CSIRO PUBLISHING

www.publish.csiro.au/journals/app *Australasian Plant Pathology*, 2005, **34**, 165–173

Plant hosts of the phytoplasmas and rickettsia-like-organisms associated with strawberry lethal yellows and green petal diseases

Claire Streten[A,D], *Mark E. Herrington*[B], *Don G. Hutton*[B], *Denis M. Persley*[C], *Geoff K. Waite*[B] *and Karen S. Gibb*[A]

[A]Charles Darwin University, Darwin, NT 0909, Australia.
[B]Department of Primary Industries and Fisheries, Horticulture and Forestry Science, Maroochy Research Station, Nambour, Qld 4560, Australia.
[C]Department of Primary Industries and Fisheries, Horticulture and Forestry Science, 80 Meiers Road, Indooroopilly, Qld 4068, Australia.
[D]Corresponding author. Email: claire.streten@cdu.edu.au

Abstract. *Candidatus* Phytoplasma australiense (*Ca*. P. australiense) is associated with the plant diseases strawberry lethal yellows (SLY), strawberry green petal (SGP), papaya dieback (PDB), Australian grapevine yellows (AGY) and *Phormium* yellow leaf (PYL; New Zealand). Strawberry lethal yellows disease is also associated with a rickettsia-like-organism (RLO) or infrequently with the tomato big bud (TBB) phytoplasma, the latter being associated with a wide range of plant diseases throughout Australia. In contrast, the RLO has been identified only in association with SLY disease, and *Ca*. P. australiense has been detected only in a limited number of plant host species. The aim of this study was to identify plant hosts that are possible reservoirs of *Ca*. P. australiense and the SLY RLO. Thirty-one plant species from south-east Queensland were observed with disease between 2001 and 2003 and, of these, 18 species tested positive using phytoplasma-specific primers. The RLO was detected in diseased *Jacksonia scoparia* and *Modiola caroliniana* samples collected at Stanthorpe. The TBB phytoplasma was detected in 16 different plant species and *Ca*. P. australiense Australian grapevine yellows strain was detected in six species. The TBB phytoplasma was detected in plants collected at Nambour, Stanthorpe, Warwick and Brisbane. *Ca*. P. australiense was detected in plants collected at Nambour, Stanthorpe, Gatton and Allora. All four phytoplasmas were detected in diseased *Gomphocarpus physocarpus* plants collected at Toowoomba, Allora, Nambour and Gatton. These results indicated that the vector(s) of *Ca*. P. australiense are distributed throughout south-east Queensland and the diversity of phytoplasmas detected in *G. physocarpus* suggests it is a feeding source for phytoplasma insect vectors or it has a broad susceptibility to a range of phytoplasmas.

Additional keyword: *Candidatus* Phytoplasma australiense.

Introduction

The tomato big bud (TBB) phytoplasma is infrequently detected in strawberry plants with lethal yellows (SLY) disease while *Candidatus* Phytoplasma australiense (*Ca*. P. australiense; Davis *et al*. 1997) is consistently associated with SLY disease (Padovan *et al*. 1998; Padovan *et al*. 2000*b*). In Australia, *Ca.* P. australiense is also associated with the diseases strawberry green petal (SGP) (Padovan *et al*. 2000*b*), papaya dieback (PDB; Gibb *et al*. 1996; Liu *et al*. 1996), Australian grapevine yellows (AGY; Padovan *et al*. 1996) and mung bean witches' broom (MBWB; Schneider *et al*. 1999). More recently, *Ca.* P. australiense has been implicated as a causal agent of pumpkin yellow leaf curl (PYLC; Streten *et al*. 2005*a*) and periwinkle phyllody (Davis *et al*. 2003). *Ca.* P. australiense is also associated with plant diseases in New Zealand including strawberry lethal yellows (SLY; Andersen *et al*. 1998), *Phormium* yellow leaf (PYL; Liefting *et al*. 1998), *Cordyline australis* (cabbage tree) sudden decline (CSD; Andersen *et al*. 2001) and *Coprosma* lethal decline (CLD; Andersen *et al*. 2001).

Although *Ca*. P. australiense has been consistently associated with a range of crop species, only a few non-crop host species have been identified during phytoplasma disease

 10.1071/AP05014 0815-3191/05/020165

surveys in Australia (Davis *et al*. 1997, 2003; Schneider *et al*. 1999). The plant host range of a phytoplasma generally reflects the number of natural vector species that are capable of transmitting the organism and their feeding behaviour (Lee *et al*. 2000). Results to date suggest that the vector of *Ca*. P. australiense has a narrow host range or a limited number of species are susceptible to this phytoplasma. In Australia, no vectors have been identified for *Ca*. P. australiense whereas in New Zealand, the planthopper, *Oliarus atkinsoni* transmits this phytoplasma (Boyce and Newhook 1953; Liefting *et al*. 1997). *O. atkinsoni* is a monophagous species that feeds on *Phormium* sp. and is essentially limited to New Zealand (Liefting *et al*. 1998; Andersen *et al*. 2001), which means that it is unlikely to transmit *Ca*. P. australiense in Australia. Identification of possible alternative hosts of *Ca*. P. australiense may provide insight into the identity of its vectors in Australia. Furthermore, the detection of phytoplasmas in plants in the vicinity of strawberry farms would implicate these plant species as possible reservoirs of *Ca*. P. australiense when strawberry plants are not being grown in the field.

In contrast to *Ca*. P. australiense, the TBB phytoplasma has a wide host range including native and introduced plant species (Davis *et al*. 1997; Schneider *et al*. 1999; Padovan and Gibb 2001; Davis *et al*. 2003). These TBB phytoplasma-associated diseases occur throughout Australia (Davis *et al*. 1997, 2003; Schneider *et al*. 1999; Padovan and Gibb 2001). The TBB phytoplasma has provisionally been assigned the Candidate species name, *Candidatus* Phytoplasma australasia (White *et al*. 1998). The wide host range of the TBB phytoplasma possibly reflects the feeding habits of its insect vector, the common brown leafhopper, *Orosius argentatus*, which is widely distributed throughout Australia (Hill 1943).

A rickettsia-like-organism (RLO) is also associated with SLY disease (Greber and Gowanlock 1979). Little is known about this RLO, as until recently there was no diagnostic test. The development of a PCR diagnostic test, which amplifies the flavoprotein subunit succinate dehydrogenase (*sdhA*) gene of the SLY RLO (Streten *et al*. 2005*b*) and the papaya bunchy top (PBT) RLO, has facilitated the identification of other hosts and possible vectors for this organism. The only known vector of an RLO is the leafhopper, *Empoasca papayae*, which transmits the PBT RLO (Davis *et al*. 1998). The identification of alternative hosts for the SLY RLO may indicate its host range and provide a focus for subsequent vector studies.

This study aimed to identify other host plants of *Ca*. P. australiense by conducting disease surveys near strawberry farms where SLY disease has been recorded. To determine whether the phytoplasmas or RLO associated with SLY disease are limited to the strawberry growing districts, diseased plant host species were also collected 50–200 km from any strawberry farm.

Methods

Source and location of samples

Diseased and asymptomatic plants were collected on, or within, 50–100 m of strawberry runner beds in the Nambour and Stanthorpe districts of south-east Queensland, between March 2001 and January 2003 (Table 1). Diseased plants were also collected in Allora, Gatton, Toowoomba and Warwick districts of south-east Queensland, which are located 50–200 km from any strawberry farm (Table 1). A single collection was made from Adelaide in South Australia (Table 1).

Screening for phytoplasmas and rickettsia-like-organisms

Total DNA was extracted from plant samples according to Doyle and Doyle (1990) using a modified CTAB buffer (Padovan *et al*. 1995). Deoxyribonucleic acid quality was determined by subjecting the samples to electrophoresis in a 1% agarose gel, which was then stained with ethidium bromide and viewed by UV trans-illumination.

Plant samples were screened for phytoplasmas using the primer pairs fP1 / rP7 (Deng and Hiruki 1991; Schneider *et al*. 1995) and fU5 / m23sr (Lorenz *et al*. 1995; Padovan *et al*. 1995), which amplify the phytoplasma 16S rRNA gene and 16S–23S spacer region. The PCR reactions were prepared according to Schneider *et al*. (1997) and subjected to 35 cycles of 95°C / 1 min; 55°C / 1 min and 72°C / 1.5 min. One microlitre of undiluted DNA or DNA diluted 1 : 10 or 1 : 50 in water was used as DNA template in PCR.

Symptomatic and asymptomatic samples were also screened using the fTufAy and rTufAy primers according to Schneider *et al*. (1997). These primers amplify the Tu elongation factor (*tuf*) gene of phytoplasmas assigned to the aster yellows and stolbur groups, which includes *Ca*. P. australiense but not the TBB phytoplasma.

Deoxyribonucleic acid samples were also tested using the PCR primers that amplify the flavoprotein subunit of succinate dehydrogenase (*sdhA*) gene (PBTF1 and PBTR1) of the RLO associated with lethal yellows (Streten *et al*. 2005*b*) and PBT disease (Davis *et al*. 1998). PCR reactions were prepared according to Davis *et al*. (1998). Amplification conditions used for the PBTF1 and PBTR1 primers were 94°C / 1 min, 52°C / 1.5 min and 72°C / 1 min for 40 cycles.

Identification of phytoplasmas and rickettsia-like-organisms

PCR products amplified from diseased and asymptomatic plants using primers specific for the phytoplasma 16S rRNA gene or the RLO *sdhA* gene were digested with the restriction enzymes *Alu*I and *Rsa*I. *Tuf* genes were digested with *Hpa*II and *Hind*III (Schneider *et al*. 1997). All digestions were in buffer supplied by the manufacturer, 1 U enzyme (Promega, Sydney, Australia), 5 µL of PCR product and sterile distilled water. Reactions were incubated overnight at the specified temperature and subsequently separated in a 12% polyacrylamide gel. The gels were then stained with ethidium bromide and visualised on a UV trans-illuminator.

Results

Rickettsia-like-organism detection in plant host species

The RLO was detected in only one diseased *Jacksonia scoparia* sample and one diseased *Modiola caroliniana* sample; both were collected at Stanthorpe (Tables 1 and 3). The *sdh*A gene amplified from these diseased plants all had the same restriction banding patterns as the reference RLO associated with SLY disease (data not shown).

Table 1. Plant species in which phytoplasmas and rickettsia-like-organisms were detected

Scientific name	Common name	Location	Symptoms	P. australiense[A]	TBB[A]	RLO[A]	SPLL-V4[A]	Number of plants tested
Amaranthus sp.		Stanthorpe	Yellowing of leaves		2			2
Araujia sericifera		Gatton	Asymptomatic					3
		Gatton,	Yellowing of leaves with red margins,		1			1
		Warwick	Witches' broom		1			1
Callitris baileyi		Stanthorpe	Yellowing of branches					1
		Stanthorpe	Asymptomatic		1			1
Chenopodium carinatum		Stanthorpe	Shortening and clumping of petioles		2			2
Conzya sp.	Fleabane	Stanthorpe	Leaf distortion		3			3
		Stanthorpe	Asymptomatic					1
		Stanthorpe	Reduced leaves					1
Cucurbita maxima	Pumpkin	Stanthorpe	Asymptomatic					5
		Stanthorpe	Yellow leaf curl	3				3
		Gatton	Yellow leaf curl	10				10
		Stanthorpe	Yellowing at vine tips					1
Datura stramonium	Thornapple	Stanthorpe	Yellowing of plant		1			3
		Stanthorpe	Asymptomatic					1
Erimophyla		Stanthorpe	Leaf distortion		1			1
Exocarpus cupressiformis	Native cherry	Stanthorpe	Reduced yellow leaves	1	5			6
Gomphocarpus physocarpus	Cottonbush	In Table 2	Refer to Table 2	8	3		3	33
Hexham sp.		Toowoomba	Witches' broom, stunting	2[B]				2
Hibiscus trionum	Bladder ketmia	Stanthorpe	Yellowing of leaves		1			1
Jacksonia scoparia		Stanthorpe	Refer to Table 3	2	4	1		23
Medicago polymorpha		Stanthorpe	Refer to Table 4	4	5			16
Modiola caroliniana	Carolina mallow	Stanthorpe	Leaf distortion, yellowing and stunting			1		1
Plantago lanceolata	Ribwort	Stanthorpe	Yellow and white leaves		1			1
		Brisbane	Yellowing and smaller plant		1			1
		Stanthorpe	Crimped and cupped leaves					3
		Stanthorpe	Asymptomatic					2
		Stanthorpe	New leathery growth					1
		Stanthorpe	Branched leaves and deformed new leaves					1
Solanum nigrum	Nightshade	Stanthorpe	Leaf tip clumping					1
		Stanthorpe	Proliferation of tips and buds		1			1
Trifolium sp.	Clover	Stanthorpe	Reddening/yellowing of leaves and clumping of plant	1				1
		Adelaide	Red and yellow leaves					3
		Adelaide	Green flowers and phyllody		1			1
Total				31	34	2	3	138

[A]Number of samples positive for the specified pathogen. P. australiense, *Candidatus* Phytoplasma australiense; TBB, tomato big bud phytoplasma (*Candidatus* Phytoplasma australasia); RLO, rickettsia-like-organism; SPLL-V4, sweet potato little leaf strain V4.
[B]*Candidatus* Phytoplasma australiense *Phormium* yellow leaf (PYL) variant strain.

Phytoplasma detection in plant host species

Eighteen out of 34 diseased plant host species tested positive using phytoplasma specific primers (Tables 1–4). The *Ca*. P. australiense Australian grapevine yellows (AGY) strain was detected in 25 plants collected from Nambour, Gatton, Stanthorpe and Allora districts of south-east Queensland. The 25 plants represented six different plant species (Tables 1–4). The *tuf* gene amplified from *Hexham* sp. (Table 1), and *G. physocarpus* from Toowoomba and Nambour (Table 2 and Fig. 1) had the same RFLP banding pattern as the *Phormium* yellow leaf phytoplasma when digested with *Hpa*II and a different banding pattern when digested with *Hind*III (data not shown). This phytoplasma was designated *Ca*. P. australiense PYL variant strain.

The TBB phytoplasma was detected in 33 plants from Stanthorpe, Brisbane, Warwick and Nambour (Tables 1–4) in Queensland and in one plant from Adelaide, South Australia (Table 1). These 34 diseased samples represented 15 plant species, some of which are also hosts for *Ca*. P. australiense. A *Ca*. P. australiense and the TBB phytoplasma mixed 'infection', was detected in only one *G. physocarpus* plant. The SPLL-V4 phytoplasma was detected in single *G. physocarpus* plants from Allora and Nambour (Table 2).

Association between phytoplasma, rickettsia-like-organism and disease

All pumpkin (*Cucurbita maxima*) plants exhibiting yellow leaf curl were positive for *Ca*. P. australiense AGY strain and all asymptomatic pumpkin plants were phytoplasma negative (Table 1). All native cherry (*E. cupressiformis*) samples with small leaf symptoms were phytoplasma positive (Table 1).

Ca. P. australiense PYL variant strain was detected in all four *G. physocarpus* plants with yellowing and little leaf or

Table 2. Phytoplasmas detected in *Gomphocarpus physocarpus* (balloon cottonbush)

Location	Symptoms	P. australiense[A]	TBB[A]	SPLL-V4[A]	Total number of plants tested
Allora	Yellowing, little leaf, bunching along stem			2	2
	Asymptomatic			1	1
	Witches' broom	1			1
Gatton	Narrow red and yellow leaves	1			1
Nambour	Asymptomatic	1			4
	Reddening and yellows of stems and leaves				2
	Little leaf, proliferation of leaves at terminal ends of branches and yellowing	1	1		1
	Yellow mottling on leaves		1		3
	Yellowing and leaf distortion				5
	Clumping of leaves along stem and yellow reduced leaves	1[B]			1
	Small mottled leaves clumped along stem				2
	Older leaves with yellow mottling, young leaves reduced and yellow, curling of leaves, green petals on very reduced flowers, leaves clumping at terminal ends and leaves protruding from flowers		1		1
Stanthorpe	Asymptomatic				1
	Yellow distorted leaves				1
Toowoomba	Asymptomatic				3
	Yellowing and reduced leaves	3[B]			3
	Green plant with wilted and distorted terminals				1
Total		8	3	3	33

[A]Number of samples positive for the specified pathogen. Abbreviations as in Table 1.
[B]*Candidatus* Phytoplasma australiense *Phormium* yellow leaf (PYL) variant strain.

Table 3. Phytoplasmas and RLOs detected in *Jacksonia scoparia* (Dogwood)

Location	Symptoms	P. australiense[A]	TBB[A]	RLO[A]	Total number of plants tested
Stanthorpe	Abnormal growth at tips		1	1	7
	Abnormal branching and growth	2	1		3
	Asymptomatic				6
	Proliferation of growth at branch ends		2		4
	Wilting at branch tips				1
	Witches broom				3
Total		2	4	1	24

[A]Number of samples positive for the specified pathogen. Abbreviations as in Table 1.

Table 4. Phytoplasmas detected in *Medicago polymorpha* (Burr trefoil)

Location	Symptoms	P. australiense[A]	TBB[A]	Total number of plants tested
Stanthorpe	Reduced leaves with reddening	2		3
	Asymptomatic	1	2	5
	Reddening and curling of leaves		2	2
	Red leaf margins			1
	Reduced leaves with curling of leaf margins			1
	Yellow and red leaves	1		3
	Stunted growth with reduced leaves		1	1
Total		4	5	16

[A]Number of samples positive for the specified pathogen. Abbreviations as in Table 1.

Fig. 1. *Gomphocarpus physocarpus* infected with *Candidatus* Phytoplasma australiense *Phormium* yellow leaf strain (left) and *Jacksonia scoparia* infected with tomato big bud (TBB) phytoplasma (right).

clumping of leaves along the stem. *G. physocarpus* plants with symptoms of yellowing, little leaf and bunching along the stem and also without symptoms tested positive for the SPLL-V4 phytoplasma (Table 2). The TBB phytoplasma was detected in a single *G. physocarpus* plant with symptoms of reduced yellow leaves, green petal and clumping of growth at terminal ends, and in a plant exhibiting yellow mottling of leaves (Table 2). *Ca*. P. australiense AGY strain and the TBB phytoplasma were detected in one *G. physocarpus* plant with symptoms of little leaf and proliferation of leaves at terminal ends (Table 4). *Ca*. P. australiense AGY strain was also detected in *G. physocarpus* plants exhibiting witches' broom or narrow red / yellow leaves (Table 2). Eleven *G. physocarpus* plants exhibiting disease symptoms tested negative for phytoplasmas or RLOs (Table 2).

Phytoplasmas were detected more commonly in diseased *J. scoparia* exhibiting an abnormal branching pattern (Fig. 1) than in plants exhibiting other phytoplasma-type symptoms (Tables 1 and 3). An RLO was also detected in *J. scoparia* plants with abnormal branching symptoms (Table 3). *M. polymorpha* plants with symptoms of reddening and curling of leaves or stunted growth and little leaf tested positive for the TBB phytoplasma (Table 4). *Ca*. P. australiense AGY strain was amplified from diseased *M. polymorpha* plants with symptoms of reddened reduced leaves or symptoms of yellow and red leaves (Table 4).

Amaranthus sp., *Araujia sericifera*, *Chenopodium carinatum*, *Erimophyla* sp., *Hibiscus trionum* and *Plantago lanceolata* were rarely observed with disease so few samples were collected (Table 1). Asymptomatic samples from each of these plant species tested positive for TBB phytoplasma (Table 1). Diseased and asymptomatic *Acacia melanoxylon*, *Acacia* sp., *Asclepias curassavica*, *Echinochloa colona*, *Malva parviflora*, *Medicago sativa*, *Osothamnus diosmifolius*, *Plantago cunninghamii* and *Sonchus* sp. were also sampled during the study (Table 5). No RLO or phytoplasma was detected in these plant species (Table 5).

Discussion

In Queensland, strawberry growers producing runners are located in the Stanthorpe region while fruit is produced in

Table 5. Plant species in which phytoplasma and rickettsia-like-organisms (RLO) were not detected

Scientific name	Common name	Location	Symptoms	Total number of plants tested
Acacia melanoxylon	Blackwood	Nambour	Proliferation of deformed undifferentiated tissue at buds	1
			Asymptomatic	1
			Interveinal yellowing	1
Acacia sp.		Stanthorpe	Yellow young leaves with red leaf margins	1
Asclepias curassavica	Red Cottonbush	Stanthorpe	Young leaves distorted	1
Echinochloa colona	Swamp grass	Stanthorpe	White striping on leaves	1
			Asymptomatic	1
Glycine max	Soybean	Toowoomba	Proliferation of flowers and seeds. Yellow seed pods, necrosis of mid vein, yellowing of leaves	15
Glycine sp.		Stanthorpe	Yellowing leaves	1
			Asymptomatic	1
			Clumping of plant growth	1
Lycopersicon esculentum	Tomato	Stanthorpe	Asymptomatic	1
			Reduced leaves, yellowing along leaf veins	1
Malva parviflora	Marshmallow	Stanthorpe	Yellowing	1
Medicago sativa	Lucerne	Toowoomba	Asymptomatic	3
			Shortened internodes, smaller leaves with clumping and elongation of leaves and yellowing	2
Melaleuca sp.	Ti tree	Stanthorpe	Chlorotic terminal	1
Osothamnus diosmifolius	Sago flower	Stanthorpe	Yellowing of leaves	1
Plantago cunninghamii	Sago weed	Stanthorpe	Yellowing	1
Plantago sp.		Stanthorpe	Distorted growth	1
Solanum mauritianum	Wild tobacco	Stanthorpe	Yellowing of branch tips	1
Sonchus sp.	Milk thistle	Stanthorpe	Yellowing and reddening of leaves	2
Total				40

the areas surrounding Nambour, Caboolture, Beenleigh and Brisbane. Strawberry plants are not grown all year round, which suggests that non-crop plant species growing near strawberry fields may be reservoirs for phytoplasmas or RLO associated with SLY disease. However, growers remove weeds growing among strawberry plants on fruit production farms and south-east Queensland was experiencing a drought during the study, reducing the number of plant hosts growing on and near strawberry runner and fruit production farms. Therefore, during the survey, the only plant species with symptoms of yellows disease at fruit production farms, where *Ca*. P. australiense is often detected in SLY or SGP diseased strawberry plants (Padovan *et al*. 2000*b*), were *Gomphocarpus physocarpus* and *Acacia melanoxylon*. Diseased *G. physocarpus* were also observed at locations 50–200 km away from fruit production farms.

Ca. P. australiense AGY strain, *Ca*. P. australiense PYL variant strain, TBB and SPLL-V4 phytoplasmas were all detected in diseased *G. physocarpus* plants, which suggests that these plants are a food source for the insect vectors or this species is susceptible to a range of phytoplasmas. Diseases of *Gomphocarpus* sp. have also been reported in Italy (D'Aquilio *et al*. 2002) and the stolbur and European aster yellows phytoplasmas were detected in these plants. Symptomatic *G. physocarpus* plants were collected at different locations in south-east Queensland, which suggests that these phytoplasmas are not confined to a single location and their insect vectors are distributed throughout south-east Queensland. Based on frequency of phytoplasma detection, the symptoms of green petal, little leaf, and reduced leaves appear to be a phytoplasma disease in *G. physocarpus* plants. The other symptoms observed for *G. physocarpus* plants may have been due to nutritional deficiencies, lack of water or another pathogen being present.

In this study, diseased *G. physocarpus*, *M. polymorpha* and *J. scoparia* were most frequently observed but other plant species with phytoplasma-type symptoms were also collected. Pumpkin plants with yellow leaf curl were observed at Gatton and *Ca*. P. australiense AGY strain was detected in these samples, thus confirming this previously reported phytoplasma associated disease (Streten *et al*. 2005*a*).

Ca. P. australiense or the TBB phytoplasma were detected in diseased *M. polymorpha*, *E. cupressiformis*, *J. scoparia* and no mixed phytoplasma 'infections' were detected for these samples. Although *Ca*. P. australiense and the TBB phytoplasma were identified as having these common host plants, *Ca*. P. australiense and TBB are unlikely to have the same vector because they only shared a limited number of host plant species and host range generally reflects the feeding habits of the vector (Lee *et al*. 2000). Therefore *M. polymorpha*, *E. cupressiformis* and *J. scoparia* are possibly food sources for a range of insect vectors.

The TBB phytoplasma was detected in diseased *Amaranthus* sp., *C. baileyi*, *C. carinatum*, *Conzya* sp., *D. stramonium*, *E. cupressiformis*, *Erimophyla* sp., *H. trionum*, *J. scoparia*, *M. polymorpha*, *Plantago lanceolata* and *S. nigrum* plants that were located near runner production farms in the Stanthorpe district. Therefore, there is an abundant supply of TBB phytoplasma inoculum in the vicinity of runner production farms. Despite this, the TBB phytoplasma is infrequently detected in plants with SLY disease (Padovan *et al*. 2000*b*). The low occurrence of TBB phytoplasma-associated strawberry disease in Stanthorpe where there is a range of sources of inoculum in the surrounding area suggests that the vector, *O. argentatus*, is either not prevalent in the region, or strawberry plants are not a preferred food source for this leafhopper species, or strawberry plants are not highly susceptible to the TBB phytoplasma.

The SPLL-V4 phytoplasma, which is closely related to the TBB phytoplasma (Padovan *et al*. 2000*a*), was only detected in diseased *G. physocarpus* plants collected at Allora. This is in contrast to previous disease surveys in northern Australia, which showed that the SPLL-V4 phytoplasma is associated with diseases that occur in a wide range of plant species (Davis *et al*. 1997, 2003; Schneider *et al*. 1999; Padovan and Gibb 2001). The identification of a single plant species positive for the SPLL-V4 phytoplasma compared with the TBB phytoplasma (16 species) suggests that in south-east Queensland, these phytoplasmas may not have a common vector and that the vector for the SPLL-V4 phytoplasma is not prevalent. If the vector for the SPLL-V4 phytoplasma and incidence of the disease is not abundant in south-east Queensland, it is unlikely that strawberry plants will be inoculated with the SPLL-V4 phytoplasma.

The TBB and SPLL-V4 phytoplasmas were detected more frequently in non-crop plants growing in or near Stanthorpe than were the *Ca*. P. australiense AGY or PYL variant strains. *Ca*. P. australiense AGY and PYL variant strains were detected in different *G. physocarpus* plants collected at Nambour and these plants were growing within 100 m of SLY diseased plants that tested positive for *Ca*. P. australiense AGY strain (Streten *et al*. 2005*b*). *Ca*. P. australiense PYL variant strain has previously been identified as being associated with SLY diseased at Caboolture and it was thought that this strain represented an isolated population within Australia (Streten *et al*. 2005*b*). The detection of *Ca*. P. australiense New Zealand strain at Nambour, Toowoomba and Caboolture in *G. physocarpus* and *Hexham* sp. plants showed that this phytoplasma is more widespread than previously thought. These plant species may be reservoirs for this phytoplasma and they may also be a source of inoculum for SLY disease if its vector is present.

The vector of the Australian RLOs is still unknown and this study provided limited insight into the nature of the insect that transmits these organisms. RLO-associated disease was only identified in two non-crop plant hosts in the area surrounding runner production farms where this organism is the most

common agent associated with SLY disease (Streten *et al*. 2005*b*). This suggests that the vector has a limited host range or only a limited number of plants are susceptible to the RLO (Lee *et al*. 1998; Lee *et al*. 2000). *G. physocarpus* plants could not be screened for an RLO because, after PCR amplification, all samples including healthy controls gave a band the same size as the reference RLO strain. We, therefore, do not know if the RLO is associated with this host species.

In conclusion, results from this study suggest that *G. physocarpus* is a food source for insect vectors of phytoplasmas and, therefore, a possible source of phytoplasma for SLY disease. Diseased pumpkin is also a possible source of *Ca*. P. australiense for strawberry plants. *Ca*. P. australiense AGY strain was also detected in diseased *M. polymorpha* plants that were collected on runner production farms and these plants are likely to be a reservoir of phytoplasmas for strawberry plants because they are located in the strawberry growing region and on strawberry farms. Although nine plant species collected on or near runner production farms were positive for the TBB phytoplasma, this may not be significant because this phytoplasma is detected only occasionally in SLY diseased plants. The Australian RLO was detected in diseased *M. caroliniana* and *J. scoparia* plants, which were growing on runner production farms in the Stanthorpe district. Thus, these plants may act as reservoirs of the RLO if the insects that feed on these species can acquire and transmit the RLO, and use strawberry as a food source.

This study of phytoplasma and RLO host range, while not intended as a systematic survey of all other host plants in the 200 km area surrounding commercial strawberry farms, has identified some key non-crop species that are hosts for the RLO and a range of phytoplasmas. Future studies should focus on these species and candidate insect vectors.

Acknowledgements

This research was supported by the Cooperative Research Centre for Tropical Plant Protection (Brisbane, Australia); and the Better Berries Program, Queensland Department of Primary Industries and Fisheries (Nambour, Australia) led by Mr Neil Greer.

References

Andersen MT, Beever RE, Sutherland PW, Forster RLS (2001) Association of '*Candidatus* Phytoplasma australiense' with sudden decline of cabbage tree in New Zealand. *Plant Disease* **85**, 462–469.

Andersen MT, Longmore J, Liefting LW, Wood GA, Sutherland PW, Beck DL, Forster RLS (1998) Phormium yellow leaf phytoplasma is associated with strawberry lethal yellows disease in New Zealand. *Plant Disease* **82**, 606–609.

Boyce WR, Newhook FJ (1953) Investigations into yellow-leaf disease of Phormium. I. History and symptomatology. *New Zealand Journal of Science Technology* **34A** (supplement 1), 1–11.

D'Aquilio M, Boarino A, Bozzano G, Marzahi C, Roggero P, Boccardo G (2002) First report of phytoplasmas infecting swan plants (*Gomphocarpus physocarpus*) in Liguria, Italy. *Plant Pathology* **51**, 796. doi: 10.1046/j.1365-3059.2002.00766.x

Davis MJ, Ying Z, Brunner BR, Pantoja A, Ferwerda FH (1998) Rickettsial relative associated with papaya bunchy top disease. *Current Microbiology* **36**, 80–84. doi: 10.1007/s002849900283

Davis RE, Dally EL, Gundersen DE, Lee I-M, Habili N (1997) '*Candidatus* Phytoplasma australiense', a new phytoplasma taxon associated with Australian grapevine yellows. *International Journal of Systematic Bacteriology* **47**, 262–269.

Davis RI, Jacobson SC, De La Rue SJ, Tran-Nguyen L, Gunua TG, Rahamma S (2003) Phytoplasma disease surveys in the extreme north of Queensland, Australia, and the island of New Guinea. *Australasian Plant Pathology* **32**, 269–277. doi: 10.1071/AP03020

Davis RI, Schneider B, Gibb KS (1997) Detection and differentiation of phytoplasmas in Australia. *Australian Journal of Agricultural Research* **48**, 535–544. doi: 10.1071/A96114

Deng S, Hiruki C (1991) Amplification of 16S rRNA genes from culturable and non-culturable Mollicutes. *Journal of Microbiological Methods* **14**, 53–61. doi: 10.1016/0167-7012(91)90007-D

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus (San Francisco, Calif.)* **12**, 13–15.

Gibb KS, Persley D, Schneider B, Thomas JE (1996) Phytoplasmas associated with papaya diseases in Australia. *Plant Disease* **80**, 174–178.

Greber RS, Gowanlock DH (1979) Rickettsia-like and Mycoplasma-like organisms associated with two yellow-type diseases of strawberries in Queensland. *Australian Journal of Agricultural Research* **30**, 1101–1109. doi: 10.1071/AR9791101

Hill AV (1943) Insect transmission and host plants of virescence (big bud of tomato). *Journal of the Council for Scientific and Industrial Research Australia* **16**, 85–90.

Lee I-M, Gunderson-Rindal DE, Bertaccini A (1998) Phytoplasma: ecology and genomic diversity. *Phytopathology* **88**, 1359–1366.

Lee I-M, Davis RE, Gundersen-Rindal DE (2000) Phytoplasmas: Phytopathogenic mollicutes. *Annual Review of Microbiology* **54**, 221–255. doi: 10.1146/annurev.micro.54.1.221

Liefting LW, Beever RE, Winks CJ, Pearson MN, Forster RLS (1997) Planthopper transmission of *Phormium* yellow leaf phytoplasma. *Australasian Plant Pathology* **26**, 148–154.

Liefting LW, Padovan AC, Gibb KS, Beever RE, Andersen MT, Newcomb RD, Beck DL, Forster RLS (1998) '*Candidatus* Phytoplasma australiense' is the phytoplasma associated with Australian grapevine yellows, papaya dieback and *Phormium* yellow leaf diseases. *European Journal of Plant Pathology* **104**, 619–623. doi: 10.1023/A:1008693904427

Liu B, White DT, Walsh KB, Scott PT (1996) Detection of phytoplasmas in dieback, yellow crinkle and mosaic diseases of papaya using polymerase chain reaction techniques. *Australian Journal of Agricultural Research* **47**, 387–394. doi: 10.1071/AR9960387

Lorenz K-H, Schneider B, Ahrens U, Seemüller E (1995) Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and nonribosomal DNA. *Phytopathology* **85**, 771–776.

Padovan AC, Firrao G, Schneider B, Gibb KS (2000*a*) Chromosome mapping of sweet potato little leaf phytoplasma reveals genome heterogeneity within the phytoplasmas. *Microbiology* **146**, 893–902.

Padovan AC, Gibb KS (2001) Epidemiology of phytoplasma diseases in papaya in Northern Australia. *Journal of Phytopathology* **149**, 649–658. doi: 10.1046/j.1439-0434.2001.00688.x

Padovan AC, Gibb KS, Bertaccini A, Magarey PA, Sears BB (1995) Molecular detection of the Australian grapevine yellows phytoplasma and comparison with grapevine yellows phytoplasma from Italy. *Australian Journal of Grape and Wine Research* **1**, 25–31.

Padovan AC, Gibb KS, Daire X, Boudon-Padieu E (1996) A comparison of the phytoplasma associated with Australian grapevine yellows to other phytoplasmas in grapevine. *Vitis* **35**, 189–194.

Padovan AC, Gibb KS, Persley D (1998) Phytoplasmas associated with diseases in strawberry. *Australasian Plant Pathology* **27**, 280.

Padovan AC, Gibb KS, Persley D (2000*b*) Association of '*Candidatus* Phytoplasma australiense' with green petal and lethal yellows disease in strawberry. *Plant Pathology* **49**, 362–369. doi: 10.1046/j.1365-3059.2000.00461.x

Schneider B, Gibb KS, Padovan AC (1997) Sequence and RFLP analysis of the elongation factor Tu gene used in differentiation and classification of phytoplasmas. *Microbiology* **143**, 3381–3389.

Schneider B, Padovan AC, De La Rue S, Eichner R, Davis R, Bernuetz A, Gibb K (1999) Detection and differentiation of phytoplasmas in Australia: an update. *Australian Journal of Agricultural Research* **50**, 333–342.

Schneider B, Seemüller E, Smart CD, Kirkpatrick BC (1995) Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas. *Molecular and Diagnostic Procedures in Mycoplasmology* **1**, 369–380.

Streten C, Conde B, Herrington M, Moulden J, Gibb K (2005*a*) *Candidatus* Phytoplasma australiense is associated with pumpkin yellow leaf curl disease in Queensland, Western Australia and the Northern Territory. *Australasian Plant Pathology* **34**, 103–105.

Streten C, Waite GK, Herrington ME, Hutton DG, Persley DM, Gibb KS (2005*b*) Rickettsia-like-organisms and phytoplasmas associated with diseases in Australian strawberries. *Australasian Plant Pathology* **34**, 157–164.

White DT, Blackall LL, Scott PT, Walsh KB (1998) Phylogenetic positions of the phytoplasmas associated with dieback, yellow crinkle and mosaic diseases of papaya, and their proposed inclusion in *Candidatus* Phytoplasma australiense and a new taxon, *Candidatus* Phytoplasma Australasia. *International Journal of Systematic Bacteriology* **48**, 941–951.

Received 30 July 2004, accepted 25 October 2004