

Mycorrhizal colonisation of three hybrid papayas (*Carica papaya*) under mulched and bare ground conditions

K. B. Walsh^{A,C} and S. Ragupathy^{A,B}

^APlant Sciences Group, Central Queensland University, Qld 4702, Australia.

^BCurrent address: Biodiversity Institute of Ontario, Department of Integrative Biology, University of Guelph, ON N1G 2W1, Canada.

^CCorresponding author. Email: k.walsh@cqu.edu.au

Abstract. The use of straw mulching has been demonstrated to decrease soil loss and to improve soil moisture and soil organic matter content in conjunction with papaya (*Carica papaya*) cultivation. Mulching may also benefit soil biota. In this study, mulching was demonstrated to significantly ($P < 0.05$) improve arbuscular mycorrhizal fungal colonisation of papaya roots (by a factor of 2.4), but decreased spore density and species diversity (by a factor of 1.5), compared with cultivation in bare ground. The genera *Glomus*, *Acaulospora*, *Gigaspora* and *Sclerocystis* dominated in both mulched and bare ground systems. The increased mycorrhizal activity in the mulched treatments was matched by an increase in leaf phosphorus in 1995 but not in 1997.

Additional keywords: nutrients, pH.

Introduction

The cropping systems of central Queensland are characterised by opportunistic cropping due to patchy distributed rainfall, with a loss of soil and nutrients after high intensity rainfall events. Papaya cropping has traditionally involved a form of shifting agriculture, with clearing of dry scrubs on hillsides to access well drained, fertile soils. Recently, the use of organic (hay) mulches has been promoted for papaya cultivation, with the following advantages cited: (i) improved weed control; (ii) lower soil temperatures; (iii) reduced water and soil loss; (iv) better use of total soil volume with increased soil water availability; (v) improved root activity; (vi) increased levels of available K, P and B in soil and (vii) reduced root-knot nematode populations (Elder *et al.* 2000). Elder *et al.* (2002) also postulated that mulching would improve conditions for soil mycorrhiza, and thus plant nutrition through association with mycorrhiza.

Arbuscular mycorrhizal (AM) fungi are not host specific. The species composition of a given soil has been primarily attributed to abiotic factors such as soil pH, soil moisture, temperature, salinity (Ragupathy and Mahadevan 1991) and total soil C and N (Johnson *et al.* 1991). The diversity of AM fungal communities tends to diminish when natural ecosystems are converted into agro-ecosystems (Sieverding 1991; Siqueria *et al.* 1989), particularly under monoculture (Allen and Bossalis 1983; Sieverding 1991). In researching an observed decline in crop production following long fallow periods on the Darling Downs (south-eastern Queensland), Thompson (1987) noted the dependence of some crops (i.e. sunflowers, wheat) on AM fungi for maximum yield, and observed that AM fungi abundance in the crop was affected by the different crop rotation cycles.

Arbuscular mycorrhizal colonisation of papaya root has been reported (Cruz *et al.* 2003; Ragupathy and Mahadevan 1993), and an increase in the growth of papaya seedlings in nursery conditions was obtained by introducing AM fungi (Reddy *et al.* 1996). In glasshouse seedling trials, Trindade *et al.* (2001) noted a linear response in growth of non-AM-inoculated plants to applied P (0–240 mg P/dm³), while AM-inoculated plants displayed a quadratic response (with a large response at low soil P levels). Working with 1.5-year-old papaya plants, Mamatha *et al.* (2002) reported an increased fruit yield in the AM inoculated plants. Plants provided with 50% of the recommended P fertilisation essentially failed to provide a crop when plants were not inoculated. When inoculated, plants produced fruit biomass equivalent to plants receiving 100% recommended P. Co-inoculation of plants with *Bacillus coagulans* as a helper bacterium, improved the extent of AM infection of papaya roots, but there was no synergistic effect on fruit yield of co-inoculation.

In the present study we characterise mycorrhizal diversity and abundance in soil and in papaya roots and soil in the mulching trials reported by Elder *et al.* (2002), as a potential contributing factor to the improvement in yield noted with hay mulching. This study represents this first report of AM fungi associated with papaya in Australian production systems.

Materials and methods

Site description

Hay mulching treatments were installed in June 1994 on the property in Yarwun, central Queensland, Australia as reported by Elder *et al.* (2002). Papaya plants were grown in double rows

Table 1. Soil analysis

All elemental concentrations reported in units of mg/kg DW. Electrical conductivity (EC, dS/m) and pH were assessed using a 1:5 soil–water mix ($n = 3$). Treatments were imposed in July 1994. Values within a row followed by the same letter are not significantly different ($P = 0.05$)

Chemical property	Before planting (1994)	After planting (1997)	
		Mulched treatment	Bare ground treatment
pH	7.2a	6.9a	7.2a
EC	0.06a	0.16a	0.05a
Organic carbon	—	21a	9.0b
N	8.7a	55.3b	11.2a
P ^A	9.7a	74.4b	73.2b
K	0.89a	0.77a	0.61a

^AColwell P extraction (Colwell 1963).

on a 2.9 m (between rows) by 1.8 m (in row) grid, with 5.5 m between the centres of the double rows (i.e. plant population of about 2000 plants/ha). Briefly, the experimental layout consisted of three *Carica papaya* varieties (hybrids 11, 13, 29) with and without mulch, arranged in a randomised complete block design with three replicates (three varieties \times two mulch conditions \times three replicates, giving 18 plots). Each replicate plot consisted of 80 plants. Coarse grass hay was applied to mulched plots at a soil depth of 10 cm just before planting, and replenished at 6-monthly intervals. The treatment area had previously been planted to papaya. Annual rainfall was 621, 1112 and 797 mm in 1995, 1996 and 1997, respectively. At the initiation of the trial in 1994, soil pH was near-neutral and soil conductivity was low, but the soil was relatively poor in macroelements (NPK) and organic carbon (Table 1). As reported by Elder *et al.* (2000), KNO₃ was supplied to the site at the rate of 20 g/site.week throughout the growing season (September to May).

Estimation of mycorrhizal colonisation

Soil cores (25 mm in diameter) were taken of surface soil (0–200 mm depth). Six samples were taken randomly within each replicate plot in late July 1997. Fine roots (<1 mm diameter) from these cores were fixed in 50% ethyl alcohol (Brundrett *et al.* 1996), and mycorrhizal infection assessed

according to Phillips and Hayman (1970) (10 mm length root pieces, treated for 1–2 h in 10% potassium hydroxide at 90°C, washed, treated with hydrogen peroxide until bleached, acidified with 5% HCl and finally rinsed in distilled water; with this procedure repeated three times). Root segments were stained in 0.05% trypan blue in lactoglycerol. For each replicate, 100 root segments were examined for mycorrhizal colonisation using brightfield microscopy.

Arbuscular mycorrhizal fungal species abundance in soil (spore density and species abundance)

Three soil samples (1 kg) from the surface soil (0–200 mm) were taken at random from each treatment replicate in late July 1997. Samples were homogenised and AM fungal spores were obtained from 100-g soil samples by wet-sieving of a 1:5 soil–water mix (Gerdermann and Nicolson 1963). After mixing, the slurry was allowed to settle for 1 h and was then decanted through a 700- μ m sieve, followed by further decanting through 450, 250, 75 and 45- μ m sieves. Residues from the 450, 250, 75 and 45- μ m sieves were collected to measure sporocarps, large spores and small spores, respectively. The residues were suspended in 50% sucrose, then centrifuged for 1–2 min (~500g) to separate the spores. Residues were mounted in lactoglycerol and examined using brightfield microscopy. Isolated spores that appeared healthy were counted and separated according to their type. The spores were broken in order to count the number of wall layers, which is a character used in identification (Berch and Trappe 1991; Schenck and Pere'z 1987).

Mycorrhizae bioassay

Sorghum was used as a bait plant in a bio-assay of mycorrhizal colonisation of field papaya plants. Arbuscular mycorrhizal-colonised papaya root samples from field plants were washed and surface sterilised with chloramine-T and Tween 20. Clean roots (5 g fresh weight) were cut into 20-mm pieces and used as inoculum for pre-germinated sorghum in 1.5 kg pots containing sterilised sand, perlite and vermiculite (v/v/v 2:1:1). A control treatment was also included in which no papaya roots were added to the sorghum pots. Sorghum plants were lightly fertilised with the commercially available nutrient solution 'Thrive' (NPK 4.8:1.6:1, weight basis). The trial involved

Table 2. Arbuscular mycorrhizal (AM) fungal colonisation (percentage of 10-mm root segments infected), soil AM spore density, soil AM species diversity and papaya leaf phosphorus (P) content in relation to mulching treatment

For P content (of the most recently matured leaf), five leaves from each block were bulked for analysis (data of Elder *et al.* 2002). Values within a column followed by differing letters were significantly different at the $P = 0.05$ significance level. When ANOVA indicated no significant effect of hybrid on an attribute level, a mean value for the mulching treatment only is presented

Hybrid	AM colonisation (%)	AM spore density/100 g soil	AM species diversity/100 g soil		Leaf P (%) content	
			Genus	Species	1995	1997
Mulched	3.22a	—	3.22a	9.11a	0.25a	0.19a
Hybrid-11	—	534b	—	—	—	—
Hybrid-13	—	428a	—	—	—	—
Hybrid-29	—	800bc	—	—	—	—
Bare ground	1.33b	—	3.56a	13.8b	0.19b	0.18a
Hybrid-11	—	1575cd	—	—	—	—
Hybrid-13	—	745b	—	—	—	—
Hybrid-29	—	1026cd	—	—	—	—

21 pots, using three papaya hybrids and two mulch conditions, and a non-inoculated control (each with three replicates),

Three months after sowing, a root and a soil sample from each pot was taken to verify colonisation (following Phillips and Hayman 1970) and to identify AM species (from fungal spores; by wet-sieving and decanting; Gerdemann and Nicolson 1963).

Statistical analysis

The software package Genstat (VSN International, Hertz, UK) was used to undertake a general linear model analysis of variance on the effect of mulching and genotype on several indices of mycorrhizal colonization, while *t*-tests were used in comparison of soil parameters. Comparisons are reported significant at a $P = 0.05$ significance level.

Results

Mycorrhizal abundance was assessed in 1997, 3 years after mulching treatments were initiated. None of the root samples were observed to possess ectomycorrhizal associations. During this time, soil organic carbon and N content increased under mulching, relative to non-mulched treatments (Table 1). The increased AM mycorrhizal activity in the mulched treatments was matched by an increase in leaf phosphorus (P %) in 1995 but not in 1997 (Table 2).

There was no significant effect of replicate block on the parameters of percentage root infectivity, soil spore density, species diversity of leaf P content (ANOVA, data not shown), nor was there a significant effect of hybrid type on these parameters, except for soil spore density (Table 2). Mulching significantly ($P < 0.05$) improved AM fungal colonisation within roots (percentage roots infected), but soil AM species diversity was not affected in terms of number of genera, and slightly decreased in terms of number of species (Table 2). In all cases, spore density observed in soil samples increased under bare ground cultivation conditions (mean across hybrids of 587 and 1116 spores/100 g soil in mulched and bare ground treatments, respectively), but the extent of this increase varied between hybrids (noted as a significant interaction between genotype and mulching condition in the ANOVA). Soils associated with hybrids 11 and 29 exhibited higher spore densities than soils associated with hybrid 13, although there was no significant difference between the hybrids in terms of root colonisation.

About 35 species of mycorrhizal fungi, including six unidentified isolates, were observed in soil samples collected from the rhizosphere of the 18 study plots (combined data for all hybrids) (Table 3). These species belonged to genera *Glomus*, *Acaulospora*, *Gigaspora*, *Sclerocystis* and *Scutellospora*, with 22 species (63% of total number of species isolates) being of the genus *Glomus*. *Glomus ambisporum*, *G. heterosporum* and *G. claroideum* were generally the most prevalent species in all blocks. There were 11 isolates common to both mulched and bare ground treatments.

In the sorghum trap trials, control sorghum plants, which were not inoculated with field papaya roots, remained free of mycorrhizal infection. All AM fungal species identified in inoculated sorghum pots were present in the field soil samples, but only 18 species were present (of 35 in field samples) (Table 4). Some AM fungal species like *Gigaspora albida*,

Glomus aggregatum, *G. fasciculatum* and *G. ambisporum* were trapped in all mulched and bare ground plots, from all papaya hybrids, while other species were restricted to some hybrids under both mulched and bare ground conditions. Species diversity in the trap sorghum pots was lower when hybrid 13 and 29 roots from the mulched treatments were used as inoculum relative to bare ground treatment roots.

Discussion

Arbuscular mycorrhizal diversity and abundance

Papaya cultivation was not associated with a reduced diversity of AM fungal species in the soil. The spore density measured in soil sampled from papaya plantations was similar to that reported for many other crops (e.g. cacao, Cuneca and Meneses 1996), although lower than reported with cassava (Sieverding 1991). Of AM species identified as present in soil, >50% were indicated to be present in papaya roots, as indicated by the sorghum trap study.

Table 3. Arbuscular mycorrhizal fungi isolated from soils under papaya cultivation

Presence and abundance indicated by: ++++ very common; +++ common; ++ rare; + very rare; — absent

Isolate	Treatment	
	Mulched	Bare ground
<i>Acaulospora</i> sp. 1 (longula type)	+	+++
<i>Acaulospora</i> sp. 2 (orange)	+	+
<i>Acaulospora gerdemannii</i>	—	++++
<i>Entrophospora</i> sp. 1	++	—
<i>Entrophospora</i> sp. 2	++	—
<i>Entrophospora</i> sp. 3	++	—
<i>Gigaspora albida</i>	++	++
<i>Gigaspora gigantea</i>	++	++
<i>Glomus aggregatum</i>	+++	+++
<i>G. albidum</i>	++	++
<i>G. ambisporum</i>	++	++++
<i>G. australe</i>	—	++
<i>G. boreale</i>	—	++
<i>G. botryoides</i>	—	++
<i>G. citricolum</i>	++	—
<i>G. claroideum</i>	+	++
<i>G. clarum</i>	+	+++
<i>G. deserticola</i>	—	++
<i>G. fasciculatum</i>	+++	+++
<i>G. heterosporum</i>	+++	++++
<i>G. lacteum</i>	—	++
<i>G. macrocarpum</i>	—	++
<i>G. magnicaule</i>	++	—
<i>G. microaggregatum</i>	—	++
<i>G. multisubstansum</i>	++	—
<i>G. occultum</i>	++	—
<i>G. pansihalos</i>	++	—
<i>G. pubescens</i>	—	++
<i>G. pustulatum</i>	—	++
<i>G. reticulatum</i>	—	++
<i>Sclerocystis microcarpus</i>	—	++
<i>S. pachycaulis</i>	—	++
<i>S. sinosa</i>	++	—
<i>S. rubiformis</i>	++	—
<i>Scutellospora</i> sp.	—	+

Table 4. Arbuscular mycorrhizal fungi identified in sorghum root ‘trap’ cultures

Presence indicated by ‘+’, absence indicated by ‘—’

Trapped species	Mulched treatment			Bare ground treatment		
	Hybrid-11	Hybrid-13	Hybrid-29	Hybrid-11	Hybrid-13	Hybrid-29
<i>Acaulospora</i> sp. 1 (longula)	—	+	+	—	+	—
<i>Acaulospora</i> sp. 2 (orange)	+	+	+	+	—	—
<i>Acaulospora gerdemannii</i>	—	—	—	—	+	+
<i>Entrophospora</i> sp. 2	—	+	+	—	—	—
<i>Entrophospora</i> sp. 3	—	+	+	—	—	—
<i>Gigaspora albida</i>	+	+	+	+	+	+
<i>Gigaspora gigantea</i>	—	+	—	—	—	+
<i>Glomus aggregatum</i>	+	+	+	+	+	+
<i>G. ambisporum</i>	+	+	+	+	+	+
<i>G. botryoides</i>	+	—	—	—	—	—
<i>G. clarum</i>	—	+	+	—	—	+
<i>G. fasciculatum</i>	+	+	+	+	+	+
<i>G. heterosporum</i>	—	+	+	—	+	—
<i>G. microaggregatum</i>	+	+	+	—	+	+
<i>G. pustulatum</i>	+	—	+	+	—	—
<i>G. reticulatum</i>	—	—	+	+	—	—
<i>Sclerocystis microcarpus</i>	+	+	+	+	—	—
<i>S. rubiformis</i>	—	—	+	+	—	—

There was, however, a decrease in soil spore abundance with mulching treatment. This result was attributed primarily to the influence of mulch on soil moisture. The production of resilient spores is a response to declining or variable soil moisture regimes. Consistent with this interpretation, it was noted that upper slope positions, which consisted of a freer draining soil more prone to less water holding capacity, contained higher soil spore densities (data not shown). Further, the difference in soil spore density between mulched and bare ground treatments was accentuated on the upper slope plots (591–618 spores/100 g soil in mulched treatments compared with 763–1718 spores/100 g soil in bare ground treatments; data not shown).

The production of resilient spores may also be a response to non-optimal soil pH. Soil pH of mulched and bare ground treatments was 6.9 and 7.2, respectively. In comparison, the optimum pH range for the effective proliferation of AM fungi is reported as 6.0–6.8. (Ragupathy and Mahadevan 1991). Decreased soil spore counts under mulching treatments, could also be a response to soil organic carbon. Mohankumar and Mahadevan (1987) have reported that high organic carbon reduced the mycorrhizal spore abundance in the soil.

Arbuscular mycorrhizal root colonisation frequency and crop benefit

The enhanced frequency of AM colonisation of plant roots under mulching treatments, compared to the bare ground treatments, is consistent with a role for mycorrhizal fungi in the observed increase in growth and fruit yield of papayas in mulched treatments (Elder *et al.* 2000, 2002). Mycorrhizal colonisation may affect the mineral nutrition of the host plant by enhancing plant growth through nutrient acquisition by the fungus or indirectly modifying transpiration and the composition of rhizosphere microflora. External hyphae can deliver up to 80% of plant P, 25% of plant N, 10% of plant K, 25% of plant Zn and 60% of plant Cu (Gianinazzi 1991).

However, higher P content in the leaves of papaya grown in mulched treatments was noted in 1995 but not in 1997 (Table 2). Thus, mycorrhizal colonisation did not consistently improve P status, a result attributable to the relatively high fertilisation levels maintained (Elder *et al.* 2002). The major effect of mulching on plant growth and fruit yield is likely to have been through plant water status. Future studies should consider reducing fertiliser inputs (in particular, P) in conjunction with mulching treatments, to take advantage of the increased mycorrhizal colonisation of papaya roots. Further, while little difference was noted in the hybrids trialled in terms of percentage root colonisation, any future work using a broader genetic base should consider varietal differences in mycorrhizal colonisation.

Acknowledgements

We acknowledge the encouragement of the late Prof. A Mahadevan, Director, Centre for Advanced Study in Botany, University of Madras, and funding support from Central Queensland University.

References

- Allen MF, Bossalis MG (1983) Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat. *The New Phytologist* **93**, 67–76. doi:10.1111/j.1469-8137.1983.tb02693.x
- Berch SM, Trappe JM (1991) ‘Revision of Trappe’s (1982) synoptic keys to Genera and species of Endogonaceae.’ (Oregon State University: Corvallis, OR)
- Brundrett M, Ashwath N, Jasper D (1996) Mycorrhizas in the Kakadu region of tropical Australia. Part I. Propagules of mycorrhizal fungi and soil properties in natural habitats. *Plant and Soil* **184**, 159–171. doi:10.1007/BF00029285
- Colwell JD (1963) The estimation of phosphorus fertiliser requirement of wheat in southern New South Wales by soil analysis. *Australian Journal of Experimental Agriculture and Animal Husbandry* **3**, 190–197. doi:10.1071/EA9630190
- Cruz AF, Ishii T, Matsumoto I, Kadoya K (2003) Evaluation of the mycelial network formed by arbuscular mycorrhizal hyphae in the rhizosphere of

- papaya and other plants under intercropping system. *Brazilian Journal of Microbiology* **34**, 72–76. doi:10.1590/S1517-83822003000100015
- Cuenca G, Meneses E (1996) Diversity patterns of arbuscular mycorrhizal fungi associated with cacao in Venezuela. *Plant and Soil* **96**, 359–362.
- Elder RJ, Macleod WNB, Reid DJ, Gillespie RL (2000) Growth and yield of 3 hybrid papayas (*Carica papaya* L.) under mulched and bare ground conditions. *Australian Journal of Experimental Agriculture* **40**, 747–754. doi:10.1071/EA99107
- Elder RJ, Reid DJ, Macleod WNB, Gillespie RL (2002) Post-ratoon growth and yield of three hybrid papayas (*Carica papaya* L.) under mulched and bare-ground conditions. *Australian Journal of Experimental Agriculture* **42**, 71–81. doi:10.1071/EA01032
- Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society* **46**, 235–244.
- Gianinazzi S (1991) Vesicular-arbuscular (endo) mycorrhizas: cellular, biochemical and genetic aspects. *Agriculture Ecosystems & Environment* **35**, 105–119. doi:10.1016/0167-8809(91)90047-2
- Johnson NC, Pflieger FL, Crookston RK, Simmons SR, Copeland PI (1991) Vesicular-arbuscular mycorrhizas respond to corn and soybean cropping history. *The New Phytologist* **117**, 657–663. doi:10.1111/j.1469-8137.1991.tb00970.x
- Mamatha G, Bagyaraj DJ, Jaganath S (2002) Inoculation of field-established mulberry and papaya with arbuscular mycorrhizal fungi and a mycorrhiza helper bacterium. *Mycorrhiza* **12**, 313–316. doi:10.1007/s00572-002-0200-y
- Mohankumar V, Mahadevan A (1987) Vesicular-arbuscular mycorrhizal association in plants of Kalakad reserve forest, India. *Angew Botany* **61**, 255–274.
- Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**, 158–161.
- Ragupathy S, Mahadevan A (1991) Ecology of vesicular arbuscular mycorrhizal (VAM) fungi in a coastal tropical forest. *Indian Journal of Microbial Ecology* **2**, 1–9.
- Ragupathy S, Mahadevan A (1993) Distribution of vesicular-arbuscular mycorrhizae in plants and rhizosphere soils of the tropical plains, Tamil Nadu, India. *Mycorrhiza* **3**, 123–136. doi:10.1007/BF00208920
- Reddy B, Bagyaraj DJ, Mallesha BC (1996) Selection of efficient VA mycorrhizal fungi for papaya. *Biological Agricultural and Horticulture* **13**, 1–6.
- Schenck NC, Perez Y (1987) 'Manual for the identification of VA mycorrhizal fungi.' (University of Florida: Gainesville, FL)
- Sieverding E (1991) 'Vesicular-arbuscular mycorrhiza management in tropical agrosystems.' (GTZ: Eschbom, Germany)
- Siqueria JO, Colozzi-Filho A, Oliveira E (1989) Ocorrência de micorrizas vesículo-arbusculares em agro e ecossistemas naturais do estado de Minas Gerais. *Pesquisa Agropecuária Brasileira* **24**, 1499–1506.
- Trindade AV, Siqueira JO, de Almeida FP (2001) Mycorrhizal dependency of papaya commercial varieties. *Pesquisa Agropecuária Brasileira* **36**, 1485–1494.
- Thompson JP (1987) Decline of vesicular-arbuscular mycorrhizae in long fallow disorder of field crops and its expression in phosphorus deficiency of sunflower. *Australian Journal of Agricultural Research* **38**, 847–867. doi:10.1071/AR9870847

Received 24 November 2005, accepted 23 May 2006