Epidemiology of Phytoplasma-Associated Papaya Diseases in Queensland, Australia

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ABSTRACT

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Three phytoplasma-related diseases of papaya (*Carica papaya*), dieback, yellow crinkle, and mosaic, are recognized within Australia. Immature leaf material was sampled every week for 8 months from a cohort of 60 female plants, located within a commercial papaya plantation, to determine the minimum time between infection and symptom expression. Phytoplasma DNA was detected using the polymerase chain reaction (PCR) with primers specific for phytoplasmas in general, and for the stolbur group of phytoplasmas. The dieback-associated phytoplasma was detected 1 week prior to (four cases) or the same week (nine cases) as symptom expression, while phytoplasma DNA was detected between 3 and 11 weeks prior to expression of mosaic symptom (six cases). Lateral shoot regrowth on the lower stem of plants which had suffered dieback disease failed to generate stolbur-specific PCR products in 15 cases. A dual infection with dieback and yellow crinkle or mosaic was diagnosed in a further two cases, using restriction fragment length polymorphism digests, and both cases were interpreted as secondary infections by the dieback-associated phytoplasma. Regrowth in three of seven cases of yellow crinkle- and three of nine cases of mosaic-affected plants tested positive for phytoplasma-specific DNA. Ratooning of dieback-affected plants and removal of yellow crinkle- or mosaic-affected plants is suggested for the management of these diseases.

Additional keywords: mollicute, mycoplasma-like organisms (MLO), papaw, papaya dieback, papaya mosaic, papaya yellow crinkle, residency time

Three diseases of papaya in Australia have been associated with phytoplasmas: dieback, yellow crinkle, and mosaic (3,7,16). Papaya dieback causes plant losses of between 10 and 100% in any one season, while yellow crinkle and mosaic are less severe diseases (9). DNA sequence analysis of the 16S ribosomal RNA (rRNA) gene has shown that while the yellow crinkle- and mosaic-associated phytoplasmas are the same (at the 16S rRNA gene and 16S-23S spacer region), the dieback-associated phytoplasma is clearly different (8,28,29). The dieback-associated phytoplasma falls into subclade xii (the stolbur subgroup of the aster yellows strain cluster) while the yellow crinkle- and mosaic-associated phytoplasmas are within subclade iii (29). Primers have been designed for the specific detection of

these papaya pathogens, facilitating epidemiological studies (8).

Management of these diseases would benefit from knowledge of the incubation period between inoculation and symptom expression, the insect vectors of the disease, and alternate plant hosts of these vectors (potential "reservoirs" of phytoplasma outside papaya plantations). Current management is limited to ratooning. In this practice, plants are cut (0.75 m height) as soon as external symptoms of any of the three phytoplasma diseases are recognized in the shoot, in an attempt to reduce spread of the pathogen. Lateral shoots are allowed to grow on these stumps, being pruned to one or two branches which form a reasonably acute angle with the old trunk (mechanically stable, and not liable to break under the weight of shoot and fruit growth), replacing the lost production potential. This management strategy is based on the assumption that the phytoplasma is localized to the upper shoot.

Of the three diseases, dieback has the most seasonal distribution in terms of symptom expression. Two hypotheses are consistent with the observation that dieback epidemics occur in two periods within the year, October to November and March to April (1,9,11): (i) plants may be infected throughout the year, but symptom expression occurs only during the two periods, due to a change in environmental

conditions which evokes a toxicity within the disease complex; or (ii) the disease has a short incubation period, with inoculation occurring in association with the two periods of symptom expression due to increased insect vector activity.

Phytoplasmas reside in the phloem tissue of the plant and many are known to be transmitted by phloem-feeding insects such as leafhoppers and planthoppers (13). The *Phormium* (New Zealand flax) yellow leaf phytoplasma, which genetically is closely related to the phytoplasma associated with papaya dieback (8,29), is vectored by a planthopper (15). The insect vector or vectors of the three phytoplasma-associated papaya diseases have not been identified, although it was noted that the periodic epidemics of yellow crinkle usually occur after hot, dry weather, which favors the breeding and movement of the leafhopper *Orosius argentatus* (20).

Reports of the lag or "incubation" period between inoculation, first detection of phytoplasma within the plant, and onset of visual symptoms vary according to the plant, phytoplasma, and symptom severity. Also, methods used to detect phytoplasma, which include DNA stain 4,6-diamidino-2-phenylindole (DAPI; 5,22,26), DNA probes and hybridization (12,19), indirect enzyme-linked immunosorbent assay (ELISA), or immunohistochemistry (14) are not equally sensitive, and can be expected to give different estimates of the time of first detection of phytoplasma within the plant. However, in all the above cases, phytoplasma are generally detected within the plant less than 3 weeks after inoculation, and visible symptoms are apparent between 2 and 8 weeks after inoculation (2,5,12,14,19,26). This lag period was attributed to a period of translocation and multiplication of the phytoplasma within the plant (5). In contrast, detection of visual symptoms of ash yellows, a phytoplasma disease causing slow decline, lagged 1 to 2, and up to 4, years behind detection by DAPI (26). Similarly, following infection of *Vicia faba* shoots by the flavescence dorée (FD) phytoplasma through a leafhopper vector, phytoplasma was first detected (by ELISA) in the roots, not the stem, 17 days after inoculation (14). Phytoplasma then multiplied within the roots, and were subsequently detected in the shoot. Symptom expression in the shoot was evident at 24 to 28 days, with strong ex-

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pression of symptoms evident 35 days after inoculation.

Two studies have attempted to estimate the incubation time of the papaya yellow crinkle and dieback diseases. Greber (10) noted symptom expression in tomato 7 to 9 weeks following transfer of infected dodder from yellow crinkle-affected papaya. Symptom expression in papaya was noted 9 to 13 weeks following transfer of infected dodder from infected tomato (10). Harding (11) noted dieback symptoms within 5 weeks of transplanting disease-free, glasshouse-reared, papaya plants to the field.

It has been suggested that papaya yellow crinkle and mosaic result from a disease complex involving the same phytoplasma strain, differentiated by other factors (such as another microbiological agent, age of host plant, or level of inoculum at infection; 7). Comparison of the incubation period would be useful in the characterization of the two diseases, but an estimate for mosaic currently is not available.

Knowledge of the time of infection, as opposed to the time of symptom expression, is useful in the quest to identify insect vectors of phytoplasmas. In this study, we aimed to improve the estimate of the incubation time of the papaya phytoplasma diseases. A reliable means of transmitting the dieback-associated phytoplasma to papaya plants has not been established; therefore, we adopted a field study approach involving regular monitoring of a cohort of plants within a commercial papaya plantation for the presence of phytoplasma. The carry-over of phytoplasma into lateral shoot growth of plants ratooned following observation of external symp-

toms was also monitored, allowing comment on the efficacy of ratooning as a management strategy for control of this disease.

MATERIALS AND METHODS

Sample collection. A total of 60 field-grown papaya plants (tissue culture clones of a dioecious variety, 'hybrid 14'), consisting of every fourth female plant within rows of approximately 30 plants within a commercial plantation at Yarwun, central Queensland, Australia, were sampled every week from September 1996 until May 1997. Lobes of six immature (not fully expanded) leaves were sampled from each plant. Immature leaves were previously demonstrated to be a reliable indicator tissue for the presence of dieback-associated phytoplasma within the plant (25). Leaf samples were frozen at -20°C until analyzed. One-third of each leaf lobe was subsampled from each sample. This material was pooled across 10 samples, giving 6 combined samples for each week of sampling. When a positive polymerase chain reaction (PCR) result was recorded, or when visible disease symptoms were noted, the individual samples and all previous samples of the affected individual were analyzed.

Diseased plants were immediately ratooned at approximately 0.75 m height, following current grower practice. Leaf lobes of lateral shoot regrowth material from papaya plants that had been ratooned following dieback (11 plants), mosaic (9 plants), and yellow crinkle (7 plants) were sampled in July and October 1997 and January 1998. Stem material was also sampled from some of these plants (two

dieback, one mosaic, and two yellow crinkle). Leaf lobes of regrowth material and stem tissue from six papaya plants that had not been ratooned following dieback were sampled in February 1998.

Amplification of DNA. DNA was extracted from the midribs of papaya leaves using the cetyltrimethylammonium bromide (CTAB) method of Doyle and Doyle (6). Total PCR volumes were 25 μl containing $1\times$ DNA polymerase reaction buffer (Boehringer Mannheim GmbH, Mannheim, Germany), 0.2 mM of each dATP, dCTP, dGTP, and dTTP (Boehringer Mannheim GmbH), 0.4 μM of each primer, 4 ng bovine serum albumin (BSA; Sigma Chemical Co., St Louis), 1 U Red Hot DNA polymerase (Advanced Biotechnologies Ltd., Surrey, UK), and 50 to 100 ng template DNA. The phytoplasma-specific primers, P1 (4) and P7 (23), were used to amplify an approximately 1.8-kilobase (kb) fragment in a region encompassing the 16S rRNA gene and the 16S-23S rRNA intergenic spacer region. A phytoplasma-specific primer, fU5, (17) and a primer specific for the stolbur group of phytoplasmas, AGY2 (8), were used to amplify a region of the 16S rRNA gene and the 16S-23S rRNA intergenic spacer region approximately 1.3 kb in length. PCRs were performed in a minicycler (M. J. Research, Watertown, MA). The reaction mixture without BSA and DNA polymerase was heated to 95°C for 5 min. Following addition of BSA and DNA polymerase, 35 cycles of denaturation (94°C ; 1 min), annealing (55°C , or 53°C for fU5 and AGY2; 1 min), extension (72°C ; 1.5 min), and a final cycle (72°C ; 5 min) were performed. PCR products were analyzed by electro-

Table 1. Polymerase chain reaction (PCR) detection of dieback and mosaic phytoplasma in young leaf tissue relative to time of appearance of visual symptoms

Disease	Date ^b	Weeks prior to visual symptoms ^a												
		0	1	2	3	4	5	6	7	8	9	10	11	12
Dieback	24 Oct 96	+	...	—	—	—	—	—	—	...	...	...	...	...
Dieback	24 Oct 96	+	...	—	—	—	—	—	—	...	...	...	...	...
Dieback	24 Oct 96	+	...	—	—	—	—	—	—	...	...	...	...	...
Dieback	24 Oct 96	+	...	—	—	—	—	—	—	...	...	...	...	...
Dieback	24 Oct 96	+	...	—	—	—	—	—	—	...	...	...	...	...
Dieback	16 Jan 97	—	—	...	...	...	...	...	...	...	...	...	...	...
Dieback	6 Feb 97	+	—	—	—	—	...	...	...	...	...	...	...	...
Dieback	13 Feb 97	+	—	—	—	—	...	...	...	...	...	...	...	...
Dieback	13 Mar 97	—	—	—	—	—	...	...	...	...	...	...	...	...
Dieback	10 Apr 97	+	+	—	—	—	...	—	—	—	—	...	...	...
Dieback	23 Apr 97	+	+	—	—	—	—	—	...	—	—	—	...	...
Dieback	1 May 97	...	+	—	—	—	...	...	...	...	...	...	...	...
Dieback	7 May 97	+	—	—	—	—	—	—	...	...	...	...	...	...
Dieback	22 May 97	+	...	—	...	—	—	—	—	—	—	...	...	...
Dieback	1 June 97	...	+	—	—	—	—	—	...	...	...	...	...	...
Mosaic	17 Apr 97	+	+	+	+	—	—	...	—	—	—	—	...	...
Mosaic	23 Apr 97	+	+	+	+	—	—	—	—	—	...	...	...	...
Mosaic	23 Apr 97	+	+	+	+	+	—	—	...	—	—	—	—	...
Mosaic	15 May 97	+	+	...	+	+	+	—	—	—	...	...	...	...
Mosaic	19 June 97	+	+	+	+	+	...	...	—	...	+	+	+	—
Mosaic	3 July 97	+	+	+	...	...	+	+	...	...	...	...	+	...

^a Data is derived from lobes of young leaves from a cohort of 60 female trees, monitored at weekly intervals. + indicates positive PCR (fU5 or AGY2 dieback; P1 or P7, mosaic); — indicates negative PCR product; ... indicates no data available.

^b Date of symptom appearance.

phoresis through a 1% (wt/vol) agarose gel in 1× Tris-acetate-EDTA (TAE) buffer (21), stained with ethidium bromide (EtBr).

Restriction fragment length polymorphism analysis. The P1 and P7 amplimers (10 µl) were digested using the restriction endonucleases *Alu* I and *Tru* 9 I in their respective buffers, as supplied by the manufacturer (Boehringer Mannheim GmbH). Digestions were incubated overnight at 37°C. The reactions were stopped by heating at 65°C for 20 min. The fragments were separated by electrophoresis through a 3% agarose gel and visualized by staining with EtBr. Known dieback, yellow crinkle, and mosaic samples were used as positive controls.

RESULTS

Disease incidence (external symptoms). A total of 22 of the 60 cohort papaya plants displayed symptoms of phytoplasma-associated diseases over the 8-month (September to May) study period. Of those 22, 15 plants exhibited dieback symptoms and 7 exhibited mosaic symptoms. No yellow crinkle was observed in this group of plants, although nearby plants displayed symptoms typical of this disease. The majority of dieback cases (11 of 15) occurred within the periods October and April to May, while mosaic cases occurred between April and June.

Disease incidence (PCR detection). DNA extracted from plants with symptoms

of the mosaic disease was amplified with the phytoplasma-specific primers (P1 and P7) but not with the stolbur-specific PCR (fU5 and AGY2), while DNA extracted from plants with symptoms of dieback plants was amplified with fU5 and AGY2 and gave faint or no product with the P1 and P7 primers. Gibb et al. (8) reported poor amplification of phytoplasma DNA in dieback material with P1 and P7, and suggested that this was due to a low titer of phytoplasma associated with this disease. Of the 15 dieback symptomatic plants, 2 did not amplify with either primer pair. DNA extracted from papaya plants which did not exhibit symptoms during the 8-month study (38 cases) gave no product with either primer pair.

Using the stolbur-specific PCR, amplimers were obtained from tissue samples collected either the week of, or the week prior to, visual symptom expression of dieback. In contrast, DNA associated with the mosaic phytoplasma was first detected between 3 and 11 weeks prior to visual symptom expression. First detection within the cohort plants of the phytoplasma associated with mosaic occurred over a 6-week period from 27 March to 7 May, with symptom expression noted over a wider time span (11 weeks, 17 April to 3 July; Table 1).

Persistence of phytoplasma in regrowth. Leaf from lateral shoots growing on stems of papaya plants which had suffered dieback failed to give a PCR product

with either set of primers (P1 and P7; fU5 and AGY2) in 15 cases (nine plants ratooned, six plants left intact). Tissue from the main trunk of the plant, sampled from the six non-ratooned plants, also failed to give PCR product. In two dieback-ratooned plants, stem tissue gave product with the P1 and P7 primers, while leaf material gave products with both primer sets. Where sufficient products existed to undertake a restriction digest (1 leaf sample, 2 stem samples), the pattern was matched to the yellow crinkle and mosaic controls.

Regrowth material from three of nine papaya plants that had been ratooned following mosaic symptom expression gave a product with the P1 and P7 primer pair (Table 2). In two of these three plants, a lateral shoot exhibited the typical dieback symptom of bending of the apex and “X-Y” patterning of the leaves, and gave a product with the stolbur-specific PCR (fU5 and AGY2). In both cases, however, another lateral shoot on the same stump gave no product with the stolbur-specific PCR. In one of these cases, the other lateral shoot gave a PCR product with the P1 and P7 primer pair and, when digested, gave the same pattern as the yellow crinkle and mosaic controls.

Regrowth material from three of seven papaya plants that had been ratooned following yellow crinkle symptom expression gave a PCR product with the P1 and P7 primer pair, while all seven samples, in-

Table 2. Presence of phytoplasma as indicated by polymerase chain reaction (PCR) in lateral shoot regrowth of papaya trees which were ratooned following appearance of external symptoms associated with a phytoplasma-associated disease

Original disease	Ratooned	No. plants	Time ^a	Tissue sampled	P1 or P7 ^b	fU5 or AGY ^b	Comments
Dieback	+	9	0.4–8	Leaf	–	–	In 5 cases, original disease verified by PCR test (plant of cohort group represented in Table 1)
Dieback	–	6	4	Leaf	–	–	Leaf and stem samples taken from each plant
				Stem	–	–	
Dieback	+	1	4	Leaf	+	+	In 1 case, original symptoms verified by PCR
				Leaf	+ RFLP	+	
				Stem	–	–	
				Stem	+ RFLP	+	
Dieback	+	1	4	Leaf	+	+	Different lateral shoots emerging from the one stump; dieback symptoms apparent in *; original symptoms verified by PCR
				Leaf	+	+	
				Stem	–	–	
				Stem	+ RFLP	–	
Mosaic	+	6	1–3	Leaf	–	–	In 1 case, original symptoms verified by PCR
Mosaic	+	1	1	Leaf	+	–	
Mosaic	+	1	4	Leaf	–	–	
				Leaf *	+	+	Different lateral shoots emerging from the one stump; dieback symptoms apparent in *
Mosaic	+	1	3.5	Leaf	+ RFLP	–	
				Leaf *	+	+	
Yellow crinkle	+	2	6	Leaf	–	–	In 1 case, regrowth displayed claw-like leaves, a symptom characteristic of mosaic
Yellow crinkle	+	3	6.5	Leaf	+	–	
Yellow crinkle	+	2	NK	Leaf	–	–	Two samples tested for each tissue
				Stem	–	–	

^a Time from symptoms (months); NK = not known.

^b Two PCRs were run for each sample, one primed with P1 or P7 (primers specific for all phytoplasmas), and the other with fU5 or AGY2 (primers specific for the stolbur group of phytoplasma). + indicates the production of amplimer of the appropriate size in the PCR reaction; – indicates the absence of product; RFLP = restriction fragment length polymorphism. Where sufficient P1 or P7 product supported a restriction digest (RFLP), the resultant pattern was characteristic of that associated with yellow crinkle- or mosaic-associated phytoplasma.

cluding stem samples, failed to give a product with the fU5 and AGY2 primer set (Table 2).

DISCUSSION

Incubation period—from infection to symptom appearance. The dieback-associated phytoplasma was detected only 1 week before visual symptoms, while the mosaic-associated phytoplasma was detected up to 11 weeks before visual symptoms were apparent (Table 1). These periods represent a minimum estimate of incubation time, because phytoplasma may not be detected due to an uneven distribution of the phytoplasma within the plant body (7,13), and the sensitivity of the PCR.

While the pathogen responsible for yellow crinkle has been transmitted between hosts by dodder (10), the pathogens involved in the dieback and mosaic diseases have not been experimentally transmitted between host plants, either by insect, grafting, or dodder. In transmission studies of other phytoplasma diseases, phytoplasma are detectable by PCR or DAPI about 2 to 3 weeks after phytoplasma inoculation, while the time to visual symptom expression varies considerably (2,5,12,14,19,26). Assuming a 2- to 3-week lag period between inoculation and phytoplasma DNA detection, there may be a total delay of 3 to 4 weeks from inoculation to onset of visual symptoms for the dieback disease, and 5 to 14 weeks for the mosaic disease.

The duration of an incubation period will be influenced by plant vigor. For example, Greber (10) reported a shorter incubation period for yellow crinkle in papaya grown under favorable (greenhouse) conditions relative to a field planting (9 to 12 and 11 to 13 weeks, respectively). Nevertheless, the estimate of incubation period for the dieback disease obtained in the current study is consistent with Harding's (11) deduction of less than 5 weeks, based on the minimum time for symptoms to appear in plants moved from insect-proof to open field conditions. The incubation period of 9 to 13 weeks reported for yellow crinkle (10) is similar to that estimated for mosaic in the current study.

We have shown that by the time of the first symptom expression of dieback (stem tip bending), phytoplasma is well dispersed within the host, although not present in high titer because it could not be observed via transmission electron microscopy (25). Together with the observation of a short period between first detection and symptom expression noted in the current study, these results are consistent with the rapid spread of the dieback phytoplasma within the plant body following an inoculation event (leafhopper feeding), coupled with a limited multiplication capacity.

The spread of phytoplasma within the plant may be limited by movement within the phloem, or by the number of phyto-

plasma cells. Certainly the number of phytoplasma cells associated with the dieback disease is low (25). The rate of phloem solute movement axially within the plant is in the order of 30 cm h⁻¹, depending on source-sink relationships (18), sufficient for movement of soluble phloem contents from the upper stem to the lower roots within 1 day in a mature papaya plant. Phytoplasma are presumably carried with this flux, although restricted by passage through the sieve plate pores. Phytoplasma must constrict to pass these pores, and it is not established if this is a passive or active process. An improved understanding of the process of movement and multiplication of phytoplasma within the phloem is required to interpret epidemiological information of the type presented in this study.

Time of infection. Knowledge of the time of infection, as opposed to the time of symptom development, is important in determining the insect vectors and possible environmental triggers for the diseases. The incidence of visible symptoms of dieback peaks at two periods during the year, October to November and late March to late May (1). In contrast, the incidence of visible symptoms of yellow crinkle peaks within one period during the year, varying between January and March, and the incidence of mosaic is generally fairly evenly spread through the year (R. J. Elder, *personal communications*).

The first detection of mosaic-associated phytoplasma within plants varied from late March until early May (seven cases; 100% of total detected; Table 1). During this period, five cases (38% of total) of dieback-associated phytoplasma were detected. This overlap is consistent with the possibility that both phytoplasmas are transmitted by the same insect. However, mosaic-associated phytoplasma DNA was not detected in plants in October, when there were five cases (38%) of dieback.

Dieback, yellow crinkle, and mosaic phytoplasmas appear within a season in papaya plantations established in new growing areas. We infer that a reservoir of disease agents exist in other vegetation. "Co-incident" transfer of dieback, mosaic or yellow crinkle could occur if the phytoplasmas were present in the same reservoir plant, or were in different plants that were all hosts for a common vector. The lack of co-incidence of DNA detection and symptom expression between mosaic and dieback in papaya during October suggests the involvement of different alternate hosts and possibly different vectors for the two diseases. In parallel studies, we have monitored the incidence of insects feeding on papaya, and are testing potential alternate hosts in the vicinity of the papaya plantings for phytoplasma.

Persistence of infection. The presence of phytoplasma within stem regrowth may represent either a carryover from the origi-

nal infection, with phytoplasma surviving within the ratooned stem, or a secondary infection of the new shoot material. Reported cases involving carryover of phytoplasma involve slow decline disorders, more comparable to papaya yellow crinkle and mosaic than dieback. For example, in X-disease (5), blueberry stunt (22), apple proliferation and pear decline (24), and ash yellows (27), the shoot, but not root, of the affected plant is reported to become free of phytoplasma during the winter. Phytoplasma are considered to reinfect the shoot from the root, via the phloem, in the new growing season.

The dieback-associated phytoplasma was detected throughout the plant body within 1 week of noting visible symptoms (25), only 2 to 3 weeks after first detection of phytoplasma within the plant. Distribution of phytoplasma DNA within the plant decreased as shoot necrosis progressed (25). This result was interpreted as a hypersensitive response by the plant, causing the death of phytoplasma throughout the plant body. Consistent with this suggestion, we noted in the current study that, in six cases, phytoplasma DNA was not present in either the main stem or regrowth of plants which were not ratooned (dead apex still present). We interpret the two cases in which phytoplasma DNA was detected in regrowth of dieback-affected plants as secondary infection events of the new shoot growth.

The higher incidence of detection of phytoplasma DNA within regrowth of plants which had suffered yellow crinkle or mosaic is consistent with the survival of mosaic- and yellow crinkle-associated phytoplasma within the stem and roots, reinfecting later lateral shoot growth. However, phytoplasma DNA was not detected in regrowth material of all former yellow crinkle and mosaic plants (Table 2), suggesting either uneven distribution or some remission of the phytoplasma within the stump. Studies of the distribution of phytoplasma within the host plant during the progression of mosaic and yellow crinkle diseases would test these interpretations.

We conclude that the grower action of ratooning as visible symptoms of any of the three diseases become apparent does not have the intended effect of preventing phytoplasma cells from reaching the lower stem, though it must reduce the number of such cells. Ratooning of dieback-affected plants is, however, warranted to promote lateral shoot growth within 0.75 m of ground level, replacing the lost production potential. In contrast, if yellow crinkle and mosaic phytoplasma survive within the trunk and roots of the plants, ratooning is not an effective strategy.

Dual infection. Gibb et al. (8) have recently reported the design of primers which allow specific detection of the yellow crinkle or mosaic phytoplasma, facili-

tating future epidemiological studies. In the current study, the P1-P7 and fU5-AGY2 primer sets did not allow unique identification of the yellow crinkle or mosaic phytoplasma in the presence of the dieback-associated phytoplasma. Therefore, restriction fragment length polymorphism analysis of the P1-P7 product was undertaken to differentiate between the two types of phytoplasma.

In both plants ratooned following appearance of dieback symptoms in which phytoplasma DNA was detected in regrowth and main stem (Table 2), only some tissues gave product with the fU5-AGY2 primer pair, and the pattern of the restriction digest of the P1-P7 product was characteristic of the phytoplasma associated with yellow crinkle or mosaic. These observations are consistent with a dual infection of the plant with dieback and yellow crinkle or mosaic phytoplasmas. A case of dual infection of papaya with dieback and yellow crinkle or mosaic phytoplasma was also recently reported by Gibb et al. (8).

Similarly, in each of two plants which developed mosaic symptoms and were ratooned, a lateral shoot was noted with dieback symptoms, and this material tested positive with both primer sets (Table 2). A second shoot from one of these plants tested positive with the P1 and P7 primers and not with the stolbur-specific PCR, and the pattern of the restriction digest of the P1-P7 product was characteristic of yellow crinkle and mosaic. We conclude that a dual infection had occurred, with secondary infection by the dieback phytoplasma.

In one plant which exhibited yellow crinkle symptoms and was ratooned, lateral shoot regrowth exhibited symptoms of mosaic (Table 2). This observation is consistent with either the hypothesis that a single phytoplasma is involved in these diseases, with differential symptom development triggered by other factors, or that two infection events occurred, one for each disease.

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