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Biological studies of soils in paired old and new land sites growing sugarcane

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Summary. The growth of sugarcane in soils from land monocultured with sugarcane, and from land which had either never been cropped with sugarcane, or just recently cropped, was compared under glasshouse conditions. In general, cane growth in new land soils was greater than in monocultured soil (shoot growth 7.4%, root growth 21.4%). Responses to soil pasteurisation were investigated in some soils and were greater in monocultured soils suggesting that root growth constraints were larger in the monocultured soil (210% response in monocultured soils *v.* 64% in new land soils). Assays for sugarcane root pathogens suggested that *Pachymetra chaunorhiza* was a major contributor to the old/new land growth responses, but it is unlikely that *Pythium* spp. were factors in the growth differences. Monitoring of other groups of organisms in soil from one site suggested that sugarcane monoculture may affect populations in the broader biological community.

Introduction

Yield decline of sugarcane, defined as the diminishing ability of caneland to produce sugar per harvested hectare (Magarey 1994), is an important productivity constraint for the Australian sugar industry. The subject has been reviewed recently (Magarey 1996) and has many similarities to replant diseases in horticultural crops. In most instances where sugarcane monoculture is established, some form of soil-borne disease occurs, and disease is viewed as an intimate part of sugarcane yield decline.

In this paper, glasshouse experiments examining sugarcane (*Saccharum* interspecific hybrid) growth in 'new' (land planted for the first time to sugarcane) and 'old' (monocultured to sugarcane) land soils, and growth responses to soil pasteurisation are reported. The objectives of the research were to examine root health in old and new land soils, to compare growth responses to soil pasteurisation, and to assay old and new land soils for known soil pathogens. The experiments were conducted between 1985 and 1993 at Tully Sugar Experiment Station, Tully (17°9'S, 145°9'E) in northern Queensland. Associated studies on crop growth and yield (Garside and Nable 1996), soil chemical properties (Bramley *et al.* 1996), soil physical properties (Ford and Bristow 1995), soil microbial biomass (J. A. Holt pers. comm.) and soil organic matter (J. A. Skjemstad, J. A. Taylor, L. J. Janik and S. Marvanek pers. comm.) sites have been conducted.

Materials and methods

Details of some of the paired old and new land sites have been described elsewhere (Bramley *et al.* 1996). Briefly, sites were chosen where land had been under sugarcane cultivation for at least 18 years ('old land') and had adjacent land which only recently (<1 crop cycle) had been planted for the first time to sugarcane ('new land'). At 1 site (Fortini), soil was obtained from land under sugarcane for 18, 5, <1 and 0 years, and at another, [Bureau of Sugar Experiment Stations (BSES), Tully], soil was taken from old caneland, and adjacent grassed headland and undisturbed rainforest. Nine sites were located in northern Queensland, (BSES Tully, Cristiano, Edwards, Ghidella, Grasso, LoMonaco, Mizzi, Toigo, Turnbull), 2 in the Herbert River District (Fortini, Kangas), 2 in the Burdekin River district (Kalamia Estate, Pegoraro), 3 in the central district (Fordyce, Valmadre, Vella), and 1 in southern Queensland (Heck). Climatic conditions ranged from the wet tropics (mean annual rainfall >4000 mm), to the humid tropics (annual rainfall 2000 mm), to the dry tropics (annual rainfall about 1100 mm), to the subtropics (annual rainfall about 1500 mm). More specific site details are given in Table 1. Soil samples were collected from each site to a depth of 20–25 cm, sieved (0.5 cm aperture) to remove rocks, and mixed thoroughly by hand. Moist soil (about 1.4 kg, equal dry weight between paired old and new land soils) was weighed into 15 cm diameter terracotta pots. Plants

Table 1. Location and site description of paired sugarcane old and new land sites from which soil was obtained for glasshouse experiments

Site	Region	Location	Annual rainfall (mm)	New land vegetation	Site planted to sugarcane (and in first crop cycle)
BSES, Tully	Northern Queensland	17°9'S, 145°9'E	3000–3500	Rainforest	No
Cristiano	Northern Queensland	17°4'S, 145°9'E	3500–4000	Grassed headland	Yes
Edwards	Northern Queensland	17°5'S, 146°E	3500–4000	Rainforest	Yes
Ghidella	Northern Queensland	17°4'S, 146°E	3500–4000	Grassed headland	Yes
Grasso	Northern Queensland	17°5'S, 146°E	3500–4000	Grassed headland	Yes
LoMonaco	Northern Queensland	17°8'S, 146°E	3000–3500	Grassed headland	Yes
Mizzi	Northern Queensland	17°5'S, 146°E	3500–4000	Grassed headland	Yes
Toigo	Northern Queensland	17°5'S, 146°E	3500–4000	Grassed headland	Yes
Turnbull	Northern Queensland	17°5'S, 146°E	3500–4000	Grassed headland	Yes
Fortini	Herbert River	18°6'S, 146°E	1500	Savannah woodland	No
Kangas	Herbert River	18°5'S, 145°8'E	1500–2000	Grassed headland	Yes
Kalamia Estate	Burdekin	19°6'S, 147°4'E	1100	Grassed headland	Yes
Pegoraro	Burdekin	19°6'S, 147°1'E	750–1000	Pumpkin rotation	No
Valmadre	Prosperpine	20°4'S, 148°5'E	750–1000	Grassed headland	Yes
Fordyce	Mackay	21°2'S, 148°9'E	1000–1500	Grassed headland	Yes
Vella	Mackay	21°2'S, 149°E	1000–1500	Grassed headland	No
Heck	Southern Queensland	27°8'S, 154°3'E	1000–1500	Grassed headland	Yes

for the experiment were pregerminated from single-bud cuttings of the sugarcane cultivar Q90, (Q114 was used at BSES Tully), and grown in University of California potting mix type BII (Baker 1957). When plants were 10–20 cm high, they were transplanted, 1 per pot, into the terracotta pots. Each plant was fertilised with 0.343 g of K_2HPO_4, and 0.153 g of NH_4NO_3 at the time of transplanting. Plants were maintained for 6 weeks on air-conditioned benches (Reghenzani 1984) operating between 25 and 30°C. Pots were subirrigated using 2-cm deep clay saucers; water was maintained in the saucers with a drip irrigation system. Seven experiments were conducted; some included soil from 1 location only while others contained a number of soils.

At harvest, roots were washed free of soil and examined for disease symptoms. Shoot and root dry weight was recorded. Some of the soil from BSES Tully, Fortini, Kalamia Estate and Pegoraro (experiments 4–7), was pasteurised (100°C, 90 min) and 0.335 g urea was added at the time of transplanting along with a basal trace element dressing (1.65 g per pot of Hortico Trace Element Mixture which contains 22% potassium, 2% magnesium, 1% iron, 1% manganese, 0.8% copper, 0.8% zinc, 0.2% boron, 0.1% molybdenum, 13% sulfur).

Pathogen assays

Known sugarcane root pathogens in Queensland include *Pachymetra chaunorhiza* (Croft and Magarey 1989), *Pythium arrhenomanes* (Croft and Magarey 1984) and various nematode species (Magarey and Croft 1995). Soils were assayed for *Pachymetra chaunorhiza* by assessing the percentage of rotted primary shoot roots (Croft and Magarey 1984; Magarey 1986) and in some

cases by assessing soil oospore populations (Magarey 1989a, 1989b). *Pythium arrhenomanes* was assayed by isolation from sugarcane root systems (Croft and Magarey 1984) or using a sorghum bait bioassay (Croft 1987). Parasitic nematodes were counted after extraction from soil or roots using the Whitehead tray technique (Whitehead and Hemming 1965).

Table 2. Harvest measurements for glasshouse experiments conducted with old and new land soils

Site	Shoot DW (g)		Root DW (g)	
	Old	New	Old	New
Experiment 1				
Edwards	3.9	4.5	2.2	4.3
LoMonaco	3.9	5.2	2.7	3.2
Toigo	3.9	5.1	1.0	1.8
Turnbull	3.7	3.4	2.4	2.3
Vella	7.5	7.1	2.3	3.6
l.s.d. (P = 0.05)	2.34		n.s.	
Experiment 2				
Fordyce	2.8	2.8	1.5	0.9
Heck	5.8	7.4	2.2	3.3
l.s.d. (P = 0.05)	3.25		n.s.	
Experiment 3				
Cristiano	9.0	6.7	3.2	3.4
Ghidella	7.0	6.8	4.1	3.5
Grasso	6.2	6.6	3.1	3.5
Kangas	6.5	7.1	4.0	5.2
Mizzi	7.1	6.6	3.1	3.8
Turnbull	5.1	5.7	4.2	4.4
Valmadre	3.4	6.1	2.8	4.5
l.s.d. (P = 0.05)	1.22		0.99	
Mean (all experiments)	5.4	5.8	2.8	3.4

Table 3. Harvest measurements for glasshouse experiments conducted with old and new land soils from sites at Fortini, Kalamia Estate, and Pegoraro, and with old, grassed headland and undisturbed rainforest soils from the BSES Tully site

Response is defined as yield in (pasteurised soil–untreated soil)/untreated soil x 100

Soil	Shoot DW (g)		Response (%)	Root DW (g)		Response (%)
	Untreated soil	Pasteurised soil		Untreated soil	Pasteurised soil	
Experiment 4 (Fortini)						
18 years cane	15.62	27.81	78	8.14	18.79	131
5 years cane	13.08	18.11	38	8.21	14.10	72
<1 years cane	14.44	23.05	60	9.92	13.90	40
New land	14.51	17.63	22	8.60	12.33	43
	l.s.d (P = 0.05): soil = 3.12, treatment = 2.25			l.s.d (P = 0.05): soil = 2.43, treatment = 1.72		
Experiment 5 (Pegoraro)						
Pumpkin rotation soil	19.89	29.21	47	6.52	14.57	123
>20 years cane	16.30	20.74	27	7.07	11.05	56
	l.s.d. (P = 0.05) = 3.14			l.s.d. (P = 0.05) = 1.67		
Experiment 6 (Kalamia Estate)						
Old land	9.79	19.56	100	3.63	9.55	164
New land	8.73	14.19	63	3.83	8.00	109
	l.s.d. (P = 0.05) = 3.84			l.s.d. (P = 0.05) = 1.77		
Experiment 7 (BSES Tully)						
Old land	10.70	15.53	45	2.81	12.25	336
Headland	9.79	14.71	50	6.24	10.99	76
Rainforest	13.23	20.54	55	9.51	13.45	41
	l.s.d (P = 0.05): soil = 1.59, treatment 1.30			l.s.d (P = 0.05): soil = 1.58, treatment = 1.29		

General biology

Vesicular arbuscular mycorrhizae (VAM) were assayed in experiments 4–7 by assessing the percentage of root colonisation using the gridline intersect method (Kormanik and McGraw 1982). Six cores were collected from each of the different field sites at the BSES Tully site using an Edelman auger (4 cm diameter) to a depth of 20 cm. The cores were bulked, mixed thoroughly, and subsampled for old land, grassed headland, and undisturbed rainforest sites. Populations of fungi (Martin 1950), actinomycetes (Williams and Davies 1965), fluorescent *Pseudomonas* spp. (Sands and Rovira 1970) and bacteria (King *et al*. 1954) present in the soil were estimated using dilution plate counts.

Statistical analyses

Plant harvest parameters were subjected to ANOVA using Statistix software 3.0 (NH Analytical Software, Roseville, Minneapolis, USA). Pathogen assays were analysed using log-linear analyses examining the likelihood ratio and, where appropriate, using ANOVA.

Results
Plant harvest measurements

Plant harvest measurements are detailed in Tables 2 and 3. In experiments 1–3, shoot growth in new land soils was 7.4% greater, and root growth 21.4% greater than in the old land soils (Table 2), though in individual experiments, responses to growth in new land soils were not always significant (P<0.05). Growth responses to soil pasteurisation occurred in both old and new land soils but

Table 4. Assay data for the sugarcane root pathogens *Pachymetra chaunorhiza* (as percentage rotted primary shoot roots) and *Pythium arrhenomanes* [as present (+) or absent (–)] in root systems at harvest in experiments 1–3

Likelihood ratio (*P. chaunorhiza*) = 87.4 (P<0.001, df = 13)
Analysis of variance: site (P<0.001, df = 27, F = 11.65); old/new land status (P<0.05, df = 27, F = 4.09)

Site	P. chaunorhiza		P. arrhenomanes	
	Old	New	Old	New
Experiment 1				
Edwards	74.1	43.1	—	—
LoMonaco	0	0	—	—
Toigo	76.1	77.8	—	—
Turnbull	3.7	0	—	+
Vella	19.0	0	—	+
Experiment 2				
Fordyce	57.7	13.2	—	—
Heck	0	0	+	+
Experiment 3				
Cristiano	74.0	68.7	—	—
Ghidella	80.4	57.1	—	—
Grasso	18.3	54.3	+	+
Kangas	26.2	1.6	—	—
Mizzi	60.7	25.9	—	—
Turnbull	4.9	0	—	—
Valmadre	0	0	—	—
Mean (all experiments)	35.4	24.4	14.3[A]	28.6[A]

[A] Percentage of samples with *P. arrhenomanes*.

Table 5. Nematode populations in soil and sugarcane root systems from some sites in experiments 1–3

Nematode assays on soil were conducted at harvest

Likelihood ratio (*Pratylenchus* in roots) = 3764.1 (*P*<0.001)

Analysis of variance: site (*P*<0.05, df = 9)

Site	Soil	Nematode species			
		Pratylenchus	*Criconemoides*	*Helicotylenchus*	*Rotylenchus*
		Soil (no. of nematodes/kg soil)			
Edwards	Old	—	—	—	—
	New	—	—	—	—
LoMonaco	Old	—	1460	—	—
	New	800	—	—	—
Toigo	Old	—	—	117	117
	New	—	—	—	—
Turnbull	Old	350	—	—	—
	New	1625	—	—	—
Vella	Old	—	—	—	—
	New	—	—	—	—
		Roots (no. of nematodes/100 g root fresh weight)			
Edwards	Old	8550	—	225	—
	New	10 525	—	850	—
LoMonaco	Old	500	—	—	—
	New	2100	—	—	—
Toigo	Old	1400	—	—	50
	New	6100	—	—	—
Turnbull	Old	12 425	—	75	—
	New	11 250	—	—	—
Vella	Old	8250	—	—	—
	New	13 800	—	150	—

tended to be greater in old land. This was particularly evident in root dry weight data from BSES Tully (Table 3). Shoot growth responses to pasteurisation were 74% in old land and 47% in new land while root growth responses were 210% in old land and 64% in new land. Discoloration (reddening, general browning) of the root system, probably indicative of the activity of soil pathogens, occurred in all untreated soils except the rainforest soil. Root systems in all pasteurised and rainforest soils appeared healthy with no root lesions evident; young roots were white, and older roots a pale tan colour.

Pathogen assays

Analysis of pathogen data (Tables 4 and 5) showed significant likelihood ratios with each pathogen, suggesting significant variation across sites and old/new land status. *Pachymetra chaunorhiza* occurred at higher levels in old land soils than new land soils (Table 4), which confirms an earlier study (Magarey 1991) in which pachymetra root rot was not detected in 14 new (which had never grown sugarcane) land soils adjacent to infested old land sites. *Pachymetra chaunorhiza* has only been observed in soils which have grown sugarcane; non-canegrowing soils in these experiments (Fortini, BSES Tully rainforest) showed no evidence of Pachymetra root rot. New land soils which had grown cane for more than 1 year did show some *Pachymetra*

chaunorhiza infestation. *Pythium arrhenomanes* status was not related to old or new land and the pathogen was present in only a few soils. It is interesting to note that the most distinct pythium root rot symptoms were seen

Table 6. Fungal, bacterial, fluorescent pseudomonad, actinomycete, *Pachymetra chaunorhiza*, *Pythium* spp. and nematode populations in old and new land (non-rhizosphere) soils from BSES Tully

Likelihood ratio = 471.8 (*P*<0.001, df = 12)

Analysis of variance: old/new land status (n.s.)

Organism	Old land	Headland	Rainforest
Total fungi x (10^6/g)	4.2	2.2	3.4
Total bacteria x (10^8/g)	4.1	3.7	4.1
Total actinomycetes x (10^6/g)	5.4	48.0	21.8
Fluorescent *Pseudomonas* spp. x (10^4/g)	2.0	0	14.0
Fungal pathogens			
Pachymetra chaunorhiza [spores/g soil (DW)]	36	0	0
Pythium spp. (% baits colonised)[A]	17	33	17
Nematodes			
Pratylenchus zeae (nematodes/kg)	273	0	0[B]
Helicotylenchus spp. (nematodes/kg)	273	0	0

[A] *Pythium arrhenomanes* present only in headland soil.

[B] Unrecognised plant parasitic species present.

Table 7. Mycorrhizal (VAM) root colonisation (as percentage of roots colonised) in root systems growing in old and new land soils in experiments 4–7
Likelihood ratio (untreated soils only) = 6.03 (n.s.)

Site	VAM colonisation of roots (%)	
	Untreated soil	Pasteurised soil
Experiment 4 (Fortini)		
18 years cane	19.8	0
5 years cane	5.8	0
<1 years cane	29.7	0
New land	6.4	0
Experiment 5 (Pegoraro)		
Pumpkin rotation soil	15.3	0
> 20 years cane	25.4	0
Experiment 6 (Kalamia Estate)		
Old land	38.5	0
New land	41.4	0
Experiment 7 (BSES Tully)		
Old land	20.9	0
Grassed headland	11.6	0
Rainforest	—	—

in root systems growing in a new land soil (Vella). Nematode assays suggested the common occurrence of the parasitic species *Pratylenchus zeae* which was present in all soils in experiments 1–3 (Table 5). There were significantly (*P*<0.05) different populations of *Pratylenchus* in root systems between sites. Populations were high in root systems but, as with pythium root rot, this did not appear to be related to old or new land status. In experiment 7, parasitic nematodes were present only in the old land soil (Table 6). Undetermined plant parasitic nematodes were present in the rainforest soil; presumably these are parasites of rainforest species.

General biology

Assays for populations of fungi, actinomycetes, bacteria and fluorescent *Pseudomonas* spp. (Table 6) at the BSES Tully site suggested that populations of fungi varied slightly between old and new land being greatest in the old land. Bacterial populations were similar across all soils. Actinomycete and fluorescent *Pseudomonas* populations tended to be higher in the rainforest soil than the old caneland soil but this trend was not statistically significant.

Assays for mycorrhizae suggested that root systems were partially colonised in all untreated soils and that soil pasteurisation was effective in eliminating these fungi (Table 7). Colonisation did not vary significantly between sites or with old or new land status.

Discussion

Plants growing in new land soils outyielded those growing in monocultured soils thus reproducing a common field observation, that crop yields are higher in new land soils. It was also evident that root health was

poor not only in monocultured but also in some new land soils, particularly where grasses had been the dominant vegetation. This is most probably due to the presence of root pathogens associated with graminaceous species (Garside *et al*. 1995). In contrast, root systems growing in untreated rainforest soil appeared healthy. The BSES Tully results highlight the acute effect of yield decline resulting from sugarcane monoculture, with root growth being particularly poor in the monocultured soil compared with the grassed headland and rainforest soils. Root growth in pasteurised soils was comparable suggesting that this treatment eliminated the effects of yield decline and suggests a major biological component to yield decline. Field experimentation by Magarey and Croft (1995) has shown that similar responses occur in the field; growth responses to soil fumigation throughout Queensland have also implicated soil biology as an important factor in yield decline.

Responses to soil pasteurisation were not confined to old land soils and a significant shoot growth response occurred in rainforest soil. Although poor root health is likely to be a major contributing factor to responses in old land soils, these results suggest that the pasteurisation response may have another component. Further research is required to investigate this and should include considering the release and immediate availability of plant nutrients. Research investigating the role of nitrogen in the response suggested that nitrogen release was not the main contributing factor to the fumigation response (R. C. Magarey and A. P. Hurney unpublished data).

Plant growth data suggest that in some cases, sugarcane monoculture may have improved the nutritional condition of old land soils. For example, at the relatively infertile Fortini site, continuous monoculture of sugarcane for 18 years led to the best sugarcane growth (untreated soil), compared with periods under sugarcane of 0, <1 and 5 years. Application of calcium and trace elements may have contributed to these responses. In contrast, the pasteurisation response was far greater in the monocultured soil suggesting that large and significant root growth constraints were present. It appears that nutritional amendments have improved the potential for growth, but the net effect was minor in comparison with the effects of yield decline.

Pathogen assays indicate that *Pachymetra chaunorhiza*, but not *Pythium arrhenomanes*, contributed to old and new land growth responses. This is consistent with other research with sugarcane yield decline (Magarey 1986, 1996; Magarey *et al*. 1995). Pachymetra root rot does not solely contribute to yield decline; significant differences in root health and plant growth were noted between old and new land, and untreated and pasteurised soils even where pachymetra root rot was absent. The presence of pachymetra root rot in new land

soils was most likely a result of contamination of these areas through movement of soil with cultivation. Many of the new land sites were adjacent to old caneland and movement of equipment occurred directly from old to new land.

Although present in high numbers in some root systems, parasitic nematodes did not appear to explain the growth differences in these glasshouse experiments. Further field studies are currently being conducted to examine more closely the effect of nematodes under commercial conditions. Magarey *et al.* (1995) have recently implicated dematiaceous fungi as a group of minor pathogens contributing to poor root health and poor cane growth. It seems probable that there are other pathogens besides *Pachymetra chaunorhiza* which contribute significantly to sugarcane yield decline.

Differences in the general biology of old and new land soils were noted at the BSES Tully site. Groups of organisms containing recognised biocontrol agents, including actinomycetes and fluorescent *Pseudomonas* spp., tended to be higher in the rainforest soil than in the soil monocultured to sugarcane. This reflects changes in the soil biological community occurring with sugarcane monoculture. Organic matter levels in new land soils tend to be higher than in soils monocultured with sugarcane (A. P. Hurney pers. comm.). It is likely that cultivation and oxidation of organic matter leads to changes in the populations and types of organisms in the biological community. Lower populations of fluorescent *Pseudomonas* spp. and actinomycetes in monocultured soils may contribute to a build up in harmful organisms and have an indirect impact on root health. The non-significant difference observed may reflect the small sample size since the assays are time consuming.

The reasons for poor root health, and the role poor root health plays in growth constraints associated with sugarcane monoculture, should be a central focus of future yield decline research as it is one characteristic of yield decline which is consistent across all sites and environments. Soil biology and root pathogens appear to be an issue central to root health but other factors may interact to produce the growth constraint. Data on the biological community gathered from the BSES Tully site indicate that there may be changes in the soil biological community associated with sugarcane monoculture. Yield decline control strategies may include altering the biological community rather than the elimination of one or several pathogens. Future research should seek to further clarify the etiology of yield decline and to identify strategies which favourably alter the soil biological community.

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