A Technique for Screening Sugarcane Cultivars for Resistance to Pachymetra Root Rot

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ABSTRACT

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A glasshouse technique for screening sugarcane cultivars for resistance to root rot caused by *Pachymetra chaunorhiza* was developed. University of California potting mixture was infested with oospores of the fungus produced on cornmeal agar, at 2×10^4 or 1×10^5 oospores per kilogram, and pregerminated single-eye cuttings were grown in the potting mixture for 6–8 wk. The numbers of healthy and diseased primary roots were then determined. The cultivars Q78, Q114, Q138, 77N631, 77N636, and H55-3426 were highly resistant to Pachymetra root rot. The correlation between glasshouse infection levels and field reactions was highly significant ($P < 0.01$), and the glasshouse infection levels of 27 cultivars tested at three seasons of the year at two inoculum levels were significantly correlated ($P < 0.05$). A highly significant correlation was found between the percentage of rotted roots (probits) of the susceptible cultivar Q90 and the logarithm of the number of oospores per kilogram.

Pachymetra root rot of sugarcane (*Saccharum* interspecific hybrid), caused by *Pachymetra chaunorhiza* Croft & Dick (7), is a major disease of sugarcane in Queensland, Australia. This disease is a component of the poor root syndrome (PRS) complex of sugarcane (9). *P. chaunorhiza* belongs to the recently recognized family Verrucalvaceae Dick (8) and is closely related to *Verrucalvus flavofaciens* Wong & Dick, the cause of kikuyu yellows in kikuyu grass (*Pennisetum clandestinum* Hochst. ex Chiov.) (18). Infected sugarcane develops a soft, flaccid rot of the primary and secondary roots, and infected roots contain high numbers of the characteristic verrucose oogonia of *P. chaunorhiza* (12). Up to 80% of primary roots can be affected in susceptible cultivars (11), and the underground portions of stalks (stubble) and the root system can become uprooted if the sugarcane lodges. When the stubble and roots are exposed above the soil surface, mechanical harvesters pick them up, and the roots and attached soil cause processing problems at sugar mills (9). The stubble of the previous crop provides the lateral buds from which ratoon crops develop. Therefore, if the stubble has been removed by the harvester, gaps form in ratoon crops, reducing the yield. The yield is also reduced by the effects of the root rot on growth. Other root pathogens commonly found associated with *P. chaunorhiza* are *Pythium graminicola* Subr. (12) and nematodes (4). Infection by *P. graminicola* can restrict infection by *P. chaunorhiza* (5).

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Soil fumigation and solarization can control Pachymetra root rot in the field (6) but are uneconomical for large-scale application. There are no known fungicides that can control *Pachymetra* when applied at nonphytotoxic rates (6). Cultivar resistance has been used to control root rot of sugarcane caused by *Pythium arrhenomanes* Drechs. (13) and has been widely used to control root pathogens in other crops (3,10,15). Field evaluation of cultivars for general root health in PRS-affected fields failed to identify any cultivars with significant resistance to the disease complex (9). Variation in disease levels within fields and difficulty in removing, washing, and rating root systems in the field make field rating laborious and unreliable. Also, interactions with other PRS pathogens in the field may confound the results. In the experiments reported here, the development of a glasshouse screening technique for resistance to Pachymetra root rot and the correlation of field and glasshouse reactions of cultivars are described.

MATERIALS AND METHODS

Pathogen. *P. chaunorhiza* was isolated from diseased sugarcane roots by techniques described previously and maintained on potato-dextrose agar (PDA; 200 g of fresh potato, 20 g of glucose, 1 L of water, and 20 g of agar) under sterile distilled water at room temperature. Inoculum was grown for 3–4 wk on PDA, V-8 juice broth (17), or cornmeal agar (CMA; Difco Laboratories, Detroit, MI) in the dark at 28 C. V-8 juice broth cultures were grown in 250-ml conical flasks with 100 ml of broth per flask on a reciprocal shaker at 100 rpm. PDA and CMA cultures were grown in 9-cm plastic petri dishes or aluminum trays with clear plastic autoclavable lids enclosed within clear autoclavable plastic bags.

The cultures of the fungus were blended in water for 30 sec (GE Instablend), and the number of oospores per milliliter of the suspension was determined in a nematode-counting chamber (Hawksley, England).

Pot trial procedure. One-bud sugarcane cuttings were pregerminated in University of California (UC) potting mixture, type BII (1), in plastic pots, 7.5 cm in diameter. After 2–3 wk, uniform plants were transferred to 1.4 kg of UC mix in clay pots, 15 cm in diameter. The inoculum was mixed with the UC mix for each pot individually with a hand beater. In later trials, the inoculum was mixed with the potting mixture in a cement mixer. The pots were placed in sealed air-conditioned benches (14), set to maintain the pots at 28 ± 2 C, in a glasshouse at the Tully Sugar Experiment Station, in northern Queensland. The clay pots were subirrigated by clay saucers kept filled with water. The plants were grown for 6–8 wk, and then the potting mixture was washed from the roots, and the healthy and *Pachymetra*-infected primary roots were counted.

Inoculum density and cultivar resistance. The effect of varying inoculum density on root rot development in the susceptible cultivar Q90 was investigated in five experiments with inoculum produced on CMA, PDA, and V-8 juice broth. In two experiments there were five replicates; in one experiment, six replicates; and in two experiments, 10 replicates. Inoculum levels ranged from 8×10^2 to 2×10^6 oospores per kilogram of potting mixture.

Another experiment included 14 cultivars with four levels of inoculum: 0, 5×10^3, 1×10^4, and 2×10^4 oospores per kilogram of potting mixture.

Seven cultivars were selected from the initial experiment as potential standards for future resistance-screening experiments. These were included in 19 experiments, with 10 replicates for each cultivar. The experiments were conducted over 3 yr, from 1984 to 1987.

The repeatability of infection levels obtained from the glasshouse experiments conducted during three seasons of the year and at two inoculum levels was tested during 1987. The experiments were conducted during March–April (monsoon season, with average high and low

ambient temperatures of 29.2 and 21.5 C and average daily total radiation of 4.54 kW·hr·m^{-2}), July–August (winter season, with average high and low ambient temperatures of 25.3 and 14 C and average daily total radiation of 4.19 kW×hr·m^{-2}), and October–November (summer season, with average high and low ambient temperatures of 30.0 and 18.8 C and average daily total radiation of 5.05 kW·hr·m^{-2}).

Correlation between resistance ratings in the glasshouse and in the field. The reactions of 18 cultivars to *P. chaunorhiza* at three levels of inoculum (5×10^3, 2×10^4, and 4×10^4 oospores per kilogram) were determined in the glasshouse. The 18 cultivars were also planted in a PRS-affected field at Babinda, in northern Queensland, in April 1984. One-bud cuttings were pregerminated in UC mix in peat pots, 7.5 cm in diameter, in the glasshouse until they were 6 wk old. The plants were then transferred to the field and planted in a 20-cm-deep drill, with CK44 fertilizer (8.7% N, 9.11% P, and 26.2% K) at 375 kg/ha placed near them. Chlorpyrifos (Lorsban 50EC) at 2 L a.i./ha was sprayed around the plants to control symphilids. Soil was raked into the drill to just cover the peat pots. There were four replicates of each cultivar, arranged in randomized complete blocks, with three plants per replicate. The plants were planted 30 cm apart with 1.5-m spacings between rows. The inoculum density in the field in which the trial was conducted was 1×10^5 oospores per kilogram of soil (R. C. Magarey, *personal communication*). The plants were dug out 9 wk after transplanting into the field, and the percentage of rotted primary roots was determined.

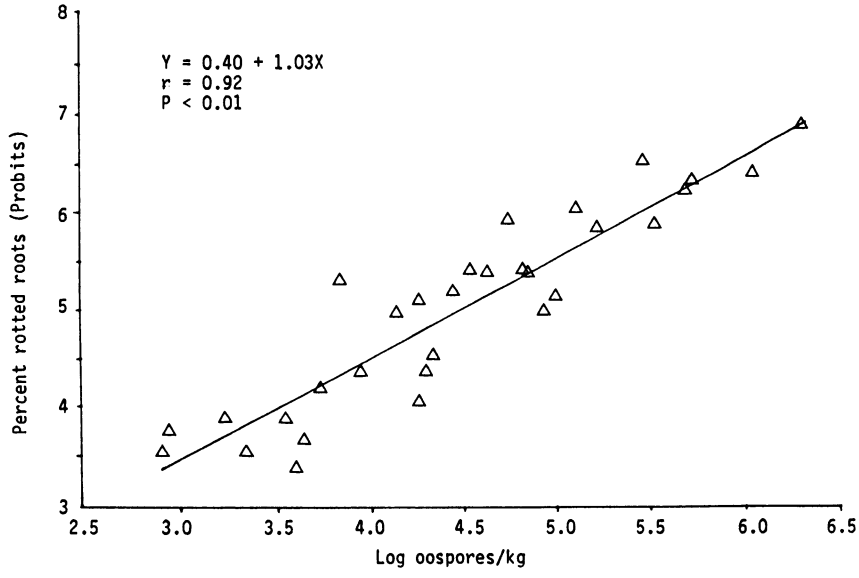

Fig. 1. Relationship between the percentage of rotted roots (probits) of cultivar Q90 and the logarithm of the number of oospores of *Pachymetra chaunorhiza* per kilogram of artificially infested potting mixture.

Table 1. Percentage of rotted roots of 14 sugarcane cultivars grown for 8 wk in the glasshouse in potting mixture infested with *Pachymetra chaunorhiza*

	Inoculum level (oospores/kg)[z]			
Cultivar	**0**	**5×10^3**	**1×10^4**	**2×10^4**
Q77	0 a	33 (34) ab	47 (43) bc	48 (44) bcd
Q78	0 a	2 (3) d	2 (5) f	0 (2) f
Q83	0 a	45 (42) a	72 (61) a	67 (55) ab
Q90	0 a	37 (35) ab	39 (38) bc	66 (55) ab
Q107	0 a	27 (31) ab	51 (46) ab	60 (51) abc
Q113	0 a	17 (24) bc	21 (26) cde	41 (39) cd
Q114	0 a	3 (7) cd	2 (4) f	1 (4) f
Q117	0 a	8 (8) cd	10 (14) def	26 (30) de
Q118	0 a	19 (22) bc	51 (46) ab	61 (51) abc
Q120	0 a	10 (18) bcd	15 (20) def	28 (31) de
Q121	0 a	27 (30) ab	55 (48) ab	72 (59) a
Q122	0 a	19 (22) bc	22 (25) cde	54 (47) abc
H56-752	0 a	14 (18) bcd	7 (9) ef	17 (24) e
Triton	0 a	30 (33) ab	23 (28) cd	26 (30) de

[z]The data are the mean percentages of rotted roots in five replicates and, in parentheses, their arc sine values. Figures in the same column followed by the same letter are not significantly different ($P < 0.05$) by the least significant difference technique applied to the arc sine values. Differences are given for between-cultivar comparisons only.

RESULTS

Inoculum density and cultivar resistance. There was a highly significant relationship between the percentage of rotted roots (probits) in cultivar Q90 and the logarithm (base 10) of the number of oospores per kilogram of potting mixture ($P < 0.01$; $r = 0.92$) in the combined results of five experiments (Fig. 1). The inoculum density for 50% infection was 7×10^4 oospores per kilogram.

Oospores produced on PDA were smaller than those produced in roots and generally did not have the distinctive verrucose ornamentation on the oogonial wall. In some batches of V-8 juice broth, *P. chaunorhiza* did not produce oospores, and when this mycelium was used as inoculum, no infection was obtained. Oospores formed on CMA closely resembled those formed in sugarcane roots. Consequently, inoculum produced on CMA was used in all subsequent experiments.

In the experiment with cultivars at four levels of inoculum, Q78 and Q114 were significantly more resistant than the other cultivars ($P < 0.05$) at 2×10^4 oospores per kilogram (Table 1). Very few roots of these cultivars (less than 4%) became infected, and when infection did occur, it was restricted to small sections of roots. Cultivars Q83, Q90, Q107, Q118, Q121, and Q122 all had more than 50% rotted roots at 2×10^4 oospores per kilogram, and the rot generally extended along large sections (50–100 mm) or complete roots. The other cultivars, Q77, Q113, Q117, Q120, H56-752, and Triton were intermediate in reaction. Infection increased with increasing inoculum level in the susceptible and intermediate cultivars, but not in the resistant cultivars.

Among the seven cultivars selected from the initial experiment to give a range of reactions, cultivars Q78 and

Table 2. Percentage of rotted roots of seven sugarcane cultivars grown for 6–8 wk in the glasshouse in potting mixture infested with oospores of *Pachymetra chaunorhiza*

Cultivar	**Percentage of rotted roots (arc sine)[z]**
Q83	62 (52) a
Q90	52 (47) b
Q113	26 (27) c
Q117	24 (25) cd
Q120	19 (22) d
Q78	10 (13) e
Q114	7 (10) e

[z]Values are the means of 19 experiments, each with 10 replicates. Figures followed by the same letter are not significantly different ($P < 0.05$) by the least significant difference technique applied to arc sines.

Q114 were significantly more resistant than the others ($P < 0.05$); Q113, Q117, and Q120 were intermediate in reaction; Q90 was susceptible; and Q83 was highly susceptible (Table 2). In five experiments in this series, the inoculum level was 1×10^5 oospores per kilogram; in the other 14 experiments, it was 2×10^4 oospores per kilogram. The ranking of the cultivars remained the same at both inoculum levels.

There were significant correlations ($P < 0.05$) between the levels of root rot recorded in 27 cultivars tested at three seasons during the year (Table 3). The reactions of cultivars at the two inoculum levels (Table 4) were also significantly correlated ($P < 0.05$). In these experiments, Q78, Q114, 77N631, 77N636, and H55-3426 were consistently highly resistant to *P. chaunorhiza*. Although the ratings were significantly correlated, the reactions of some cultivars varied between trials.

Correlation of glasshouse and field reactions. The correlation between the field reactions of 18 cultivars and their glasshouse reactions was highly significant ($P < 0.01$) at inoculum levels of 2×10^4 and 4×10^4 oospores per kilogram, but not at 5×10^3 oospores per kilogram. The best relationship between the percentages of rotted roots (transformed to arc sines) in the field and in the glasshouse was obtained at 2×10^4 oospores per kilogram (Fig. 2). Cultivars rated as intermediate in the glasshouse tended to be more susceptible in the field.

DISCUSSION

This is the first report of sugarcane cultivar resistance to *P. chaunorhiza*. The cultivars Q78, Q114, and Q138 were resistant in both the glasshouse and the field. Other cultivars with a high level of resistance in the glasshouse were 77N631, 77N636, and H55-3426. A number of cultivars with moderate levels of resistance were also identified.

The technique for screening cultivars for resistance to *P. chaunorhiza* in the glasshouse is both repeatable and correlated with field reaction. Many cultivars can be screened with relatively little labor, compared to digging and rating plants in the field. Also, the glasshouse technique enables resistance to one component of the PRS complex to be determined under standard conditions without the complication of interactions with other pathogens. Inoculum densities used in the glasshouse experiments were similar to inoculum densities recorded in fields of susceptible cultivars in northern Queensland (2×10^4–2×10^5 oospores per kilogram of soil; Magarey, *personal communication*). Some cultivars with intermediate levels of infection in the glasshouse experiments had relatively high levels of infection in the field. The correlation between glasshouse and field reactions needs to be confirmed by further research.

Cultivar resistance to root diseases caused by other oomycetes has been tested with dry weight of mycelium (10) and zoospores (3) as the unit of inoculum. *P. chaunorhiza* failed to produce any infection when mycelium containing no oospores was used as inoculum, and zoospore release by this fungus has not been observed (5). Oospores have been used as the unit of inoculum for studies of *Pythium* species (16). Oospores of *P. chaunorhiza* appear to be the infective propagule in soil and

Table 3. Correlation coefficients for comparisons of percentages of rotted roots (transformed to arc sines) of 27 sugarcane cultivars grown for 6–8 wk in the glasshouse in potting mixture infested with *Pachymetra chaunorhiza* at two levels of inoculum during three seasons

		Monsoon		Winter		Summer
		2×10^4 [w,x]	1×10^5	2×10^4	1×10^5	2×10^4
Monsoon	1×10^5	0.77**				
Winter	2×10^4	0.56**	0.69**			
	1×10^5	0.46*	0.62**	0.83**		
Summer	2×10^4 [y]	0.69**	0.70**	0.67**	0.66**	
	1×10^5 [z]	0.77**	0.74**	0.72**	0.69**	0.86**

[w] Inoculum level in oospores per kilogram.
[x] * = Significant at $P < 0.05$; ** = significant at $P < 0.01$.
[y] In the summer experiment at 2×10^4 oospores per kilogram, only 26 cultivars were available.
[z] In the summer experiment at 1×10^5 oospores per kilogram, only 22 cultivars were available.

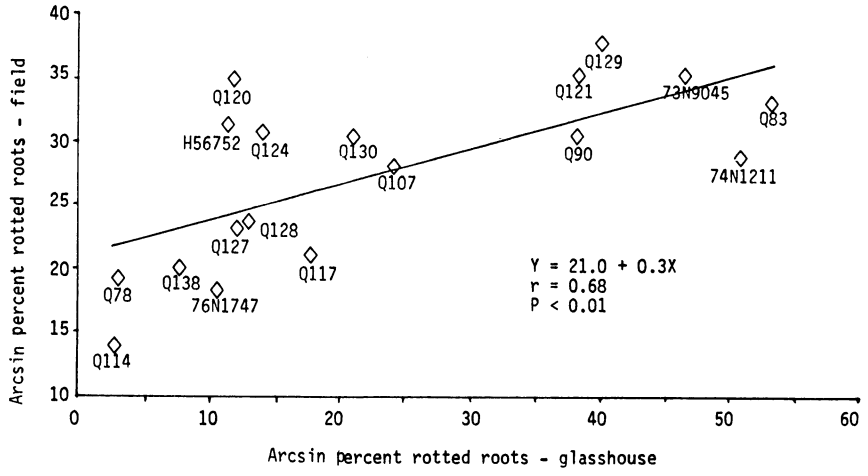

Fig. 2. Relationship between the arc sine of the percentage of rotted roots of 18 sugarcane cultivars grown in a field naturally infested with *Pachymetra chaunorhiza* and the arc sine of the percentage of rotted roots of the same 18 cultivars grown in the glasshouse and inoculated with 2×10^4 oospores of *P. chaunorhiza* per kilogram of potting mixture.

Table 4. Percentage of rotted roots of 27 sugarcane cultivars grown for 6-8 wk in the glasshouse in potting mixture infested with *Pachymetra chaunorhiza*

	Inoculum level (oospores/kg)[x]	
Cultivar	2×10^4	1×10^5
Q78	7 (12)	15 (19)
Q83	57 (50)	71 (60)
Q90	60 (51)	65 (55)
Q113	24 (25)	35 (34)
Q114	6 (8)	12 (17)
Q117	24 (25)	31 (31)
Q120	10 (19)	30 (31)
58N829	14 (18)	23 (25)
77N631	5 (8)	5 (5)
77N636	3 (6)	6 (8)
73N9045	43 (41)	58 (51)
76N1747	6 (8)	22 (25)
H55-3426[y]	7 (11)	7 (12)
Triton	29 (29)	46 (44)
Pelorus	33 (33)	44 (39)
Q63	43 (40)	47 (43)
Q94[z]	61 (54)	42 (38)
Q96	34 (33)	48 (44)
Q107	35 (35)	51 (45)
Q121	45 (41)	61 (53)
Q124[y]	33 (32)	41 (37)
Q126	43 (39)	51 (47)
Q128	15 (16)	38 (36)
Q130	25 (26)	43 (41)
Q132	35 (35)	47 (42)
Q134[y]	26 (26)	26 (27)
Q138	17 (19)	20 (16)
LSD ($P < 0.05$)	(11)	(12)

[x] The data are mean percentages of rotted roots and, in parentheses, their arc sine values, in three experiments, conducted at three seasons of the year (monsoon, winter, and summer). There were five replicates for each cultivar in each experiment, except for the first seven cultivars listed, which had 10 replicates.
[y] Cultivars not available for the summer experiment at 1×10^5 oospores per kilogram.
[z] Cultivar not available for the summer experiment at 2×10^4 and 1×10^5 oospores per kilogram.

therefore should be the preferred inoculum for cultivar screening.

The relationship between the percentage of rotted roots (probits) and the logarithm of the number of oospores per kilogram had a slope of 1.03 in experiments reported in this paper, which is lower than the theoretical slope of 1.31 calculated by Baker (2) for nonmotile inoculum and a moving infection court. No attempt was made to determine the degree of dormancy or the viability of the oospores used as inoculum in this study.

Since there is no economic chemical or cultural control available for Pachymetra root rot, cultivar resistance appears to be the only alternative available. In areas of northern Queensland affected by Pachymetra root rot, some of the intermediate and resistant cultivars identified in this study are being widely planted and made up 70% of the total area harvest in 1987.

Research is currently in progress to confirm the correlation between glasshouse and field reactions of cultivars and to determine the yield losses in susceptible, intermediate, and resistant cultivars at a range of inoculum densities.

Cultivars used as parents in the Bureau of Sugar Experiment Stations' plant-breeding program and cultivars selected for potential commercial release are currently rated for resistance to Pachymetra root rot in glasshouse trials.

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