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A comparison of the phytoplasma associated with Australian grapevine yellows to other phytoplasmas in grapevine

by

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S u m m a r y : The phytoplasma associated with Australian grapevine yellows (AGY) was compared to other phytoplasma diseases of grapevine using the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. Comparison of eight different Australian isolates suggests that only one type of phytoplasma is associated with this disease. Based on RFLP analysis of the 16S rRNA gene, it was shown that AGY is different from the tomato big bud and sweet potato little leaf phytoplasma strains which are widespread in Australia and that it represents the only other phytoplasma strain recorded in Australia to date. Restriction profiles of grapevine phytoplasmas using *Mse* I suggest that AGY is unique but most closely resembles those phytoplasmas associated with grapevine diseases in the stolbur group. Sequence analysis of the 16S rRNA gene and adjacent spacer region supports this association. The uniqueness of AGY was confirmed by PCR assays using non-ribosomal primers; the primer pair STOL11f1/r2 specific for stolbur phytoplasmas did not result in amplification products in grapevines affected with AGY; the primer pair fMLO1/rMLO1 which amplifies a region of the *tuf* gene from phytoplasmas in the aster yellows cluster, amplified AGY DNA confirming its association within this phylogenetic group. RFLP analysis of the *tuf* PCR product again highlighted a distinction between AGY and other stolbur phytoplasmas occurring in grapevine. The only other phytoplasma in Australia which is in the stolbur group is associated with dieback in papaya, and it has the same RFLP profile of the *tuf* PCR product as AGY.

K e y w o r d s : phytoplasma, Australian grapevine yellows, PCR, RFLP.

Introduction

Phytoplasmas occur in many countries (MCCOY *et al.* 1989) and they represent a major group of plant pathogens in terms of host range, distribution and damage to cultivated plant species (HULL 1971). Studies in Europe, the USA and the Asian continent have shown that a number of different phytoplasma strains can occur in a particular region (AHRENS and SEEMÜLLER 1992; LEE *et al.* 1993; NAKASHIMA and MURATA 1993; NAMBA *et al.* 1993). In view of this, when work on phytoplasmas began in 1992 in our laboratory at the Northern Territory University, one of the primary aims was to determine how many phytoplasma strains were present in Australia. This question was particularly interesting because Australia is isolated from other large land masses, and while plant material has been brought into the country, strict quarantine procedures have prevented many plant diseases from being introduced. Initial results from this project showed that only two types of phytoplasmas were present, the sweet potato little leaf (SPLL) phytoplasma and the tomato big bud (TBB) phytoplasma. Restriction fragment length polymorphism (RFLP) and sequence analysis of the 16S rRNA gene from SPLL and TBB shows that they are almost identical and that they are most closely related to phytoplasmas from south-east Asia (GIBB *et al.* 1995; PADOVAN unpublished). Continuation of this Australian survey has revealed the presence of phytoplasmas in almost 40 plant hosts, and RFLP analysis of the 16S rRNA gene suggests that they are all genetically related, belonging to the sunn-hemp group of phytoplasmas (GIBB *et al.* unpublished).

A phytoplasma etiology had also been suspected in Australian grapevines showing downward rolling and yellowing of leaves, shoot tip dieback, shrivelling of young bunches and non-lignification of affected shoots. A phytoplasma association was suggested due to natural heat therapy of infected vines, sensitivity to tetracycline, fluorescence microscopy showing intense fluorescence in phloem of symptomatic vines (MAGAREY and WACHTEL 1986 a and b) and electron microscopy showing phytoplasma-like bodies in phloem cells of symptomatic vines but not in symptomless tissue (MAGAREY *et al.* 1988). This disease was first reported in 1975 and termed Australian grapevine yellows (AGY). In 1995, a phytoplasma was detected in infected grapevine by the polymerase chain reaction (PCR) assay using phytoplasma specific primers (PADOVAN *et al.* 1995). The aim of the present study was to determine the genetic relatedness of the phytoplasma associated with AGY.

Grapevine yellows diseases have been reported in Europe, Israel, Chile, New Zealand and the USA (MAGAREY 1986). Studies have shown that three different phytoplasma groups are associated with these diseases: (1) elm yellows, (2) western-X disease and (3) stolbur. Flavescence dorée which occurs in France and northern Italy belongs to the elm yellows group (DAIRE *et al.* 1993). Within the X-disease group are phytoplasmas isolated from grapevines in northern Italy and from New York (CHEN *et al.* 1993), and PRINCE *et al.* (1993) also showed the presence of a phytoplasma from the X-disease group occurring in grapevines from Virginia in the USA. Phytoplasmas from the stolbur group have been reported in grapevines from France (called

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bois noir), northern Italy, Sicily and Israel (DAIRE *et al.* 1994), Germany (called Vergilbungskrankheit, MAIXNER *et al.* 1994), Spain (LAVINA *et al.* 1995), and central Italy (aster yellows group I-G, DAVIS *et al.* 1993). Our initial studies showed that AGY was similar to a phytoplasma from grapevine in central Italy. The work presented here describes a more detailed comparison using key phytoplasmas from the three representative groups to characterise AGY using PCR/RFLP analysis of the 16S rRNA gene and adjacent spacer region. Sequencing of this region has been done for the AGY phytoplasma to determine the level of homology with other key phytoplasmas. In addition, genomic primers have been used to confirm these results; non-ribosomal stolbur-specific primers were used to detect phytoplasmas in yellowing grapevines and the *tuf* gene, which codes for the elongation factor Tu required for protein translation, was targeted for PCR/RFLP analysis to differentiate AGY from the European phytoplasmas. Finally, studies have shown that a phytoplasma recently found associated with dieback in papaya (PPDB) is related to the stolbur group of phytoplasmas (GIBB *et al.* 1996). In this paper, AGY and PPDB are compared by RFLP analysis of the *tuf* gene PCR product.

Materials and methods

Sources of DNA from infected grapevines: *Vitis vinifera* cv. Riesling and Chardonnay showing typical AGY symptoms were collected from vineyards near Loxton (South Australia) and Mildura (Victoria). Asymptomatic vines from Riesling were also collected as healthy controls. DNA was extracted from these tissues using either of two methods (AHRENS and SEEMÜLLER 1992; DAIRE *et al.* 1992). Except for Fig. 2 where AGY isolates were compared, AGY refers to a phytoplasma from a Riesling vine collected near Loxton, South Australia. This isolate was used for sequencing.

Purified DNA from other diseased vines was provided by the following researchers: grapevine yellows in Chardonnay vines from Emilia-Romagna (central Italy) (A. BERTACCINI, Università degli Studi, Bologna, Italy); Vergilbungskrankheit from Riesling vines (M. MAIXNER, BBA, Bernkastel-Kues, Germany); grapevine yellows phytoplasmas in Chardonnay from France, northern Italy (Udine region), Israel and Spain (X. DAIRE, INRA, Dijon, France).

Sources of DNA from other plants: Flavescence dorée (FD) maintained in broad bean (*Vicia faba*) from southern France was obtained from E. BOUDON-PADIEU and X. DAIRE (INRA, Dijon, France) and the Green Valley strain of the western X-disease (GVX) maintained in periwinkle (*Catharanthus roseus*) from the USA was obtained from E. SEEMÜLLER (BBA, Dossenheim, Germany) as a representative of the western-X phytoplasma group. The phytoplasma associated with SPLL in sweet potato was also used as a reference phytoplasma for initial comparative studies and this was collected from Darwin, Northern Territory. Papaya (*Carica papaya*) leaf and petiole samples from plants with dieback symptoms were collected from Queensland and the DNA from both sweet potato and papaya was extracted by the method of AHRENS and SEEMÜLLER (1992).

Amplification of DNA: After DNA extraction, the quality of the DNA was determined by electrophoresis on an agarose gel. 1 µl of undiluted DNA (ca. 50-100 ng) was used in a 50 µl PCR reaction containing 0.1 mM each dNTP, 0.4 µM each primer, 0.2 U thermostable DNA polymerase (Advanced Biotechnologies Ltd, Surrey, U.K.) and buffer supplied with the enzyme. Thermocycling conditions included a hot start of 92 °C for 1 min followed by 40 cycles of 92 °C for 45 s, 55 °C for 30 s and 72 °C for 1 min. PCR products were analysed by electrophoresis of a 3-5 µl aliquot on a 1 % agarose gel containing 0.5 µg/ml ethidium bromide and viewed on a UV transilluminator. Primers used for amplification of the 16S rRNA gene and spacer region were P1 (DENG and HIRUKI 1991) and P7 (SCHNEIDER *et al.* 1995) which gave a 1800 bp product. The primer pair STOL11f1/r2 (provided by X. DAIRE and E. BOUDON-PADIEU) was used for specific detection of stolbur phytoplasmas giving a 900 bp fragment. The primer pair fMLO1 and rMLO1 was used to amplify a region of the *tuf* gene (SCHNEIDER and SEEMÜLLER 1996) to give a 1000 bp product. Using these *tuf* primers, only phytoplasmas in the aster yellows strain cluster are amplified (B. SCHNEIDER, personal communication).

RFLP analysis: A total reaction volume of 10 µl containing 3-6 µl of PCR product was digested with a restriction endonuclease according to the manufacturers instructions. The enzymes used were *Alu* I, *Mse* I, and *Sau* 3AI (New England Biolabs, Beverly, MA, USA). Digestions were allowed to proceed overnight at 37 °C and products were analysed by either agarose electrophoresis using 3 % Ultra High Res Agarose (Progen Industries Ltd, Darra, QLD, Australia) in 0.5 X TBE or 5 % polyacrylamide gel electrophoresis in 1 X TBE.

Sequencing: The sequence of the 16S rRNA gene and adjacent spacer region of AGY was determined directly from spin column-purified PCR products (Wizard PCR Preps, Promega Corp., Madison, WI, USA) using the Amplicycle Sequencing Kit (Perkin-Elmer Corp., Norwalk, CT, USA). The DNA in the sequencing reactions was labelled with α-^{33}P-dATP. Primers used for the sequencing reactions were the same as those used in the PCR for amplifying the 16S rRNA gene. Additional internal primers were used: fU5 (AHRENS *et al.* 1994); 16R723f (PADOVAN *et al.* 1995); P3 (SCHNEIDER *et al.* 1995); rP1 (WEISBURG *et al.* 1991) and R16F2 (LEE *et al.* 1993). Thermocycling conditions were 30 cycles of 95 °C for 1 min, 45 °C for 1 min and 72 °C for 1 min, with a hot start of 95 °C for 1 min. The sequence was compared to other phytoplasma 16S rRNA sequences available in the databank (EMBL and Genebank) or kindly supplied by C. SMART (for spacer region sequences) using Gap for pairwise comparisons or PileUp for multiple sequence alignments (GCG University of Wisconsin via the Australian National Genomic Information Service, University of Sydney). Accession numbers for sequences used are X76433 (SUNHP), X90591

(SPLL), X76427 (STOL), X95706 (AGY), X68373 (AAY), X76425 (PD), X68375 (AT), X76431 (BAWB), X68376 (ULW), X76560 (FD), X68339 (ASHY), X76429 (BVK), X76432 (SCWL), X76430 (VAC), X76428 (VK), D12569 (MLO-I), M30970 (OAY), M86340 (SAY), L33767 (AY1) and L33762 (CPh). The 16S rRNA and spacer region sequence of Phormium yellow leaf (PYL) was kindly supplied by L. LIEFTING, University of Auckland, N.Z. (accession number U43571).

Results

16S rDNA amplification: The phytoplasma 16S rRNA gene and spacer region were amplified from grapevine using the primer pair P1/P7. Fig. 1 shows PCR products from a reference phytoplasma sweet potato little leaf in sweet potato (SPLL), and grapevine with yellows (AGY). Controls of asymptomatic sweet potato (HSP) and grapevine (HG) gave no PCR products. The extraction methods from both DAIRE *et al.* (1992) and AHRENS and SEEMÜLLER (1992) gave DNA suitable for PCR analysis (results not shown).

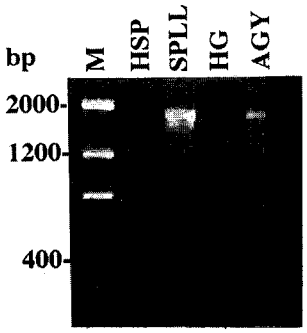

Fig. 1: PCR amplification using primer pair P1/P7 to give a 1800 bp product. M=DNA size markers (DNA mass ladder, Life Technologies); HSP=healthy sweet potato; SPLL=sweet potato little leaf; HG=healthy grapevine; AGY=Australian grapevine yellows.

16S rDNA RFLP analysis: Four isolates collected from Riesling vines in Mildura, Victoria (AGY 1-4), 4 isolates from Loxton in South Australia (2 from Riesling vines (AGY 5-6) and 2 from Chardonnay vines (AGY 7-8)) were amplified using the primers P1/P7 and digested with *Alu* I for RFLP analysis (Fig. 2). The RFLP profile generated showed no polymorphisms among the AGY samples. This profile is different from that obtained with the reference phytoplasma SPLL. The same results were found when *Mse* I was used (results not shown).

The restriction profiles generated for AGY and the 3 representative groups of grapevine phytoplasmas using *Mse* I is shown in Fig. 3. The profile obtained with AGY showed more restricted bands in common with the phytoplasma from Emilia-Romagna in Italy (GY-It) and Germany (VK). These are different from the profiles obtained with FD and GVX. Similarly, when the PCR product was digested with *Alu* I, AGY was most similar to both GY-It and VK (which still have the same pattern as each other) and is clearly different from FD and GVX (results not shown).

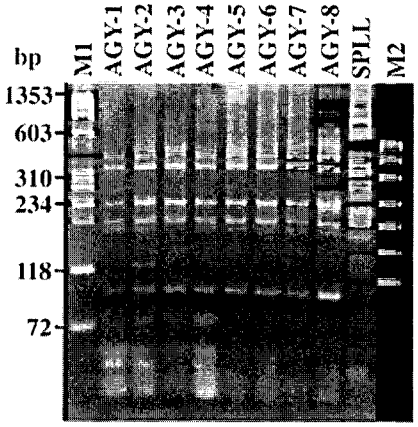

Fig. 2: RFLP analysis of P1/P7 PCR products of 8 AGY isolates (AGY 1-8) compared to SPLL using *Alu* I. M1=øX174/*Hae* III; M2=pUC19/*Hpa* II. AGY 1-4 from Riesling vines in Mildura (Victoria), AGY 5-8 from vines in Loxton (South Australia), AGY 5-6 Riesling and AGY 7-8 Chardonnay.

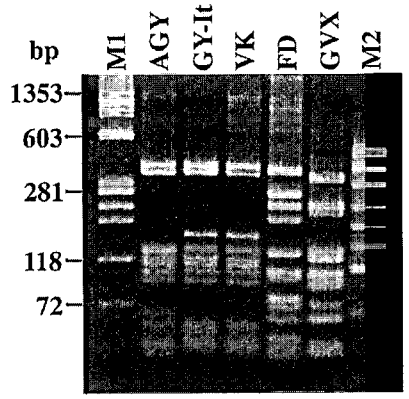

Fig. 3: RFLP analysis of P1/P7 PCR products of grapevine phytoplasmas using *Mse* I. M1=øX174/*Hae* III; M2=pUC19/*Hpa*II; AGY=Australian grapevine yellows, GY-It=grapevine yellows from Italy (Emilia-Romagna), VK=Vergilbungskrankheit from Germany, FD=flavescence dorée from France, and GVX=green valley X strain in periwinkle from the USA.

16S rRNA sequence analysis: The AGY 16S rRNA gene and adjacent spacer region sequences (accession number X95706) were compared to published sequences of key phytoplasmas representing the major phylogenetic groups using PileUp. The resulting tree guide for the 16S rRNA sequences (Fig. 4) shows that AGY is most similar to sequences in the aster yellows strain cluster, and is most closely related to the stolbur group of phytoplasmas. Identical results were obtained when spacer region sequences were used in the PileUp analysis (results not shown). The percent similarity between the 16S rRNA gene sequence of AGY and other aster yellows type phytoplasmas was determined using Gap. The AGY 16S rRNA sequence showed most similarity to Phormium yellow leaf (99.5 %) from New Zealand followed by a stolbur phytoplasma (97.6 %) from France and Vergilbungskrankheit (97.5 %) from Germany (Table).

Stolbur-specific DNA amplification: Non-ribosomal primers (STOL11f1/r2) specific for the stolbur group of phytoplasmas were used to amplify DNA from grapevine with yellows from southern France

(GF1 and GF2), northern Italy (Udine) (G-It), Spain (GS), Israel (GIs) as well as AGY. Fig. 5 shows that GF2, GS and GIs were positive indicating a stolbur phytoplasma presence in these samples. This primer pair did not amplify GF1, G-It and the AGY phytoplasma. Healthy grapevine (HG) was not amplified.

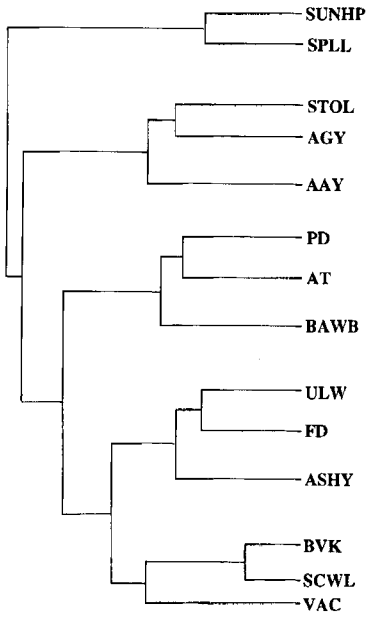

Fig. 4: Guide tree diagram representing a PileUp multiple sequence alignment of 16S rRNA gene sequences. Key phytoplasmas included were sunn-hemp witches' broom (SUNHP), sweet potato little leaf (SPLL), stolbur (STOL), Australian grapevine yellows (AGY), American aster yellows (AAY), pear decline (PD), apple proliferation (AT), black alder witches' broom (BAWB), elm yellows (ULW), flavescence dorée (FD), ash yellows (ASHY), phytoplasma from a leafhopper (BVK), sugarcane white leaf (SCWL), and vaccinium witches' broom (VAC). GapWeight 5.0, GapLengthWeight 0.5.

Table

Percent similarity, in descending order, between AGY and other phytoplasmas in the aster yellows cluster, as determined by Gap analysis of the 16S rRNA gene sequence

	PYL	STOL	VK	MLO-I	OAY	SAY	AY1	CPh
AGY	99.5	97.6	97.5	96.7	96.7	96.4	94.7	94.4

PYL = Phormium yellow leaf; STOL = stolbur; VK = Vergilbungskrankheit; MLO-I = onion yellows; OAY = oenothera aster yellows; SAY = severe aster yellows; AY1 = Maryland aster yellows; CPh = clover phyllody.

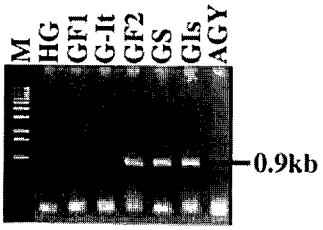

Fig. 5: PCR amplification of grapevines showing yellows symptoms using non-ribosomal stolbur-specific primers. M=DNA marker (1 kb ladder, BRL); HG=healthy grapevine; GF1=grapevine with yellows from France infected with the flavescence dorée phytoplasma, G-It=Chardonnay with yellows from northern Italy (Udine), GF2=grapevine with yellows from France infected with a stolbur phytoplasma, GS=Grenache with yellows from Spain, GIs=Chardonnay with yellows from Israel, and AGY=Australian grapevine yellows (Riesling).

tuf DNA amplification: Using the primers fMLO1 and rMLO1, only those phytoplasmas belonging to the aster yellows strain cluster (AGY, GY-It (Emilia-Romagna), VK and bois noir (BN)), were amplified to give the expected 1000 bp product (Fig. 6). The phytoplasmas representing the elm yellows group (FD) and the western-X group (GVX) were not amplified. There were no amplification products in the negative controls (results not shown).

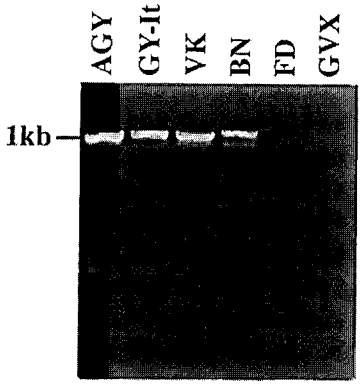

Fig. 6: PCR amplification of the *tuf* gene from selected grapevine phytoplasmas. The PCR product was approximately 1000 bp. AGY=Australian grapevine yellows, GY-It=grapevine yellows from Italy (Emilia-Romagna), VK=Vergilbungskrankheit from Germany, BN=bois noir, FD=flavescence dorée from France, GVX=green valley X strain in periwinkle from the USA.

tuf RFLP analysis: Digestion of the *tuf* PCR products with *Sau* 3AI (Fig. 7) showed that AGY was different from the grapevine phytoplasmas in the stolbur group. Another Australian phytoplasma which has recently been found to be associated with dieback in papaya (PPDB) (GIBB *et al.* 1996) was also compared by *Sau* 3AI digestion of the *tuf* PCR product. PPDB gave the same profile as AGY suggesting that the two phytoplasmas are genetically related. Similarly, when *Hind* III was used as the restriction enzyme, AGY and PPDB gave the same profile, which was different to that for the European grapevine phytoplasmas (results not shown).

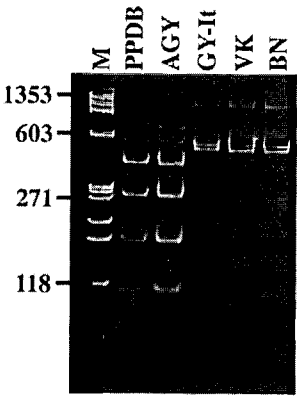

Fig. 7: RFLP analysis of the *tuf* PCR products using *Sau* 3AI. M=DNA marker (øX174/*Hae* III). PPDB=papaya dieback; AGY=Australian grapevine yellows, GY-It=grapevine yellows from Italy (Emilia-Romagna), VK=Vergilbungskrankheit from Germany, and BN=bois noir.

Discussion

When AGY was first reported in Australia, the similarity of symptoms between AGY and flavescence dorée (FD) in France led to the suggestion that the same organism was involved with both diseases. It was known that FD was associated with a phytoplasma (CAUDWELL 1983) and it was therefore thought that the same phytoplasma was also associated with AGY. It has since been shown that a phytoplasma is involved with AGY, but serological studies (KUSZALA *et al.* 1993) and more recent molecular studies (PADOVAN *et al.* 1995) have revealed that these phytoplasmas are different even if they are associated with similar symptoms.

Grapes are grown extensively in the southern regions of Australia, and while the disease has been reported in most of these regions, only a few samples have been used in differentiation studies. The limited survey of 2 regions in southern Australia described here suggests that only one type of phytoplasma is associated with AGY, however, different symptom expressions indicate the possibility of other phytoplasmas being involved with grapevine diseases in Australia. The symptoms associated with AGY which are first seen in spring from flowering onwards, have been well documented (MAGAREY and WACHTEL 1986 a and b). These reports have also described another symptom type in grapevine which occurs in the cooler regions of southern Australia and which appears to be less severe and affects fewer vines. These symptoms appear towards the end of the growing season, affecting the whole vine rather than individual spurs and the condition has been called late season AGY to distinguish it from the typical early season AGY symptoms (BONFIGLIOLI *et al.* 1996). In the study described here, only vines exhibiting the early season symptoms were used, and it is not yet known whether the same phytoplasma is involved with the late season symptoms. A more comprehensive study is being undertaken to determine whether other phytoplasmas are associated with grapevine diseases in Australia (GIBB, unpublished).

The RFLP profiles of AGY 16S rDNA and the representative grapevine yellows phytoplasmas show that the AGY phytoplasma is most similar to the Italian grapevine yellows (aster yellows group I-G) and the German Vergilbungskrankheit (stolbur group). In fact, the latter two could not be differentiated in this study and were found to be identical by RFLP analysis of the small ribosomal RNA gene and the *tuf* gene. Recent phylogenetic analyses using 16S rRNA sequences have shown that the stolbur phytoplasmas form a subgroup within the aster yellows cluster (SEEMÜLLER *et al.* 1994). When the 16S rRNA sequence of AGY was compared to key phytoplasma sequences representing the different phylogenetic groupings, AGY was most similar to the stolbur phytoplasmas. This was also the case when the spacer region sequence was analysed. When all available 16S rRNA sequences within the aster yellows cluster were compared to each other in more detail using pairwise comparisons, AGY was found to be most similar to Phormium yellow leaf (PYL) showing 99.5 % homology. PYL is a phytoplasma associated with yellowing of native flax plants in New Zealand. The next closest relatives to AGY were stolbur from France and Vergilbungskrankheit from Germany.

The 16S ribosomal gene has been used extensively for the classification of phytoplasmas, however, because this gene is highly conserved, closely related strains can be difficult to differentiate. More variable non-ribosomal DNA can therefore be very useful in distinguishing closely related strains, either as primers in specific PCR reactions or as probes in dot blots and Southern hybridisations. In this study, in addition to analysis of the ribosomal gene, non-ribosomal primers were used to further understand the genetic relatedness between AGY and phytoplasmas in the aster yellows strain cluster. Using non-ribosomal stolbur-specific primers in a PCR reaction, no amplification of AGY occurred suggesting that even though AGY is closely related to the stolburs, sufficient differences exist at the genome level to prevent the oligonucleotides from priming the reaction. Furthermore, DNA amplification using *tuf* gene primers that are specific for phytoplasmas in the aster yellows cluster followed by RFLP analysis of PCR products showed that AGY was not identical with any phytoplasma within this cluster. While *tuf* gene analysis is not intended to replace the current ribosomal-based classification system for phytoplasmas, it provides a useful tool for differentiating aster yellows phytoplasmas from other phytoplasmas in a single PCR reaction, and for further differentiation within the aster yellows group by RFLP analysis (SCHNEIDER and SEEMÜLLER 1996).

A survey of phytoplasmas in Australia has shown that the TBB and SPLL phytoplasmas occur in a wide range of different plant species distributed over the continent and that they are most closely related to phytoplasmas from southeast Asia belonging to the sunn-hemp and peanut witches'-broom groups (GIBB *et al.* unpublished). This was not unexpected due to the proximity of the two continents. More recently, witches'-broom disease of lime (WBDL) from the Sultanate of Oman and the United Arab Emirates has also been placed in this group (ZREIK *et al.* 1995). It was therefore a surprise when the AGY phytoplasma was shown to be quite distinct from the widespread TBB and SPLL phytoplasmas. It is not known whether the phytoplasma associated with AGY was originally introduced from infected European vine stocks, or whether it arose as a separate phytoplasma within Australia. It is interesting that the closest relative to AGY outside Australia is a phytoplasma from New Zealand, PYL. This suggests that there may be an "indigenous" phytoplasma occurring in the southern Pacific region which has remained localised, although a more extensive survey of phytoplasmas in this region, in both cultivated and native species, needs to be done to support the idea that AGY arose within Australia rather than being a recent introduction.

Within Australia, RFLP analysis of both the 16S rRNA gene (GIBB *et al.* 1996) and *tuf* gene PCR products has shown that AGY is closely related to the phytoplasma associated with dieback in papaya (PPDB). It is interesting to note that papaya and grapevines are generally grown in different regions, the former growing in the northern tropical and subtropical regions of Australia, while the latter grows in the more southern regions with a temperate cli-

mate. PPDB was first reported in 1922 (GLENNIE and CHAPMAN 1976) over 50 years before AGY was recorded. Neither papaya nor grapevines are native to Australia and no alternate hosts have yet been identified.

While this study aimed to characterise the AGY phytoplasma at the molecular level, it is evident that epidemiological studies are required to understand the role of AGY in Australian vines. It is known that the leafhopper *Orosius argentatus*, which occurs throughout Australia is able to transmit the SPLL phytoplasma (GIBB *et al.* 1995) but it is not known whether it also transmits AGY, or whether another vector is responsible. In Germany, the vector of Vergilbungskrankheit has been identified as *Hyalesthes obsoletus* (MAIXNER 1994) and in New Zealand, the vector of PYL is *Oliarus atkinsoni* (CUMBER 1952), both planthoppers from the family Cixiidae. It is possible that a planthopper may also be involved in the transmission of AGY within Australia. Potential reservoirs also need to be identified as these have an important role to play in maintaining leafhopper populations and providing a pathogen reservoir during winter.

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