SHORT, NON-REFEREED PAPER

A NEW METHOD FOR ASSESSING SUGARCANE VARIETY TOLERANCE TO NEMATODES

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Abstract

A new pot trial method was developed to assess sugarcane variety tolerance to nematodes under controlled conditions. Pre-germinated single-budded setts were planted into pots containing sterilised soil or sterilised soil inoculated with a cocktail of nematodes. The nematode community was dominated by *Meloidogyne,* and also contained *Pratylenchus*, *Helicotylenchus*, *Xiphinema*, *Paratrichodorus*, *Scutellonema* and *Tylenchorhynchus*. Shoot height, shoot and root biomass and nematode numbers in the roots at harvest were recorded. Two trials with varieties N12 (tolerant) and N51 (susceptible) were planted using the above method. In Trial 1, no significant differences in growth were noted between treatments, largely due to low nematode numbers in the roots. In Trial 2, the number of nematodes used for inoculation was doubled. This significantly increased nematode numbers in the roots of the inoculated treatments at harvest. No significant differences in growth were observed between the sterilised control and the sterilised and inoculated treatment for N12, confirming tolerance to plant parasitic nematodes. For N51, all growth parameters tested were significantly lower in the inoculated treatment, confirming the susceptibility of this variety. This new pot trial method will be used to assign nematode tolerance ratings for varieties in future; however, further refinement is required.

Keywords: sugarcane, variety, tolerance, nematodes, pot trials

Introduction

To effectively manage nematodes within the South African sugar industry, an integrated management approach is promoted. An essential component of that system is varietal tolerance to nematodes. Due to the high diversity of nematodes associated with sugarcane, tolerance is a more practical approach than resistance (Cadet and Spaull, 2005). The effective use of tolerant varieties to manage nematodes has been demonstrated previously in South Africa (Rutherford *et al*., 2002; Spaull and Cadet, 2003).

In the past, nematode tolerance was determined using varietal response to nematicide in field trials. If a variety responded to a nematicide, it was termed susceptible and vice versa. However, due to the inherent variability associated with field trials, variable responses to nematicide were observed across environments, making ratings difficult. A pot trial method under controlled conditions was thus investigated.

Materials and Methods

Trial details

Two pot trials using varieties N12 (tolerant) and N51 (susceptible) were conducted at the South African Sugarcane Research Institute.

Trial 1 was planted on 20 January 2017 and was terminated on 19 June 2017, five months after planting. There were two treatments: sterilised soil and sterilised soil inoculated with a cocktail of nematodes. The nematode community was dominated by *Meloidogyne* but also contained *Pratylenchus*, *Helicotylenchus*, *Xiphinema*, *Paratrichodorus*, *Scutellonema* and *Tylenchorhynchus*. Approximately 500 nematodes per pot were used for inoculation. Setts were pre-germinated by placing onto wet tissue paper in an incubator at 25°C. To maximise the effect of nematodes on the plant, setts were removed when the buds were swollen but before root development. Setts were then planted into 10 cm pots containing the respective treatments. Each treatment was replicated ten times and arranged in a randomised complete block design. The pots were placed in a temperature-controlled glasshouse maintained at 25°C. After one month, the seedlings, together with the soil, were transplanted into 37 cm pots containing sterilised soil and the pots transferred to a shadehouse facility.

Trial 2 was planted on the 22 September 2017 with the following modifications:

1. The sterile soil in each 10 cm pot was inoculated with approximately 1 100 nematodes.
2. The seedlings and soil were transferred from the 10 cm pots into 25 cm pots after one month.
3. The trial was maintained in a temperature-controlled glasshouse for the duration of the experiment.

Trial 2 was harvested on 19 December 2017, three months after planting.

For both trials, shoot height, dry shoot and root weight as well as nematode numbers in the roots were recorded at harvest. Monthly shoot height was also recorded (data not shown).

Data Analysis

Root nematode data were analysed per trial using a general analysis of variance in randomised blocks. Where data were not normal, a log (x+1) transformation was used. Means were separated at the 5% significance level using the Sidak test.

Growth data were analysed per variety and within each trial. Data were subjected to a two tailed t-test and significant differences determined at the 5% significance level. Equality of variance was tested using the F-test.

Results and Discussion

Root nematode data

In Trial 1, there was no significant difference between varieties when inoculated with nematodes. A significant increase in *Pratylenchus* due to nematode inoculation was observed for variety N12, compared to the sterilised control (Table 1). *Pratylenchus* numbers were highest in this treatment, with 123 nematodes per gram dry root weight recorded. Variety N51 (inoculated) had 81 *Pratylenchus* per gram dry root weight in (Table 1). Stirling and Blair (2000) suggested that numbers of <100 *Pratylenchus* per gram of dry root pre-season and <250 mid-season have little chance of reducing sugarcane yield. Since these numbers were determined at the end of the trial, it is inferred that *Pratylenchus* numbers in the roots were too low to have a significant effect on sugarcane yield. The number of *Meloidogyne* in the roots were lower with a maximum of 29 per gram of dry root weight in variety N12 (inoculated) and negligible numbers in variety N51 (Table 1). Stirling and Blair (2000) suggested numbers in excess of 100 *Meloidogyne* per gram of dry root pre-season and 200 mid-season, would be required to have an effect on sugarcane yield. It is likely that *Meloidogyne* numbers were also too low to cause a yield reduction.

Table 1. Average number of *Pratylenchus*, *Helicotylenchus*, *Meloidogyne* and total plant parasitic nematodes found in the roots at harvest for varieties N12 and N51 grown either in sterilised soil or sterilised soil inoculated with a cocktail of nematodes, harvested at five months after planting (Trial 1) and three months after planting (Trial 2). Means with the same letter are not significantly different at the 5% significance level, determined using the Sidak test.

TRIAL 1				
Treatment	***Pratylenchus***	***Helicotylenchus***	***Meloidogyne***	**Total plant parasitic nematodes**
N12 Sterilised	21[a]	0*	0*	22[a]
N12 Sterilised and Inoculated	123[b]	1*	29*	137[a]
N51 Sterilised	18[a]	1*	1*	20[a]
N51 Sterilised and Inoculated	81[ab]	2*	0*	83[a]
TRIAL 2				
Treatment	***Pratylenchus***	***Helicotylenchus***	***Meloidogyne***	**Total plant parasitic nematodes**
N12 Sterilised	1[a]	0*	2[a]	3[a]
N12 Sterilised and Inoculated	739[b]	0*	51[b]	791[b]
N51 Sterilised	3[a]	0*	0[a]	3[a]
N51 Sterilised and Inoculated	615[b]	0*	468[b]	1085[b]

* Data could not be normalised with transformation. Data did not match the parameters required for ANOVA and were therefore not analysed statistically.

Trial 2 was inoculated with twice the number of nematodes. Consequently, nematode numbers were higher the second trial with significant increases in *Pratylenchus*, *Meloidogyne* and total nematode numbers due to nematode inoculation being observed. Numbers of *Pratylenchus* were >600 nematodes per gram dry root weight and numbers of *Meloidogyne* were >450 for variety N51 (inoculated with nematodes) (Table 1). According to the thresholds proposed by Stirling and Blair (2000) these numbers are likely to have a 5-20% effect on yield depending on environmental conditions. It must be noted that the thresholds proposed by Stirling and Blair (2000) were based on field sampling for nematodes to inform nematode management measures. Consistent with Trial 1, there was no difference in the nematode populations between varieties in the inoculated treatment (Table 1).

Growth parameters

Due to the low nematode numbers in the roots at harvest (Table 1), no significant differences in growth were observed between treatments in Trial 1 (Table 2).

Despite the low nematode numbers in Trial 1 (Table 1), visual differences in height were evident between treatments in the susceptible variety N51, up to three months after planting. These differences became less obvious in the subsequent months, possibly due to the controls becoming pot bound with an associated slowing of growth. For this reason, the second pot trial was harvested at three months instead of five months.

Consistent with the significant increase in nematode numbers in the roots (Table 1), a corresponding significant decrease in growth parameters was noted for variety N51 due to nematode inoculation (Table 2). This confirms the susceptibility of variety N51 to nematodes. Conversely, despite significant increases in nematode numbers in the roots in variety N12 (Table 1), no corresponding significant difference in growth was noted (Table 2). The

population of *Meloidogyne* was lower in variety N12 (inoculated) compared to variety N51 (inoculated) but this difference was not significant (Table 1). This confirms the tolerance of this variety to nematodes.

Table 2. Shoot height and dry root and shoot weight at harvest for varieties N12 and N51 grown either in sterilised soil or sterilised soil inoculated with a cocktail of nematodes harvested at five months after planting (Trial 1) and three months after planting (Trial 2). Significant differences were determined per variety and within each trial. Means with the same letter are not significantly different at the 5% significance level, determined by using a two tailed t-test.

TRIAL 1						
Treatment	N12			N51		
	Shoot height (cm)	Dry root weight (g)	Dry shoot weight (g)	Height (cm)	Dry root weight (g)	Dry shoot weight (g)
Uninoculated	45.5[a]	15.8[a]	85.9[a]	53.3[a]	20.8[a]	87.7[a]
Inoculated	50[a]	14.4[a]	82.6[a]	53.4[a]	27.2[a]	99.5[a]
TRIAL 2						
Treatment	N12			N51		
	Shoot height (cm)	Dry root weight (g)	Dry shoot weight (g)	Height (cm)	Dry root weight (g)	Dry shoot weight (g)
Uninoculated	23.6[a]	1.03[a]	11.6[a]	21.7[b]	1.13[b]	11.41[b]
Inoculated	23.0[a]	1.07[a]	8.57[a]	17.2[a]	0.53[a]	5.98[a]

Conclusion and further work

The pot trials described above allowed both the development and refinement of a new method which will ultimately be used to assign nematode tolerance ratings for released varieties. Based on the results from previous field studies N12 is considered tolerant to nematode infestation while N51 is considered susceptible. The results from this pot trial support these ratings and these varieties could therefore be used as standards in future nematode screening trials. More trials are required to confirm this result. Future work will also focus on identifying the optimal thermal time at which plants should be harvested before they become pot bound to ensure repeatability and consistency across seasons.

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