Phytoplasmas Associated with Papaya Diseases in Australia

K. S. Gibb, Faculty of Science, Northern Territory University, Darwin, Northern Territory, 0909, Australia, **D. M. Persley,** Plant Protection Unit, Queensland Department of Primary Industries, Meiers Road, Indooroopilly, Queensland, 4068, Australia, **B. Schneider,** Faculty of Science, Northern Territory University, Darwin, Northern Territory, 0909, Australia, and **J. E. Thomas,** Plant Protection Unit, Queensland Department of Primary Industries, Meiers Road, Indooroopilly, Queensland, 4068, Australia

ABSTRACT

Gibb, K. S., Persley, D. M., Schneider, B., and Thomas, J. E. 1996. Phytoplasmas associated with papaya diseases in Australia. Plant Dis. 80:174-178.

Leaf, petiole, and crown samples were taken from papaya with dieback or mosaic diseases, which have an unknown etiology, and from yellow crinkle disease, which has been associated with a phytoplasma. Using the polymerase chain reaction (PCR) and phytoplasma-specific primers, an amplification product of the expected size (1,076 bp) was observed in samples from papaya with yellow crinkle, mosaic, and dieback, and in none of the samples from asymptomatic plants. A second set of phytoplasma-specific primers was used with samples from each of the three diseases to produce a PCR product of 1,800 bp for restriction fragment length polymorphism (RFLP) analysis of phytoplasma relatedness. The phytoplasmas associated with yellow crinkle and mosaic were identical by RFLP analysis using three restriction enzymes, but the phytoplasma from dieback disease differed from the other two. The phytoplasma associated with dieback is related to the Stolbur phytoplasmas that form part of the aster yellows phytoplasma strain cluster. The phytoplasma associated with yellow crinkle and mosaic, along with sweet potato little leaf, appears to be related to a phytoplasma, crotalaria witches'-broom from Thailand, which in turn is related to the western X-phytoplasma.

Papaya (*Carica papaya* L.) is an important perennial fruit crop throughout tropical and subtropical regions. In Queensland, Australia, papaya is grown in coastal regions, with major centers of production located in the subtropical southeast and central regions and in tropical northern Queensland. The papaya industry in Queensland is affected by three diseases known locally as dieback, yellow crinkle, and mosaic (28).

Dieback, the most serious of these, has been known since 1922 and frequently causes losses from 10 to 100% of trees in plantations in southern and central Queensland (9). This disease is the most serious limiting factor of the Australian papaya industry (9), and the etiology of the disease remains unresolved despite extensive research. The disease has not been consistently associated with nutritional or environmental factors, nor have fungal or bacterial pathogens been isolated from affected plants (9). Although a viral etiology has been postulated, detailed work has failed to detect viral infection, and five plant viruses common in Queensland failed to induce dieback symptoms when inoculated to papaya seedlings (15–17).

Corresponding author: K. Gibb
E-mail: gibbk@darwin.ntu.edu.au

Accepted for publication 14 October 1995.

Publication no. D-1995-1206-04R
© 1996 The American Phytopathological Society

A typical symptom of dieback is a bunched appearance of the inner crown leaves, with one or more of these leaves shriveling and dying. The larger crown leaves rapidly develop chlorosis, then necrosis. The entire crown dies within 1 to 4 weeks, and the stem gradually dies back from the top. Affected plants also develop necrosis of the phloem and laticifers and a marked reduction in latex flow (9,15,28). While young plants invariably die from dieback, older plants may have a recovery phase if the affected stem is cut, allowing the development of apparently healthy side shoots from lateral buds (28). This apparent "recovery" raises a number of interesting questions about the plant-disease association. If the causal agent is a microorganism, this recovery may be a reflection of an uneven distribution of the agent in the host plant.

Worldwide, a number of decline or dieback diseases have been reported from papaya. A dieback disease in Israel (7) has a suspected mollicute etiology, although viruses or phytoplasmas have not been detected by electron microscopy and the causal agent remains unknown. Other diseases in papaya with a suspected phytoplasma etiology are bunchy top disease in the Caribbean region (31) and, more recently, papaya nonripening disease in Hawaii (3). The etiology of a dieback disease of papaya in South Africa is unknown (2), but four dieback-like diseases are caused by viruses. In Hawaii, tomato spotted wilt virus has been isolated from papaya (10), and rhabdoviruses are associated with papaya apical necrosis and papaya droopy necrosis in Venezuela and Florida, respectively (32). Papaya decline caused by tobacco ringspot virus occurs in Texas (18) and Nigeria (19). None of the viruses have been detected in papaya plants with dieback disease in Australia (14), and young papaya plants failed to develop typical dieback symptoms following sap inoculation with tobacco ringspot, tomato spotted wilt, tobacco streak, lettuce necrotic yellows, and *Solanum nodiflorium* mottle viruses (16).

Mycoplasmalike organisms (MLOs), now called phytoplasmas, have been found in phloem cells of plants with yellow crinkle by electron microscopy of ultrathin sections (11). The causal agent of yellow crinkle was transmitted by a dodder (*Cuscuta* sp.) from symptomatic papaya to tomato (*Lycopersicon esculentum*), white clover (*Trifolium repens*), and jimson weed (*Datura stramonium*), in which it caused symptoms typical of phytoplasma diseases (12). Periodic epidemics occur, usually after hot, dry weather, which favors the breeding and movement of the leafhopper *Orosius argentatus* (23). The initial symptoms of yellow crinkle disease are a pronounced yellowing of leaves about halfway up the canopy, accompanied by bending of the petioles. The crown leaves develop thin, translucent areas along the margins and between the main veins. These areas become necrotic and tattered as growth proceeds. The crown leaves eventually become clawlike in appearance, and the older leaves dry and fall, leaving the tree a bare pole with a few stunted leaves at the top. Floral parts may show phyllody and proliferation of axillary leaves, which are straplike and thickened.

Mosaic is sporadic in occurrence and is generally the least important of the three diseases, and its etiology is unknown (23). Virus particles have not been detected by electron microscopy, and virus-specific bands were not detected following dsRNA extraction of tissue from diseased and healthy papaya plants (A. W. Geering and D. M. Persley, *unpublished data*). Plants affected by mosaic disease have stunted, yellow young leaves with translucent areas around the margins. Affected plants are often stunted, producing multiple side shoots. In field-grown papaya, it is often difficult to differentiate yellow crinkle from mosaic disease, but the water-soaked

streaks on the petioles and upper portion of the stem and reduced or absent latex flow (28) are characteristic of mosaic disease. A distinguishing feature of yellow crinkle is phyllody (23,28), which is not observed in papaya with mosaic disease.

The recent introduction of polymerase chain reaction (PCR) assays has greatly enhanced the capacity to detect and identify phytoplasmas, and it seemed appropriate to reinvestigate the three diseases in papaya using this molecular tool. Using primers based on sequences of cloned phytoplasma DNA fragments, a number of groups have used PCR to amplify phytoplasma-specific DNA from infected plant tissue (4,6,24). The 16S rRNA region from a phytoplasma has been sequenced (21), and PCR assays using primers based on the 16S rRNA region to amplify phytoplasma specific DNA fragments allow detection of a wide range of phytoplasmas (20). Restriction fragment length polymorphism (RFLP) analysis of the amplified PCR product is being used to study genetic relatedness and is useful, in combination with biological information, for differentiating phytoplasmas (1).

As part of a current program to detect and characterize phytoplasma diseases in Australia (8), samples of dieback, mosaic, and yellow crinkle from papaya in geographically separate areas of Queensland were examined using molecular techniques to determine their etiology.

MATERIALS AND METHODS

Source of phytoplasmas. Leaves, petioles, and crowns from eight papaya with papaya yellow crinkle disease, four with papaya mosaic disease, and four asymptomatic papaya were collected near Caboolture, Queensland, in February 1994. Samples from four papaya with yellow crinkle, four with mosaic, and one asymptomatic papaya were collected near Caboolture in February 1995. Samples from four papaya with papaya dieback disease and one asymptomatic papaya were collected at Redlands Research Station, Cleveland, Queensland (64 km from Caboolture), in February 1995. One sample each of papaya with yellow crinkle and dieback from Rockhampton, Queensland (670 km from Caboolture) was provided by R. Elder, Queensland Department of Primary Industries, Rockhampton. Sweet potato with sweet potato little leaf (SPLL) disease was collected near Darwin, Northern Territory, in 1991.

Additional phytoplasmas, which have been grouped on the basis of their 16S ribosomal DNA restriction patterns and nucleotide sequence (25,27), were included in this study to help group the unknown phytoplasmas from Australia. These phytoplasmas are referred to as the reference phytoplasmas. All phytoplasmas were transmitted to periwinkle. The sources of Stolbur of *Lycopersicon esculentum* (STOLF) from France, American aster yellows (AAY) from Florida, sunn hemp or crotalaria witches'-broom (SUNHP) from Thailand, witches'-broom of *Vaccinium myrtillus* (VAC) from Germany, Blütenverkleinerung (BVK) from Germany, and ash yellows (ASHY) from New York are as described previously (25). Sesame phyllody (SEPT), phyllody of *Cleome viscosa* (CLP), and Crotalaria phyllody (CROP) originated from Thailand.

Extraction of DNA and PCR. DNA was isolated from field-collected plants using a phytoplasma enrichment procedure (1). The nucleic acid pellet was resuspended in 50 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), and 5 µl of the sample was subjected to gel electrophoresis in a 1% agarose gel in 0.5× TBE buffer followed by staining with ethidium bromide and photographing on a UV transilluminator. The quality and quantity of the DNA was estimated from the gel, and the nucleic acid was either used without further dilution or diluted 1:10 before being used in the PCR.

The primer pair used to screen all papaya and sweet potato samples was 16R723f and m23SR, which amplified approximately 1,076 bp of the 16S rRNA gene through the 16S/23S spacer region and into the start of the 23S rRNA gene (22). A second set of primers, P1 (5) and P7 (sequence provided by C. Smart and B. Kirkpatrick, University of California, Davis), amplified a larger region of approximately 1,800 bp comprising the 16S rRNA gene, the spacer region, and the beginning of the 23S rRNA gene. These primers were used in RFLP analysis.

For the PCR, each reaction contained 0.2 mM each dNTP, 0.4 µM each primer, 1× DNA polymerase buffer supplied with the enzyme, 0.2 U thermostable DNA polymerase (Advanced Biotechnologies Ltd., Surrey, U.K.), and 1 µl of undiluted or 1:10 diluted nucleic acid sample. The total reaction volume was 50 µl in a Corbett FTS-320 thermocycler (Corbett Research, Mortlake, NSW, Australia). A manual hot start PCR at 92°C for 1 min was followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 30 s, and extension at 72°C for 30 s except for the final cycle, when extension was for 90 s. After amplification, 5 µl from each sample was subjected to electrophoresis in a 1.0% agarose gel and visualized by staining with ethidium bromide and UV illumination. Total nucleic acid extracted

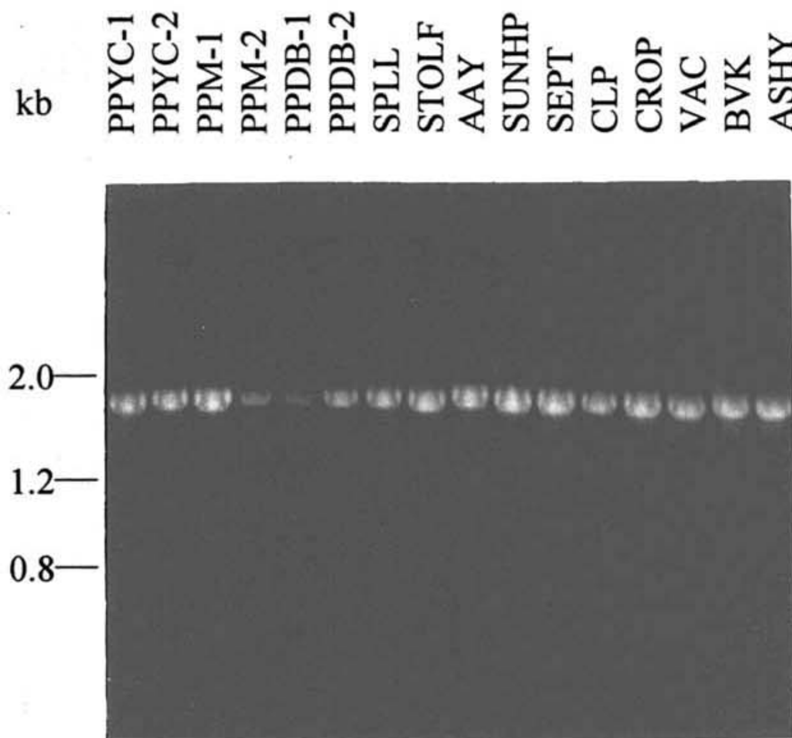

Fig. 1. Polymerase chain reaction (PCR) amplification of phytoplasmas from papaya and from sweet potato using the primer pair P1/P7, which amplifies the entire 16S rRNA gene and the spacer region between the 16S rRNA and 23S rRNA genes. DNA markers are not shown but are indicated by the sizes given in kilobase pairs. PPYC-1 = papaya yellow crinkle from Caboolture; PPYC-2 = papaya yellow crinkle from Rockhampton; PPM-1 = papaya mosaic from Caboolture; PPM-2 = papaya mosaic from Caboolture; PPDB-1 = papaya dieback from Redlands Research Station, Cleveland; PPDB-2 = papaya dieback from Rockhampton; SPLL = sweet potato with sweet potato little leaf; STOLF = Stolbur of *Lycopersicon esculentum* from France; AAY = American aster yellows from Florida; SUNHP = sunn hemp or crotalaria witches'-broom from Thailand; SEPT = sesame phyllody from Thailand; CLP = phyllody of *Cleome viscosa* from Thailand; CROP = Crotalaria phyllody from Thailand; VAC = witches'-broom of *Vaccinium myrtillus* from Germany; BVK = Blütenverkleinerung from Germany; and ASHY = ash yellows from New York.

from asymptomatic plants was subjected to the PCR as a negative control, and in some experiments water controls were included, in which no plant nucleic acid was added to the PCR reaction mix.

RFLP analysis. Five μl of the PCR products amplified using the P1/P7 primers were digested separately using three restriction enzymes, *Alu* I, *Rsa* I, and *Mse*I, in buffers supplied by the manufacturer (New England Biolabs, Beverly, MA). Digestions were incubated overnight at 37°C, and the fragments were separated by electrophoresis in a 3% UltraHigh Resolution Agarose gel (Progen Industries, Darra, Queensland, Australia) in 0.5× TBE buffer and visualized by staining with ethidium bromide and photographing on a UV transilluminator.

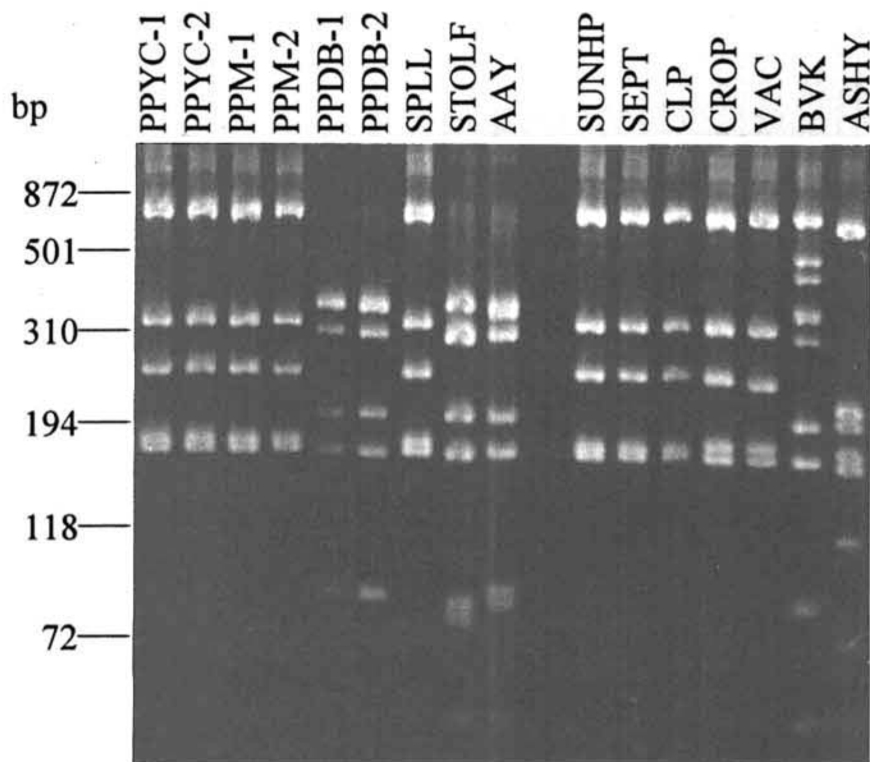

Fig. 2. Restriction digests of the polymerase chain reaction (PCR) products described in Figure 1. The restriction enzyme was *Alu* I. DNA markers are not shown but are indicated by the sizes given in base pairs. Abbreviations are the same as for Figure 1.

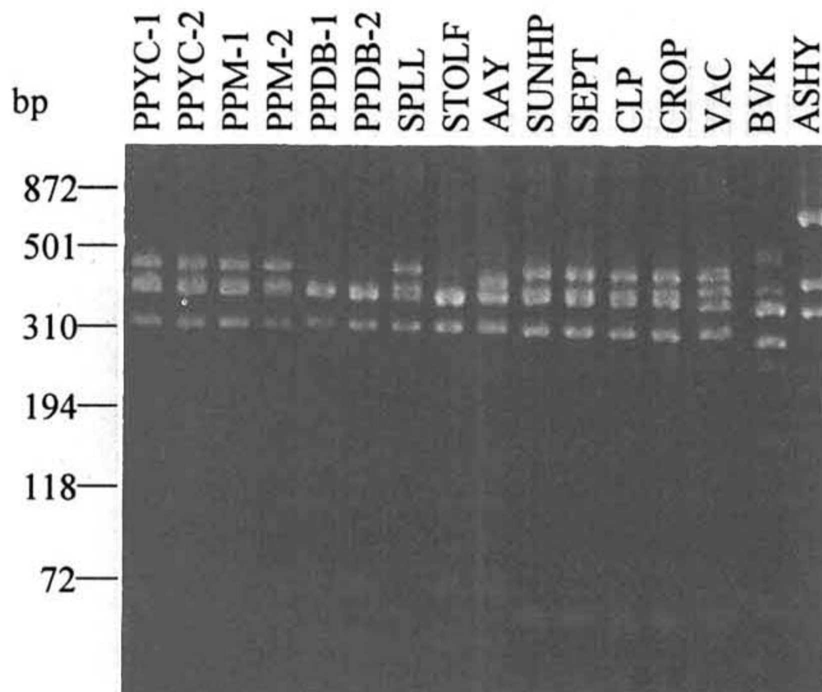

Fig. 3. Restriction digests of the polymerase chain reaction (PCR) products described in Figure 1. The restriction enzyme was *Rsa* I. DNA markers are not shown but are indicated by the sizes given in base pairs. Abbreviations are the same as for Figure 1.

RESULTS

PCR detection of phytoplasma DNA in papaya. Initial screening of samples from papaya with dieback, yellow crinkle, and mosaic with the primer pair 16R723f and m23SR gave the expected PCR product of 1,076 bp. All diseased samples consistently gave a PCR product, with the exception of one dieback sample from Cleveland, Queensland, which gave no positive signal. All diseased samples were also subjected to the PCR using the primer pair P1/P7, and the expected 1,800-bp PCR product was obtained for each sample. Figure 1 shows the 1,800-bp PCR product for two representative papaya samples from each disease (dieback, yellow crinkle, and mosaic), SPLL, and each of the reference phytoplasmas. On no occasion were PCR products observed with the water controls or with asymptomatic plants.

RFLP analysis of the 1,800-bp PCR products. Following digestion of the 1,800-bp PCR product with the restriction enzymes *Alu* I, *Rsa* I, and *Mse* I, the yellow crinkle, mosaic, and SPLL samples all gave the same restriction pattern. RFLP profiles for only two representative papaya samples from each disease are shown (Figs. 2–4), but all diseased samples were in fact subjected to RFLP analysis. For each restriction enzyme, the pattern for yellow crinkle, mosaic, and SPLL was identical to SUNHP, SEPT, and CLP, all from Thailand (Figs. 2–4). Following digestion with *Alu* I or *Rsa* I, these phytoplasmas were identical to CROP from Thailand and VAC from Germany, (Figs. 2 and 3). CROP and VAC were, however, differentiated from each other and from SUNHP, SEPT, and CLP by digestion with *Mse* I (Fig. 4).

For each restriction enzyme, the two representative dieback samples gave the same restriction pattern, but this pattern was different from yellow crinkle and mosaic (Figs. 2–4). Following digestion with *Alu* I, the pattern for dieback was similar but not identical to STOLF and AAY (Fig. 2). Following digestion with *Rsa* I, the pattern for dieback was the same as that for STOLF but different from that for AAY (Fig. 3); and following digestion with *Mse* I, dieback was more similar to STOLF than it was to AAY, but as with *Alu* I, it was not identical to either (Fig. 4).

DISCUSSION

Papaya with yellow crinkle, mosaic, and dieback diseases collected from widely

separated geographic areas in Queensland consistently gave a PCR product of the expected size using primers specific for phytoplasma DNA. No PCR products were observed with asymptomatic papaya. This result confirms the reported association between yellow crinkle and a phytoplasma, but this is the first time that mosaic and dieback diseases have been associated with phytoplasmas. This result with dieback is especially significant because it is such an important disease for the Australian industry and much research effort has been devoted to it. Harding (14) suggested a viral or phytoplasmal etiology of the disease following specific autofluorescence in phloem cells and laticifers of affected plants. Electron microscopy of ultrathin sections of various plant organs failed to detect virus particles or phytoplasmas (14; D. H. Gowanlock and R. S. Greber, *personal communication*). The failure to detect phytoplasmas may have been because the assay was not sufficiently sensitive, especially if the phytoplasmas were at very low levels and unevenly distributed throughout the plant. Alternatively, this failure may have been due to the often rapid lethal decline of plants following the onset of symptoms preventing the extensive reproduction of phytoplasmas in affected cells. Application of tetracycline antibiotics to diseased plants in the field failed to cause remission of dieback symptoms, with treated plants declining at the same rate as untreated plants (14). Although these data do not support a phytoplasmal etiology for dieback, an alternative explanation is that the antibiotic treatments were ineffective due to the rapid death of affected plants. With lethal yellowing disease of palm, which is associated with a phytoplasma, it is known that antibiotic treatment of affected plants is seldom effective if applied after 25% of the canopy has yellowed (29).

One could argue that the phytoplasmas associated with mosaic and dieback diseases may be the yellow crinkle phytoplasma in combination with other disease agents, which results in a different disease phenotype. To study this further, it was important to characterize the phytoplasmas from each disease by RFLP analysis. Results showed that the phytoplasma(s) associated with yellow crinkle and mosaic diseases could not be distinguished from each other or from the SPLL phytoplasma, which has been detected in a wide range of host plant species throughout Australia (8). The yellow crinkle, mosaic, and SPLL phytoplasma gave the same RFLP patterns as SUNHP, SEPT, and CLP, all from Thailand. The SUNHP phytoplasma was found to be most closely related to members of the western X-disease strain cluster based on restriction site and sequence analysis of the 16S rRNA gene (25,27,30). Schneider et al. (26) showed by sequence analysis that SUNHP is closely related to faba bean phyllody (FBP), and that when they were included in the phylogenetic analysis, they were clearly differentiated from the western X-disease group (group C-III) and formed a distinct cluster of their own. FBP was differentiated from SUNHP, SEPT, and CLP on the basis of restriction site analysis of in vitro–amplified ribosomal DNA, which included the 16S/23S rDNA spacer region. Strains FBP and SUNHP represent a group of phytoplasmas that are sufficiently different from the strains of the western X-disease to form a distinct cluster of their own, and the name "faba bean phyllody strain cluster" (group C-VI) is proposed for the new group (26). The Australian tomato big bud strain (TBB) is closely related to the strains in the faba bean phyllody strain cluster (26), and TBB is closely related to SPLL (8). In this study, the representative of the western X-disease strain cluster was VAC, which was differentiated from SUNHP by *Mse* I. Another, as yet ungrouped, phytoplasma used in this study was CROP from Thailand, and this was also differentiated by *Mse* I.

The RFLP results presented here suggest that the same phytoplasma may be associated with different disease phenotypes, yellow crinkle and mosaic. It is possible, however, that the mosaic disease results from a disease complex that may include the yellow crinkle phytoplasma but also some other as yet unknown factor. At Caboolture, papaya with mosaic were growing alongside the same cultivars with yellow crinkle, which suggests that the differences are probably not due to cultivar differences. It is also possible that this phytoplasma is associated with mosaic, and the different disease phenotype is a reflection of the age of host plant at infection or the level of inoculum at infection. The lack of differentiation may also be a reflection of the tests used. It is possible that in this case the 16S rRNA gene and spacer region does not show sufficient variation to distinguish the yellow crinkle and mosaic phytoplasmas. Other approaches, such as the use of randomly cloned probes in DNA hybridization assays, may allow differentiation of the phytoplasmas from papaya with yellow crinkle and mosaic.

Following digestion with *Alu* I, *Rsa* I, and *Mse* I, the phytoplasma associated with dieback was different from that associated with yellow crinkle and mosaic. Following digestion with *Alu* I, the phytoplasma associated with dieback gave similar but not identical RFLP patterns to STOLF from France and AAY from Florida from group A-I (27) or group I (13). Following digestion with *Rsa* I, the pattern for dieback was the same as that for STOLF but different from that for AAY; and following digestion with *Mse* I, dieback was more similar to STOLF than it was to AAY, but it was not identical to either. This indicates that the phytoplasma associated with dieback is similar but not identical to the Stolbur phytoplasmas within the aster yellows strain cluster. On the basis of RFLP patterns, however, this phytoplasma is not different from the Australian grapevine yellows phytoplasma (22).

Although this is the first time papaya with dieback or mosaic were shown to be

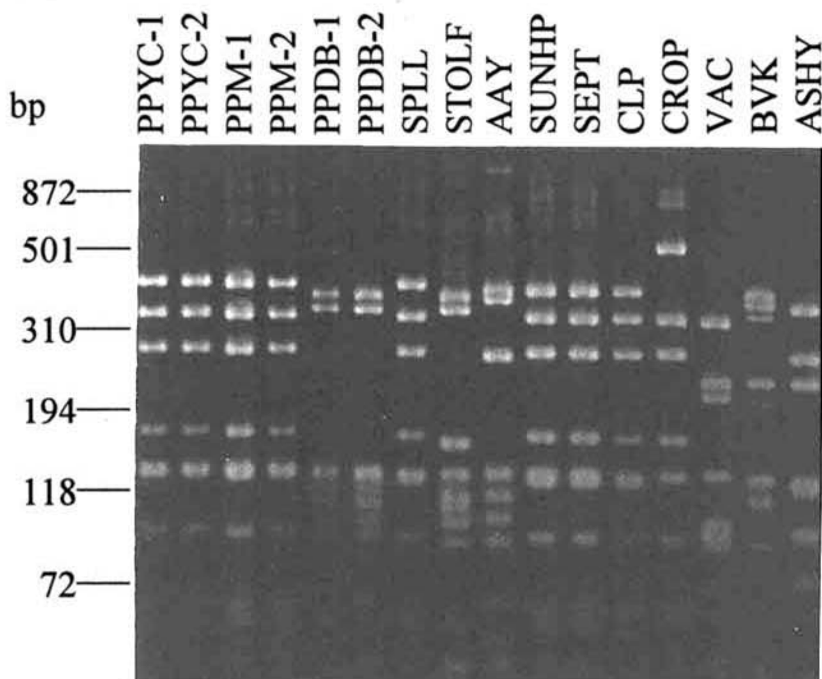

Fig. 4. Restriction digests of the polymerase chain reaction (PCR) products described in Figure 1. The restriction enzyme was *Mse* I. DNA markers are not shown but are indicated by the sizes given in base pairs. Abbreviations are the same as for Figure 1.

associated with a phytoplasma, further research, including a comprehensive survey of diseased plants and transmission studies, will be needed before either disease could be definitively attributed to a phytoplasma.

ACKNOWLEDGMENTS

We thank the Australian Centre for International Agricultural Research and the Northern Territory University internal grants scheme for financial support, and Richard Davis for collection of papaya samples.

LITERATURE CITED

1. Ahrens, U., and Seemüller, E. 1992. Detection of DNA of plant pathogenic mycoplasmalike organisms by a polymerase chain reaction that amplifies a sequence of the 16S rRNA gene. Phytopathology 82:828-832.
2. Allen, P., Da Graca, J. V., French, T. N., Martin, M., and Lavers, J. 1985. Possible virus-like infection of 'honey gold' papaws. Pages 210-213 in: Proc. Annu. Congr. S. Afr. Soc. Crop Prot., 15th. Review of Plant Pathology (1988) 67:2529.
3. Borth, W. B., Hu, J. S., Ullman, D., and Jones, V. 1994. PCR detection of mycoplasma-like organisms in Hawaii. (Abstr.) Phytopathology 84:1127.
4. Deng, S., and Hiruki, C. 1990. Enhanced detection of a plant pathogenic mycoplasma-like organism by polymerase chain reaction. Proc. Jpn. Acad. 66 Ser. B:140-144.
5. Deng, S., and Hiruki, C. 1991. Amplification of 16S rRNA genes from culturable and nonculturable Mollicutes. J. Microbiol. Meth. 14:53-61.
6. Deng, S., and Hiruki, C. 1991. Genetic relatedness between two nonculturable mycoplasmalike organisms revealed by nucleic acid hybridization and polymerase chain reaction. Phytopathology 81:1475-1479.
7. Franck, A., and Bar-Joseph, M. 1992. Use of netting and whitewash spray to protect papaya plants against Nivun Haamirimide dieback disease. Crop Prot. 11:525-528.
8. Gibb, K. S., Padovan, A. C., and Mogen, B. D. 1995. Studies on sweet potato little-leaf phytoplasma detected in sweet potato and other plant species growing in northern Australia. Phytopathology 85:169-174.
9. Glennie, J. D., and Chapman, K. R. 1976. A review of dieback - a disorder of the papaw (*Carica papaya* L.) in Queensland. Queensl. J. Agric. Anim. Sci. 33:177-188.
10. Gonsalves, D., and Trujillo, E. E. 1986. Tomato spotted wilt virus in papaya and detection of the virus by ELISA. Plant Dis. 70:501-506.
11. Gowanlock, D. H., Greber, R. S., Behncken, G. M., and Finlay, J. 1976. Electron microscopy of mycoplasma-like bodies in several Queensland crop species. Aust. Plant Pathol. Soc. Newsl. 5 (Suppl.) Abstr. 223.
12. Greber, R. S. 1966. Identification of the virus causing papaw yellow crinkle with tomato big bud virus by transmission tests. Queensl. J. Agric. Sci. 23:147-153.
13. Gundersen, D. E., Lee, I.-M., Rehner, S. A., Davis, R. E., and Kingsbury, D. T. 1994. Phylogeny of mycoplasmalike organisms (phytoplasmas): A basis for their classification. J. Bacteriol. 176:5244-5254.
14. Harding, R. M. 1989. Investigations into the cause and control of papaw dieback disease. Ph.D. thesis. University of Queensland, Brisbane, Australia.
15. Harding, R. M., and Teakle, D. S. 1988. Autofluorescence of necrotic phloem cells, and a reduced latex flow; new symptoms for papaw dieback disease in Australia. Aust. J. Agric. Res. 39:857-862.
16. Harding, R. M., and Teakle, D. S. 1993. Failure of five viruses to cause typical Australian papaw dieback disease. Australas. Plant Pathol. 22:62-67.
17. Harding, R. M., Teakle, D. S., and Dale, J. L. 1991. Double-stranded RNA in *Carica papaya* is not associated with dieback disease and is unlikely to be of viral origin. Aust. J. Agric. Res. 42:1179-1186.
18. Lambe, R. C. 1963. Terminal necrosis and wilt of papayas. J. Rio Grande Valley Hortic. Soc. 17:128-129.
19. Lana, A. F. 1980. Transmission and properties of viruses isolated from *Carica papaya* in Nigeria. J. Hortic. Sci. 55:191-197.
20. Lee, I.-M., Gundersen, D. E., Hammond, R. W., and Davis, R. E. 1994. Use of mycoplasmalike organism (MLO) group-specific oligonucleotide primers for nested-PCR assays to detect mixed-MLO infections in a single host plant. Phytopathology 84:559-566.
21. Lim, P.-O., and Sears, B. 1989. 16S rRNA sequence indicates that plant-pathogenic mycoplasmalike organisms are evolutionarily distinct from animal mycoplasmas. J. Bacteriol. 171:5901-5906.
22. Padovan, A. C., Gibb, K. S., Bertaccini, A., Vibio, M., Bonfiglioli, R. E., Magarey, P. A., and Sears, B. B. 1995. Molecular detection of the Australian grapevine yellows phytoplasma and comparison with grapevine yellows phytoplasmas from Italy. Aust. J. Grape Wine Res. 1:25-31.
23. Peterson, R. A., Coates, L. M., and Persley, D. M. 1993. Papaya diseases. Pages 70-76 in: Diseases of Fruit Crops. D. M. Persley, ed. Department of Primary Industries, Brisbane.
24. Schaff, D., Lee, I.-M., and Davis, R. E. 1992. Sensitive detection and identification of mycoplasma-like organisms in plants by polymerase chain reactions. Biochem. Biophys. Res. Comm. 186:1503-1509.
25. Schneider, B., Ahrens, U., Kirkpatrick, B., and Seemüller, E. 1993. Classification of plant-pathogenic mycoplasma-like organisms using restriction-site analysis of PCR-amplified 16S rDNA. J. Gen. Microbiol. 139:519-527.
26. Schneider, B., Cousin, M.-T., Klinkong, S., and Seemüller, E. Taxonomic relatedness and phylogenetic positions of phytoplasmas associated with diseases of faba bean, sunn hemp, sesame, soybean, and eggplant. Phytopathol. Z. 102:225-232.
27. Seemüller, E., Schneider, B., Mäurer, R., Ahrens, U., Daire, X., Kison, H., Lorenz, K.-H., Firrao, G., Avinent, L., Sears, B. B., and Stackebrandt, E. 1994. Phylogenetic classification of phytopathogenic mollicutes by sequence analysis of 16S ribosomal DNA. Int. J. Syst. Bacteriol. 44:440-446.
28. Simmonds, J. H. 1965. Papaw diseases. Queensl. Agric. J. 91:666-677.
29. Simone, G. W. 1991. Lethal yellowing disease. Pages 16-20 in: Diseases and Disorders of Ornamental Palms. A. R. Chase and T. K. Broschat, eds. American Phytopathological Society, St. Paul, MN.
30. Smart, C., Sears, B., and Kirkpatrick, B. 1994. Analysis of evolutionary relationships between MLOs and other members of the class mollicutes based on 16S/23S rRNA intergenic sequences. Pages 269-270 in: Int. Congr. Int. Org. Mycoplasmol. (IOM), 10th.
31. Story, G. E., and Halliwell, R. S. 1969. Association of a mycoplasma-like organism with the bunchy top disease of papaya. Phytopathology 59:1336-1337.
32. Zettler, F. W., and Wan, S. H. 1994. Papaya droopy necrosis and papaya apical necrosis. Page 66 in: Compendium of Tropical Fruit Diseases. R. C. Ploetz, ed. American Phytopathological Society, St. Paul, MN.