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Field evaluation of selected introgression clones for their resistance to root-knot nematodes

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Abstract Sugarcane nematodes, root-knot (RKN) and root-lesion (RLN), cause an estimated loss of over $80 million per year to the Australian sugar industry. In particular, RKN is a major problem if sugarcane is planted in sandy soil. No effective control method is available for sugarcane nematodes in Australia. Crop rotation and fallowing provide only short-term control and nematode populations usually bounce back within 12 months after these control methods. The use of nematicides is restricted due to inconsistent results, difficulty in application and the highly toxic nature of the chemicals to humans and the environment. No commercial cultivars are resistant to sugarcane nematodes. Recent glasshouse trials in Australia suggested that clones from introgression populations, originating from crossing between commercial canes and *Saccharum spontaneum* or *Erianthus arundinaceus*, possessed good resistance to root-knot nematodes. Field trials were established to determine the reliability of glasshouse resistance-screening results. Eight introgression clones that showed resistance to RKN in glasshouse trials were evaluated in a field in Wallaville, north of Childers. Test clones were planted in plots with high and low nematode populations and maintained up to the second-ratoon crop. Trial plots were assessed for nematodes each year 6 weeks after planting and ratooning. Three years of results showed that 7 of 8 introgression clones consistently maintained low numbers of RKN until the end of the trial period, and significantly ($P<0.05$) lower numbers of RKN (195 nematodes per kg of soil) compared to commercial standards (1500 nematodes per kg of soil). There was no difference in numbers of nematodes high and low nematode treatments after the second ratoon. These results suggested that the glasshouse-screening trials are reliable and can predict the field resistance of clones for RKN, and that introgression clones are a valuable source of resistance to these important pathogens/pests. Commercial cultivars Q208$^{\Phi}$ and Q240$^{\Phi}$ maintained high yield despite very high numbers of RKN, suggesting that these clones might be tolerant to RKN.

Key words Sugarcane nematodes, root-knot nematode, *Meloidogyne javanica*, *Saccharum spontaneum*, *Erianthus arundinaceus*, nematode resistance

INTRODUCTION

Plant-parasitic nematodes are a major restriction on sugarcane production worldwide (Ramouthar and Bhuiyan 2018). In Australia, they cause up to 20% yield loss per year (Blair and Stirling 2007). Nematode diversity in sugarcane is higher than any other agricultural crop, with more than 400 species and 48 genera of nematodes having been recorded from sugarcane roots and/or rhizosphere. The most important nematode pathogens in Australia are root-lesion nematode (*Pratylenchus* spp., predominately *P. zeae*) (RLN) and root-knot nematode (*Meloidogyne* spp.) (RKN) (Stirling and Blair 2000). RKN has been a major issue for sugarcane crops grown on sandy soil. Cadet and Spaull (2005) reported that resistance to nematodes was rare in commercial sugarcane clones, and this was confirmed for Australia by Blair (2005) and Stirling *et al.* (2011), who were unable to find any commercial cultivar with resistance to RKN.

Modern commercial cultivars are derived from crosses between noble cane *Saccharum officinarum*, and its wild relative *S. spontaneum*, which were then backcrossed to *S. officinarum* or other complex hybrids (Cox *et al.* 2000). These interspecific hybridisations provided high yield, resistance to diseases and tolerance to drought and water logging (Cox *et al.* 2000). In this process, selected hybrid progenies go through a series of backcrosses with noble canes to improve sugar content, a process known as 'nobilisaton' (Jeswiet 1930). The early hybrids formed the basis of sugarcane-breeding programs around the world.

A collaborative program between Australia and China in early 2000 used new sources of *Erianthus* spp. and *S. spontaneum* clones to generate new introgression families (Foreman *et al.* 2007). *E. arundinaceus* and *S. spontaneum* clones have several valuable traits for expanding the Australian germplasm collection, including high vigour, drought and waterlogging tolerance, and resistant to diseases. In previous studies, 10 *E. arundinaceus* clones were rated highly resistant to pachymetra root rot (Magarey and Croft 1996), and introgression clones originating from *E. arundinaceus* and *S. spontaneum* resistant to RKN in glasshouse screening trials have been identified (Croft *et al.* 2015; Bhuiyan *et al.* 2016). Reliability of the glasshouse screening trials under field conditions is unknown.

Here, we report on results of some selected introgression clones for resistance to RKN when planted in the field. We aimed to confirm glasshouse results by determining the field resistance and tolerance of a few progenies from *S. spontaneum* and *E. arundinaceus* crossing and of commercial cultivars against RKN. The multiplication of RKN populations was plotted against the threshold for root-knot nematode according to the SRA disease manual (Magarey 2013).

MATERIALS AND METHODS

Pre-treatment

Before the field trial was established, susceptible and resistant soybean hosts for root-knot nematodes were planted in split plots in order to create high and low nematode treatment plots. Soybean varieties A6785 and Leichardt were planted to suppress and increase the nematode populations, respectively. In addition to the cover crop pre-treatment, a nematicide, fenamiphos (Nemacur® 10G), was applied at the rate of 3 g of product per metre of row to the low nematode treatment plots to further reduce the number of nematodes as much as possible. The nematicide was incorporated into soil by sprinkler irrigation after the application.

Planting, harvesting and data collection

Eight introgression clones and six commercial cultivars were sourced from SRA Bundaberg and Mackay research stations (Table 1). The trial was established at Wallaville (25°5.577'S, 151°59.789'E) in August 2015 in a sandy loam soil, using a split-plot design of four replicates with two nematode treatments (low and high) arranged as main plots and clones as subplots. Each subplot comprised five rows by 10 m with a 1-m gap between subplots, and 1.6 m row spacing. The site had a history of high RKN populations, and soil samples were collected from each subplot before planting to determine the initial nematode population.

The plant, first- and second-ratoon crops were harvested in September 2016, 2017 and 2018, respectively. Subplot weight and a six-stalk sample for juice lab analysis were collected during harvest. Cane yield (TCH, t/ha), percentage commercial cane sugar (CCS) and sugar yield (TSH, t/ha) were calculated from this information.

Soil samples were collected 6 weeks after harvest of each crop and sent to SRA Woodford Pathology laboratory for nematode assessment; these were timed to coincide with the start of build-up of populations after ratooning (G. Stirling per. comm.). Soil samples were collected from three inner rows, approximately 1 m from the plot ends. Each sample was a composite of eight subsamples ('cores'), collected in a systematic zigzag pattern, on the row in amongst the roots to 25 cm deep. Soil core samples were then mixed together to get a representative sample, and approximately 300 g were weighed out. Nematodes were extracted from the soil samples using a modified Whitehead tray method (Whitehead and Hemming 1965). Approximately 250 g of the soil sample were placed on double-layered tissue paper on a steel mesh set in a flat tray. The soil and roots were almost covered in water and left for 48 h at 25°C. Nematodes were collected on a 38 µm sieve and the extract then poured into a 30 mL plastic vial. Extracted nematodes were stored at 6°C until they were counted under a compound microscope (10–40x) using a Hawksley slide counting chamber of 1 mL capacity.

Table 1. Introgression clones and commercial cultivars in the field trial at Wallaville.

Clone	Female parent	Male parent	Type[1]
MQB88-10825	MQB72-12011	Unknown	Ss BC1
KQB09-30117	QBYC05-20721	QC91-580	Ss BC2
KQB09-20048	KQ228	QBYC04-10577	Ss BC1
QBYN05-20563	YN2000-113	YueTang93-159	Ss BC1
KQ08-6014	QN80-3425	QBYC06-30415	Ea BC3
KQ08-1359	QN80-3425	QBYC06-30296	Ea BC3
KQ08-1348	QN80-3425	QBYC06-30415	Ea BC3
KQ08-1347	QN80-3425	QBYC06-30415	Ea BC3
KQ228ϕ	QN80-3425	CP74-2005	Comm
Q240ϕ	QN81-289	SP78-3137	Comm
Q232ϕ	QN80-3425	QS72-732	Comm
Q208ϕ	Q135	QN61-1232	Comm
Q200ϕ	QN63-1700	QN66-2008	Comm
Q135	NCo310	QN54-7096	Comm

[1] Ss BC1 = *S. spontaneum* backcross one; Ss BC2 = *S. spontaneum* backcross two; Ea BC3 = *E. arundinaceus* backcross three; Comm = commercial cultivar.

Statistical analysis

We fitted a linear mixed model to all datasets using PROC MIXED in SAS version 9.4 (SAS Institute, Cary) and performed a split-plot analysis with clone, treatments (treated and untreated) and their interaction effects were treated as fixed effects. Block (replication), interaction among blocks, and the error term (residual) were treated as random effects. There were no significant differences in nematode numbers between the treated and untreated main plots. Subsequently, the trial was re-analysed ignoring the main plot effects. Crop class (plant, first and second ratoon), clone and their interactions effects were treated as fixed effects. All possible interactions of the fixed effects were also included in the model. Degrees of freedom were adjusted using the Kenward–Roger method (Kenward and Roger 1997) and normality of residuals was tested using PROC UNIVARIATE of SAS. Nematode data were log-transformed (ln(x+1)) before analysis. Estimated log-transformed values were then back-transformed for presentation of the results. For the appropriate significant factors, protected-mean comparisons of all possible pairwise differences of the means were tested at alpha = 0.05, using Fisher's protected LSD test. *PDMIX800* SAS Macro was used to convert mean separation output to letter groupings (Saxton 1998).

RESULTS

Most of the introgression clones were highly resistant to RKN, maintaining nematode populations well below the threshold level (Magarey 2013) over the 3 years (Figure 1). Two *S. spontaneum* BC1 clones, MQB88-10825 and QBYN05-20563, showed highest level of resistance, maintaining the RKN population between 1 and 30 nematodes per 200 g soil over the three crops. Two *E. arundinaceus* BC3 clones were the second best, maintaining the RKN population between 1 and <100 nematodes per 200 of soil. All commercial clones supported high number of RKN, with the second-ratoon crop supporting the highest number of RKN compared to plant and first-ratoon crop. The highest nematode numbers were on Q232ϕ (>1200 nematodes per 200 g of soil), followed by Q208ϕ (>1100 nematodes per 200 g of soil) in the second-ratoon crop.

Other species of nematodes were recorded in only small numbers, except for root-lesion nematodes (*Pratylenchus zeae*) (Table 2). Introgression clone MQB88-10825 supported the highest number of root-lesion nematodes in the second ratoon.

There were significant differences in cane yield, CCS and sugar yield among crop classes and clones (Table 3), with the interaction of crop class and clone significant for cane yield and CCS, but not for sugar yield.

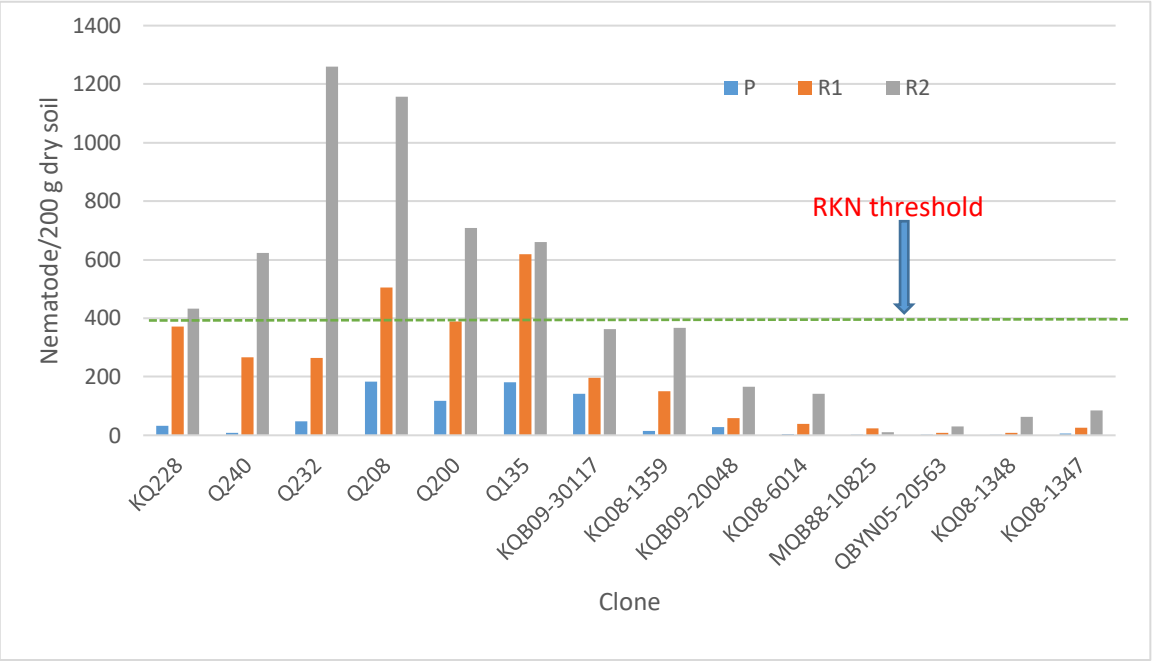

Figure 1. Root-knot nematodes (RKN) per 200 g of soil in the field trial at Wallaville from 2016 to 2018 in plant (P), first-ratoon (R1) and second-ratoon (R2) crops.

Table 2. Numbers[1] of root-lesion (RLN) and other nematodes (per 200 g of dry soil) present in the field trial at Wallaville from 2016 to 2018 in plant (P), first-ratoon (R1) and second-ratoon (R2) crops.

Clone	RLN			Other nematodes		
	P	R1	R2	P	R1	R2
Q208(t)	215 a	373 a	192 a	14 a	28 a	25 a
KQB09-30117	189 a	347 a	194 a	29 ab	49 a	14 b
Q135	182 a	233 a	285 a	36 a	81 a	32 a
KQ08-1348	170 a	175 a	360 a	24 a	45 a	13 a
MQB88-10825	129 b	140 b	793 a	18 a	44 a	41 a
Q240(t)	117 a	159 a	271 a	5 b	42 a	19 a
KQ08-1359	112 a	217 a	227 a	66 a	31 ab	14 b
KQ08-1347	95 b	127 ab	280 a	33 a	48 a	31 a
KQ228	62 a	119 a	99 a	17 a	30 a	27 a
KQ08-6014	60 b	155 a	261 a	17 a	31 a	10 a
KQB09-20048	54 b	158 a	319 a	11 b	49 a	22 ab
Q200(t)	45 b	187 a	127 a	35 a	38 a	18 a
Q232(t)	39 b	119 a	98 a	16 b	73 a	46 ab
QBYN05-20563	22 b	187 a	312 a	15 a	27 a	9 a

[1]Values are the means of four replications, and those followed by same letter(s) in each row for each type of nematode are not significantly different according to Fisher's Protected LSD test (*P<0.05*).

Table 3. Type-3 test of fixed effects of yield components for crop class (plant, first- and second-ratoon crops) and clones/controls and their interactions.

Effect	Numerator DF	Denominator DF	Cane yield (t/ha)		CCS		Sugar yield (t/ha)	
			F value	Pr > F	F value	Pr > F	F value	Pr > F
Crop	2	291	16.13	<0.0001	114.75	<0.0001	22.04	<0.0001
Clone/Controls	13	291	10.1	<0.0001	75.67	<0.0001	16.97	<0.0001
Crop*Clone	26	291	1.67	0.0241	1.79	0.0123	0.95	0.5364

Two popular cultivars Q208(ɸ) and Q240(ɸ) maintained consistently high sugar yields across crops (Table 4). Other commercial cultivars had lower sugar yields in the second ratoon than in the plant and first-ratoon crops. As expected most of the introgression clones had low sugar yields, except for KQB09-20048 that yielded about the same as Q208(ɸ) and Q240(ɸ). Most of the introgression clones maintained similar sugar yields across the three crops.

Table 4. Cane yield[1], percentage commercial cane sugar[1] (CCS) and sugar yield[1] of clones in the plant, first-ratoon and second-ratoon crops.

Clone	Cane yield (t/ha)			CCS			Sugar yield (t/ha)		
	P	R1	R2	P	R1	R2	P	R1	R2
KQ228(ɸ)	135 a	110 b	93 b	13.6 b	16.1 a	14.6 b	18.1 a	17.6 a	13.7 b
Q240(ɸ)	89 a	98 a	105 a	13.9 b	16.3 a	14.5 b	11.9 b	15.5 a	15.5 a
Q232(ɸ)	113 a	106 a	90 a	13 b	15.4 a	13.9 b	14.7 ab	16.2 ab	12.7 b
Q208(ɸ)	117 a	107 a	103 a	13.5 b	16.2 a	14.5 b	15.5 a	17.4 a	14.9 a
Q200(ɸ)	97 a	91 ab	72 b	12.2 c	15.4 a	13.5 b	11.5 ab	13.9 a	9.6 b
Q135	117 a	100 ab	78 b	12 c	15.1 a	13.7 b	13.7 ab	14.7 a	10.7 b
MQB88-10825	106 b	149 a	126 ab	5.4 b	6.2 a	6.1 a	5.6 b	9.2 a	7.8 ab
KQB09-30117	110 a	104 a	75 b	11.6 c	14.4 a	13.1 b	12.3 ab	14.8 a	9.8 b
KQB09-20048	110 a	120 a	101 a	11.5 b	13.9 a	13.7 a	12.4 b	16.4 a	13.8 ab
QBYN05-20563	98 a	106 a	95 a	10.7 b	12.5 a	9.3 c	10.4 ab	13 a	9.1 b
KQ08-6014	73 a	78 a	59 a	10.3 b	13.1 a	12.8 a	7.5 a	9.9 a	7.4 a
KQ08-1359	84 a	63 ab	56 b	10.6 b	14.3 a	13.4 a	8.7 a	8.9 a	7.4 a
KQ08-1348	109 a	104 a	90 a	11.9 b	14.6 a	13.4 a	13.1 a	15 a	12.1 a
KQ08-1347	122 a	101 ab	96 b	10.5 b	13.5 a	11.5 b	12.6 a	13.5 a	11.1 a

[1]Values are the means of four replications, and values followed by same letter(s) in each row for each yield component are not significantly different according to Fisher's Protected LSD test (*P<0.05*)

DISCUSSION

RKN is one of the detrimental nematode pests in the Australian sugar industry and in many horticultural crops. Here, we have shown that introgression clones provide potential resistance to RKN, as most of the introgression clones that we tested maintained much lower RKN populations that did the commercial cultivars. This confirmed results of the glasshouse screening trials for RKN resistance (Croft *et al.* 2015; Bhuiyan *et al.* 2016), and it is the first report from Australia showing sugarcane clones highly resistant to RKN under field conditions. We also showed that some of the current commercial sugarcane cultivars grown in Australia possess low levels of resistance to RKN.

One of our objectives was to identify if any of the introgression clones possess tolerance to RKN. In order to achieve this, we tried to create high and low nematode populations. However, there were no significant differences in nematode populations between the two treatments. We found that the treatment of a combination of susceptible and resistant cover crops and subsequent application of nematicides did not change the nematode numbers – there were low nematode counts prior to establishment of the trial. Tolerant clones would have minimal yield response differences between the low and high nematode treatments. More works needs to be done to achieve a suitable experimental system for testing tolerance.

Nematode populations were exceptionally high in the second ratoon, in particular, in commercial cultivars. Sugar yield for most of the major cultivars also decreased significantly over crops. This could be, at least partly, because the nematode population increased to well over the threshold level of 400 RKN per 200 g of soil (Magarey 2013; Ramouthar and Bhuiyan 2018). Interestingly, Q208(ɸ) and Q240(ɸ) maintained their high yields despite the increasing RKN numbers over crops. Those two cultivars appear to be tolerant to RKN but further investigation should be conducted to confirm these results and identify further tolerant varieties for the Australian sugar industry. In South Africa, growing tolerant varieties increased yields by 25–124% over those of a susceptible variety and nematode-tolerant varieties can sustain yield over time through continued production of high-yielding ratoon crops (Spaull and Cadet 2003). In Louisiana, the nematode tolerant clone CP70-321 comprised 20% of the cane area grown, and in Brazil and South Africa when growers switched from susceptible to tolerant cultivars yield losses decreased by 15–47% (Cadet and Spaull 2005). Limited information is available on the tolerance of Australian sugarcane cultivars to nematodes.

Introgression clone KQB09-20048 maintained high cane and sugar yields and CCS across the three crop classes and at the same time maintained the nematode population at very low levels. This clone, with intermediate resistance to sugarcane smut and pachymetra root rot, has potential to become a commercial cultivar with the added benefit of nematode resistance. Most of the other introgression clones kept RKN population at very low levels, but yield performance was poor. These clones could be used as parents for breeding clones for nematode resistance. Nematode-resistant sugarcane crops could be used as a rotation crop with nematode-susceptible vegetable and grain crops where nematodes are a problem and would provide a huge benefit for sugarcane growers.

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