***Saccharum spontaneum*, a Novel Source of Resistance to Root-Lesion and Root-Knot Nematodes in Sugarcane**

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Abstract

Root-lesion nematode (*Pratylenchus zaei*) and root-knot nematode (*Meloidogyne javanica*) are two important pathogens of sugarcane (*Saccharum* hybrid). No commercial cultivars are resistant to these nematodes in Australia. Twenty accession lines of *S. spontaneum*, a wild relative of sugarcane, were tested against these two nematode species. *S. spontaneum* lines were tested twice for resistance to root-lesion nematode and three times for root-knot nematode. Reproduction (final population/starting population) of root-lesion nematodes was significantly lower in 17 of the 20 *S. spontaneum* accession lines tested in two experiments compared with two commercial cultivars. Four *S. spontaneum* lines supported a significantly lower number of root-lesion nematodes per gram of root than that of two commercial

sugarcane cultivars. Reproduction of root-knot nematodes was significantly lower in 16 *S. spontaneum* lines compared with two commercial cultivars. Fourteen of the *S. spontaneum* lines tested supported significantly fewer eggs per gram of root compared with two commercial cultivars. This study showed that *S. spontaneum* lines possessed resistance for root-lesion and root-knot nematodes. Targeted crossing with commercial hybrid parental lines should be conducted to introduce nematode resistance into sugarcane cultivars for the Australian sugar industry.

Keywords: field crops, *Saccharum spontaneum*, *Meloidogyne javanica*, *Pratylenchus zaei*, nematode resistance

In Australia, nematodes cause 5 to 20% yield loss per year with an estimated cost of >\$80 million (Blair and Stirling 2007). Plant-parasitic nematodes are considered to be a major constraint to sugarcane production worldwide (Ramouthar and Bhuiyan 2018). The most important nematode pathogens in Australia are root-lesion nematode (*Pratylenchus* spp., predominately *P. zaei*) and root-knot nematode (*Meloidogyne* spp., particularly *M. javanica*) (Blair et al. 1999a). Ramouthar and Bhuiyan (2018) reported that resistance to nematodes was rare in commercial sugarcane cultivars, and this was confirmed for Australian cultivars by Blair (2005) and Stirling et al. (2011), who were unable to find any commercial cultivar with resistance to either root-lesion or root-knot nematodes.

P. zaei and *M. javanica* are widespread in all Australian cane-growing regions, where *M. javanica* is abundant in sandy soil and *P. zaei* occurs in all soil types (Blair and Stirling 2007; Blair et al. 1999a, b). In Australia, sugarcane is a semiperennial crop commonly harvested annually or biannually for 4 to 6 years before replanting. Rotation crops, such as soybean and peanut, and short-term fallowing only reduce nematode numbers for about 4 to 6 months and nematode population densities increase to their original level within 12 months when sugarcane is replanted (Stirling 2008; Stirling et al. 2001). Nematicides are relatively expensive and only reduce nematode populations for a few months (Blair and Stirling 2007). It is difficult to prevent crop loss attributable to nematodes because current cultivars have limited resistance (Stirling 2006) and there are no economically effective alternative control measures.

The genetic base of modern sugarcane cultivars is narrow. Cultivars are derived from only a relatively small number of interspecific

hybridization events involving a handful of *Saccharum officinarum* and *S. spontaneum* accessions (Arceneaux 1967; Roach 1989). The commercial cultivars were developed from the intercrossing between early hybrids and backcrossing with *S. officinarum*, termed “nobilization” (Yu et al. 2018). These hybrids have been repeatedly used for developing new sugarcane cultivars, and it has been suggested that this has led to diminishing genetic gain in terms of yield and pest and disease resistance in recent years (Kandel et al. 2018). Wild relatives of sugarcane, such as *Erianthus* spp. and *S. spontaneum*, have a number of valuable traits, including high vigor, drought and water-logging tolerance, and resistance to diseases (Bonnert and Henry 2011; Jackson and Henry 2011). Stirling et al. (2011) identified a number of lines of *E. arundinaceus* and progenies derived from crosses between *E. arundinaceus* and commercial sugarcane with useful resistance to root-knot and root-lesion nematodes. The main drawbacks of *Erianthus* spp. are that they seldom produce fertile progeny when crossed with commercial cultivars, and it is harder to retain the target traits because of the rapid loss of *Erianthus* chromosomes within one or two backcross generations (Piperidis et al. 2000). In early 2000, backcrossed families derived by crossing Chinese accessions of *S. spontaneum* and commercial sugarcane cultivars in China were imported to Australia. Some of these families contained lines that possess some degree of resistance to root-knot and root-lesion nematodes (Bhuiyan et al. 2016; Croft et al. 2015). This provided evidence that *S. spontaneum* could be a good source of resistance to nematodes. There have been limited efforts to identify nematode resistance in collections of *S. spontaneum*.

A *Saccharum* germplasm collection at Sugar Research Australia (SRA) Meringa maintains a number of *S. spontaneum* accession lines collected from various countries. The aim of this study was to compare the root-lesion and root-knot nematode resistance of 20 *S. spontaneum* lines from the SRA germplasm collection to the resistance of two commercial cultivars and three resistant lines of *E. arundinaceus* (Bhuiyan et al. 2014, 2016; Croft et al. 2015; Stirling et al. 2011).

Materials and Methods

Plant materials. Twenty *S. spontaneum* lines were collected from the SRA Meringa germplasm collection in March 2013. These lines were then maintained in 5-liter pots at SRA Woodford Pathology

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Station. Table 1 lists the accession lines, the collection location, and chromosome number. Two commercial cultivars previously shown to be susceptible to both species of nematodes and three *E. aurundinaceus* lines previously shown to be resistant were included for comparison. Stalks of commercial sugarcane and wild accession lines were stripped of leaves and leaf sheaths, cut into one-budded cuttings using an electric saw, and washed in running water to remove dirt and extraneous materials. The cuttings were then treated with hot water at 50°C for 30 min, planted in trays of vermiculate, and germinated at 31°C for 2 weeks.

Root-lesion nematode. *Experiments 1 and 2.* *S. spontaneum* lines were screened for resistance to *P. zeae* in 2017 (experiment 1) and in 2018 (experiment 2). Germinated cuttings were planted in 700-ml plastic pots (one plant per pot) in sterilized Searles seed raising mix (containing an unknown quantity of plant mulch, topsoil, sand, wood dust, manure, and chemical fertilizers; J. C. & A. T. Searle Pty Ltd, Kilcoy, QLD, Australia) in April. Pots were maintained on an air-conditioned bench in a glasshouse. The temperature within the pots was maintained at $26 \pm 2^\circ\text{C}$ for the duration of the trial. *P. zeae* nematodes were reared on sterile carrots in clear plastic bottles for approximately 2 months as described previously (Kagoda et al. 2010; Moody et al. 1973). Nematodes were washed from the carrot cultures with deionized water and stored in a beaker before inoculation. Approximately 4 weeks after planting, the plants were inoculated with a mixture of juvenile and adult *P. zeae*. The 10 most vigorous plants of each line were inoculated with approximately 2,000 nematodes per plant. Nematodes were added in 5 ml of deionized water at the base of the plant in two holes using a pipette. Accession lines IK76-41 and MOL1032 were not included in experiment 2 because of germination failure.

Root-knot nematode. *Experiment 3, 4, and 5.* The *S. spontaneum* lines were screened for resistance to *M. javanica* in 2016 (experiment 3), 2017 (experiment 4), and 2018 (experiment 5). The procedures for germination of the cuttings and growth of the plants were the same as for the root-lesion nematode experiments, except the 700-ml plastic pots were filled with sterilized fine white washed sand instead of

potting mix (Caboolture Gravel & Landscapes, Morayfield, QLD, Australia). *M. javanica* nematodes were reared in the roots of tomato plants in a glasshouse as described by Van den Bergh et al. (2002). *M. javanica* eggs were extracted from the tomato plants using the bleach method (Stirling et al. 2011). Approximately 2 months after inoculation, roots of the tomato plants were washed free of sand, cut into 2-cm pieces, and soaked in 0.5% sodium hypochlorite (NaOCl) for 5 min. Egg suspensions were passed through a 150- μm sieve to remove debris and were retrieved on a 38- μm sieve. Each plant was inoculated with 5,000 eggs of *M. javanica*, using the same method as for *P. zeae*. Accession lines IK76-41 and MOL1032 were not included in experiment 5 because of germination failure.

Trial design and assessment. Experiments for both nematodes were established separately in a glasshouse at SRA Woodford Pathology Research Station (26.55° S, 152.46° E). Each experiment was set out on air-conditioned benches using a randomized complete block design in 10 replicate pots and one plant per replication. Two data loggers (Thermodata Pty Ltd, Brisbane, QLD, Australia) were included in each trial by burying them approximately 5 cm deep in seed raising mix or sand to record the temperature of the potting media during the trial period. All experiments were harvested approximately 11 weeks after inoculation (Bhuiyan et al. 2014). For measuring shoot biomass, shoots were cut near the base of the plant and were then cut into 6- to 10-cm pieces and put into a brown paper bag. The samples were maintained in a drying cabinet (forced draft) for 2 weeks at 65°C and then weighed. For measuring root biomass, the whole root system was weighed and then a subsample was collected, weighed, and dried as described for shoots. The final weight of the root samples was measured and then the biomass of the whole root system was estimated. The remainder of the root system was used to assess nematode population.

P. zeae was extracted from a mixture of root and potting mix using a modified Whitehead tray method (Whitehead and Hemming 1965). Approximately 250 g of the mixture was placed on double-layered

Table 1. List of *Saccharum spontaneum* lines, commercial cultivars, and *Erianthus arundinaceus* lines

Accession line	Species	Origin	Chromosome number ^a	Comments
Q135	<i>Saccharum</i> hybrid	Australia	–	Sugar Research Australia (SRA) commercial cultivar; susceptible to <i>Pratylenchus zeae</i> and <i>Meloidogyne javanica</i>
Q208 ^A	<i>Saccharum</i> hybrid	Australia	–	SRA commercial cultivar; susceptible to <i>P. zeae</i> and <i>M. javanica</i>
IK76-41	<i>S. spontaneum</i>	Indonesia	–	–
MOL1032	<i>S. spontaneum</i>	Unknown	64	Aitken et al. (2018)
MOL5904	<i>S. spontaneum</i>	Unknown	80	Aitken et al. (2018)
US56-15-2	<i>S. spontaneum</i>	Thailand	–	Aitken et al. (2018)
NG28-101	<i>S. spontaneum</i>	Papua New Guinea	114, 80	Aitken et al. (2018)
IK76-86	<i>S. spontaneum</i>	Indonesia	–	Aitken et al. (2018)
IS76-173	<i>S. spontaneum</i>	Indonesia	–	Aitken et al. (2018)
OKINAWA	<i>S. spontaneum</i>	Japan	112	Aitken et al. (2018)
IMP1944	<i>S. spontaneum</i>	Indonesia	–	Aitken et al. (2018)
DJANTOER_2	<i>S. spontaneum</i>	Indonesia	112	Aitken et al. (2018)
IK76-49	<i>S. spontaneum</i>	Indonesia	–	Aitken et al. (2018)
SES343	<i>S. spontaneum</i>	India	64	Aitken et al. (2018)
GLAGAH-1286	<i>S. spontaneum</i>	Indonesia	58	Aitken et al. (2018)
SES208A	<i>S. spontaneum</i>	India	64	Aitken et al. (2018)
US71-4-1	<i>S. spontaneum</i>	Unknown	–	Aitken et al. (2018)
SES182	<i>S. spontaneum</i>	India	52	Aitken et al. (2018)
MANDALAY	<i>S. spontaneum</i>	Indonesia	96	Aitken et al. (2018)
COIMBATORE	<i>S. spontaneum</i>	India	64	Aitken et al. (2018)
SES516	<i>S. spontaneum</i>	Thailand	96	Aitken et al. (2018)
KLOET	<i>S. spontaneum</i>	Indonesia	112 to 114	Aitken et al. (2018)
IJ76-370	<i>E. arundinaceus</i>	Indonesia	60	Besse et al. (1997); resistant to <i>P. zeae</i> and <i>M. javanica</i>
IJ76-333	<i>E. arundinaceus</i>	Indonesia	60	Resistant to <i>P. zeae</i> and <i>M. javanica</i>
IK76-48	<i>E. arundinaceus</i>	Indonesia	60	Besse et al. (1997); resistant to <i>P. zeae</i> and <i>M. javanica</i>

^a Dashes indicate no data available.

Kleenex tissue paper (Kimberly-Clark Australia Pty, Mission Point, NSW, Australia) 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 ± 1°C. Nematodes were collected on a 38-µm sieve and then the extract was poured into a 30-ml plastic vial.

M. javanica extracted from roots was washed free of sand. For the extraction of eggs, roots were submerged in 1% NaOCl and agitated for 5 min. The NaOCl solution was then poured through two sieves (a 150-µm sieve over a 38-µm sieve), with eggs being collected in the lower sieve, and washed into a 30-ml plastic vial.

Extracted nematodes and eggs samples were stored at 6°C until counting. Counting of *P. zeae* or eggs of *M. javanica* was performed under a compound microscope (10 to 40×) using a Peters counting chamber (Chalex Corporation, Wallowa, OR, USA) (Peters 1952) of 1-ml capacity.

Table 2. *Pratylenchus zeae* reproduction factor (RF), nematode per gram of root, and shoot and root biomass for experiment 1 for the two commercial cultivars, 20 *Saccharum spontaneum* lines, and three *Erianthus arundinaceus* lines

Accession line	RF ^x	Nematode/g root	Shoot biomass (g)	Root biomass (g)
Q135 ^y	35 a	1,138 ab	22.01 a	26.61 abcdefg
Q208 ^{Ay}	28 a	896 abcde	19.54 ab	32.29 abcd
IK76-41	16 b	379 cdefgh	16.17 abc	36.09 abc
MOL5904	15 bc	944 abcd	6.24 gh	16.66 defghij
IK76-86	13 bcd	1,015 abc	10.04 cdefg	15.52 efghij
US56-15-2	13 bcd	724 abcdef	11.6 cdef	18.21 cdefghij
DJANTOER_2	12 bcd	679 abcdefg	10.21 cdefg	20.33 cdefghi
NG28-101	11 bcde	911 abcde	6.96 fgh	16.62 defghij
IK76-49	11 bcde	983 abc	8.35 efgh	12.66 hij
IMP1944	10 cde	1,010 abc	10.26 cdefg	14.91 fghij
SES343	10 cdef	1,169 ab	10.96 cdef	10.4 ij
MOL1032	10 def	1,513 a	7.64 efgh	9.34 j
MANDALAY	9 def	462 bcdefg	10.36 cdefg	19.98 cdefghi
US71-4-1	9 def	321 efghi	14.36 abcd	27.6 abcdefg
IS76-173	9 def	1,230 ab	6.93 fgh	9.85 ij
SES182	9 def	139 hij	15.39 abcd	45.31 ab
OKINAWA	9 def	538 abcdefg	7.38 efgh	17.61 defghij
SES208A	9 def	249 ghi	11.99 bcde	29.27 abcdef
COIMBATORE	8 def	688 abcdefg	9.57 defg	14.1 ghij
GLAGAH-1286	8 efg	300 fghi	14.43 abcd	24.26 bcdefgh
KLOET	7 fg	333 defghi	8.59 efg	22.73 bcdefgh
SES516	5 g	121 ij	19.12 ab	30.83 abcde
IJ76-333 ^z	3 h	122 ij	4.92 h	26.88 abcdefg
IK76-48 ^z	2 h	33 k	20.34 a	50.23 a
IJ76-370 ^z	2 h	58 jk	7.88 efgh	33.11 abcd

^x Data are mean values of the respective accession line across the row. Data were log-transformed ($\ln[x + 1]$) before analysis. Back-transformed data are presented. Means followed by the same letter(s) in a column are not significantly different ($P = 0.05$) using Fisher's protected least significant difference test.

^y Commercial cultivar.

^z *Erianthus arundinaceus* line.

Table 3. Pearson correlation coefficients to compare plant biomass and nematode counts for *Pratylenchus zeae* in experiments 1 and 2

Parameters	RF ^z	Nematode/g root
Experiment 1		
Shoot biomass	0.18*	-0.14
Root biomass	0.20*	-0.44***
n	150	150
Experiment 2		
Shoot biomass	0.05	-0.30**
Root biomass	-0.24*	-0.55***
n	110	110

^z Reproduction factor (RF) = proportion of final nematode population after harvesting and initial population used for inoculation. Significant at * $P \leq 0.05$, ** $P < 0.001$, and *** $P < 0.0001$.

Data collection and statistical analysis. The number of nematodes (*P. zeae*) or eggs (*M. javanica*) per plant was calculated, and the reproduction factor (RF) was determined as follows: $RF = Pf/Pi$, where Pf is the final nematode population or egg number after extraction and Pi is the initial nematode population or number of eggs used for inoculation. The number of nematodes or eggs per gram of dry root was estimated from the total number of nematodes or eggs present in the total root biomass divided by the weight of the root system. A linear mixed model was fitted to all data sets using PROC MIXED in SAS version 9.4 software (SAS Institute, Cary, NC). For each experiment, accession lines were treated as a fixed effect. Block (replication) and the error term (residual) were treated as random effects. Interactions of the fixed effects were 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 in SAS. Values were log-transformed ($\ln[x + 1]$) before analysis. Estimated log-transformed values were then back-transformed for presentation in the results. For the appropriate significant factors, protected-mean comparisons of all possible pairwise differences of the means were tested at alpha equal to 0.05, using Fisher's protected least significant difference test. The PDMIX800 SAS macro was used to convert mean separation output to letter groupings (Saxton 1998). PROC CORR in SAS was used to calculate correlations between observed values for root and shoot biomass and nematode or egg counts. Furthermore, correlations between nematode or egg counts among experiments were determined.

Results

Root-lesion nematode. *Experiment 1.* The two commercial cultivars (Q208^A and Q135) had significantly higher RF values (≥ 28) than the *S. spontaneum* lines, which were significantly higher than the *E. arundinaceus* lines (Table 2). There was significant variation in RF values between *S. spontaneum* lines, with RF values ranging from 5 (SES516) to 16 (IK76-41). The number of nematodes per gram of root varied from 121 (SES516) to 1,513 (MOL1032) for *S. spontaneum* lines. Seven *S. spontaneum* lines supported significantly fewer nematodes per gram of root compared with the commercial cultivar Q135, and four lines supported significantly fewer nematodes per gram of root compared with the commercial cultivar Q208^A. Three *E. arundinaceus* lines had the lowest nematode numbers per gram of root. There were significant differences for both root and shoot biomass between the *S. spontaneum* lines and between some of the *S. spontaneum* lines and the two commercial cultivars and the *E. arundinaceus* lines. Shoot biomass for *S. spontaneum* lines varied from 6 (MOL5904) to 19 g (SES516) and root biomass ranged from 9 (MOL1032) to 45 g (SES182). There were significant correlations between shoot and root biomass and RF values and a highly significant negative correlation between root biomass and nematodes per gram of root (Table 3). The correlation between shoot biomass and nematodes per gram of root was not significant.

Experiment 2. Experiment 2 was similar to experiment 1 in that 17 of the 18 *S. spontaneum* lines had significantly lower RF values compared with the two commercial cultivars and the RF values for the three *E. arundinaceus* lines were significantly lower than 16 of the 18 *S. spontaneum* lines and the commercial cultivars. Nine *S. spontaneum* lines supported a significantly lower number of nematodes per gram of root (17 to 78) compared with the two commercial cultivars (>300) (Table 4). The shoot and root biomass was in general greater in experiment 2 than experiment 1 and there was significant variation between lines. Root biomass was negatively correlated with RF values and nematodes per gram of root (Table 3). In experiment 2, shoot biomass was not significantly correlated with RF value but was negatively correlated with nematodes per gram of root.

There were strong correlations between experiment 1 and experiment 2 for RF values ($r = 0.97$) and nematodes per gram of root ($r = 0.67$) (Table 5).

Root-knot nematodes. *Experiment 3.* Nineteen of the 20 *S. spontaneum* lines and the three *E. arundinaceus* lines had significantly

lower RF values than the two commercial cultivars Q135 and Q208^A (Table 6). Thirteen of the *S. spontaneum* lines and the three *E. arundinaceus* lines had fewer nematode eggs at the end of the experiment than were used to inoculate the pots at the commencement of the experiment. Seventeen of the 20 *S. spontaneum* and the three *E. arundinaceus* lines had a significantly lower number of *M. javanica* eggs per gram of root compared with the two commercial cultivars. Shoot biomass was significantly higher in two *E. arundinaceus* lines (IJ76-370 and IK76-48) and *S. spontaneum* line SES208A than the two commercial cultivars. Shoot and root biomass were significantly negatively correlated with eggs per gram of root but showed no significant correlation with RF (Table 7).

Experiment 4. *M. javanica* egg production in the two commercial cultivars was higher in experiment 4 than in experiment 3. Seventeen of the 20 *S. spontaneum* lines and the three *E. arundinaceus* lines had significantly lower RF values than the two commercial cultivars (Table 8). Thirteen of the 20 *S. spontaneum* lines and one *E. arundinaceus* line had fewer nematode eggs at the end of the experiment than were used to inoculate the pots at the commencement of the experiment. Fifteen of the 20 *S. spontaneum* lines and two *E. arundinaceus* lines had a significantly lower number of *M. javanica* eggs per gram of root compared with the two commercial cultivars. Shoot and

Table 4. *Pratylenchus zeae* reproduction factor (RF), nematode per gram of root, and shoot and root biomass in experiment 2 for the two commercial cultivars, 19 *Saccharum spontaneum* lines, and three *E. arundinaceus* lines

Accession line	RF ^x	Nematode/g root	Shoot biomass (g)	Root biomass (g)
Q135 ^y	23 a	612 a	20.36 abcde	49.12 def
Q208 ^{Ay}	17 ab	322 abc	25.64 a	83.1 abcde
MOL5904	12 bc	283 abcd	15.45 efg	43.88 efgh
US56-15-2	9 cd	343 abc	16.23 efg	40.31 fgh
NG28-101	8 cde	159 bcde	15.62 efg	53.36 bcdef
IK76-86	8 de	154 bcde	13.07 gh	59.87 bcdef
IS76-173	7 cdef	639 a	14.51 efgh	19.92 h
OKINAWA	7 def	382 ab	11.18 h	23.15 gh
IMP1944	7 def	176 bcde	24.75 abc	53.20 cdef
DJANTOER_2	6 defg	219 abcde	17.27 defg	46.66 efg
IK76-49	6 defg	48 fgh	18.57 bcdef	72.41 abcdef
SES343	6 efg	75 efg	22.11 abcd	96.07 abcd
GLAGAH-1286	5 fgh	78 efg	15.54 efg	69.59 abcdef
SES208A	4 fgh	46 fgh	18.27 cdef	57.10 bcdef
US71-4-1	4 fgh	40 fgh	24.02 abc	82.95 abcde
SES182	4 gh	26 hi	17.02 defg	128.80 a
MANDALAY	4 ghi	117 cdef	13.89 fgh	43.23 efgh
COIMBATORE	3 hij	102 defg	25.51 ab	40.49 fgh
SES516	3 hijk	34 gh	17.49 defg	78.29 abcdef
KLOET	2 ijkl	17 hij	16.16 efg	108.02 ab
IJ76-370 ^z	2 jkl	16 hij	12.94 gh	101.08 abc
IJ76-333 ^z	2 kl	10 ij	15.03 efgh	126.89 a
IK76-48 ^z	1 l	7 j	16.94 defg	126.67 a

^x Data are mean values of the respective accession line across the row. Data were log-transformed ($\ln[x + 1]$) before analysis. Back-transformed data are presented. Means followed by the same letter(s) in a column are not significantly different ($P = 0.05$) using Fisher's protected least significant difference test.

^y Commercial cultivar.

^z *Erianthus arundinaceus* line.

Table 5. Pearson correlation coefficients to compare RF values and number of *Pratylenchus zeae* per gram of root between experiments 1 and 2

Parameter	Experiment 2 ^z	
	RF	Nematode/g root
Experiment 1	0.97*	0.67**
<i>n</i>	23	23

^z Significant at * $P < 0.0001$ and ** $P = 0.0007$.

root biomass varied significantly among the lines. The only significant correlation was between shoot biomass and RF value (Table 7).

Experiment 5. *M. javanica* egg production in the two commercial cultivars in experiment 5 was lower than in experiment 4. Sixteen of the 18 *S. spontaneum* lines and the three *E. arundinaceus* lines had significantly lower RF values than the two commercial cultivars (Table 9). The *S. spontaneum* line, MOL5904, had a significantly higher RF value than all other lines and its number of eggs per gram of root was higher than all other lines except for IK76-49. Nine *S. spontaneum* lines and one *E. arundinaceus* line had fewer

Table 6. *Meloidogyne javanica* reproduction factor (RF), eggs per gram of root, visual rating, and shoot and root biomass in experiment 3 for the two commercial cultivars, 20 *S. spontaneum* lines, and three *Erianthus arundinaceus* lines

Accession line	RF ^x	Eggs/g root	Shoot biomass (g)	Root biomass (g)
Q135 ^y	9 a	22,429 a	10.6 fg	2.1 ghij
Q208 ^{Ay}	8 a	10,159 a	13.2 cdef	4.3 cdefg
MOL5904	5 ab	18,777 a	11.3 efg	1.4 ij
MOL1032	4 bc	10,313 abc	10.5 efg	2.6 fghij
IK76-49	3 bc	8,634 ab	7.9 gh	1.9 ghij
SES182	2 c	1,396 bcd	17.1 bcde	5.9 bcdef
SES343	2 c	1,139 cd	18 bcd	3.6 efgh
KLOET	1 d	201 def	10.6 fg	9.1 bc
US56-15-2	1 d	311 de	17.3 bcde	5.2 bcdef
IS76-173	0 d	679 d	9.1 fgh	1.6 hij
DJANTOER-2	0 d	1,155 cd	3.9 i	1 j
OKINAWA	0 d	5 ij	4.3 i	1.3 ij
IK76-86	0 d	76 efg	18.4 bcd	8.1 bcd
GLAGAH-1286	0 d	18 ghij	17.7 bcde	4 defgh
MANDALAY	0 d	8 hij	11.3 efg	6.6 bcdef
SES516	0 d	2 j	20.1 abc	5.9 bcdef
IJ76-333 ^z	0 d	42 fgh	18.1 bcd	9.8 b
US71-4-1	0 d	16 ghij	19.7 abc	3.6 efgh
IJ76-370 ^z	0 d	10 hij	21.5 ab	22 a
IMP1944	0 d	1,301 bcd	11.9 defg	1.3 j
COIMBATORE	0 d	1,413 bcd	5.9 hi	1.4 ij
SES208A	0 d	31 fghi	21.2 ab	7.3 bcde
NG28-101	0 d	587 de	12.2 defg	2.9 fghij
IK76-48 ^z	0 d	12 ghij	29.1 a	10.4 b
IK76-41	0 d	34 fghi	10.2 fg	3.5 efghi

^x Data are mean values of the respective accession line across the row. Data were log-transformed ($\ln[x + 1]$) before analysis. Back-transformed data are presented. Means followed by the same letter(s) in a column are not significantly different ($P = 0.05$) using Fisher's protected least significant difference test.

^y Commercial cultivar.

^z *E. arundinaceus* line.

Table 7. Pearson correlation coefficients to compare shoot and root biomass, RF values, and *Meloidogyne javanica* eggs/g of root in experiments 3, 4, and 5

Parameters	RF ^z	Eggs/g root
Experiment 3		
Shoot biomass	-0.07	-0.20*
Root biomass	-0.12	-0.27**
<i>n</i>	122	122
Experiment 4		
Shoot biomass	0.24**	0.06
Root biomass	0.09	-0.10
<i>n</i>	125	125
Experiment 5		
Shoot biomass	0.28**	0.09
Root biomass	-0.14	-0.20*
<i>n</i>	113	113

^z Reproduction factor (RF) = proportion of final nematode population after harvesting and initial population used for inoculation. Significant at * $P \leq 0.05$ and ** $P < 0.001$.

nematode eggs at the end of the experiment than were used to inoculate the pots at the commencement of the experiment. Fifteen of the 18 *S. spontaneum* lines and the three *E. arundinaceus* lines had a significantly lower number of *M. javanica* eggs per gram of root compared with the two commercial cultivars. There were significant differences between lines for shoot and root biomass. The only significant correlations were between shoot biomass and RF value and root biomass and the eggs per gram of root (Table 7).

Strong correlations (0.76 to 0.93) were observed among experiments 3, 4, and 5 in relation to RF value and eggs per gram of root (Table 10).

Discussion

Our study identified new sources of resistance to root-knot (*M. javanica*) and root-lesion (*P. zeae*) nematodes in accessions of the wild sugarcane relative *S. spontaneum*. To our knowledge, this is the first reported study demonstrating that nematode resistance is common and in diverse *S. spontaneum* lines. The response of *S. spontaneum* lines to both types of nematode ranged from very low reproduction to reproduction similar to the commercial cultivars. This study indicated that *S. spontaneum* has wide variability in relation to nematode resistance, which could be attributed to the high genetic diversity among *S. spontaneum* lines as demonstrated elsewhere (Besse et al. 1997).

Our study indicated that the majority of the *S. spontaneum* lines suppressed reproduction of *M. javanica* to below or close to the initial starting population. However, there were exceptions and two *S. spontaneum* lines, MOL5904 and IK76-49, consistently had RFs for *M. javanica* similar to or greater than the commercial cultivars in the three experiments. Reproduction of *P. zeae* in most of the *S. spontaneum* lines was significantly lower than the commercial cultivars but *P. zeae* was still able to multiply in these lines in contrast to

M. javanica, where multiplication was very restricted in most lines. The resistance mechanisms of *S. spontaneum* to the two nematodes are likely to be different. Even though both *M. javanica* and *P. zeae* are obligate endoparasites, they parasitize their host plant using entirely different mechanisms. *M. javanica* induces host cells to transform into specialized feeding sites and becomes sedentary by remaining on the feeding site for rest of its life (Fuller et al. 2008). *P. zeae* is a migratory, polyphagous, intracellular endoparasite. It does not form obvious feeding patterns, is mobile and “breaks and enters” into the cortical tissue, and leaves host roots if required (Jones and Fosu-Nyarko 2014).

Resistance to multiple species of nematodes in a single host is rare, but the frequency of resistance to a single species of nematode is more common (Peng and Moens 2003). Our study indicated that a few of the *S. spontaneum* lines are resistant to both types of nematode. For example, accession lines KLOET, OKINAWA, GLAGAH-1286, SES516, US71-4-1, and SES208A possessed very high to moderate resistance to both types of nematode and conversely the line MOL5904 is susceptible to both nematodes.

There were strong correlations between two root-lesion experiments (experiments 1 and 2) as well as among three root-knot experiments (experiments 3, 4, and 5) in relation to RF values and

Table 8. *Meloidogyne javanica* reproduction factor (RF), eggs per gram of root, visual rating, and shoot and root biomass in experiment 4 for the two commercial cultivars, 20 *S. spontaneum* lines, and three *E. arundinaceus* lines

Accession line	RF ^x	Eggs/g root	Shoot biomass (g)	Root biomass (g)
Q135 ^y	28 a	24,141 a	21.83 abcd	5.85 cd
IK76-49	20 ab	23,752 a	17 bcdefghi	4.43 cdef
Q208 ^{ay}	17 b	8,336 ab	21.51 abcde	10.6 ab
MOL5904	15 b	31,285 a	14.59 ghij	2.45 fg
MOL1032	14 b	25,823 a	15.31 efghi	2.77 efg
SES182	7 c	5,543 bc	20.52 abcdefg	6.47 bcd
SES343	2 d	2,292 bcd	21.17 abcdef	4.19 cdef
IJ76-333 ^z	1 de	2,313 bcd	10.24 kl	3.05 efg
IJ76-370 ^z	1 def	1,623 cde	12.71 ijk	3.84 def
IS76-173	1 efg	1,532 cde	14.92 ghij	2.42 fg
US56-15-2	1 efgh	774 def	23.33 ab	4.2 cdef
KLOET	0 fghi	272 fghi	15.15 fghi	7.29 abc
COIMBATORE	0 ghi	724 def	15.37 efghi	2.46 fg
NG28-101	0 ghi	518 efg	17.85 bcdefghi	2.72 efg
US71-4-1	0 ghi	321 fgh	19.63 bcdefgh	3.73 def
DJANTOER_2	0 ghi	565 def	15.94 defghi	1.74 g
MANDALAY	0 hi	250 fghi	10.52 jkl	2.81 efg
IK76-86	0 ghi	123 ghij	16.24 cdefghi	4.78 cde
SES208A	0 i	67 ijk	16.7 bcdefghi	4.41 cdef
IK76-41	0 i	45 jk	22.8 abc	5.98 cd
IK76-48 ^z	0 i	18 k	28.4 a	11.92 a
OKINAWA	0 i	100 hij	8.14 l	1.83 g
IMP1944	0 i	70 ijk	21.92 abcd	2.54 efg
GLAGAH-1286	0 i	40 jk	14.42 hijk	4.02 def
SES516	0 i	32 jk	16.9 bcdefghi	3.84 def

^x Data are mean values of the respective accession line across the row. Data were log-transformed ($\ln(x + 1)$) before analysis. Back-transformed data are presented. Means followed by the same letter(s) in a column are not significantly different ($P = 0.05$) using Fisher's protected least significant difference test.

^y Commercial cultivar.

^z *E. arundinaceus* line.

Table 9. *Meloidogyne javanica* reproduction factor (RF), eggs per gram of root, visual rating, and shoot and root biomass in experiment 5 for the two commercial cultivars, 18 *S. spontaneum* lines, and three *E. arundinaceus* lines

Accession line	RF ^x	Eggs/g root	Shoot biomass (g)	Root biomass (g)
MOL5904	32 a	37,633 a	21.7 bcdef	4.6 h
IK76-49	21 b	13,700 ab	15.9 ghi	7.4 defgh
Q135 ^y	16 bc	10,751 b	22.5 bcde	7.6 defgh
Q208 ^{ay}	13 c	6,746 bc	29.5 a	10.4 bcdef
SES182	6 d	2,067 d	19.6 cdefg	14.9 ab
SES343	5 d	2,412 cd	24.2 abcd	9.6 bcdef
COIMBATORE	2 e	1,328 d	19.8 cdefg	6.4 fgh
US56-15-2	2 ef	1,380 d	23.7 abcd	5.4 gh
KLOET	1 efg	306 ef	19.4 defg	21.4 a
IS76-173	1 efgh	754 de	17.4 fgh	6 fgh
DJANTOER_2	1 fghi	395 ef	24.3 abcd	9.7 bcdef
IJ76-333 ^z	1 ghij	324 ef	16.9 gh	9.2 bcdefg
IJ76-370 ^z	1 hij	193 fg	13 i	14.5 abc
NG28-101	0 hijk	322 ef	17.9 efgh	4.6 h
IMP1944	0 ijk	152 fg	27 ab	6.6 efgh
SES208A	0 jk	68 gh	18.6 efgh	11.7 bcd
IK76-86	0 jk	86 gh	16.2 ghi	8.5 cdefg
IK76-48 ^z	0 k	30 hi	18 efgh	10.1 bcdef
US71-4-1	0 k	19 i	24.6 abc	9.4 bcdefg
MANDALAY	0 k	13 i	13.2 i	12.4 bcd
GLAGAH-1286	0 k	12 i	14.6 hi	11.2 bcde
SES516	0 k	10 i	19.5 cdefg	10.8 bcdef
OKINAWA	0 k	11 i	13.3 i	8.3 defg

^x Data are mean values of the respective accession line across the row. Data were log-transformed ($\ln(x + 1)$) before analysis. Back-transformed data are presented. Means followed by the same letter(s) in a column are not significantly different ($P = 0.05$) using Fisher's protected least significant difference test.

^y Commercial cultivar.

^z *E. arundinaceus* line.

Table 10. Pearson correlation coefficients to compare reproduction factor (RF) values and *Meloidogyne javanica* eggs per gram of root among experiments 3, 4, and 5^z

Parameter	RF		Eggs/g root	
	Experiment 4	Experiment 5	Experiment 4	Experiment 5
Experiment 3	0.93*	0.76*	0.91*	0.81*
Experiment 4	–	0.83*	–	0.91*
n	25	23	25	23

^z Significant at * $P < 0.0001$.

nematode per gram of root. This gives us confidence that the resistance measures are repeatable and that the traits can be selected for in a targeted breeding program to introduce nematode resistance.

Stirling et al. (2011) previously suggested that shoot and root biomass can be used to assist in comparisons between sugarcane lines when screening for nematode resistance. We measured shoot and root biomass of accession lines after harvesting in order to secure as much information as possible. Shoot biomass was not useful in determining the nematode resistance in our glasshouse trials. This could be attributable to significant variations of shoot biomass among the *S. spontaneum* lines, which was reflected by the inconsistent correlations of shoot biomass with RF values and nematodes or eggs per gram of root. On the other hand, there were strong negative correlations between root biomass and root-lesion nematode per gram of root. This parameter can be used to select resistant lines for root-lesion nematodes in a sugarcane breeding program. However, the relationship between root biomass and eggs per gram of root for root-knot nematodes was not definitive and needs further investigation.

Accession lines from *E. arundinaceus* were consistently resistant against both types of nematode. This is in agreement with previous findings demonstrating that *E. arundinaceus* is highly resistant to biotic and abiotic stresses (Foreman et al. 2007; Magarey and Croft 1996; Stirling et al. 2011). Unfortunately, it has been difficult to introduce these attractive traits of *E. arundinaceus* into commercial sugarcane cultivars because most of the progenies from *Saccharum* × *Erianthus* crosses are sterile, and even for the fertile progenies, the loss of chromosomes in backcrossing of early hybrid generations (Piperidis et al. 2000).

Modern sugarcane cultivars are derived from crosses originally made between noble cane *S. officinarum* and its wild relative *S. spontaneum*, which were then backcrossed to *S. officinarum* or other hybrids (Cox et al. 2000). These interspecific hybridizations provided high yield, resistance to diseases, and tolerance to drought and water logging (Cox et al. 2000). Nematode resistant traits in *S. spontaneum* lines tested in our experiment, and probably more, would be easier to transfer and retain in modern sugarcane cultivars compared with other wild species. D'Hont et al. (2008) found that 15 to 25% of the chromosomes of modern cultivars are derived from *S. spontaneum* via whole chromosomes and chromosomes that have recombined with *S. officinarum*.

We used two parameters, reproductive factor (RF) and number of nematodes or eggs per gram of root, to measure resistance of accession lines to nematodes. The RF value has been used widely to measure the reproductive capabilities of nematodes on test plants in nematode resistance screening trials (Stirling et al. 2011; Thompson et al. 2009). This parameter was a good indicator of host resistance in our experiments, because it provided separation of resistance among test lines. This parameter is also readily measured and results are easy to interpret. However, this value was sometimes not as effective as nematodes or eggs per gram of root in identifying resistance among test lines with variable root weights. For example, *S. spontaneum* line COIMBATORE had the lowest RF values for *M. javanica* and had a small root biomass (Tables 5, 6, and 8). On the other hand, COIMBATORE supported a relatively high number of eggs per gram of root. In the study such as ours where accession lines had a diverse range of root biomass, nematodes or eggs per gram of root may be a better indicator of resistance. As indicators for resistance, parameters related to nematode numbers that are not corrected for root weight may be more effective when the root biomass of the test lines is fairly similar.

Our study provided valuable information about the nematode resistance genes in these wild accessions in SRA's collection, and targeted crossing with commercial hybrids should be conducted to increase nematode resistance in sugarcane cultivars for the Australian sugar industry. The methods developed for screening lines for resistance to the two nematode species will help breeders maintain resistance in backcross populations while selecting for key traits such as sugar content and yield.

In Louisiana, sugarcane mosaic virus resistance was successfully introduced into cultivated sugarcane by crossing between *S.*

spontaneum and commercial cultivars in the early 1980s (Legendre 1989). Similar work has been conducted in other crops and has resulted in improved resistant cultivars. For example, Thompson et al. (2011) reported that increased resistance to root-lesion nematodes was achieved in Australian chickpea by hybridizing a commercial cultivar (*Cicer arietinum*) with wild relatives (*C. reticulatum* and *C. echinospermum*). Wild relatives of sugarcane were also reported to be highly resistant to *Pachymetra* root rot (Croft et al. 2015; Magarey and Croft 1996). Thus, introgression of resistant genes from *S. spontaneum* and other wild relatives of sugarcane has the potential to provide the industry with improved cultivars that could help manage a number of difficult-to-control soil pathogens.

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