A SURFACE MULCH OF CROP RESIDUES ENHANCES SUPPRESSIVENESS TO PLANT-PARASITIC NEMATODES IN SUGARCANE SOILS

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ABSTRACT

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Most Australian sugarcane crops are harvested green, with the crop residues left behind after harvest remaining on the soil surface as mulch, a process known as green cane trash blanketing. Sampling in trash-blanketed sugarcane fields showed that roots were present to a depth of 150 cm, but that more than 90% of the root biomass was in the upper 30 cm of the soil profile. Many of these roots were concentrated in a layer just below the trash blanket and they were unusually healthy, presumably because population densities of *Pratylenchus zeae*/g root were 5-16 times lower than in roots a few cm further down the profile. Results of a microcosm experiment indicated that mulching soil with sugarcane residue increased soil C, microbial activity and numbers of free-living nematodes, and enhanced suppressiveness to *Meloidogyne javanica* and *P. zeae* to a greater extent than incorporating the residue into soil. It is hypothesized that roots immediately beneath the trash blanket remain healthy because C inputs from root exudates and organic matter on the soil surface sustain a soil food web capable of suppressing root pathogens, including plant-parasitic nematodes.

Key words: biological control, mulch, *Meloidogyne javanica*, organic amendments, plant-parasitic nematodes, *Pratylenchus zeae*, root distribution, sugarcane, suppression, trash blanket

RESUMEN

Stirling, G.R., Halpin, N.V. and M.J. Bell. 2011. Una cubierta de residuos de cultivo aumenta la supresividad a nematodos fitoparásitos en suelos de caña de azúcar. Nematropica 41:109-121.

En Australia, la mayoría de la caña de azúcar se cosecha verde, dejando los residuos de cultivo en el campo como cubierta, en un proceso que se conoce como cubierta de desechos de caña verde. En un muestreo de campos de caña de azúcar con este sistema de cubierta de desechos se observó que las raíces se encuentran hasta una profundidad de 150 cm, pero que más del 90% de la masa de raíces se encuentra en los 30 cm superiores del perfil del suelo. Muchas de estas raíces se concentran en la capa inmediatamente debajo de la cubierta de desechos y se observó que se encontraban inusualmente saludables, presumiblemente porque la densidad de población de *Pratylenchus zeae*/g de raíz era de 5 a 16 veces menor que en las raíces halladas unos pocos centímetros más abajo. Los resultados de un experimento de microcosmos demostraron que la cubierta de residuos de caña aumenta el C del suelo, la actividad microbial y la cantidad de nematodos de vida libre, y que mejora la supresividad a *Meloidogyne javanica* y *P. zeae* en mayor medida que incorporando el residuo en el suelo. Proponemos la hipótesis de que las raíces inmediatamente debajo de la cubierta de residuos permanecen saludables porque los ingresos de C de los exudados de raíces y de la materia orgánica del suelo sostienen una red alimentaria supresora de patógenos de raíces que incluyen a los nematodos fitoparásitos.

Palabras clave: caña de azúcar, control biológico, cubierta de desechos, distribución de raíces, enmiendas orgánicas, *Meloidogyne javanica*, nematodos fitoparásitos, *Pratylenchus zeae*, supresión .

INTRODUCTION

More than 310 species of plant-parasitic nematodes in 48 genera have been recorded from the roots and rhizosphere of sugarcane, and at least five of these genera are usually present in most sugarcane fields (Stirling and Blair, 2000; Cadet and Spaull, 2005). Although some of these nematodes cause crop losses in particular soil types or regions, species of *Pratylenchus* and *Meloidogyne* are widespread and particularly damaging to sugarcane, and are therefore economically important world-wide. In Australia, *P. zeae* and *M. javanica* are the key pests in a nematode community that is responsible for about 15 and 12% yield loss in plant and ratoon crops, respectively (Blair and Stirling, 2007).

Comprehensive surveys of sugarcane soils in Australia (Blair *et al*., 1999a; b) showed that there are regional differences in nematode occurrence, and that the distribution of pest nematodes also varies with soil texture and cultivar. The survey in south Queensland also showed that crop age had a major impact on nematode population density (Blair *et al*., 1999a). Populations of *P. zeae* (measured as nematode numbers/g root) were three times lower in third and later ratoons than in plant crops. A similar decline in nematode population densities with crop age was observed for *M. javanica*. The gradual decline in crop vigor and yield that occurs during the sugarcane cropping cycle was thought to have been partly responsible for this result, as root health in low yielding ratoon crops was likely to have been poorer than in higher-yielding plant crops, and this may have affected the quality of food available to the nematodes. However, Blair *et al.* (1999a) also suggested that natural enemies of nematodes may have built up during the sugarcane cropping cycle, thus gradually increasing the soil's suppressiveness to nematodes.

In considering the soil management factors that could possibly be associated with differences in suppressiveness between plant and ratoon crops, the tillage that is associated with removing the old crop and preparing for replanting warrants some attention. Tillage is known to disrupt the soil food web and is particularly detrimental to larger soil organisms (e.g. predatory nematodes and arthropods) that prey on nematodes (Wardle, 1995). Given that sugarcane soils were extensively ripped and cultivated in preparation for planting during the period when Blair *et al*. (1999a) conducted their survey, it is likely that some of the organisms that regulate populations of plant-parasitic nematodes were destroyed during the tillage process. More recent evidence suggests that this does occur, as populations of both *P. zeae* and *M. javanica* resurge more strongly when sugarcane is planted into conventionally tilled soil than into non-tilled soil (Stirling *et al*., 2010a). Thus it is possible that tillage-induced changes to the soil biota are responsible for the rapid increase in nematode populations in plant crops, and that population decline in subsequent years is due to the gradual return of predators.

A second factor possibly associated with increased suppressiveness in ratoon crops is the annual deposition of a blanket of sugarcane residue on the soil surface. Most Australian sugarcane crops are harvested green and the crop residues that remain after harvest are left on the soil surface as mulch, a process that is known as green cane trash blanketing (GCTB). Given the role of organic matter in enhancing suppressiveness to nematodes and other soil-borne pathogens (Stirling, 1991; Stone *et al*., 2004; Oka, 2010), it is possible that C inputs from crop residues promote the development of a soil food web capable of limiting populations of plant-parasitic nematodes. Such a possibility is supported by experimental evidence which shows that suppressiveness to *P. zeae* and other plant-parasitic nematodes is enhanced when residues from sugarcane crops are incorporated into soil (Stirling *et al*., 2003; 2005).

Over the last 10 years, a sugarcane farming system based on minimum tillage, controlled traffic, legume break crops and residue retention has been introduced into the Australian sugar industry (Garside *et al*., 2005; Stirling, 2008). Since the amount of tilled sugarcane land is now declining and a permanent cover of crop residues is an integral component of the new farming system, it is clearly time to look at the impact of minimum tillage and trash blanketing on suppressiveness to plant-parasitic nematodes. Thus the objectives of this work were to compare the suppressiveness of soil just under the trash blanket with soils further down the profile, and to determine whether sugarcane residue and other organic materials enhance suppressiveness to plant-parasitic nematodes when they are used as mulch.

MATERIALS AND METHODS

Distribution of sugarcane roots and nematodes with depth

Data were collected from a field trial near Bundaberg, Queensland that was established in a light-textured soil (clay content of 7.3% in the upper 10 cm of the profile) to evaluate the effects of cropping history, tillage practice and N inputs on soil properties and their subsequent effects on growth and yield of sugarcane. The site was planted to sugarcane variety Q151 in September 2006 and details of treatments, together with results of agronomic, soil chemical and nematological evaluations, have been published previously (Bell *et al*., 2010; Stirling *et al*., 2010a). In this component of the work, soil samples were collected in the first ratoon crop from four replicate plots of the soybean/direct drill/0N treatment (i.e. sugarcane had been planted following a soybean rotation crop and did not receive fertilizer N during either the plant or first ratoon crop).

In October 2008, immediately after the ratoon crop was harvested, soil samples collected to monitor soil nitrogen dynamics were also used to study nematode distribution in the soil profile to a depth of 150 cm. Two cores 48 mm in diameter were collected with a hydraulically-operated sampling tube and separated into seven depth intervals (0-10, 10-20, 20-30, 30-60, 60-90, 90-120, 120-150 cm). Roots retrieved by passing the soil through a 5 mm sieve were then dried at 80°C and weighed, while nematodes were extracted by incubating 200 g of the sieved soil for 2 days (Whitehead and Hemming, 1965).

Nematode distribution in the upper 20 cm of the soil profile was determined in March 2008 (in the middle of the growing season and about 6 months after the plant crop was harvested) and in October 2008 (at the end of the growing season and at the same time as the soil profile samples to a depth of 150 cm were collected). At both sampling times, the trash blanket was moved aside from an area about 10 cm from the base of a plant, two 20 × 20 cm holes were dug to a depth of 20 cm and soil from various increments in the profile was retained. In March 2008, two of these increments were near the surface (0-2 and 2-5 cm) and others were further down the profile (10-15 and 15-20 cm). Roots were sieved from each soil sample and then nematodes were extracted by incubating roots in a mist chamber for 3 days or by spreading soil on a tray as described previously. Total soil organic C and total N were analyzed in a combustion analyzer using the Dumas dry combustion method; labile C (33 mM permanganate-oxidizeable C) according to Moody *et al*., (1997) and water soluble C (1:2 water extract) using the chromic oxidation method of Heanes, (1984). In October 2008, samples from five depths (0-2, 2-5, 5-10, 10-15 and 15-20 cm) were retained and nematodes were extracted in the same way from soil and roots. Root health was also assessed using a 1-5 rating scheme where 1= roots severely diseased, with no tertiary roots; 2= roots highly diseased, with tertiary roots erratically distributed and contributing <20% of total root length; 3= intermediate levels of root disease, with tertiary roots contributing 20-50% of total root length; 4= healthy root system, with large numbers of functional tertiary roots; 5= very healthy root system, with a uniform spread of functional tertiary roots contributing >90% of total root length.

Impact of different mulches on nematode populations near the soil surface

This experiment was established in September 2008 on a sandy clay loam soil about 5 km from the previous site. Twelve plots each 20 m long and 9 m wide (consisting of five sugarcane rows on 1.8 m centers) were set up to accommodate four mulch treatments (untreated control, mill mud, sugarcane residue, and sugarcane residue + mill mud) replicated three times and arranged in a randomized block design. A third ratoon crop of sugarcane (variety Q138) was growing at the site and the sugarcane residue treatments were achieved by either removing the residues remaining after the recent harvest (equivalent to 10.75 t dry matter/ha) or leaving them on the soil surface as a GCTB. Mill mud (a by-product of the sugar milling industry) was spread on the surface of appropriate plots at 150 t/ha (the equivalent of 15 t dry matter/ha). The sugarcane was then allowed to ratoon and was irrigated regularly, as is standard practice for the region. About seven months after mulch treatments were established (22 April 2009), a soil sample (a composite of ten 44.5 mm-diameter cores to a depth of 10 cm) was collected from each plot and nematodes were extracted from 200 g sub-samples as described previously.

A second set of samples was collected about two months later (13 June 2009) to check nematode and root distribution with depth. Five 44.5 mm diameter cores/plot were collected about 30 cm from the base of the plant at depths of 0-2, 2-5 and 5-10 cm. Cores from each depth were combined, the samples were weighed and the soil moisture content was determined. Roots were then sieved from each sample and weighed before being cut into small pieces and returned to the sample from which they were collected. Nematodes were extracted from 200 g sub-samples of the soil using the method described previously.

Microcosm experiment comparing suppressiveness from incorporated and mulched residues

In preparation for this experiment, sandy loam soil from the sugarcane field used for the cropping history× tillage practice × N experiment mentioned previously was sealed in large plastic bags and fumigated with methyl iodide (11 µL iodomethane/100 L soil). Two organic materials were also obtained for use in the experiment: leaves and stalks of sugarcane that had been packaged for use as garden mulch (C), and soybean residue that was collected from the soil surface after a soybean crop had been harvested (S). The C/N ratio of the two materials was 88:1 and 29:1, respectively.

On 25 July 2006, sections of open-ended pipe 11cm long and 10.4 cm in diameter (volume = 934 mL) were filled with 1.02 kg of fumigated soil that had been amended with either sugarcane residue (C) or a 1:1 mixture of sugarcane and soybean residue (CS), or left untreated (Nil). The amount of crop residue mixed with the soil (20.8 g dry matter/kg dry soil) was the equivalent of 25 t dry residue/ha. The pipes (microcosms) were then placed upright in an 11 cm deep hole in the field from which the soil had been collected. Gaps between pipes were filled with field soil and then half the pipes were covered with a layer of sugarcane residue 3 cm deep (to simulate the mulch layer normally present in the GCTB system), while the remainder were covered with about 1 cm of soil. Thus, the factorial experiment consisted of three incorporated amendment treatments (C, CS, and Nil) × two mulch treatments (mulch and no mulch). Temperatures at a depth of 10 cm under the

mulched and non-mulched treatments were recorded with a Tiny Tag Plus™ data logger (Hastings Data Loggers, Port Macquarie, NSW, Australia).

The site received rainfall while the pipes were buried and was also watered when the adjacent crop of sugarcane was irrigated, but otherwise was subject to normal environmental conditions. After 18 weeks (4 December 2006) and again at 27 weeks (2 February 2007), four replicate pipes were retrieved to assess the chemical and biological status of the soil in the microcosms. The soil or mulch that covered a further eight replicate pipes of each treatment was then removed, a saucer was placed under each pipe so that it could be used as a pot and the soils were assayed in the glasshouse for suppression to root-knot nematode (*M. javanica*) and lesion nematode (*P. zeae*) by comparing nematode multiplication rates on plants grown in the pots. In the *Meloidogyne* assay, a tomato seedling (cv. Tiny Tim) was planted in each pot and four replicate pots were inoculated with 6,000 eggs of *M. javanica*. After 7 weeks in the glasshouse, roots were rated for galling on the 0-10 scale of Zeck (1971), and then roots were immersed in 1% NaOCl for 5 minutes and eggs were retrieved on a 38 µm sieve and counted. In the *Pratylenchus* assay, a single seed of maize (cv. H5) was planted in each pot and four replicate pots were inoculated with 2,000 *P. zeae*. After 7 weeks in the glasshouse, roots were placed in a mist cabinet and the number of nematodes recovered was counted after 5 days.

The chemical status of the soil at the time microcosms were retrieved from the field was assessed using methods described previously. At both sampling times, the biological status of the soil was also assessed by measuring microbial activity and counting free-living nematodes. Microbial activity was estimated by allowing soil enzymes to hydrolyze fluorescein diacetate (FDA) to water-soluble fluorescein, and measuring the end product with a spectrophotometer (Schnürer and Rosswall, 1982). Readings were corrected for background absorbance and appropriate standard curves (Chen *et al*., 1988) were used to calculate microbial activity (expressed as µg FDA hydrolyzed/g dry soil/min). Nematodes were extracted from 200 mL samples using the method described previously and total numbers of free-living nematodes were counted at a magnification of 40X. Nematodes were then fixed in formalin-acetic acid and a sample of at least 100 randomly-selected specimens was identified to family or genus level at a magnification of 400X and assigned a trophic group and colonizer-persister (c-p) value according to Ferris *et al*, (2001).

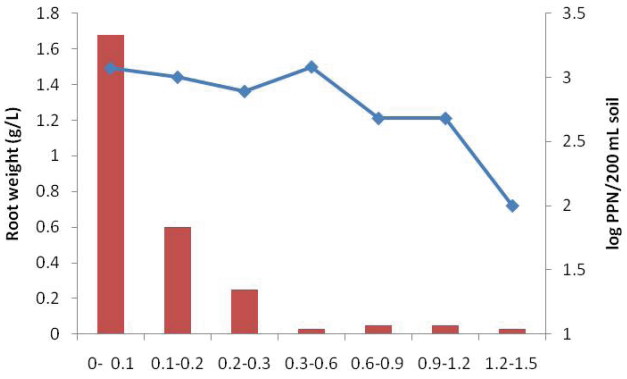

Fig. 1. Distribution of sugarcane roots (bars) and total numbers of plant-parasitic nematodes (line) at various depths in the soil profile to 150 cm under trash blanketed sugarcane immediately after harvest of the first ratoon crop

Statistical analysis

Nematode data from pot and field experiments were transformed ($\log_{10}$ no. nematodes +1) and subjected to analysis of variance using Genstat 8.2, with a repeated-measures procedure used for data obtained from different depths in the soil profile. Back-transformed means are presented in the tables of results. Relationships between various parameters (nematode population densities, root biomass, root health, soil chemistry) and depth were examined using regression analysis.

RESULTS

Distribution of sugarcane roots and nematodes with depth

When a ratoon crop was sampled to a depth of 150 cm, free-living nematodes and six species of plant-parasitic nematodes were found throughout the profile. Population densities of all nematodes declined with depth (Table 1), with regression analyses indicating that this relationship was significant for total plant-parasitic nematodes, total free-living nematodes and all plant parasites except *Helicotylenchus dihystera* and *Rotylenchulus parvus*. Most of the roots were near the surface, with 63% of the total root biomass in the upper 10 cm of the profile and a further 22% and 9% in the 10-20 and 20-30 cm zones, respectively (Table 1). Root biomass declined significantly with depth, but populations of each genus of plant-parasitic nematode were not strongly correlated with root biomass (r ranging from 0 to 0.49). Also, total populations of plant parasites were not closely related to root distribution (Table 1; Figure 1).

Samples collected from the upper 20 cm of the soil profile in March 2008 indicated that a large proportion of the roots in that zone were concentrated

Table 1. Distribution of roots, free-living nematodes (FLN), various species of plant-parasitic nematodes (TPPN), and relationships with depth, in the soil profile to 150 cm under trash blanketed sugarcane immediately after harvest of the first ratoon crop.

Depth (cm)	Root dry wt. (g/L)	Nematodes/200 mL soil							
		FLN	*Pratylenchus zeae*[z]	*Meloidogyne javanica*	*Rotylenchulus parvus*	*Helicotylenchus dihystera*	*Xiphinema elongatum*	*Paratrichodorus minor*	TPPN
0-10	1.68	1181	311	435	240	64	19	109	1178
10-20	0.60	566	294	138	214	116	11	235	1008
20-30	0.25	560	228	18	106	200	6	227	784
30-60	0.03	192	114	25	966	34	1	63	1203
60-90	0.05	68	151	1	285	28	1	17	482
90-120	0.05	86	102	3	21	29	1	11	166
120-150	0.03	56	50	1	3	39	1	7	100
Sig.[z]	***	***	**	*	ns	ns	*	*	**
R^2	0.39	0.63	0.32	0.20	0.02	0.06	0.16	0.29	0.32

[z] The last two rows of the table indicate the significance of the regression between each parameter and depth (ns = not significant; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$), and the corresponding R^2 value.

just under the trash blanket (Table 2) and that roots in the 0-2 cm zone showed few signs of the lesions and blackening usually observed on sugarcane roots. Concentrations of soil N and all forms of soil C (total, labile and water-soluble) were highest in surface soil and declined significantly with depth, and populations of free-living nematodes followed a similar pattern (Table 2). In contrast, *P. zeae* had a different distribution, with populations lowest in the 0-2 cm zone and not declining significantly with depth (Table 2). Additional sampling of upper soil layers in October 2008 indicated that the distribution of nematodes and roots was similar to the previous sample (Table 3). A dense layer of fine roots was present just under the trash blanket, with roots in the 0-2 cm zone rated as much healthier than roots further down the profile (Table 3).

Impact of different mulches on nematode populations near the soil surface

Seven months after mulches were applied, populations of *P. zeae* were significantly lower in plots mulched with sugarcane residue (with or without mill mud) than in non-mulched plots or plots mulched with mill mud alone (Table 4). However, populations of free-living nematodes and other plant parasites were not affected by mulching. Many of the free-living nematodes (but not *P. zeae*) were parasitized by an oomycete similar to *Myzocytium* (particularly those from the mill mud + sugarcane mulch treatment), suggesting that the nematode population in some treatments may have been limited by this parasite. Ciliate protozoans were also observed in the samples, with numbers highest in the mill mud + sugarcane mulch treatment and lowest in plots without mulch (data not presented).

Nine months after mulches were applied, populations of free-living nematodes were not affected by mulching (Table 5). However, the mulch effect was almost significant ($P = 0.059$), with the data showing a similar trend to the previous sampling time (i.e. more free-living nematodes in plots mulched with sugarcane residue than in non-mulched plots). Detailed analyses of the nematode community were not carried out, but observations of the omnivorous and

predatory component at depths of 0-2 and 5-10 cm in the sugarcane mulch treatment indicated that there were 107±13 Mononchidae and 317±17 Dorylaimida/200 g soil at a depth of 0-2 cm and only 3±2 Mononchidae and 26±3 Dorylaimida/200 g soil at 5-10 cm. The predatory nematode (identified as a species of *Coomansus*) was seen feeding on nematodes in water suspensions. As observed 7 months after treatment, populations of *P. zeae* were lowest in plots mulched with sugarcane residue (with or without mill mud), but in this case, differences between mulches were only significant when populations were compared on the basis of the number of nematodes /g root (Table 5). Populations of *H. dihystera* and *T. annulatus* were again not affected by mulch treatment. Data obtained from samples collected at different depths showed that bulk density was lower and root biomass was higher in soil from 0-2 cm than further down the profile. Populations of free-living nematodes were highest in surface soil whereas plant-parasitic nematodes did not respond in the same way to depth. Populations of *P. zeae* were much lower at 0-2 cm than further down the profile, whereas depth had the opposite effect on *H. dihystera* and no effect on *T. annulatus* (Table 5).

Microcosm experiment comparing suppressiveness from incorporated and mulched residues

Covering the soil surface with sugarcane residue reduced temperatures at a depth of 10 cm and markedly

Table 2. Distribution of roots, free-living nematodes (FLN), *Pratylenchus zeae*, soil C and soil N, and relationships with depth, in the upper 20 cm of the soil profile in mid-season samples taken from a trash blanketed, first ratoon crop of sugarcane.

Depth (cm)	Root dry wt. g/L soil	FLN /200 mL soil	*Pratylenchus* /200 mL soil	*Pratylenchus* /g root	Total C g/kg	Total N g/kg	Labile C g/kg	Water soluble C mg/kg
0-2	2.47	2395	290	136	18.9	1.03	2.27	91
2-5	0.59	1930	795	1066	14.9	0.73	1.65	84
10-15	0.28	1210	908	1528	11.8	0.58	1.05	65
15-20	0.35	900	488	848	9.4	0.45	0.76	51
Sig. [z]	**	**	ns	ns	***	***	***	***
R^2	0.55	0.52	0.03	0.16	0.83	0.73	0.91	0.80

[z] The last two rows of the table indicate the significance of the regression between each parameter and depth (ns = not significant; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$), and the corresponding R^2 value.

Table 3. Distribution of roots, free-living nematodes (FLN) and *Pratylenchus zeae*, together with root health ratings and relationships with depth, in the upper 20 cm of the soil profile after harvest of a trash blanketed, first ratoon crop of sugarcane.

Depth (cm)	Root dry wt. g/L soil	Root health rating	FLN /200 mL soil	*Pratylenchus* /200 mL soil	*Pratylenchus* /g root
0-2	4.4	4.4	4735	142	117
2-5	1.8	2.4	2395	703	453
5-10	1.2	1.6	1393	830	554
10-15	1.0	1.5	1128	823	545
15-20	0.5	1.4	765	378	293
Sig. [z]	***	***	***	ns	ns
R^2	0.60	0.73	0.64	0.04	0.03

[z] The last two rows of the table indicate the significance of the regression between each parameter and depth (ns = not significant; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$), and the corresponding R^2 value.

Table 4. Effects of mulching with sugarcane residue and mill mud on populations of free-living and plant-parasitic nematodes on sugarcane at depths of 0-10 cm in a field experiment at Bundaberg, Queensland in April 2009, 7 months after mulch treatments were imposed

	Nematodes extracted/200 g soil[z]			
Mulch	Free-living nematodes	*Pratylenchus zeae*	*Tylenchorhynchus annulatus*	*Helicotylenchus dihystera*
No mulch	1049 a	2138 a	174 a	390 a
Mill mud	1153 a	1849 a	326 a	427 a
Sugarcane	1845 a	835 b	248 a	615 a
Sugarcane + mill mud	970 a	410 c	279 a	650 a

[z]Numbers in each column followed by the same letter are not significantly different ($P = 0.05$)

Table 5. Main effects of surface mulch and depth within the soil profile on bulk density, root biomass and populations of free-living nematodes, *Pratylenchus zeae, Helicotylenchus dihystera* and *Tylenchorhynchus annulatus* in a field experiment at Bundaberg, Queensland in June 2009, 9 months after mulch treatments were imposed.

				Nematodes/200 g soil				
		Bulk density	Fresh wt. roots/200 g soil	Free-living nematodes	*P. zeae*	*H. dihystera*	*T. annulatus*	*P. zeae* /g root
Mulch	No mulch	1.42 a[z]	0.35 a	1061 a	1383 a	479 a	299a	5668 a
	Mill mud	1.34 a	0.32 a	1327 a	988 a	266 a	234 a	3917 ab
	Sugarcane	1.38 a	0.35 a	1927 a	503 a	666 a	588 a	1820 c
	Sugarcane + mill mud	1.31 a	0.26 a	1527 a	670 a	502 a	268 a	3155 b
Depth	0-2 cm	1.22 c	0.56 a	2460 a	349 b	716 a	252 a	658 c
	2-5 cm	1.34 b	0.27 b	1361 b	1333 a	446 b	433 a	5457 b
	5-10 cm	1.53 a	0.12 c	916 c	1202 a	295 c	313 a	10592 a

[z]Numbers in each column followed by the same letter are not significantly different ($P = 0.05$)

Table 6. Effect of three crop residue treatments incorporated into fumigated soil (sugarcane residue [C], sugarcane + soybean residue [CS] and no crop residue [Nil]) and two mulch treatments (sugarcane residue covering the soil as mulch, and no mulch) on soil carbon and nitrogen status in microcosms that were left in the field for 18 or 27 weeks

		C (g/kg)		N (g/kg)		Labile C (g/kg)	
Factor		18 wk.	27 wk.	18 wk.	27 wk.	18 wk.	27 wk.
Incorporation	Nil	9.8 b[z]	9.5 b	0.55 b	0.53 b	0.84 c	0.7 b
	CS	12.2 a	11.8 a	0.68a b	0.62 ab	1.27 b	1.17 a
	C	12.7 a	12.2 a	0.74 a	0.68 a	1.47 a	1.15 a
Mulch	No mulch	10.8 b	10.6 b	0.59 b	0.57 a	1.13 a	0.92 b
	Mulch	12.3 a	11.6 a	0.72 a	0.64 a	1.25 a	1.1 a

[z]Main effects are presented for each parameter at each sampling time, as incorporation × mulch interactions were not significant. Means in each column followed by the same letter are not significantly different ($P = 0.05$).

reduced diurnal fluctuations in temperature. In microcosms covered with soil, the average temperature increased from about 18°C (daily range of 7-9°C) in August 2006 to about 32°C (daily range 10-12°C) in January 2007. The comparable figures for microcosms covered with mulch were 17°C (daily range <1°C) in August 2006 and 28°C (daily range 1-1.5 °C) in January 2007.

Incorporating crop residues into soil increased soil C (particularly the labile component), and the N content (Table 6). This in turn impacted on microbial activity and populations of free-living nematodes, with both parameters consistently higher in the two incorporated residue treatments than in the corresponding control at 18 and 27 weeks (Tables 7 and 8). Mulching the soil surface with sugarcane residue also increased soil C and N at both sampling times, although the effect was less than when residues were incorporated and was not always significant (Table 6). Microbial activity was higher in mulched than non-mulched microcosms at 18 weeks but at 27 weeks there was a significant incorporation × mulch interaction, with the mulch effect only apparent when sugarcane residue had been incorporated into soil (Table 7). Populations of free-living nematodes did not respond in the same way as microbial activity, with mulching reducing nematode populations across all treatments at 18 weeks and in residue-incorporated treatments at 27 weeks (Table 8).

Indices derived from nematode community analyses were inconsistent, with the coefficient of variation for many indices (across the four replicate samples of each treatment) often greater than 20% and sometimes as high as 60%. Thus despite the impacts of incorporated residues and mulching on total numbers of free-living nematodes, there was no tangible evidence that nematode communities differed between treatments. The predominant nematodes in all treatments were bacterial feeders with c-p values of 2 and 3 (i.e. *Cephalobidae, Prismatolaimus* and *Rhabdolaimus*), and there were also small numbers of Rhabditidae and fungal-feeding *Aphelenchus* and *Aphelenchoides*. Predatory nematodes (Mononchidae) were absent and the omnivorous and carnivorous Dorylaimida rarely comprised more than 1% of the nematode community.

Results for the *Meloidogyne* assay at both sampling times showed that mulching significantly reduced root galling and the number of root-knot nematode eggs recovered from tomato roots (Tables 9 and 10). The residue incorporation effect was never significant (P = 0.21 and 0.1 for gall ratings and 0.27 and 0.25 for egg numbers at 18 and 27 weeks, respectively), but the level of galling and the number of eggs was consistently lower in soil into which sugarcane residue had been incorporated than in the non-amended control. There was no incorporation × mulch interaction for either of the measured parameters at either sampling time.

In the bioassay with *P. zeae* at the 18 week sampling time, incorporating crop residues into soil and mulching both reduced the number of lesion nematodes recovered from maize roots (Table 11). The incorporation × mulch interaction was not significant. However, at 27 weeks, mulching reduced numbers of *P. zeae* only in soil that had not received incorporated crop residues (Table 11).

DISCUSSION

Sugarcane roots are generally located in upper layers of the soil profile, with root biomass declining exponentially with depth. Roots can be present at depths

Table 7. Effect of three crop residue treatments incorporated into fumigated soil (sugarcane residue [C], sugarcane + soybean residue [CS] and no crop residue [Nil]) and two mulch treatments (sugarcane residue covering the soil as mulch, and no mulch) on microbial activity in microcosms that were left in the field for 18 or 27 weeks.

Time in field	Factor	Microbial activity (µg FDA/g/min)		
18 weeks	Incorporation	Nil	CS	C
		0.287 c[z]	0.450 b	0.497 a
18 weeks	Mulch		No mulch	Mulch
			0.385 b	0.437 a
27 weeks	Incorporation × Mulch		No mulch	Mulch
		Nil	0.483 c	0.465 c
		CS	0.537 b	0.534 b
		C	0.564 b	0.664 a

[z]Main effects are presented for the 18 week sampling time, as the incorporation × mulch interaction was not significant. Means in each row followed by the same letter are not significantly different (P = 0.05). Due to a significant incorporation × mulch interaction at 27 weeks, all means are compared, and those followed by the same letter are not significantly different (P = 0.05).

greater than 4 m (Smith *et al*., 2005; Chopart *et al*., 2009), but the results of studies which characterize the sugarcane root system to a depth of 1.5 or 2 m indicate that 50-75% of the roots are usually found within 30-50 cm of the soil surface (Evans 1938; Paz-Vergara *et al*., 1980; Ball-Coelho *et al*., 1992). In this study, which was done in a soil where there were no obvious physical barriers to root growth, root distribution followed the expected pattern for sugarcane. However, the surface rooting habit was even more pronounced than has been observed elsewhere, with 94% of the root biomass in the upper 30 cm of the soil profile. Even within this zone, strong stratification was apparent, as many roots were concentrated just under the trash blanket to a depth of about 2 cm.

Given the obvious importance of roots in the surface layers of soil, it was interesting to observe that root health was better in the 0-2 cm zone than further down the profile. The root system near the surface was exceptionally healthy, being comprised of a network of white or light brown roots that showed few signs of damage due to pathogens. In contrast, roots in the zone from 2-20 cm were usually moderately or severely diseased, with rotted stubs on primary and secondary roots indicating that many of the tertiary (fine) roots had rotted away. When tertiary roots were present, discoloration, blackening or incipient lesions were apparent. Since *P. zeae* produces purplish-black lesions on sugarcane roots and they eventually expand to girdle roots and destroy the fine root system (Stirling and Blair, 2000), it is likely that the presence of these symptoms at depths greater than about 2 cm and their absence near the soil surface was associated with the distribution of this nematode. Data presented in Tables 2, 3, and 5 indicate that the population density of *P. zeae*/g root was 9, 5, and 16 times lower, respectively, in roots from 0-2 cm than in roots collected a few cm further down the profile. Populations of *P. zeae* in soil were also consistently lower near the soil surface than at depth.

Given that roots were abundant in the soil immediately beneath the trash blanket, it is unlikely that populations of *P. zeae* in upper layers of soil were limited by the availability of food resources. It is possible that the environment near the soil surface (in terms of moisture and temperature) was not suited to *P. zeae*, but the insulating and moisture retention properties of the trash blanket suggest that this was unlikely. The most likely explanation is that surface soil was suppressive to the nematode. The fact that individually and collectively, population densities of other plant-parasitic nematodes did not mirror root distribution provides additional evidence that nematode-suppressive forces were operating in the upper part of the soil profile. Plant-parasitic nematodes may have different feeding habits but they are all obligate parasites of roots, so in the absence of top-down regulatory processes, their population densities would be expected to be much higher near the surface than at depth.

The exceptional health of roots under the trash blanket prompted the establishment of an experiment to evaluate the potential of using mill mud as mulch. This readily-available waste product from the sugar milling industry contains useful quantities of plant nutrients and is therefore used as a fertilizer (Barry *et al*., 2001; Quereshi *et al*., 2001). It is normally applied to the surface of the field and then incorporated with the trash blanket. From a nematode management perspective, the question was whether mill mud would reduce

Table 8. Effect of three crop residue treatments incorporated into fumigated soil (sugarcane residue [C], sugarcane + soybean residue [CS] and no crop residue [Nil]) and two mulch treatments (sugarcane residue covering the soil as mulch, and no mulch) on the total number of free living nematodes in microcosms that were left in the field for 18 or 27 weeks.

		No. free living nematodes/200 mL soil[z]		
Time in field	Factor	Nil	CS	C
18 weeks	Incorporation	3,451 a[z]	10,162 b	14,521 b
18 weeks	Mulch		No mulch	Mulch
			8,609 a	7,396 b
27 weeks	Incorporation × Mulch		No mulch	Mulch
		Nil	1,585 d	2,000 d
		CS	4,560 b	3,069 c
		C	8,472 a	5,508 b

[z]Main effects are presented for the 18 week sampling time, as the incorporation × mulch interaction was not significant. Means in each row followed by the same letter are not significantly different ($P = 0.05$). Due to a significant incorporation × mulch interaction at 27 weeks, all means are compared, and those followed by the same letter are not significantly different ($P = 0.05$).

Table 9. Effect of three crop residue treatments incorporated into fumigated soil (sugarcane residue [C], sugarcane + soybean residue [CS] and no crop residue [Nil]) and two mulch treatments (sugarcane residue covering the soil as mulch, and no mulch) on galling caused by *Meloidogyne javanica* on tomatoes grown in microcosms that were left in the field for 18 or 27 weeks and then inoculated with the nematode.

		Root gall rating[y]		
Time in field	Factor	Nil	CS	C
18 weeks	Incorporation	3.62 a[z]	3.25 a	3.00 a
27 weeks		4.50 a	4.08 a	3.62 a
	Mulch	No mulch	Mulch	
18 weeks		3.83 a	2.75 b	
27 weeks		4.67 a	3.47 b	

[y]Based on the 0-10 scale of Zeck (1971), where 0 = no galls and 10 = severe galling
[z]Main effects are presented for each sampling time, as incorporation × mulch interactions were not significant. Means in each row followed by the same letter are not significantly different ($P = 0.05$).

Table 10. Effect of three crop residue treatments incorporated into fumigated soil (sugarcane residue [C], sugarcane + soybean residue [CS] and no crop residue [Nil]) and two mulch treatments (sugarcane residue covering the soil as mulch, and no mulch) on the number of eggs produced by *Meloidogyne javanica* on tomatoes grown in microcosms that were left in the field for 18 or 27 weeks and then inoculated with the nematode.

		No. eggs/plant[z]		
Time in field	Factor	Nil	CS	C
18 weeks	Incorporation	68,077 a[z]	43,853 a	40,458 a
27 weeks		162,180 a	204,640 a	114,550 a
	Mulch		No mulch	Mulch
18 weeks			102,329 a	23,933 b
27 weeks			249,460 a	97,273 b

[z]Main effects are presented for each sampling time, as incorporation × mulch interactions were not significant. Means in each row followed by the same letter are not significantly different ($P = 0.05$).

Table 11. Effect of three crop residue treatments incorporated into fumigated soil (sugarcane residue [C], sugarcane + soybean residue [CS] and no crop residue [Nil]) and two mulch treatments (sugarcane residue covering the soil as mulch, and no mulch) on the number of *Pratylenchus zeae* in roots of maize plants grown in microcosms that were left in the field for 18 or 27 weeks and then inoculated with the nematode.

		No. *P. zeae*/plant[z]		
Time in field	Factor	Nil	CS	C
18 weeks	Incorporation	4083 a[z]	1409 b	1538 b
18 weeks	Mulch		No mulch	Mulch
			4046 a	1057 b
27 weeks	Incorporation × Mulch		No mulch	Mulch
		Nil	46,025 a	11,510 b
		CS	17,740 b	17,660 b
		C	17,760 b	13,800 b

[z]Main effects are presented for the 18 week sampling time, as the incorporation × mulch interaction was not significant. Means in each row followed by the same letter are not significantly different (P = 0.05). Due to a significant incorporation × mulch interaction at 27 weeks, all means are compared, and those followed by the same letter are not significantly different (P= 0.05).

populations of plant-parasitic nematodes if it was simply applied to the trash blanket and then left on the surface as mulch. Seven months after such a treatment was applied experimentally, such an effect was observed, as populations of *P. zeae* in the mill mud + sugarcane residue treatment were lower than in the treatment with sugarcane residue alone. However, the addition of mill mud increased the rate at which sugarcane residue decomposed, because observations made 9 months after treatments were applied indicated that some surface residues remained in the sugarcane treatment but all residues had disappeared in the mill mud + sugarcane treatment. This change in the decomposition rate was almost certainly due to the relatively high amounts of N, P, and other nutrients present in mill mud. The eventual loss of surface residues was possibly one of the reasons that the effect of the mill mud + sugarcane residue treatment on *P. zeae* had begun to dissipate at 9 months (Table 5).

In addition to showing the effects of mill mud, sampling at both 7 and 9 months indicated that populations of *P. zeae* in the 0-10 cm zone were lower in mulched than non-mulched plots (Table 4). However, when lesion nematode population densities were measured at various depths within that zone, there were significant mulch and depth effects but no mulch × depth interaction (Table 5). Collectively, these results indicate that retaining sugarcane residues on the soil surface reduces populations of *P. zeae* in upper layers of the soil profile, but that factors other than the presence of a mulch layer contribute to this effect. Another interesting observation was that populations of the two ectoparasitic nematodes present at the site (*H. dihystera* and *T. annulatus*) were not affected by either mulch or depth, indicating that they did not respond in the same way as *P. zeae*, a migratory endoparasite.

Results of a microcosm experiment on the effect of crop residues as soil amendments clearly showed that incorporating sugarcane residue into soil enhanced suppressiveness to *P. zeae*. Such a result was not unexpected, as previous work with similar treatments in the field had shown that 47 weeks after the residue was incorporated, the number of *P. zeae* in roots was reduced by 95% relative to the non-amended control (Stirling *et al*., 2005). With regard to *M. javanica*, bioassay results showed a trend towards increased suppressiveness following the incorporation of sugarcane residue into soil, but unlike the results of previous studies (Stirling *et al*., 2003), this effect was not strong enough to be significant.

The surprising result from this experiment was the effect of sugarcane residue as mulch. Mulching improved soil C levels and increased microbial activity, but free-living nematodes did not respond in the same way, suggesting that suppressive forces were limiting nematode populations. Bioassay results also showed that mulched soil was more suppressive to root-knot and lesion nematodes than non-mulched soil, and that soil which had not previously been amended with organic matter became relatively suppressive to these nematodes after it was mulched.

A useful working hypothesis to explain results from both the field and microcosm studies is that a blanket of sugarcane residue covering the soil surface provides C inputs that help sustain a soil food web capable of suppressing root pathogens (including plant-parasitic nematodes), thereby enabling a healthy layer of roots to develop immediately beneath the mulch layer. Results from the microcosm study strongly support this hypothesis. They also suggest that one of the reasons that mulching impacts on suppressiveness is that the soil environment is more amenable to biological activity because soil temperature fluctuations are dampened. The results of some of the field studies also supported the above hypothesis, as populations of *P. zeae* were relatively low immediately under the trash blanket at one site (Tables 2 and 3) and were lower in mulched than non-mulched plots at another site (Table 4). However, there were also results that did not support the hypothesis. For example, additional sampling at the second site showed that populations of *P. zeae* in surface soil were low, regardless of the presence or absence of mulch, and that the mulch layer did not reduce populations of *H. dihystera* and *T. annulatus* near the surface (Table 5). These observations suggest that either the agents suppressing *P. zeae* are specific for certain nematodes or that other factors are also affecting the capacity of plant-parasitic nematodes to survive in surface soil.

Further work is obviously required to better understand the mechanisms regulating populations of plant-parasitic nematodes in trash-blanketed sugarcane soils, but parasitism, predation and competition (top-down processes) are almost certainly involved. However, identifying the organisms responsible for suppressing *P. zeae* and other nematodes will be a time-consuming task, as a wide range of antagonists of nematodes are likely to reside in the mulch layer and in the soil immediately beneath it. Omnivorous and predatory nematodes are one of many possible suppressive agents (Sanchez-Moreno and Ferris, 2007), and at the site where the data in Tables 4 and 5 were collected, their population densities were much higher at 0-2 cm than further down the profile. However, the lack of omnivorous and predatory nematodes in the microcosm experiment suggests that at least in this instance, other soil organisms were responsible for the suppressive effect of mulch.

From a practical perspective, the main message from this work is that the trash blanket plays an important role in maintaining root health in sugarcane. Not only does it improve the environment for root growth by reducing fluctuations in moisture and temperature, but together with exudates from roots, the trash blanket provides the C inputs required to improve soil physical and chemical properties and sustain a soil food web capable of cycling nutrients and suppressing pathogens. Although the data collected in this study

suggest that the C benefits from the trash blanket tend to be confined to the zone within a few cm of the soil surface, this situation is likely to change over time. C levels in sugarcane soils have increased after 20-25 years of trash blanketing (Thorburn *et al*., 2000), and C is likely to accumulate at an even greater rate and have an impact further down the profile when tillage is eliminated from the sugarcane production system (Stirling *et al*., 2010b). Given the key role of soil C in enhancing numerous properties associated with soil health and suppressiveness to root pathogens (Weil and Magdoff, 2004; Stone *et al*., 2004), the benefits from the trash blanket are likely to be greater in the long-term than they are today.

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