Screening Cotton for Tolerance to *Hoplolaimus columbus*

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ABSTRACT

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In order to adequately plan experiments to measure tolerance of cotton to *Hoplolaimus columbus*, coefficients of variation (CVs) and error variances of yield were examined to determine the minimum number of replicates and environments needed to measure specified differences for tolerance index (TI) and field tolerance. In two greenhouse trials with 84 genotypes, the error variance was 1,423 and would have required 231 replicates to ensure detecting a 10% genotypic difference in TI. In the 3-yr, one-location field study reported, CVs ranged from 10.0 to 25.4%, while error variances for TI ranged from 68 to 512. The pooled error variance (243) was used in calculating the minimum number of replicates and environments. There was no genotype × environment interaction for TI. To detect a 20% difference in TI, a minimum of 14 replicates in one environment or six replicates in two environments was required. Field tolerance, on the other hand, revealed a highly significant genotype × environment interaction and required as many as 23 environments with four replicates per environment to detect a 10% difference at 0.05. Choosing an α level of 0.20 instead of 0.05 would reduce the minimum number of replicates and/or environments by over one-half. This information should be useful in planning experiments to screen cotton germ plasm for tolerance to *Hoplolaimus columbus* in the future.

Additional keywords: *Gossypium hirsutum*, lance nematode

The Columbia lance nematode (*Hoplolaimus columbus* Sher) is an important pest throughout the southeastern United States but primarily in Georgia, North Carolina, and South Carolina (3,5–7). However, screening cotton (*Gossypium hirsutum* L.) germ plasm for tolerance and/or resistance to this pest has received limited attention. In a greenhouse study, 117 cotton genotypes were screened for tolerance by inoculating them with *H. columbus* adults and juveniles and observing plant responses (shoot and root weights) between inoculated and noninoculated controls (2). Eighteen genotypes were later classified as tolerant on the basis of root weights. Six of seven genotypes classified as tolerant in the greenhouse experiment were also tolerant in a subsequent field test (D. T. Bowman, *unpublished*). Shoot weight and total plant weights in the greenhouse did not correlate well with field measurements of tolerance index (D. T. Bowman, *unpublished*).

Six cotton genotypes evaluated in a field infested with Columbia lance nematodes did not exhibit tolerance to this nematode (9), although one genotype, PD3, appeared to have tolerance (10). Variation among years, locations, plots, and infestation levels and uniformity may account for inconsistent findings.

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Screening for resistance and/or tolerance to nematodes is costly and time-consuming. Therefore, knowledge of error variances and coefficients of variation (CVs) from historical data would be useful in efficiently allocating resources for evaluation of germ plasm for tolerance or resistance to Columbia lance nematode. The objective of this study was to examine historical data from screening studies to determine the minimum number of replicates and/or environments necessary to detect specified differences to assist in planning for future research.

MATERIALS AND METHODS

Greenhouse test. One greenhouse experiment with 84 genotypes with two runs of one replicate each was conducted during 1988–1990. *H. columbus*-infested soil was collected from a field in Scotland County, North Carolina. The nematodes were extracted from the soil by elutriation and centrifugal flotation (4). Clay pots (15 cm in diameter, 1,300 cm^3) were filled with a sterile sand/soil mix. Five seeds per pot were planted, and plants were later thinned to three per pot. Depressions were then made around the root zone of each plant, and 2,500 (run 1) and 2,000 (run 2) Columbia lance adults and juveniles were applied to each pot. On the basis of trials using natural populations to determine effects on yield, these inoculum densities are four to five times the damage threshold (*unpublished*). Noninfested pots served as controls. No other plant-parasitic nematodes were present in the soil. Pots were paired on the greenhouse bench.

At 90 days after inoculation, soil was carefully washed from the roots and the roots were dried and weighed. Shoots were also dried and weighed. Nematodes were extracted from the soil and roots. Tolerance for root damage was determined by calculating a tolerance index using the formula: TI = (dry weight of roots from infested pots/dry weight of roots from noninfested pots) × 100.

Field test. Yield trials were conducted in 1990, 1991, and 1992 near Gibson, Scotland County, North Carolina, in soil naturally infested with *H. columbus*. Analysis of data included only genotypes common to all three trials: Deltapine 90, La. L^oR^s Carter, and Stoneville 506. The trials included four replicates in a randomized complete block design in a split-plot arrangement of treatments. Main plots consisted of genotypes, and subplots were either treated with 1,3-dichloropropene (65 kg ha^{-1} injected 30 cm deep at least 7 days prior to planting) or not treated. This nematicide effectively controls *H. columbus* (12). Each main plot was four rows 9.1 m long and 0.97 m wide, and each subplot was two rows wide.

The trials were planted 1 May 1990, 23 May 1991, and 6 May 1992. Soil samples were taken in July of each year, and nematodes were extracted from soil by elutriation and centrifugation and from roots collected in the soil sample using a mist chamber. Midseason numbers of *H. columbus* averaged 107 and 925/500 cm^3 of soil and roots in 1990, 189 and 457 in 1991, and 71 and 504 in 1992 in the treated and nontreated plots, respectively. The soil was a Norfolk loamy sand (Fine-loamy, siliceous, thermic Typic Paleudult). Cultural practices were the same for the 3 yr. An additional trial, using only McNair 235, was established in another location in Scotland County in 1992. The planting date, harvest date, and all other cultural practices were the same as in the other 1992 test.

The two-row subplots were harvested by machine on 18 October 1990, 23 October 1991, and 15 October 1992. A tolerance index for yield was computed for each main plot using the formula: TI = (yield of nontreated subplot/yield of treated subplot) × 100.

The number of replicates necessary to reveal specified differences between genotypes was computed using: $R \geq [2(t_{\alpha/2} + t_\beta)^2\sigma^2]/d^2$, where $t_{\alpha/2}$ is the t value associated with the desired significance level of the t test, t_β is the t value associ-

ated with the probability of accepting a false hypotheses based on the t test, σ^2 is the error variance, and d is the true difference between tolerance indices (8). The formula used to calculate the minimum number of environments required to show specified differences in tolerance index between two genotypes at varying replicate levels is: $E \geq [2(t_{\alpha/2} + t_{\beta})^2(\sigma^2/R + \sigma^2_{GE})]/d^2$, where R is the number of replicates and σ^2_{GE} is the genotype × environment interaction if significant. Unless otherwise noted, the β level chosen for this study was 0.2 or power = 0.8 and the α level was 0.05.

RESULTS AND DISCUSSION

Greenhouse test. Plant resistance to nematodes is based on suppression of nematode reproduction. There is little evidence in this test that reproduction was affected significantly by any cultivar. The number of Columbia lance nematodes per 500 cm^3 of soil plus roots in that soil ranged from 124 to 690. Thus, resistance may not be a factor in the response of cotton to Columbia lance nematode. The error variance for tolerance index based on root weights from the two-run (replicate), 84-genotype greenhouse screening was 1,423. Thus, the number of replicates required to show a 10% difference was calculated to be 231. For a 20, 30, 40, or 50% genotypic difference, the number of single replicate runs would be 58, 26, 14, and 9, respectively. Therefore, greenhouse evaluation for tolerance to *H. columbus* may not be practical. The use of nine or 10 replicates in order to gain an inference at the 50% level could still serve to minimize the number of field trials.

The need to develop a more efficient greenhouse screening method is still necessary. However, at an α level of 0.20, the number of replicates needed to detect a 10, 20, 30, 40, or 50% genotypic difference would be 130, 32, 14, 8, and 5, respectively. Five replicates may not be impractical. By increasing the α level chosen, one will carry fewer genotypes along in the screening program. There is the obvious danger of dropping genotypes that are tolerant. Nevertheless, it is critical to look at large numbers in plant breeding programs, and successful breeders use high selection pressure, i.e., high α levels, because a high type I error rate is not perceived to be as critical as a high type II error rate.

Field test. For tolerance index of yield, coefficients of variation ranged from 10.0% to 25.4% and error variances ranged from 68 to 512 during the 3 yr of this research (CV = 10.0, 25.4, 15.9, and 18.8% and error variance = 68, 512, 150, and 243 for 1990, 1991, 1992, and pooled data, respectively). The highest variance occurred in 1991, when cotton was planted late because of wet soil conditions; the abnormally late planting may have contributed to the high variability compared to years when planting dates were normal. The site and cultural practices were the same for all 3 yr and probably did not affect the variation.

The error variance calculated in the separate study in 1992, using McNair 235, was 266, which was similar to that found in the combined analysis, i.e., 243. The genotype × environment interaction was not significant in the combined study.

Calculation of the minimum number of replicates required to show specified differences in tolerance index for yield between two genotypes for a single test, assuming no genotype × environment interaction, resulted in 57 replicates to show a 10% difference. The number of replicates needed to show 20, 30, 40, and 50% differences would be 14, 6, 4, and 2, respectively. A 10% difference is not unrealistic in many agronomic studies, and a 20% significant difference is very common. However, in order to detect a 20% tolerance index difference, one would need to use a minimum of 14 replicates.

By changing the α level to 0.20, one would reduce the number of replicates required to 22, 5, 2, and 1 for detecting 10, 20, 30, and 40% differences in tolerance index. Five replicates to detect a 20% difference is a reasonable number for field trials.

Environments could be substituted for replicates; two environments with seven replicates at each environment would ensure detecting a 20% difference in tolerance index (Table 1). This level would be acceptable in many screening programs because there is still a high probability of obtaining an economical gain from the use of that level of tolerance.

Table 1. Number of field environments required to show specified differences in tolerance index to *Hoplolaimus columbus* between two cotton genotypes at varying replicate levels, assuming no genotype × environment interactions

No. of replicates per environment	Genotypic differences (% of $\bar{x}$)				
	10	20	30	40	50
2	29	7	3	2	1
3	19	5	2	1	1
4	14	4	2	1	1
5	11	3	1	1	1
6	10	2	1	1	1

Table 2. Number of field environments required to show a 10% difference in seed cotton yield between two cotton genotypes at varying replicate levels in fields treated and not treated for *Hoplolaimus columbus*, assuming a genotype × environment interaction

Treatment	Replicate number						
	4	6	8	10	12	14	16
Treated	13	9	6	5	4	4	3
Not treated	23	15	11	9	8	6	6

These findings are very similar to those obtained for screening soybean (*Glycine max* (L.) Merr.) germ plasm for tolerance to soybean cyst nematode (*Heterodera glycines* Ichinohe) (11). There was no genotype × environment interaction in that study, and error variances were similar in size to those reported in this study.

Field tolerance test. Field tolerance is defined as a measure of relative performance in the presence of the disease and is the type of information requested by growers. Data collected from the nontreated plots in the tolerance index study were used to compute, on an individual environment basis and across environments, the number of replicates required to reveal a specified difference in yield. To reveal a 5% difference in yield between two genotypes, one would need to have 20 replicates in a field infested with Columbia lance nematodes and 35 replicates in a field treated with a nematicide. At the 10% level, one would need five replicates in a nematode-infested field and nine replicates in a field treated with a nematicide. One replicate would reveal a 20% difference in seed cotton yield in a nematode-infested field, whereas two replicates would be needed in a field treated with a nematicide.

When data were combined over environments, a highly significant genotype × environment interaction was detected for yield in both nontreated and treated plots. To detect a 10% difference in seed cotton yield between two genotypes in a field infested with Columbia lance nematodes, one would need 23 environments with four replicates per environment or six environments with 16 replicates per environment (Table 2). Choosing an α level of 0.20 instead of 0.05 would reduce the combined number of replicates and environments by nearly one-half to two-thirds. The magnitude of the genotype × environment interaction is less in the nematicide-treated plots, resulting in only 13 environments with four replicates per environment to detect a 10% difference in yield; the number of environments is nearly one-half that required for nontreated plots. In the analyses, genotype × environment interaction accounted for 10% of the variation in the nematicide-treated plots and 15% in the nontreated plots. Typically, in North Carolina cultivar trials, two environments with four replicates per environment would be required to detect a 10% difference in yield between two genotypes (1; D. T. Bowman, *unpublished*). Obviously, the nematode increases variability from test to test and the use of a nematicide, even though it dramatically improves precision, does not equal the precision in trials conducted in nematode-free fields. Within an environment, use of the nematicide seems to create variability above that found in nematode-infested plots.

Screening germ plasm for tolerance to nematodes will continue to be costly and time-consuming. Limitations in field space with uniform nematode infestation may dictate that initial screenings be conducted in the greenhouse, followed by confirmation studies in the field. The use of a fumigant to achieve yields equivalent to those in a noninfested field also may add to variability. We hope these findings will assist researchers in planning future Columbia lance nematode tolerance screening studies for cotton.

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