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The potential of organic and inorganic soil amendments, and a biological control agent (*Trichoderma* sp.) for the management of Phytophthora root rot of papaw in far northern Queensland

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Abstract. Non-chemical options for the management of *Phytophthora palmivora* on papaw in far northern Queensland were evaluated in pot and field experiments. In pots, sawdust (150 m^3/ha) + urea (600 kg/ha) reduced root rot, increased plant growth and reduced *Phytophthora* inoculum to undetectable levels. The addition of filterpress (400 m^3/ha) and mill ash (200 m^3/ha), reduced the damage caused by *P. palmivora*, but amendment with chicken manure (4% v/v), MinPlus (4.5 t/ha) and Trichodry (1.0 kg/m^3) plus Trichoflow (5.0 kg/ha) failed to reduce root damage. In a second pot experiment, growing brassica (Bioquire Mulch) as a green manure significantly ($P < 0.05$) reduced *Phytophthora* inoculum levels and root rot severity, whereas the incorporation and composting of soybean, brachiaria, banana and sugar-cane failed to reduce the severity of root rot in papaw compared to a bare fallow. In a field crop of papaw grown for 46 weeks on 0.75 m high mounds, the suppressive effects of sawdust (150 m^3/ha) + urea (600 kg/ha), filterpress (400 kg/ha), molasses (100 L/ha/week for 20 weeks), brassica (Bioquire Mulch, 4.5 kg/ha), soybean (cv. Leichardt, 40 kg/ha) and gypsum (5 t/ha) were compared. Soil amended with sawdust + urea had the lowest incidence of root rot. Soil amended with filterpress or molasses had the highest incidence of root rot and the highest populations of *P. palmivora*. Cumulative totals of percentage soil moisture were greatest in soil amended with filterpress or molasses, and this was evidence of the influence soil moisture retention has on root rot development.

Additional keywords: Carica papaya, sawdust, molasses, brassica, green manure, gypsum.

Introduction

The soilborne pathogen *Phytophthora palmivora*, which causes damping-off of seedlings, root rot, stem rot and fruit rot of papaw (*Carica papaya*) is a major constraint to the expansion of the papaw industry of northern Queensland (latitudes 16.48°S to 17.26°S). Of prime importance to the papaw industry has been Phytophthora root rot, which causes a decay of the taproot and eventual death of the plant (Teakle 1957).

Research conducted by Peterson *et al*. (1997) demonstrated that the chemicals metalaxyl and phosphorous acid, which are used extensively to control *Phytophthora*-induced diseases of many crops (Schwinn and Staub 1987), were largely ineffective in controlling Phytophthora root rot of papaw. However, growing papaw on single row mounds (at least 0.75 m high) significantly reduced plant losses and increased yields (Peterson *et al*. 1997).

Phytophthora is a poor saprophytic competitor (Tsao 1969) and survival of the pathogen in the soil is vulnerable to cultural practices that stimulate the activity of soil antagonists and reduce the likelihood of ponding. Therefore, the ploughing and composting of non-host green manure crops prior to planting papaw is one strategy likely to stimulate microbial antagonism against the soilborne propagules of *Phytophthora*. Composts and manures that stimulate the activity of actinomycetes, fluorescent pseudomonads, endospore-forming bacteria and certain fungi have been found to reduce the incidence and severity of *Phytophthora* diseases in crops such as avocado (*Persea americana*) (Stirling *et al*. 1992) and thryptomene (*Thryptomene calycina*) (Aryantha *et al*. 2000). Cultural practices that

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improve soil drainage, organic levels and nutritional balances have dramatically reduced the severity of Phytophthora root rot of avocado (Broadbent and Baker 1975).

This study reports on two glasshouse experiments and a field experiment that tested organic and inorganic soil amendments, crop rotation and two commercially available biological control products for their effectiveness in reducing Phytophthora root rot of papaw.

Methods

Pot experiments

These experiments examined the effect of soil amendments and crop rotation on *Phytophthora* infectivity and reproduction, root rot severity, plant height and fresh weight of papaw roots.

In the first of the glasshouse experiments, the soil amendments used included sawdust + urea, chicken manure, filterpress, mill ash and Min-plus, which are all products or by-products of local industries and are therefore readily available to north Queensland papaw growers. Filterpress, also known as filter cake or mill mud, and mill ash are by-products of the sugar industry (Barnes 1974) and are already used as an organic source of nutrients. Aryantha *et al*. (2000) showed that fresh chicken manure or chicken manure composted for 5 weeks before incorporation into potting mix infested with *P. cinnamomi* significantly reduced the pathogen's survival. Sawdust, used as a soil amendment, has the potential to encourage the development of antagonistic organisms known to lyse hyphae and spores of soilborne fungi such as *Phytophthora* (Barron 1992). However, its value is limited by its low nutrient content and a tendency to induce nitrogen deficiency problems, hence the need for a nitrogen source such as urea. Min-plus, which is produced from ground basalt rock quarried in the local area, has been promoted as a means of soil rejuvenation (Leonardos *et al.* 1987). The replenishment of soil fertility has been suggested as a means of reducing soilborne disease by increasing the host resistance to fungal attack (Broadbent and Baker 1975). The remaining treatments, Trichodry and Trichoflow, are commercial preparations of a strain of *Trichoderma* sp. (Schelling 1998). *Trichoderma* spp. have been studied extensively for their ability to act as biological control agents against a range of plant pathogens.

Soil preparation and determination of Phytophthora *levels.* A kraznozem soil collected from around the root zone of mature papaw plants heavily infected with *P. palmivora* was passed through a 10-mm-diameter sieve before being thoroughly mixed in a concrete mixer (in 7.5 kg batches) for 5 min. Kraznozem soil used in the uninfested treatment was taken from a field that had never grown papaw, and was sieved and pasteurised with aerated steam (70°C for 30 min). These soils were used to generate five replicate pots (18-cm diameter) of each treatment. Four soil samples were taken at random from each pot using a modified cork-borer (10-mm diameter) and bulked to give a 20 g composite sample that was used to assess the initial *Phytophthora* populations (Pi). The number of colony forming units (cfu) was determined by spreading five 1 mL aliquots of 1 : 2 (w/v) dilutions of soil on P_{10}ARP+H selective medium (Jeffers and Martin 1986) and counting after incubation for 72 h in the dark at 26°C.

Assessment of disease severity. Eight-week-old papaw seedlings of cv. Hybrid 29 that had been grown in pasteurised potting mix were transplanted to all pots and after 10 days, pots were placed in plastic trays filled with water to a depth of 25 mm to saturate the soil (Duniway 1979). After a further 3 days, pots were removed from the trays and the soil allowed to drain. Each pot was fertilised fortnightly with 0.3 g of Aquasol and plants were hand watered as required for the next 8 weeks. All plants in this glasshouse experiment were observed daily. Final *Phytophthora* densities (Pf), root rot severity ratings and plant growth assessments (plant height, fresh weight of roots) were conducted as plants died, and on surviving plants 10 weeks after transplanting. Root rot severity was assessed using the following scale: 1, no root rot; 2, rot of secondary roots only; 3, rot of primary root only; 4, rot of primary root and feeder roots; 5, complete root rot resulting in the death of the plant. Fresh weights of roots were recorded by excising the roots from the stem, and carefully washing and blotting them dry on absorbent paper before weighing. Sections of diseased roots and stems were surface sterilised in 70% ethanol for 1 min, blotted dry with sterile paper then transferred to P_{10}ARP+H selective media. The plates were observed for growth of *P. palmivora* from the roots and stems after incubation in the dark at 26°C for 72 h.

Assessment of soil amendments and Trichoderma*-based products.* Treatments (Table 1) were mixed by hand with the infested soil from the appropriate pots. Pots were placed in a completely randomised design on a glasshouse bench (ambient temperature 18–31°C). The various soil amendments were allowed to compost for 8 weeks before the papaw seedlings were transplanted to the soil. *Phytophthora* populations were assessed at this time as previously described. Trichodry powder had been incorporated by hand into the seedling mix (1 kg/m^3 soil) of appropriate pots at seeding, with additional applications of Trichoflow powder (5 kg/ha) applied in water to pots at transplanting and every 4 weeks thereafter. Assessments of disease severity were made as described.

Table 1. Effect of soil amendments on populations of *Phytophthora palmivora*, plant height, fresh weight of roots and root rot severity in a naturally infested kraznozem soil

Treatments	Rate of application	Log cfu/g soil[A] Pi	Pf	Plant height (cm)	Fresh wt. of roots (g)	Root rot severity[B]
Sawdust + urea	150 m^3/ha + 600 kg/ha	5.15 a[C] (172)	0.00 c (0)	39.8 a	19.23 a	1.2 d
Chicken manure	4% v/v	5.18 a (176)	5.46 ab (234)	24.1 bcd	2.95 d	4.2 ab
Filterpress	400 m^3/ha	5.28 a (195)	5.21 ab (181)	38.9 a	13.14 bc	2.8 c
MinPlus	4.5 t/ha	5.15 a (172)	5.10 ab (163)	20.5 bcd	1.37 d	4.8 ab
Trichodry + Trichoflow	1.0 kg/m^3+ 5.0 kg/ha	5.17 a (174)	5.25 ab (189)	16.3 cd	0.27 d	5.0 a
Mill ash	200 m^3/ha	5.17 a (175)	5.52 a (249)	27.5 b	8.17 c	3.6 bc
Filterpress + Trichodry	200 m^3/ha + 1.0 kg/m^2	5.19 a (179)	5.08 b (159)	15.7 d	2.13 d	4.4 ab
Untreated control		5.16 a (173)	5.12 ab (166)	20.0 bcd	0.91 d	5.0 a
Uninfested control		0.00 b (0)	0.00 c (0)	39.4 a	13.96 b	1.0 d

[A]Equivalent means are presented in parentheses, back transformed from $\log_e (x + 1)$ transformation.
[B]Phytophthora root rot assessed on a 1 to 5 scale of increasing disease severity.
[C]Means in the same column followed by the same letter are not significantly different ($P > 0.05$).

Table 2. Populations of *Phytophthora palmivora* (log_e cfu/g soil) in a naturally infested kraznozem soil in the glasshouse following rotation with banana and sugar-cane, green manure crops (brassica, soybean and brachiaria) and a bare fallow

Treatments	Plants per pot	Initial inoculum densities[A]	Inoculum densities prior to composting[A]	Inoculum densities at planting of papaw[A]	Final inoculum densities[A]
Brassica	5	3.00 a[B] (19.1)	2.18 bc (7.8)	0.00 b (0.0)	0.57 c (0.8)
Soybean	5	2.78 a (15.2)	1.43 c (1.4)	0.00 b (0.0)	2.23 b (8.3)
Brachiaria	Thick stand	2.64 a (13.03)	2.81 ab (15.6)	0.32 b (0.4)	3.37 a (28.2)
Banana	1	3.09 a (21.07)	3.45 a (30.5)	1.96 a (6.1)	3.80 a (43.5)
Sugar-cane	1	2.72 a (14.15)	2.63 b (12.9)	0.00 b (0.00)	3.32 a (26.6)
Bare Fallow	—	2.84 a (16.08)	2.17 bc (7.7)	1.21 a (2.4)	2.96 ab (18.3)
Uninfested control	—	0.00 b (0.00)	0.00 d (0.0)	0.00 b (0.0)	0.00 c (0.0)

[A]Equivalent means are presented in parentheses, back transformed from log_e $(x + 1)$ transformation.
[B]Means in the same column followed by the same letter are not significantly different ($P > 0.05$).

Assessment of crop rotation and green manure crops. In the second glasshouse experiment, the treatments involved the growing and incorporation of two green manure crops, Bioquire Mulch (BQ mulch, a mixture of *Brassica campestris,* and *B. napus*), and soybean (*Glycine max*) cv. Leichardt, the pasture brachiaria (*Brachiaria humidicola*) cv. Tully, two locally grown commercial crops, sugar-cane (*Saccharum officinarum*) and banana (*Musa acuminata*) cv. Cavendish, and a bare fallow.

Sufficient *Phytophthora*-infested and uninfested soil was placed in 18-cm-diameter pots and the initial *Phytophthora* populations assessed as previously described. Seeds of the brassica, soybean (treated with Nitrogerm 100 legume inoculant) and brachiaria treatments were sown into the potted soil, and a single tissue-cultured banana plantlet, and a single 'shot' sugar-cane sett (pre-germinated in vermiculite) were transplanted to the appropriate pots. Pots were placed in a completely randomised design on a glasshouse bench (ambient temperature 19–34°C). Newly germinated brassica and soybean seedlings were thinned to five plants/pot and a thick stand of brachiaria was obtained. All rotation crops (Table 2) were hand-watered and fertilised as previously described. Soil in the bare fallow and uninfested control pots was also kept moist. After 8 weeks, the *Phytophthora* populations were assessed and the plant tops in each pot were cut and weighed, before being mulched in a Viking electric mulcher. The soil and roots in each pot were chopped and mixed with the mulched plant tops from that pot. The soil/mulched material was composted for a further 8 weeks before being assessed for *Phytophthora* populations. Eight-week-old papaw seedlings were transplanted to each pot after the period of composting, and assessed for disease severity as described above after 8 weeks.

Field experiment

Treatments from the glasshouse experiments, considered to have the most potential in controlling root rot, were further evaluated in the field. Molasses was also included as a treatment as many growers were applying molasses to their crops in the belief that the product could help reduce the incidence of root rot. The other field treatment not previously tested in the glasshouse was gypsum. Calcium and calcareous amendments may have a positive effect in controlling *Phytophthora* by improving soil structure, drainage, aeration, calcium supply and resistance to fungal attack (Borst 1970).

The experiment was established in August 1999 in a kraznozem soil at South Johnstone Research Station. The site was divided into 28 plots, each 18 m × 12 m. Soil samples were collected and assayed for *P. palmivora* and other microbial populations using a hand corer (a stainless steel tube of 25 mm internal diameter and 300 mm long). Pre-plant core samples were collected at ten random points in each plot and a sample of approximately 500 mL was obtained. Following the planting of papaw, core samples were taken from the root zone of each datum plant. Soil samples were also collected for nutrient analysis using an auger (internal diameter 95 mm) and forwarded to the Department of Natural Resources, Agricultural Chemistry Laboratory in Mareeba. Core samples were collected from two random points in each plot, bulked and a 500 mL sub-sample taken per plot. Volumetric soil moisture content was measured using a Sentek Diviner 2000. Access tubes (75 cm long) were established in the centre of the datum row within each plot.

Intensive sampling indicated that although the field was infested with *P. palmivora*, populations of the pathogen were low and not uniformly distributed in the experimental site. To increase inoculum levels, the site was artificially infested with *P. palmivora* using the following procedure.

Production of P. palmivora *inoculum.* An isolate of *P. palmivora* was acquired from a diseased papaw plant by excising small sections of rotted tap root and plating onto P_{10}ARP+H culture media. Inoculum was produced from a single sporangium isolate (BRIP 28017) obtained from a sporulating sector of the P_{10}ARP+H colony. Sections of the colony were transferred to an appropriate number of 250 mL autoclavable polycarbonate screw-top containers containing 1% potato-dextrose agar (1% PDA). Six days later, under aseptic conditions, sterile wooden toothpicks were pushed into the *Phytophthora*-colonised PDA and incubated at 26°C for a further 2 weeks for the purpose of creating a carrier of *Phytophthora* mycelial inoculum.

Assessment of microbial populations. Microbial populations were assessed using the following soil dilution technique combined with selective media (Aryantha 1997). Populations of endospore-forming bacteria were estimated using nutrient agar plus 0.1% Nystatin (Halsall 1982), fluorescent pseudomonads using King's B medium (Dhingra and Sinclair 1994), actinomycetes using water agar (Aryantha 1997) and total fungi were estimated using PDA plus chloramphenicol. The soil dilution technique involved diluting 1 g of soil in 9 mL of sterile distilled water and vortexing for 5 s. One millilitre of this solution was used to make a dilution series of 10^{-1} to 10^{-5}. A 100 µL sample of the two consecutive dilutions most appropriate to each micro-organism group was placed on the surface of the appropriate selective medium. The appropriate dilutions had been established during a preliminary experiment. A single agar plate was used per dilution. For endospore-forming bacteria, soil dilutions were heated to 80°C for 10 min before plating (Halsall 1982).

Plates selective for actinomycetes and endospore-forming bacteria were incubated at 26°C for 5 days and 2 days, respectively. Fluorescent pseudomonads and fungal plates were incubated at 20°C for 2 days. Colony-forming units of actinomycetes, endospore-forming bacteria and fungi were confirmed and counted with the aid of a dissecting

Table 3. Treatments included in a field experiment with papaw

No.	Treatment
1.	Sawdust 150 m^3/ha + urea 600 kg/ha applied and incorporated on 7 September 2000
2.	Filterpress 400 m^3/ha applied and incorporated on 7 September 2000
3.	Molasses 100 L/ha/week applied for 20 weeks (14 November 2000–27 March 2001)
4.	Brassica (BQ Mulch) 4.5 kg/ha sown 1 June and incorporated on 7 September 2000
5.	Soybean (cv. Leichardt) 40 kg/ha sown 1 June and incorporated on 7 September 2001
6.	Gypsum 5 t/ha applied and incorporated on 7 September 2000
7.	Unamended

microscope. Fluorescent pseudomonads were counted under UV light (Sylvania Blacklight-Blue F18/blb) as the medium used was differential rather than selective.

Trial site layout, site infestation and treatment application. In November 1999, three raised beds each 1.5 m wide and 18 m long were formed in each plot. Papaw plants were planted 1.5 m apart within the row and watered with sprinkler irrigation. After 3 months, plants within the experimental area were infested with *P. palmivora* by inserting *Phytophthora*-infested toothpicks into one of the upper leaf nodes of each plant. These plants were incorporated with a rotary-hoe on 15 May 2000 and the site was ploughed, disced and rotary-hoed in preparation for the planting of the green manure treatments. One week after the site was prepared for planting, *P. palmivora* populations were assessed as described previously.

During the period June 2000 to April 2001, four replicates of the seven treatments listed in Table 1 were established in a randomised complete block design. On 1 June 2000, seeds of soybean cv. Leichardt (40 kg/ha) treated with Nitrogerm 100 legume inoculant were sown by hand in drills 0.5 m apart and 0.1 m within the row, and BQ Mulch (4.5 kg/ha) was broadcast on the appropriate plots before all the green manure plots (Table 3) were rolled with a drum roller. Due to cool, dry conditions at the time, over-head irrigation was applied to the green manure plots as required. The BQ Mulch received two side dressings of fertiliser (Nitrophoska) at the rate of 185 kg/ha as the plants grew. Plots, other than the green manure treatments (Table 3), were maintained as a clean fallow by spraying weeds with herbicide (paraquat) as they emerged. Soil samples were collected for base-line nutrient data just prior to planting the green manure crops. On 7 September, the sawdust + urea, filterpress and gypsum treatments were broadcast over the surface of the appropriate plots. All plots were then deep-ripped, rotary-hoed, and three mounds (0.75 m high) each 1.5 m wide and 18 m long were formed within each plot. On 11 November, soil samples were collected for nutrient analysis. Two days later, soil samples were collected and pre-plant populations of *P. palmivora*, actinomycetes, total fungi, fluorescent pseudomonads and endospore-forming bacteria were assessed as previously described. On 14 November, weekly applications of molasses commenced and continued for 20 weeks. Each molasses treatment (100 L/ha/week) was diluted in water (417 L/ha) and applied with a watering can along the centre of the mounds of appropriate plots.

Papaw establishment. Nine-week-old papaw seedlings (Hybrid 29) were transplanted from pasteurised potting mix into the experimental area on 23 November 2000. All plots received a basal fertiliser (12.7% N, 14.2% P, 10.9% K 2.4% S and 2% Zn) at a rate of 266 kg/ha, a side dressing of 19.3% N, 0% P and 28.4% K at the rate 336 kg/ha and two applications of urea (39 kg/ha) through the mini-sprinkler irrigation. Soil moisture was recorded twice-weekly from 8 December 2000 and continued for 15 weeks. On 12 December 2000, plots were sampled and population levels of actinomycetes, fungi, fluorescent pseudomonads and endospore-forming bacteria were determined. Assessments of the microbial populations were conducted every 6 weeks until 7 April 2001, after which the sampling interval was extended to every 12 weeks. An assessment of *P. palmivora* populations occurred on 7 April. Plant heights (cm) were recorded at 8 weeks and 14 weeks after transplanting. Plant infection counts were recorded as plants showed symptoms of root rot, and the diseased plants were cut at ground level and moved to the inter-row. The experiment was completed 46 weeks after the transplanting of papaw.

Data analyses

For the two pot experiments, ANOVA was used to analyse inoculum densities [using $\log_e(x + 1)$ transformation], root rot severity, plant height and fresh weight of roots. Similarly, ANOVA was used to analyse inoculum density data from the field experiment using $\log_e(x + 1)$ transformation, while the percentage papaw infection was analysed following a variance-stabilising transformation {arc sine [square root (x)]}. Analysis of variance was used to analyse the nutrient status of soils, cumulative soil moistures and the plant height of papaw. Where an overall treatment effect was found to be significant, pair-wise testing between treatment means was done using the protected least significance difference (LSD) test. With the antagonist population data, a repeated measures analysis of variance was used. This analysis accounts for the non-uniform covariance structure of the repeated measurements by using a correction factor (Greenhouse and Geisser 1959). The antagonist population data were transformed using a $\log_e(x + 1)$ transformation. GenStat (GenStat 5 Committee 1993) was used to analyse these data.

Results

Pot experiments

Assessment of soil amendments and biological agents. The initial *Phytophthora* populations in soils sampled from pots prior to treatments (Table 1) being imposed showed they did not differ significantly ($P > 0.05$). At the conclusion of the experiment, sawdust + urea was the only soil treatment which significantly reduced ($P < 0.05$) the populations of *P. palmivora* and increased the fresh weight of papaw roots. However, three of the seven treatments (mill ash, sawdust + urea and filterpress) reduced ($P < 0.05$) root rot severity and increased plant height compared with the untreated (Table 1). Papaw plants in filterpress and mill ash-amended soil had significantly ($P < 0.05$) more roots than those in all other treatments except sawdust + urea and the uninfested control. Trichodry plus Trichoflow chicken manure and Min-plus failed to reduce the inoculum density and root rot severity compared with the untreated control.

Assessment of crop rotation and green manure crops. Prior to commencing the experiment, there was no significant difference ($P > 0.05$) between *Phytophthora* populations in the pots allocated to each treatment (Table 2). After 8 weeks

Table 4. Effect of crop rotation and green manure crops on plant height, fresh weight of tops and roots, and root rot severity of papaw grown in the glasshouse in a kraznozem soil naturally infested with *Phytophthora palmivora*

Treatment	Plant height (cm)	Fresh wt. of tops (g)	Fresh wt. of roots (g)	Root rot severity[A]
Brassica (BQ Mulch)	32.5 b[B]	39.6 c	18.56 b	2.6 d
Soybean (Leichardt)	39.4 a	53.5 ab	19.29 b	3.0 bc
Brachiaria (Tully)	34.2 b	33.6 c	13.04 b	3.8 a
Banana (Cavendish)	39.8 a	54.7 a	21.85 b	3.2 abc
Sugar-cane	36.3 ab	43.4 bc	13.16 b	3.8 a
Bare Fallow	33.2 b	32.5 c	15.56 b	3.4 abc
Uninfested control	40.1 a	61.9 a	42.12 a	1.2 e

[A]Phytophthora root rot assessed on a 1 to 5 scale of increasing disease severity.
[B]Means in the same column followed by the same letter are not significantly different ($P > 0.05$).

Table 5. *Phytophthora palmivora* population densities in May 2000 following the artificial infestation of the field-site, in November 2000 following the incorporation of the soil amendment treatments, and in April 2001 during growth of the papaw crop, and the total percentage papaw infection

Treatment	Inoculum densities/g soil[A]			% papaw infection[B]
	May 2000	Nov. 2000	Apr. 2001	
1. Sawdust + urea	2.36 (9.6)	0.13 (0.1)	0.81 bc[C] (1.2)	0.23 c (5.0)
2. Filterpress	2.68 (13.6)	0.48 (0.6)	1.69 a (4.4)	1.05 a (75.4)
3. Molasses	2.15 (7.6)	0.13 (0.1)	1.24 ab (2.5)	0.69 b (41.0)
4. Brassica	2.42 (10.3)	0.00 (0.0)	0.64 bcd (0.9)	0.37 c (13.1)
5. Soybean	2.14 (7.5)	0.21 (0.2)	0.00 d (0.0)	0.39 bc (14.6)
6. Gypsum	2.10 (7.2)	0.00 (0.0)	0.20 cd (0.2)	0.34 c (11.2)
7. Unamended	2.46 (10.6)	0.13 (0.1)	0.20 cd (0.2)	0.42 bc (16.8)

[A]Equivalent means are presented in parentheses, back transformed from $\log_e (x + 1)$ transformation.
[B]Equivalent means are presented in parentheses, back-transformed from inverse sine transformation $[\sin^{-1}(\%/100)^{1/2}]$.
[C]Means in the same column followed by the same letter are not significantly different ($P > 0.05$).

of growing the various rotation crops and just prior to composting, *Phytophthora* populations were greatest ($P < 0.05$) in soil in which banana plants had grown. Soil in which soybean had grown had a lower inoculum density than the brachiaria, banana and sugar-cane soil at this stage. The fresh weight of tops of the various rotation crops just prior to incorporation into the infested soil were brassica, 89.2 g; soybean, 63.7 g; brachiaria, 66.1 g; banana, 157.9 g and sugar-cane, 91.9 g. The biomass of banana tops was significantly greater ($P < 0.05$) than those of the other crop types. Following the period of composting, *Phytophthora* populations in soils amended with brassica, soybean, sugar-cane and brachiaria were significantly less ($P < 0.05$) than those in banana-amended soil and the bare fallow. Final inoculum densities (Pf) were reduced ($P < 0.05$) in soil amended with Brassica or soybean (Table 2) compared with all other treatments except bare fallow, and there was significantly less ($P < 0.05$) damage to papaw roots in brassica-amended soil (Table 4). Minor feeder root damage was recorded on a single plant growing in uninfested soil. *P. palmivora* was isolated from sections of these diseased feeder roots. The best papaw growth occurred in the uninfested soil and in soil amended with either banana or soybean (Table 4).

Field experiment

In May 2000, after the artificial infestation of the trial-site, there was no significant difference ($P > 0.05$) between the inoculum density in the plots allocated to each treatment (Table 5). The mean inoculum density was approximately 9.5 cfu/g of soil. In contrast, inoculum densities obtained from samples collected just prior to planting papaw plants in November 2000 were about 0.2 cfu/g of soil. In April 2001, 6 months after planting papaw, populations of *P. palmivora* were highest ($P < 0.05$) in plots amended with filterpress or molasses. This result correlated with a significant increase ($P < 0.05$) in the incidence of root rot in filterpress-amended soil (Table 5). Soil amended with sawdust + urea had the lowest incidence of root rot.

Nutrient analyses of soil samples before treatments were imposed showed there was no significant difference in pH, EC, NH_4-N, NO_3-N and organic carbon levels across the four replicates. Soil nutrient data collected on 31 October 2000, 8 weeks after the incorporation of the soil amendments and just prior to transplanting papaw, are presented in Table 6. Of the organic amendments applied, filterpress was the only treatment to significantly ($P < 0.05$) increase the organic carbon levels. The addition of filterpress to plots also

Table 6. Nutrient status of soils sampled just prior to planting papaw in late November 2000, following the incorporation of various soil amendments, and plant height of papaw in January and March 2001

Treatment	pH	EC (mS/cm)	NH_4-N (mg/kg)	Org. C (%)	Plant heights (cm) Jan. 2001	Mar. 2001
1. Sawdust + urea	5.50 ab[A]	0.0625 ab	5.53 b	2.215 b	78.0 c	123.5 b
2. Filterpress	6.36 d	0.0895 b	11.05 c	2.897 c	94.4 a	142.8 a
3. Molasses	5.87 c	0.0540 a	3.59 ab	2.183 b	79.3 c	120.8 b
4. Brassica	5.63 b	0.0630 ab	4.50 ab	1.916 a	77.3 c	119.5 b
5. Soybean	5.48 ab	0.0620 a	2.14 a	1.982 ab	76.4 c	119.8 b
6. Gypsum	5.40 a	0.1255 c	1.16 a	1.923 a	86.4 b	127.3 b
7. Unamended	5.62 b	0.0455 a	1.84 a	2.020 ab	80.1 bc	126.8 b

[A]Means in the same column followed by the same letter are not significantly different ($P > 0.05$).

Table 7. Cumulative totals of soil moisture readings obtained twice weekly for 15 weeks, taken at 10 cm increments to a depth of 60 cm from mounds following the application of various soil amendments

Treatment	Totals of soil moisture readings (mm/100 mm soil) at 10 cm	20 cm	30 cm	40 cm	50 cm	60 cm
1. Sawdust + urea	552.3 b[A]	640.5 ab	532.5 bc	775.4 a	798.8 bc	815.7 b
2. Filterpress	488.3 b	776.9 a	772.8 a	715.2 a	718.6 c	826.8 ab
3. Molasses	747.0 a	776.3 a	536.7 bc	673.9 ab	839.4 ab	814.0 b
4. Brassica	640.6 ab	638.2 ab	608.1 ab	825.3 a	946.3 ab	901.9 ab
5. Soybean	473.6 b	529.9 b	533.7 bc	718.2 a	983.3 a	812.7 b
6. Gypsum	584.3 ab	545.0 b	379.3 c	674.1 ab	948.4 ab	995.5 a
7. Unamended	649.8 ab	539.6 b	507.5 bc	533.1 b	831.7 abc	812.1 b

[A]Means in the same column followed by the same letter are not significantly different ($P > 0.05$).

increased soil pH, and both filterpress and sawdust + urea increased NH_4-N compared with unamended soil. Gypsum increased soil EC ($P < 0.05$) compared with all other treatments. There was no significant ($P < 0.05$) treatment effect on NO_3-N levels.

The papaw crop grew normally and by January 2001, there were quantitative differences in plant growth between treatments (Table 6). Assessments conducted in January and March 2001 showed that plant height had significantly increased ($P < 0.05$) in filterpress-amended plots compared with all other treatments.

An early start to the wet season saw the majority of rain fall in November and February. Cumulative totals of soil moistures collected between December 2000 and March 2001 are presented in Table 7. Total soil moisture in the top 10 cm of soil was increased by 15% in molasses-treated soil, and there was a 48% increase in soil moisture in soil amended with filterpress and molasses compared with unamended soil at 20 cm. The addition of filterpress increased soil moisture at 30 cm by 52%. At the lower depths of 40–60 cm, soil moistures did not differ significantly ($P > 0.05$).

Filterpress and molasses significantly ($P < 0.001$) stimulated the populations of fluorescent pseudomonads, total fungi and actinomycetes compared with brassica, soybean, gypsum and the unamended treatments. Populations of fluorescent pseudomonads increased 18-fold where filterpress was applied and 9-fold in molasses-amended plots (Table 8), and there was a significant treatment × time interaction ($P < 0.001$) in the recovery of fluorescent pseudomonads (Fig. 1). The increase in the number of actinomycetes in plots amended with filterpress and molasses was five times greater than that in unamended plots and there was a significant treatment × time interaction ($P < 0.001$) in the recovery of actinomycetes (Fig. 1). Populations of endospore-forming bacteria were significantly higher ($P < 0.001$) in molasses-amended plots than in all other treatments (Table 8). There was no significant treatment × time interaction ($P < 0.001$) in the recovery of total fungi and endospore forming bacteria.

Discussion

The objective of these experiments was to determine if crop rotation and soil amendments could be integrated with mounding (Peterson *et al*. 1997) to further reduce the incidence of root rot of papaw caused by *P. palmivora*. In the pot experiment, sawdust + urea reduced *Phytophthora* inoculum to undetectable levels, which correlated with reduced damage to roots and an increase in plant growth. In the field, soil amended with sawdust + urea again had the lowest incidence of root rot. In greenhouse pot experiments, Tsao and Zentmyer (1979) reported that urea

Table 8. Overall effect of the soil amendments sawdust + urea, filterpress, molasses, brassica, soybean and gypsum on mean populations of fluorescent pseudomonads, total fungi, actinomycetes and endospore-forming bacteria in a kraznozem field soil

Treatment	Fluorescent pseudomonads[AC]	Total fungi[AC]	Actinomycetes[BC]	Endospore-forming bacteria[BC]
1. Sawdust + urea	2.191 b (7.94)	3.332 bc[C] (26.99)	2.732 c (14.36)[C]	2.225 ab (8.25)[C]
2. Filterpress	3.354 d (27.62)	4.149 d (62.37)	3.707 d (39.73)	2.315 b (9.12)
3. Molasses	2.672 c (13.47)	3.406 c (29.14)	3.751 d (41.56)	3.280 c (25.58)
4. Brassica	1.111 a (2.04)	2.837 a (16.06)	2.488 b (11.04)	2.216 ab (8.17)
5. Soybean	0.987 a (1.68)	2.743 a (14.53)	2.444 b (10.52)	2.030 ab (6.61)
6. Gypsum	0.793 a (1.21)	2.830 a (15.95)	2.158 a (7.65)	2.244 b (8.43)
7. Unamended	0.918 a (1.50)	2.998 ab (19.05)	2.287 ab (8.85)	1.934 a (5.92)

[A]Equivalent means in parentheses are of populations of fluorescent pseudomonads and total fungi $\times 10^3$/g of soil.
[B]Equivalent means in parentheses are of populations of actinomycetes and endospore-forming bacteria $\times 10^5$/g of soil.
[C]Means in the same column followed by the same letter are not significantly different ($P > 0.05$).

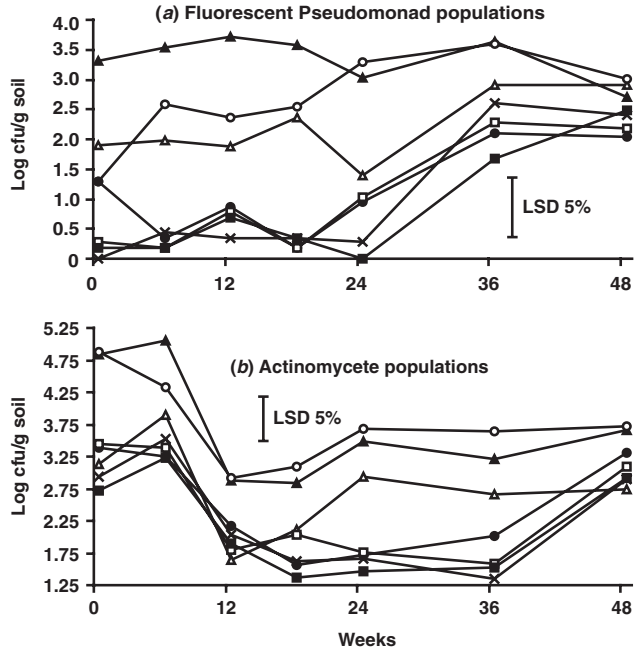

Fig. 1. Effect of soil amendments (△) sawdust + urea, (▲) filterpress, (○) molasses applied weekly for 20 weeks, (●) brassica, (□) soybean, (■) gypsum, and a bare fallow (×) on (*a*) fluorescent pseudomonad and (*b*) actinomycete populations in a field soil. Time 0 represents a soil sampling 2 weeks prior to transplanting papaw.

at 0.1% was effective as a pre-plant soil mix in controlling Phytophthora root rot on avocado seedlings. Research conducted by Vawdrey and Stirling (1997) also showed that combining sawdust and urea at the rates shown in Table 1, reduced root-knot nematode damage in tomatoes. The high rate of urea used with sawdust in our pot and field experiments was an attempt to achieve concentrations of NH_4-N in soil which were inhibitory to *P. palmivora* and to prevent any nitrogen drawdown problems which may arise with materials having a high C:N ratio (e.g. sawdust). However, such high quantities of nitrogen raise environmental concerns, such as reducing the quality of ground-water supplies.

Tsao and Oster (1981) found that amending natural soils with chicken manure inhibited the germination of *Phytophthora* propagules. Aryantha *et al*. (2000) reported that chicken manure compost significantly reduced the survival of *P. cinnamomi* and the development of disease symptoms on *Lupinus albus* seedlings. However, these findings contrast with our results, which found that composted chicken manure did not reduce either *Phytophthora* inoculum densities or the incidence of root rot on papaw. This may have been due to the maturity of the chicken manure used or the relative sensitivity of different *Phytophthora* species. Aryantha *et al*. (2000) noted that chicken manure composted for 2 weeks had the highest populations of *P. cinnamomi* and the highest microbial activity, whereas freshly composted chicken manure had the lowest microbial and pathogen populations. This was largely attributed to the heat produced in the initial phase of composting and to the release of ammonia, which are both directly toxic to *P. cinnamomi* (Tsao and Oster 1981). Aryantha *et al*. (2000) also stated that chicken manure was phytotoxic to some plant species, possibly predisposing the plants to root rot infection. The sensitivity of many plant species to the phosphorous in chicken manure is well documented, with concentrations as low as 5% v/v being recorded as phytotoxic on Proteaceous plants (Aryantha *et al*. 2000). However, symptoms of phytotoxicity were not evident in papaw seedlings in our pot experiment and plants grew vigorously following transplanting into soil amended with chicken manure.

As *Phytophthora* spp. are considered poor saprophytic competitors (Tsao 1969), growing non-host green manure crops has the potential to reduce soilborne disease inoculum. In our glasshouse and field studies, brassicas grown as a green manure crop had this effect on populations of

P. palmivora. The reduction in *Phytophthora* inoculum density was most likely due to the toxic influence of glucosinolate products released from roots during the growth of the brassica, or from roots and shoots following their decomposition (Kirkegaard *et al*. 1996). Unfortunately, the suppressive effect of brassica against *P. palmivora* populations in our pot experiment did not translate into a significant reduction in the incidence of root rot in the field.

On its own, the addition of organic matter appears a necessary but not a sufficient condition for suppressing plant pathogens in soil (Broadbent and Baker 1975). Filterpress is widely used as an organic source of nutrient (Barnes 1974) but little is known of its effect on soilborne pathogens. Although filterpress applied in the field experiment significantly increased soil organic matter levels, the levels of ammonia, EC and pH [all factors known to inhibit *P. cinnamomi* in soil and *in vitro* (Broadbent and Baker 1975; Tsao and Oster 1981)], the *P. palmivora* populations and the incidence of root rot were very high.

Increasing microbial populations through the use of organic matter has been reported by many researchers (Zentmyer 1963; Weste and Vithanage 1977; Aryantha *et al.* 2000) to be an important factor in the biological control of Phytophthora root rot diseases. However, in our field experiment, infection of papaw was highest in plots amended with filterpress and molasses, despite the fact that populations of soil antagonists were the greatest in these treatments. The increased water-holding capacity in soil amended with filterpress and molasses is the most likely cause of the high incidence of root rot associated with these amendments. Duniway (1979) concluded that the most important environmental factor influencing *Phytophthora*-related root disease was the duration of saturation, or near-saturation, of soil. Soil conditions such as these are known to favour the rapid formation of sporangia and infectious zoospores and a high level of disease. Therefore the success or failure of an organic amendment to control Phytophthora root rot is very much dependent on the moisture retention properties of the amendment.

The application rates of the soil amendments used in our experiments were based on previous research (Vawdrey and Stirling 1997; Aryantha *et al*. 2000) and supplier recommendations. In most instances, the rates of application were relatively high and offer opportunities for further research into strategies involving repeated applications of smaller quantities over longer periods. The opportunity also exists for investigations into methods of enhancing the biofumigation potential of brassicas and other glucosinolate-producing plants grown as green manures. This research should investigate methods of maximising the isothiocyanate concentration released into the soil following the incorporation of the crop. It is hoped that by integrating single row mounds with biofumigation methods, growers will attain a high level of root rot control in papaw.

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