BUREAU OF SUGAR EXPERIMENT STATIONS
QUEENSLAND, AUSTRALIA

BIOASSAY FOR COMPARING LEVELS OF *PYTHIUM GRAMINICOLA* IN SOILS

by

B.J. Croft

June, 1987

BIOASSAY FOR COMPARING LEVELS OF PYTHIUM GRAMINICOLA IN SOILS

In the study of any soil borne pathogen it is essential to have some technique for determining the level of the pathogen in the soil. The levels of Pythium species in soil have been measured by soil dilution plating on selective media (3). However species with lobulate sporangia are not frequently isolated on these media and bioassay techniques have been developed for these fungi (5). In Hawaii, pineapple roots have been used as baits in a bioassay for P. graminicola Subr. (1). However, considerable space is required to maintain pineapple plants and they are not always readily available.

Poor Root Syndrome (PRS) of sugarcane in Queensland causes serious crop losses (4). A Pachymetra sp. (formerly referred to as the root rot fungus) which rots the primary roots of the sugarcane plant, and P. graminicola which can restrict fine root development, are two pathogens consistently found in affected soils (2). The study of the role of P. graminicola in the sugarcane PRS in Queensland has been hampered by the lack of a means of quantifying the level of this species in soils.

This paper outlines the development of a sorghum bioassay (SB) for P. graminicola using sorghum seedling roots.

MATERIALS AND METHODS

Technique 1 (SB1)

The soils used in the trials were used as soon as possible after collection. Soils were passed through a 2 mm sieve and dilutions were carried out using the serial dilution technique of Tsao (6). Fifty grams of the test soil were added to 240 mL plastic cups. A layer of sterile vermiculite was then placed over the soil. Thirty to forty seeds of sorghum cultivar FS26 were then placed on the vermiculite and the seeds were covered with another layer of vermiculite. The cups were watered to soil saturation and placed in an environment chamber at 28-30°C. After two days when the seeds had germinated, the cups were placed in an incubator at 18-20°C with artificial lighting (Plantlux fluorescent tubes). The plants were kept at 18-20°C for 4-5 days and then the roots were washed in running tap water for 2-3 hours, blotted dry and embedded in potato dextrose agar plus 25 ppm rifampicin (Sigma Chemicals) and three drops of 2.5% pimaricin (Pimafucin, Gist-Brocades). After 24 and 48 hours, the number of roots with Pythium-like colonies were counted and a selection of colonies sub-cultured for species identification. Identification of species was made on the morphology of sexual and asexual structures produced in sterile distilled water with 2-3 pieces (each 10 x 20 mm) of boiled sugarcane leaves.

Technique 2 (SB2)

A variation of the above assay was developed in an attempt to prevent secondary inoculum production which was likely to occur in SB1. Sorghum seeds were pregerminated for three days at room temperature in vermiculite, then carefully removed and placed in 10 g of test soil and 20 mL of water in 90 mm diameter petri dishes and incubated at 28°C for 24 hours. The sorghum roots were then plated out on selective medium as in SB1. Test soils were prepared as described above for SB1.

Experiment 1: Autoclaved soil from a sugarcane field in Mourilyan, North Queensland (G.S.G. - Red Earth) was inoculated with varying levels of P. graminicola inoculum. The fungus was grown on potato dextrose broth for five days, the mycelium was strained from the broth, blotted dry on paper towel and weighed. A sample of the mycelium was dried at 70°C for seven days for moisture determination. The remaining mycelium was macerated in water for 30 seconds in a blender (G.E. Instablend). The hyphal suspension was added to the autoclaved soil in petri dishes to give a range of inoculum levels and then assayed by SB2.

Experiment 2: The sugarcane cultivar Q90 was grown in Mourilyan soil in sub-irrigated terra cotta pots for six weeks and the soil assayed by SB2 on two occasions.

Experiment 3: The two bioassay techniques were compared using three soils in which sugarcane cultivar Q90 had grown in sub-irrigated terra cotta pots for six weeks. The soils were originally collected from canefields in the Mourilyan and Babinda areas and on Tully Sugar Experiment Station.

RESULTS

Experiment 1: Percent infected sorghum roots and the level of inoculum of P. graminicola were highly significantly statistically related (Figure 1, Prob. $=<0.01$). The relationship was best described by the following equation:

$$Y = 66.1 + 263.7X + 8.7 \ln X$$

$$R^2 = 0.99$$

where Y = percent infected roots

X = level of inoculum (g dry wt/kg soil)

Experiment 2: There was a highly significant statistical relationship between the percent infected sorghum roots and the dilution of the test soils when the results of the two separate trials were combined (Figure 1, Prob. $=<0.01$). The relationship was best described by the following equation:

$$Y = 41.6 + 32.2X + 14.5 \ln X$$

$$R^2 = 0.88$$

where Y = percent infected roots

X = dilution

A small number of isolates of a non-pathogenic Pythium species, P. spinosum Sawada, were isolated from the soil, but only the number of P. graminicola isolates were included in the analysis of this trial.

Experiment 3: When the levels of Pythium species in three soils were measured with the two bioassay techniques, SB2 detected more P. graminicola and less P. spinosum than SB1 (Table 1). In the SB2 assay, Mourilyan soil had the highest levels of P. graminicola (73%) followed by Babinda (30%) and then Tully (27%) soil. The level of P. graminicola infection generally decreased with increasing dilution in SB2. One isolate of the RRF was recovered from the sorghum roots in Mourilyan soil in the SB1 assay and one isolate of P. acanthicum Drechs. from the Babinda soil in the SB2 assay.

DISCUSSION

Sorghum bioassay SB2 gave greater recovery of P. graminicola than SB1 and reduced the levels of P. spinosum recovered from field soils. In the SB2 assay zoospore infection is favoured since roots sit on top of the soil in water and therefore P. spinosum, which does not produce zoospores, is not as frequently isolated. Percent sorghum roots infected with P. graminicola and the level of dilution of the field soils in the SB1 assay were not significantly related.

The percent infected sorghum roots was highly related to the level of P. graminicola inoculum in inoculated soils and to the level of dilution in field soils in the SB2 bioassay. Percent infected sorghum roots in the SB2 assay can therefore be used to compare the level of P. graminicola in soils.

The RRF can infect sugarcane (Saccharum officinarum L.), S. spontaneum L. and Erianthus spp (unpublished data) but this is the first report of this fungus infecting sorghum (Sorghum bicolor L.).

The SB2 bioassay is a quick and simple way of comparing levels of P. graminicola and some other Pythium species in soil. Possible uses for the assay include determining the relationship between root damage and P. graminicola levels, comparing the effects of various treatments on P. graminicola levels and monitoring field populations of P. graminicola during a season. This assay should help to elucidate the role of P. graminicola in the poor root syndrome of sugarcane in Queensland and may have applications to the study of this pathogen in other crops.

ACKNOWLEDGEMENTS

I would like to thank Miss J. Moore for her able technical assistance.

REFERENCES

1. Adair, C. 1972. Ecological relationships of Pythium species in the rhizosphere of sugarcane - and possible significance in yield decline. Hawaii. Plant. Rec. 58:213-240.

2. Croft, B.J. and Magarey, R.C. 1984. Pathogenic fungi associated with northern poor root syndrome of sugarcane. Proc. Aust. Soc. Sugar Cane Technol. 1984 Conf. pp.55-61.

3. Dick, M.W. and Ali-Shtayeh, M.S. 1986. Distribution and frequency of Pythium species in parkland and farmland soils. Trans. Br. Mycol. Soc. 86(1):49-62.

4. Egan, B.T., Hurney, A.P., Ryan, C.C. and Matthews, A.A. 1984. A review of the northern poor root syndrome of sugarcane in North Queensland. Proc. Aust. Soc. Sugar Cane Technol. 1984 Conf., pp.1-9.

5. Stanghellini, M.E. and Kronland, W.C. 1985. Bioassay for quantification of Pythium aphanidermatum in soil. Phytopathology 75:1242-1245.

6. Tsao, P.H. 1960. A serial dilution end-point method for estimating disease potentials of citrus Phytophthoras in soil. Phytopathology 50:717-724.

Table 1

Percent sorghum roots infected with *Pythium* species in three soils using two sorghum bioassay techniques (SB1 and SB2)

Soil	1/Dilution	Percent *Pythium* spp infecting sorghum roots	
		Bioassay technique	
		SB1	SB2
Mourilyan	1	33 Pg[a]	73 Pg
		30 Pg[b]	3 Ps
		3 RRF[c]	
	2	20 Ps	37 Pg
	4	33 Pg	43 Pg
		3 Ps	
	8	3 Pg	17 Pg
	16	0	7 Pg
Babinda	1	43 Ps	30 Pg
			3 Ps
	2	23 Ps	40 Pg
			7 Pac[d]
	4	10 Pg	10 Pg
		3 Ps	
	8	3 Pg	17 Pg
	16	0	0
Tully SES block 10	1	3 Pg	27 Pg
		37 Ps	23 Ps
	2	17 Ps	13 Pg
			10 Ps
	4	10 Ps	3 Pg
			7 Ps
	8	7 Ps	3 Pg
	16	0	0

a Pg = *P. graminicola*

b Ps = *P. spinosum*

c RRF = *Pachymetra*

d Pac = *P. acanthicum*

Table 2

Percent sorghum roots infected with *P. myriotylum* in the *P. graminicola* and *P. myriotylum* inoculated plots of the *Pythium* yield loss trial

Treatment			Percent *P. myriotylum* infected sorghum roots				
			1/Dilution				
Variety	Weeks after planting	Depth (cm)	1	2	4	8	16
Q90	6	7.5-15	30	3	0	0	0
	14	7.5-15	35	10	0	-	-
		15-30	0	0	0	-	-
Q138	6	7.5-15	13	10	3	3	0
	14	7.5-15	70	20	10	-	-
		15-30	5	5	0	-	-

- Not tested

Table 3

Percent sorghum roots infected with *Pythium* species in SB2 bioassay from the untreated plots in the *Pythium* yield loss trial sampled at 6 and 14 weeks after planting

Treatment			Percent sorghum roots infected with *Pythium* spp 1/Dilution		
Variety	Weeks after planting	Depth (cm)	1	2	4
Q90	6	7.5-15	43 u[a]	7 u	0
	14	7.5-15	35 Pg 15 Pac 5 Pm 15 Px 5 Pp	10 Pg 10 Pac	5 Px
	14	15-30	31 Pg 12 Pac 31 Px	7 Pg 27 Pac 7 Px	5 Pac
Q138	6	7.5-15	15 u 23 Pac	11 u 16 Pac	
	14	7.5-15	6 Pg 39 Pac	20 Pg 10 Pac 10 Px	15 Pac
	14	15-30	18 Pg 44 Pac 9 Px	13 Pg 13 Px	5 Pm

a u = undetermined, *Pythium* spp, included *P. graminicola*, *P. myriotylum*, *P. periilum* and an unidentified *Pythium* species (Px).

Pg = *P. graminicola*

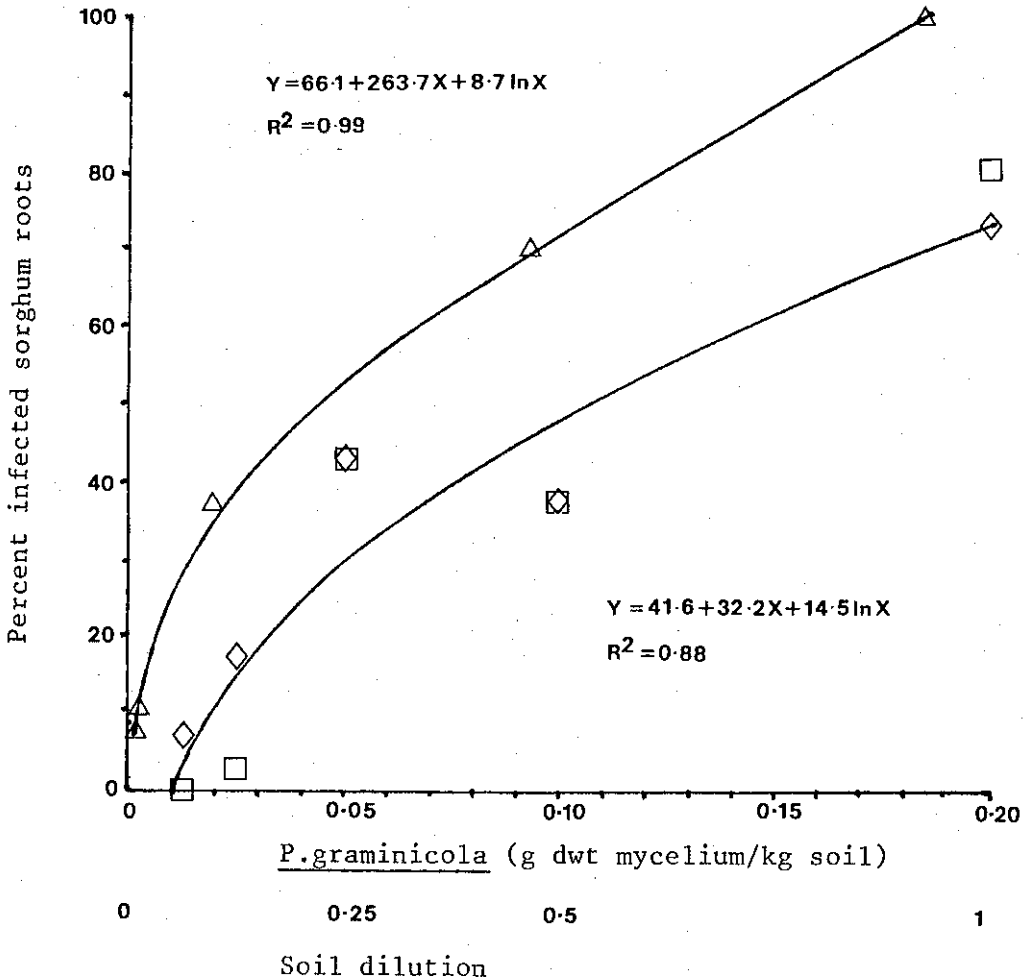
Pac = *P. acanthicum*

Pm = *P. myriotylum*

Px = unidentified *Pythium* species

Pp = *P. periilum*

Figure 1. Percent P.graminicola infected sorghum roots versus the rate of P.graminicola inoculum in autoclaved soil (△) and the level of dilution of Mourilyan field soil tested on two separate occasions (◇ and □).



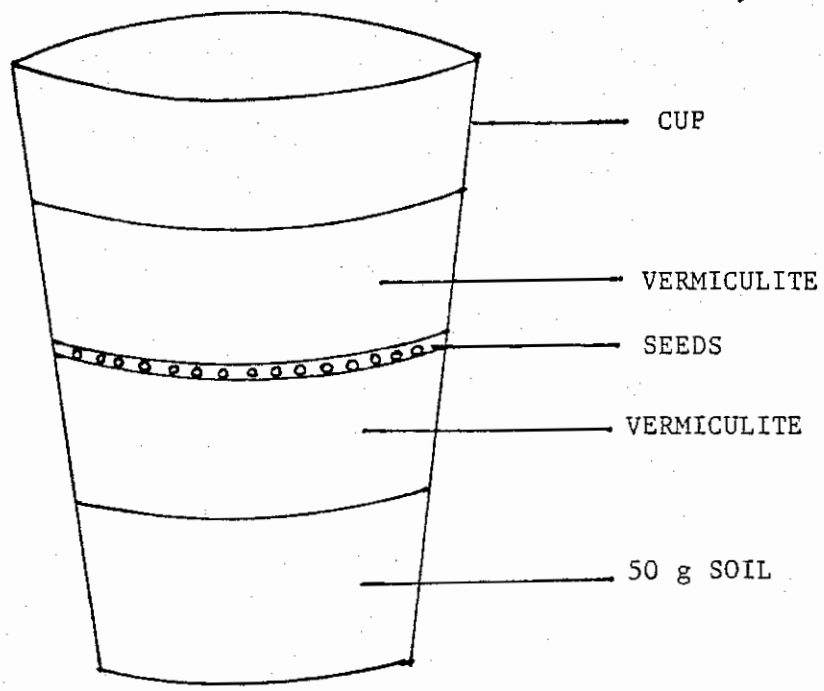


Diagram 1. Arrangement of seeds, soil and vermiculite in plastic cups used in the sorghum bioassay (SB2).