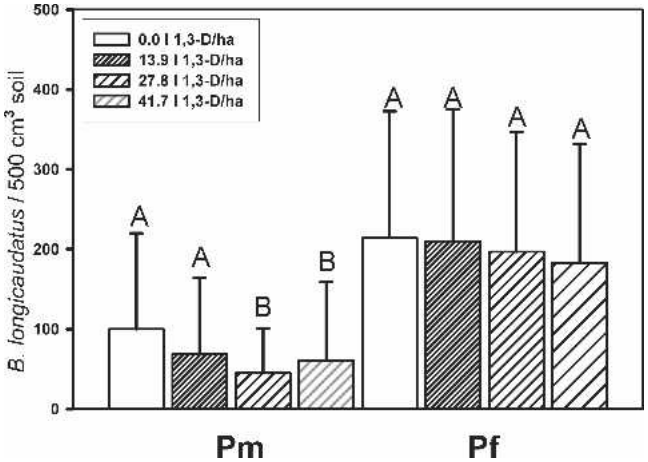

FIG. 1. Influence of in-furrow injection of 1, 3-dichloropropene (1,3-D) at four rates (0.0, 13.9, 27.8, and 41.7 l/ha) on mid-season (Pm) and final (Pf) population densities of *Belonolaimus longicaudatus* per 500 cm^3 soil in field plots in 2004 to 2005. Means are based on 72 observations for each level, and error bars show the standard deviation. Means with the same letter within a sampling date do not differ according to the Waller-Duncan k-ratio *t* test (k-ratio = 100).

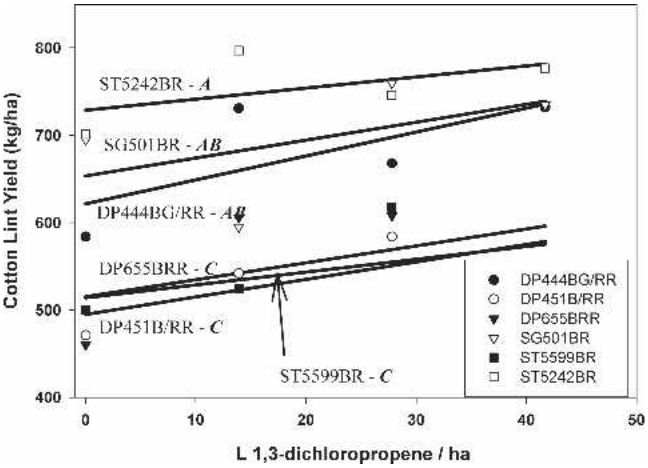

FIG. 3. Influence of in-furrow injection of 1,3-dichloropropene (1,3-D) at four rates (0.0, 13.9, 27.8, and 41.7 l/ha) on cotton lint yield of six cotton cultivars (early-to-mid maturity: DP444BG/RR SG501BR, ST5242BR; mid-to late maturity: DP451B/RR, ST5599BR, DP655BRR) in two fields infested with *Belonolaimus longicaudatus* in 2004 and 2005. Slopes do not differ according to the heterogeneity of slopes test, the cultivar x fumigant rate interaction was NS, and overall significance of the full model was $P = 0.0001$ ($R^2 = 0.71$). Cultivar names followed by the same letter in bold and italics do not differ according to Waller-Duncan k-ratio *t* test (k-ratio = 100). Regression equations for each cultivar (R^2 values based cultivar means): DP444BG/RR – $Y = 621 + 2.74 \times$ ($R^2 = 0.49$); DP451B/RR – $Y = 495 - 2.01 \times$ ($R^2 = 0.57$); DP655BRR – $Y = 515 + 1.94 \times$ ($R^2 = 0.25$); SG501BR – $Y = 654 + 2.05 \times$ ($R^2 = 0.26$); ST5599BR – $Y = 514 + 1.47 \times$ ($R^2 = 0.27$); ST5242BR – $Y = 729 + 1.27 \times$ ($R^2 = 0.30$).

reproduction of *B. longicaudatus* at mid-season either year, but the population densities of this nematode were lower on DP444BG/RR than on most other cultivars according to the Waller-Duncan k-ratio *t* test (k-ratio = 100) (Fig. 2). The relationship for cultivar and nematode Pf was the same when analyzing all plots or analyzing only nontreated plots both years.

Cotton lint yield increased linearly ($Y = 588$ kg cotton lint/ha + 1.92 l 1,3-D/ha [$R^2 = 0.72$, $P \leq 0.0001$]) with the rate of 1,3-D applied before planting. Fumigation increased ($P \leq 0.01$) the yield of both early-to-mid maturity and mid-to-late maturity cotton cultivars compared to nonfumigated plots (Fig. 3). Years differed, but first-order interactions for year and cultivar were not significant for nematode population data or yield data, and second-order interactions were not significant ($P \leq 0.10$).

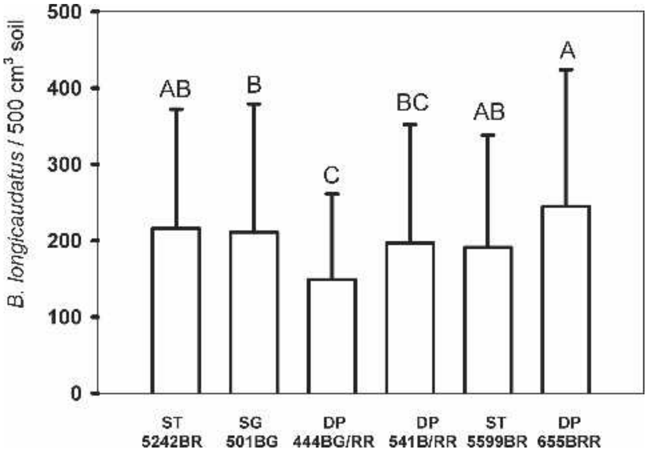

FIG. 2. Effect of cultivar on mean final population densities of *Belonolaimus longicaudatus* during 2004 and 2005. Means are based on 48 observations for each cultivar over all rates of fumigation, and error bars show the standard deviation. Means with the same letter do not differ according to the Waller-Duncan k-ratio *t* test (k-ratio = 100).

The heterogeneity of slopes' test demonstrated that cultivars did not respond differently to the rate of 1,3-D applied. Early-to-mid maturity cultivars yielded significantly more than mid-to-late maturity cultivars both years according to the Waller-Duncan k-ratio *t* test (k-ratio = 100).

DISCUSSION

The linear rate of suppression of *B. longicaudatus* mid-season population levels relative to the rate of 1,3-D injected indicated that fumigation with differing rates appeared to be effective in modifying nematode Pi for the purposes of this research. Although the effect of rate of fumigant on nematode Pi was not measured directly, the mid-season densities of *B. longicaudatus* show that the population density was reduced in an approximately linear relationship to the rate of 1,3-D applied. Also, though aldicarb, which was applied in-furrow to all plots, may have nematistatic effects on *B. longicaudatus*, the low rate applied for insect control in this research would not generally be considered to be nematicidal. The lack of impact on nematode Pf demonstrated that the reproductive rate of *B. longicaudatus* on cotton was sufficient to recover from the effects of fumigation, or alternatively, that it was able to migrate from areas that the fumigant did not reach. Small, yet significant, differences in *B. longicaudatus* Pf were noted among cultivars. These may be intrinsically related to the cultivar, but also could be related to the length of the growing season related to cotton maturity. Highest nematode reproduction occurred on DP655BRR, which was the latest maturing cultivar used in this research, whereas the lowest reproduction was on DP444BG/RR, the earliest of the cultivars used. This relationship, however, was not always consistent, as DP451B/RR is mid-to-late maturity and did not differ from DP444BG/RR for nematode reproduction.

The consistent yield response of cultivars to the rate of 1,3-D across years in the presence of *B. longicaudatus* demonstrated the pathogenicity of this nematode to all cotton cultivars evaluated. The economic return from use of 1,3-D, however, appears negative, as a liter of 1,3-D provides for an increase of only 1.92 kg/ha cotton lint yield. This translates into a cost of US$2.90 for 1 liter of 1,3-D to produce an additional US$2.54 in income, assuming cotton at US$1.32/kg, regardless of the cultivar (Anonymous, 2005).

One type of statistical evidence/definition for tolerance would be a significant interaction between nematode Pi and cultivar yield, which would indicate density-dependent tolerance to nematodes (Trudgill and Cotes, 1983; Boerma et al., 1986; Cook and Evans, 1987; Koenning et al., 1992; Koenning and Bowman, 2005). By this definition, none of the evaluated cultivars could be considered tolerant to *B. longicaudatus*, as the slopes of the regression lines do not differ. Heterogeneous slopes would indicate that the regression lines may intersect, and tolerance is thus density dependent. Some of the cotton cultivars evaluated in the current work were considered to be relatively tolerant (DP451B/RR, DP655BRR) or intolerant (SG501BR) to *H. columbus* in other research, but none of the cultivars evaluated in the current research showed evidence of a density-dependent type of tolerance to *B. longicaudatus* (Koenning and Bowman, 2005).

The higher mean yields of the early-to-mid maturity cultivars than later cultivars, especially the relatively high yields of ST5242BR and SG501BR, could be interpreted as a form of tolerance that is not density-dependent, or alternatively, that these cultivars were genetically superior in the test environment. Extension personnel and consultants would certainly recommend the use of the early-to-mid maturity cultivars rather than mid-to-late maturity cultivars where sting nematode is present, based on the current research.

Mid-to-late maturing cultivars tended to yield more and exhibit more tolerance to *H. columbus* than did early-to-mid maturity cultivars, which also were more sensitive to variation in initial Pi (Koenning and Bowman, 2005). Generally, late maturity cultivars are preferred on the sandy soils in which both *H. columbus* and *B. longicaudatus* occur because they are less determinate and more resistant to drought stress. Although the relationship between tolerance to *H. columbus* and stress is tenuous, this concept agrees with the assertion by Wallace (1987) that tolerance to nematodes evolved from tolerance to stresses in the abiotic environment, such as drought. The relationship between cultivar yields relative to cultivar maturity in the presence of *B. longicaudatus*, however, was the converse of that encountered with *H. columbus*. Earlier maturing cultivars may avoid damage from *B. longicaudatus* by maturing before the nematode population density is so great that it eliminates the benefits of additional root growth that may occur with later maturing cultivars. If future research verifies that early maturity minimizes damage from *B. longicaudatus*, then the greater yields of early-to-mid maturity cultivars, compared to a mid-to-late maturity one, might best be described as avoidance rather than tolerance. Another explanation for the lack of density-dependent tolerance to *B. longicaudatus* is that mechanisms of tolerance to ectoparasitic nematodes may be very different from the mechanisms involved in tolerance to semi-endo or endoparasitic nematodes.

Research comparing cultivar response to *B. longicaudatus* at varying population densities was achieved through differential rates of application of 1,3-D in the current research. Evaluation of tolerance at different population levels, however, requires extensive land and material for testing (Reese et al., 1988; Koenning et al., 1992). Comparison of fumigated vs. nonfumigated plots is more cost-effective than using multiple nematode levels, since more cultivars can be evaluated for the same expenditure in labor and other costs. The limitation of the latter approach is that it may not reveal other mechanisms that may minimize crop loss in response to nematode parasitism, as was the case with *B. longicaudatus* in the current research.

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