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Temporal incidence of three phytoplasma-associated diseases of *Carica papaya* and their potential hemipteran vectors in central and south-east Queensland

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Abstract. Weekly or fortnightly records of the incidences of three phytoplasma diseases of papaya (dieback, yellow crinkle and mosaic) were taken over 4 years at three sites in central and southern Queensland. Outbreaks of dieback generally occurred between October and December and yellow crinkle between November and March. Disease outbreaks appeared to be associated with weather conditions which caused the surrounding vegetation to dry. No consistent incidence pattern was evident for mosaic disease. Over three sites, the incidence of dieback was 68–85%, yellow crinkle was 2–27% and mosaic was 5–8%. When the time from infection to the expression of disease symptoms was considered, the plants would have been infected between September and November for both dieback and yellow crinkle diseases. The diseases were absent when plants were grown under insect proof netting, implying that the diseases were transmitted by aerial vectors, probably insects. Disease symptoms and PCR testing indicated that the phytoplasmas were present in plants outside the netted area but not inside. Seven different species of planthoppers and 13 species of leafhoppers were collected from papaya. Although some were present from September to November, none gave a positive PCR test for any of the three phytoplasmas. Regular sampling indicated that the insects do not stay for long or breed on the papaya plants, suggesting that papaya is not a favoured host. On this basis, we present the hypothesis that dieback and yellow crinkle are transmitted by leafhoppers or planthoppers. The insects are transported into the district by weather troughs/fronts that involve north-to-south air movement in spring (September–November). Dieback and yellow crinkle outbreaks generally occur in years with dry conditions in late winter and early spring, as the insects are attracted to green papaya plantations surrounded by unattractive dry, brown vegetation.

Additional keywords: Australia, weather influences, dieback, yellow crinkle, mosaic, plant disease, epidemiology, Cicadellidae, Fulgoroidea.

Introduction

In Australia, papaya (*Carica papaya*) (papaw) is grown commercially in coastal regions of Queensland, with major centres of production located in the subtropical south-east and central regions, and in tropical northern Queensland (145–153°E, 16–28°S). Queensland papaya crops are affected by three diseases known locally as dieback, yellow crinkle and mosaic (Simmonds 1965; Persley 1993). Dieback is the most serious disease and has been known since 1922. Epidemics start suddenly and 10–100% of trees are often affected within a few weeks (Glennie and Chapman 1976). The relative importance of yellow crinkle and mosaic has not been determined, as growers tend to treat the three diseases as a single problem and label it dieback (Drew and Considine 1995). Except for records kept between October 1992 and May 1993 at two locations (Aleemullah and Walsh 1996), only casual observations are available on the temporal incidence of dieback, while virtually nothing has been reported about the occurrence of yellow crinkle and mosaic. Aquilizan (1995), O'Hare (1995) and D. Hall (personal communication) have all noted that dieback was often associated with long dry periods followed by periods of heavy rainfall.

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The aetiology of dieback, yellow crinkle and mosaic has remained unresolved despite extensive research. Yellow crinkle appears to be caused by the same organism that causes tomato big bud, as was shown through dodder transmission experiments (Greber 1966). Phytoplasmas were later observed in plants affected by tomato big bud (Bowyer 1974). A consistent association of phytoplasma with each of the three diseases has been demonstrated using polymerase chain reaction (PCR) techniques in independent work by three groups (Davis and Teakle 1995; Gibb *et al.* 1996; Liu *et al*. 1996). Based on DNA sequence analysis of the 16S rDNA and 16S–23S rDNA spacer regions, the phytoplasmas associated with yellow crinkle and mosaic have been included in the taxon 'Candidatus Phytoplasma australasia' which also includes the phytoplasmas associated with tomato big bud (White *et al*. 1998). The dieback-associated phytoplasma belongs to the taxon 'Candidatus Phytoplasma australiense' and includes the phytoplasmas associated with Australian grapevine yellows and *Phormium* yellow leaf (White *et al*. 1998).

Most phytoplasma vectors are leafhoppers (Cicadellidae) and planthoppers (Superfamily Fulgoroidea) (Tsai 1979), both of which belong to the hemipteran suborder, Auchenorrhyncha. Liefting *et al.* (1997) found that a phytoplasma similar to dieback, *Phormium* yellow leaf phytoplasma, was transmitted by *Oliarus atkinsoni* Myers (Homoptera: Fulgoroidae Cixiidae). The tomato big bud causal agent is transmitted by the leafhopper *Orosius argentatus* (Evans) (Hill 1943), and so this hopper species is also a candidate vector for yellow crinkle. As phytoplasmas are restricted to phloem tissue, their vectors are postulated to be phloem feeders (McCoy 1979).

An understanding of relations between weather parameters, vectors and disease incidence is a basic requirement for the prediction of plant disease epidemics as well as for developing disease management strategies. This study was undertaken to obtain information on the temporal and spatial incidence of dieback and to determine any relationships between dieback incidence, site physical characteristics, papaya leafhopper and planthopper species and numbers, and weather patterns. Yellow crinkle and mosaic incidence were also recorded in order to determine their importance relative to dieback as well as to characterise their temporal patterns over time. In addition, enclosures were used to determine whether dieback, yellow crinkle and mosaic could be excluded from disease-free papaya plants, a result that would support the hypothesis that these diseases are spread by insect vectors.

Methods

Site descriptions and management

Three sites were monitored for papaya dieback, yellow crinkle and mosaic diseases. Sites 1 and 2 were located at Yarwun, central Queensland (24°S, 151°E). Site 3 was located 650 km south at Redland Bay, south-east Queensland (27°S, 153°E).

Site 1 was a field trial originally established to investigate the effect of mulching on the productivity of three papaya hybrids. Elder *et al*. (2000) provide a comprehensive description of the trial site. Briefly, two mulching treatments, mulched and bare ground, and three hybrid treatments, Hybrid 29, Hybrid 11 and Hybrid 13 (Aquilizan 1987) were compared in a randomised incomplete block design. There were three replicate blocks with two small blocks within each replicate. Each small block was divided into three hybrid plots. Plant positions were in double rows on a 2.0 m (between row) by 1.8 m (within row) grid with an average of 5.5 m between centres of the double rows. There were 79–81 plant positions in each plot resulting in a total of 1441 plant positions with four to five plants in each position. Five months after planting, these were thinned to one per position to give 90% female and 10% male plants. Thinning involved cutting back unwanted plants to stumps about 15 cm in height; these regrew slowly but were available to replace the main plant if it was removed due to disease. The mulch consisted of coarse grass hay to a depth of 10 cm and this was added just prior to planting on 7 June 1994. The hay was topped up at 6-monthly intervals to maintain a 10 cm depth of mulch. The edges of all plots were planted to bana grass (*Pennisetum purpureum* × *P. glaucum*) to provide windbreaks up to 4 m in height. The site (0.75 ha) faced north-west to north-east on steeply sloping land with an average slope of about 25%. The site was watered using trickle irrigation (T-tape) and fertilised according to standards recommended by Anon. (1994*a*, 1994*b*). Crop ratooning involved cutting plants out and leaving a 30 cm stump. Ratooning occurred on four occasions (4 September, 3 October, 7 November and 19 December 1996) reflecting commercial practice. On the first three occasions, plants were cut out if their fruit were too high to pick easily from the ground and if all fruit were at least 3 months from picking. On the last occasion (19 December 1996), the remaining plants were cut out.

Site 2 consisted of a block of 925 plant positions and comprised 90% tissue cultured female Hybrid 14 plants (Drew and Vogler 1993) and 10% male F1 Hybrid 29 plants planted on 29 March 1996. The female plant positions each had one plant. The male positions each had an average of three but were subsequently reduced to a single plant at flowering. Plant and row spacing was similar to Site 1. This site was approximately 500 m from Site 1 facing south to south-west on steeply sloping land (up to 30%). It was watered using trickle irrigation and fertilised in a similar manner to Site 1.

Prior to planting in Site 2, 71 plant positions (eight rows with either eight or nine plant positions per row) on the lower side of the block were enclosed in an insect-proof structure (white Living Shade Anti-thrips net; mesh size of 0.28 × 0.78 mm). Row 5 with eight plant positions had male F1 Hybrid 29 plants. The remaining seven rows contained tissue cultured female Hybrid 14 plants. Plants inside the enclosure were cut back to 75-cm-high stumps on three occasions when they reached the roof (height 2.5 m). Plants were trimmed away from the sides to ensure that they did not touch the mesh. The enclosure had a double entrance to exclude insects.

Site 3 consisted of tissue cultured Hybrid 2000.1 plants (Drew and Considine 1995) established on flat ground in two single rows. The rows were 2 m apart with one plant per position every 2.5 m in the row. They were fertilised (broadcast) with Q7K (Incitec, Brisbane) and watered with overhead sprinklers. A further two rows were enclosed in a 3.6-m-high, insect-proof structure (Klayman Meteor cloth; mesh size of 1.5 × 2.0 mm) with a double entry. A total of 65 plants of undetermined sex was planted outside, and 67 planted inside the enclosure in May 1996.

Disease incidence

The incidence of papaya plants affected with dieback, yellow crinkle and mosaic disease was recorded weekly from 25 October 1994 to 19 March 1998 at Site 1, from 16 May 1996 to 12 February 1998 at Site 2 and fortnightly from 1 June 1996 to 31 December 1997 at Site 3.

Disease identification was based on visual symptoms. Disease type and plant spatial position for each diseased plant were recorded at Sites 1 and 2. As there were only 65 plants outside the enclosure at Site 3, the position of diseased plants was not recorded.

Diseased plants at Sites 1 and 2 were cut to 30-cm stumps. Since these plants often regrew and may have been re-infected, a plant position may have had multiple occurrences of the diseases at these sites. Affected plants at Site 3 were not ratooned but were left intact. Total incidence for each disease at each site was calculated as the number of plant positions with at least one occurrence of the disease during the study, divided by the number of plant positions.

The effects of hybrid and mulching on the incidence of dieback, yellow crinkle and mosaic disease was determined by analysis of variance of the proportion of plant positions at Site 1 that had at least one occurrence of the disease up until ratooning (4 September 1996) and from ratooning until the end of the experiment.

Insect monitoring

Insects were collected from the foliage, flowers, fruit and trunk of the trees at Sites 1 and 2 using a petrol-driven vacuum-blower (Dolmar PB250) that had a gauze collecting bag (mesh size 0.9 × 0.3 mm) placed over the inlet pipe. Site 1 was sampled weekly from 26 September to 7 December 1995 and fortnightly from 4 January to 20 June 1996 where each of 60 randomly selected trees was sampled for 60 s. Sampling was conducted fortnightly from 5 July 1996 to 4 December 1996 at Site 2, except during September and October (the months usually preceding a dieback event) when weekly sampling was conducted. Each of ten randomly selected trees inside the enclosure and ten outside were sampled for 30 s.

Following sampling, insects trapped in the collecting bag from a single plant were transferred into a vial and stored temporarily in a cold box. Leafhoppers and planthoppers were separated from other insects and tentatively placed into species groups using the keys of Lower (1952), Fennah (1965), Evans (1966), Knight (1987) and Fletcher and Stevens (1988). Specimens of each group were sent to M. Fletcher (Orange Agricultural Institute) (Cicadellidae) and J. Donaldson (Queensland Department of Primary Industries) (Fulgoroidea) for identification. Voucher specimens were lodged in the collections of each organisation. The remaining specimens were frozen at –20°C until they could be analysed for presence of phytoplasma.

Weather

Associations of weather parameters with incidence of dieback, yellow crinkle and mosaic disease were investigated for Sites 1 and 2. The limited disease incidence data for Site 3 precluded it from investigation. Three-hourly weather data on atmospheric pressure, dewpoint, temperature, rainfall and wind direction at Gladstone (23°50′S, 151°20′E) approximately 40 km east of Yarwun, were obtained from the Australian Bureau of Meteorology (Livio Regano, personal communication). Weekly rainfall records were kept on the Yarwun property where Sites 1 and 2 were located.

It was hypothesised that the incidence of the papaya diseases may be related to the occurrence of troughs transporting vectors from other areas. The passage of weather troughs which move from west to east are accompanied by disturbed weather conditions including stronger winds and perhaps rain. To investigate this hypothesis, possible troughs were inferred from the climatic data (Livio Regano, personal communication). These possible troughs were then verified by inspection of weather maps produced by the Australian Bureau of Meteorology and published daily in 'The Australian' newspaper. In particular, troughs producing a north-south airflow were examined because anecdotal evidence suggests that there is a north to south progression of disease outbreaks over a period of about 30 days.

PCR testing of plant material and insects for phytoplasmas

A lobe was cut from each of six or seven immature leaves from 60 plants outside the enclosure at Site 2 each week between September 1996 and May 1997. Similarly, lobes were cut from each plant in the Site 2 enclosure on 15 January 1998. Leaf samples were frozen at –20°C until processed for DNA extraction. One-third of each of the six or seven lobes from each plant sample was used for DNA extraction. DNA was also extracted from leafhopper and planthopper specimens of species that occurred in sufficient numbers at Sites 1 and 2. At Site 3, immature lobes were sampled from ten selected plants outside the enclosure every 2 weeks over a 19-month period and inside the enclosure at the end of the experiment. Samples were either stored at –70°C or the DNA extracted within 2 days and stored at –20°C. The CTAB method of Doyle and Doyle (1990) was used to extract DNA from the midribs of leaf lobes and from insect bodies. DNA extracts were amplified by PCR as fully detailed by Guthrie *et al*. (1998) using the phytoplasma-specific primers, P1 (Deng and Hiruki 1991) and P7 (Schneider *et al*. 1995), and the stolbur group-specific primers, fU5 (Lorenz *et al*. 1995) and AGY2 (Gibb *et al.* 1998).

Results

Total disease incidence

Dieback incidence was high (68–85%) whereas mosaic incidence was low (5–8%) at all three sites (Table 1). Yellow crinkle incidence was variable with 27% of plants affected at Site 1 and less than 5% at Sites 2 and 3.

Of the 60 female papaya plants sampled outside the enclosure at Site 2 and tested by PCR from September 1996 until May 1997, 15 had dieback, none had yellow crinkle and seven had mosaic (Guthrie *et al*. 1998). For the duration of

Table 1. The number of plants with dieback, yellow crinkle and mosaic symptoms that were removed, and the incidence of these diseases between October 1994 and March 1998 at Site 1, May 1996 and February 1998 at Site 2, and between May 1996 and December 1997 at Site 3

Site[A]	Total no. of plant positions	Dieback			Yellow Crinkle			Mosaic		
		No. of plants affected	No. of positions affected	Incidence (%)	No. of plants affected	No. of positions affected	Incidence (%)	No. of plants affected	No. of positions affected	Incidence (%)
1	1441	1546	976	68	451	391	27	124	116	8
2	854	840	602	70	39	39	5	43	43	5
3	65	55	55	85	1	1	2	3	3	5

[A]Sites 1 and 2 had multiple plants per position and Site 3 had one plant per position.

the trials, no plants within the enclosures at either Sites 2 or 3 displayed phytoplasma disease symptoms, despite a major dieback episode at both locations in the spring of 1997 with more than 65% of plant positions affected (see Fig. 4). Phytoplasma DNA was not detected by PCR in any leaf samples taken from plants within either of the enclosures using either the P1/P7 or the fU5/AGY2 primers.

There were 755 plant positions (52%) at Site 1 and 192 positions (22%) of the 854 plant positions outside the enclosure at Site 2 with at least one healthy plant on 19 February 1998. There were only three plant positions (5%) with at least one healthy plant on 10 December 1997 at Site 3.

Records of regeneration from Sites 1 and 2 indicate that the losses due to dieback cannot be compared directly with those caused by yellow crinkle and mosaic. Plants with dieback symptoms can be successfully ratooned without the reappearance of symptoms or phytoplasma in regrown plants (Guthrie *et al*. 1998). Plants with yellow crinkle and mosaic symptoms that were ratooned usually showed symptoms in the regrowth and tested positive for phytoplasma DNA (Guthrie *et al*. 1998).

Spatial distribution of diseases

At Site 1, there was no obvious relationship between distribution of any of the three diseases and any known physical characteristic of the trial site (Fig. 1). Characteristics examined included slope, wind direction and edge effects. This was also the case when disease distribution was considered on a yearly basis (July–June). There was no significant ($P > 0.05$) effect of blocking, hybrid or mulching treatment on the proportion of plant positions with dieback, yellow crinkle or mosaic in the period to ratooning. Similarly, blocking, hybrid and mulching treatment did not affect the proportion of plant positions with dieback and yellow crinkle in the post-ratooning period. However, there was a significant ($P < 0.05$) difference among hybrids and between mulching treatments for the proportion of plant positions with mosaic. The relevance of this difference is doubtful given the overall low incidence (<5%) of mosaic. The distribution of the diseases at Site 2 was also unrelated to site characteristics (Fig. 2), with infections randomly spread throughout the trial site.

Temporal disease incidence

Temporal incidence of each of the three diseases varied throughout the survey periods at Sites 1 and 2 (Figs 3 and 4). Peaks in disease levels appeared suddenly and lasted for periods of 4 to 8 weeks for dieback and 8 to 12 weeks for yellow crinkle. Incidence of plants with mosaic was much less than dieback and yellow crinkle with infections occurring over longer periods.

Outbreaks of dieback at Sites 1 and 2 were seasonal, mainly occurring in October/November (Figs 3 and 4). The

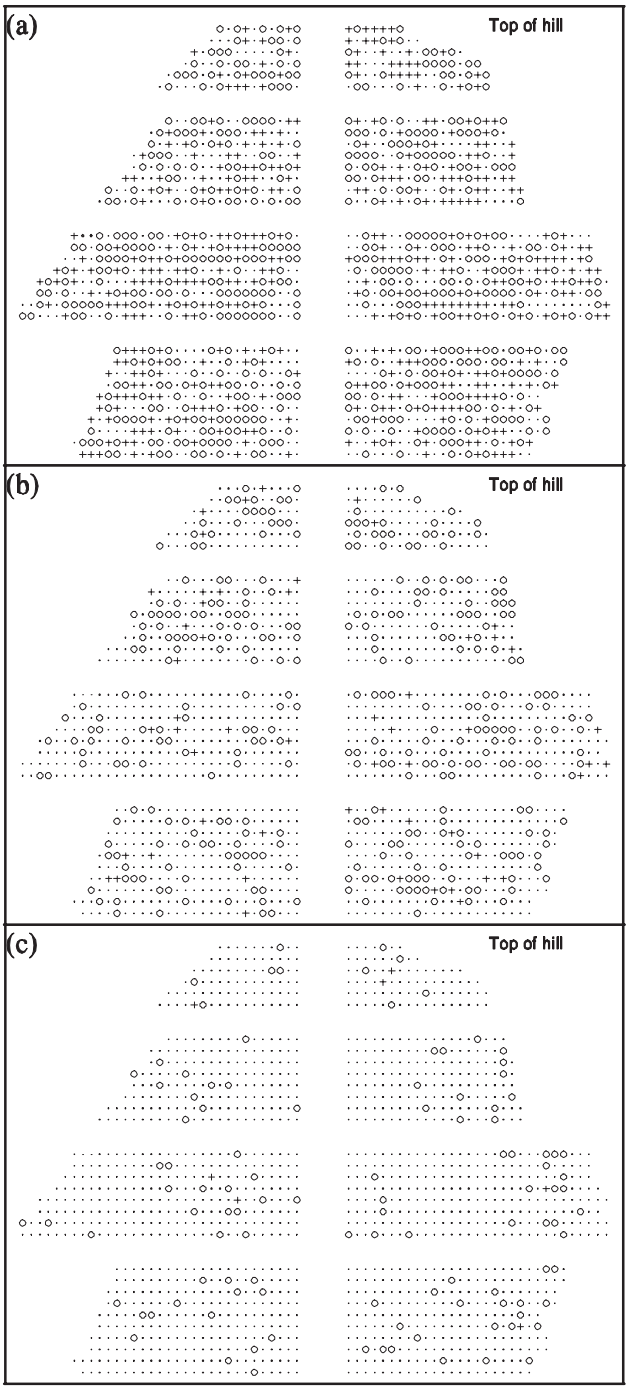

Fig. 1. Cumulative spatial distribution of (*a*) dieback, (*b*) yellow crinkle and (*c*) mosaic between October 1994 and March 1998 at Site 1 (Yarwun). Disease incidence at a plant position is indicated as did not occur (·), occurred once at a plant position (○) or occurred more than once at a plant position (+).

first occurrence of dieback at Site 3 was in mid-November 1996, 7 months after establishment of the trial, when two plants were affected. Another 11 plants were affected by dieback between January and May 1997 with no further

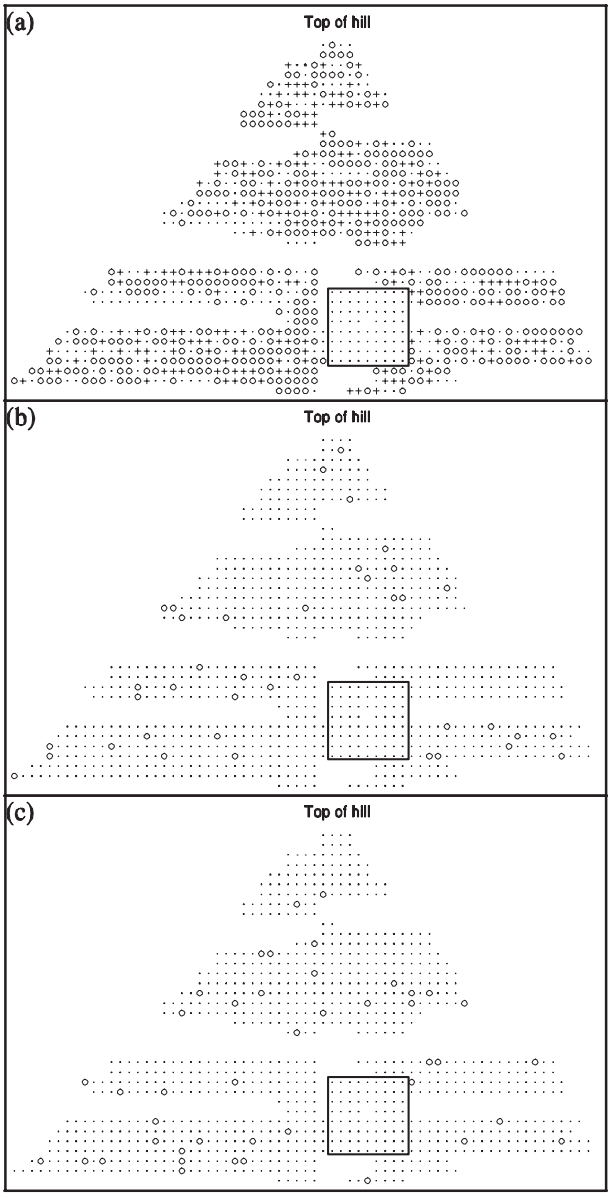

Fig. 2. Cumulative spatial distribution of (*a*) dieback, (*b*) yellow crinkle and (*c*) mosaic between May 1996 and February 1998 at Site 2. Disease incidence at a plant position is indicated as did not occur (·), occurred once at a plant position (○) or occurred more than once at a plant position (+). The small, boxed area indicates the enclosure used to exclude potential phytoplasma vectors.

occurrences until October 1997 when 42 plants were affected during October and November. Dieback outbreaks occurred in October/November for three (1994, 1995 and 1997) of the four survey years for Site 1 (Fig. 3) and for one (1997) of the two survey years for Sites 2 (Fig. 4) and 3. The outbreak of dieback in 1997 began in early- to mid-October at Sites 1 and 2 whereas it began in late-October to early-November at Site 3, the southern-most site. Only a small number of plants were affected by dieback during these months in 1996 at all sites (2, 38 and 2 plants for Sites 1, 2 and 3 respectively). An outbreak of dieback was also recorded in April/May 1995 at Site 1 (Fig. 3), but this was not characteristic of other seasons or sites.

Although the number of plants affected by yellow crinkle was less than for dieback, seasonal patterns in yellow crinkle outbreaks were evident (Figs 3 and 4). Outbreaks of yellow crinkle occurred between November and January in 1994–95 and between January and March in 1997 and 1998 at Site 1, whereas in 1995–96 only low numbers were recorded (Fig. 3). At Site 2, a similar pattern was observed with minor outbreaks in January/February 1997 and 1998 (Fig. 4). Only one plant developed yellow crinkle symptoms at Site 3.

Overall numbers of mosaic affected plants were less than for yellow crinkle and substantially less than for dieback. Although seasonal patterns were less evident for mosaic compared with dieback and yellow crinkle, the disease did tend to occur in periods of 3–6 months. In most years at Site 1, a mosaic outbreak occurred between January and June although it tended to be later in 1997. At Site 2, no mosaic was observed in 1996, whereas in 1997 an outbreak peaked in June/July. At site 3, only three plants showed mosaic symptoms during the entire monitoring period.

Insects

Seven species of planthopper and 13 species of leafhopper were collected from Sites 1 and 2 at Yarwun (Tables 2 and 3, respectively). Although species were numerous, individuals of each species were few. The greatest mean number of individuals collected for a species during any sampling was one per tree for *Zygina honiloa* at Site 2 in late September 1996. Further, all specimens were adults; nymphs were never collected. Three leafhoppers, *Orosius* spp., *Austroasca alfalfae* and *Z. honiloa*, occurred in 'reasonable' numbers (arbitrarily set at ≥ three specimens in any 1 week) at Site 1 within the 5 weeks prior to the peak of the dieback outbreak in early November 1995. A minor dieback outbreak occurred at Site 2 in October/November 1996 and one species, *Z. honiloa*, was collected in 'reasonable' numbers (≥ three specimens) within the 5 weeks prior to the outbreak. Only one hopper specimen, the cicadellid, *Z. honiloa*, was caught during the monitoring period inside the enclosure at Site 2. A rip in the ceiling, which may have allowed the hopper to enter, was noticed at the same time and immediately repaired. Phytoplasma DNA was not detected by PCR in any of the insects using either set of primers (P1/P7; fU5/AGY2).

Weather

No direct associations between disease incidence and rainfall or temperature (Figs 3 and 4), pressure or wind direction (data not shown) were evident at Sites 1 and 2. There was also no evidence to suggest that dieback outbreaks were associated with long dry periods followed by heavy rainfall.

Fronts and troughs were common throughout the year but particularly so in the months leading up to dieback outbreaks (July to October). Based on the observation that there tends to be a north-to-south progression of dieback outbreaks, troughs that produce air flow down the eastern coast of Queensland were selected. The timing of these troughs during September and October is marked on Figs 3 and 4. This form of weather system occurred two or three times during these 2 months in all 4 years although a dieback outbreak was not recorded in 1996.

Discussion

Insect vectors

Although it was not possible to conclusively determine the vector/s of dieback, yellow crinkle or mosaic from our data, there is evidence that insects, particularly planthoppers and leafhoppers, are the most likely vectors. Phytoplasmas were never detected in plants in the enclosures at Sites 2 and 3 either by PCR testing or by visual symptoms whereas up to 78% (Site 2) and 95% (Site 3) of plant positions at these sites were affected in the immediately surrounding area. Evidence from phytoplasma disease studies in other crops indicates that planthoppers and leafhoppers are the most likely transmission agents (Lee and Davis 1992). Insects in general, and planthoppers and leafhoppers in particular, were present in only small numbers and only as adults during or just before sampling when symptoms of the three diseases appeared (Tables 2 and 3) suggesting that the insects may not breed on papaya. The distribution of diseased plants at Sites 1 and 2 was widespread and could not be related to any environmental factor. This suggests that an insect vector fed and presumably transmitted the phytoplasma and then either

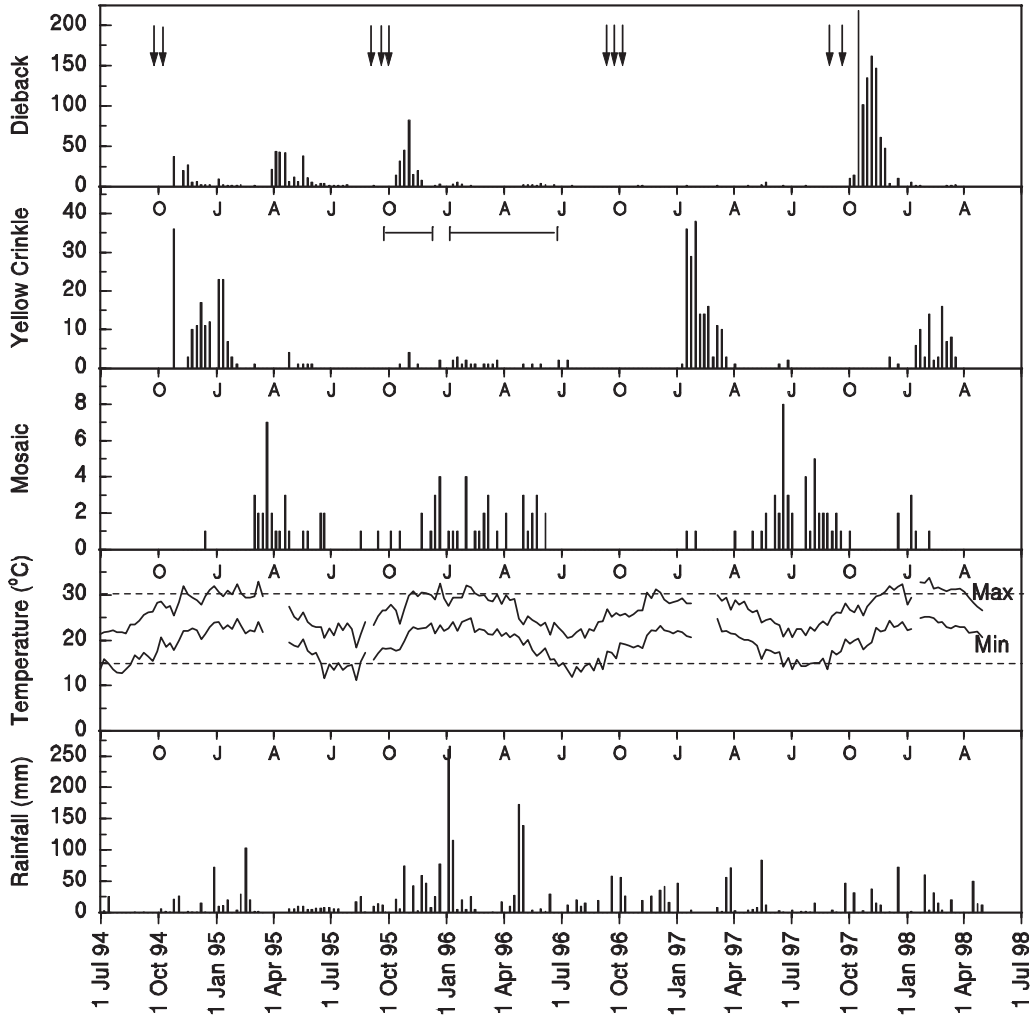

Fig. 3. Weekly incidence of plants exhibiting dieback, yellow crinkle or mosaic disease symptoms at Site 1 and mean weekly maximum and minimum temperatures and weekly rainfall at Gladstone, the closest site with complete climatic data. The vertical arrows indicate the presence and time of troughs that produce air flow down the eastern coast of Queensland. The horizontal bars in the yellow crinkle graph indicate the vacuum sampling periods for insects. Note that the scales for each disease are different and represent the number of affected plants.

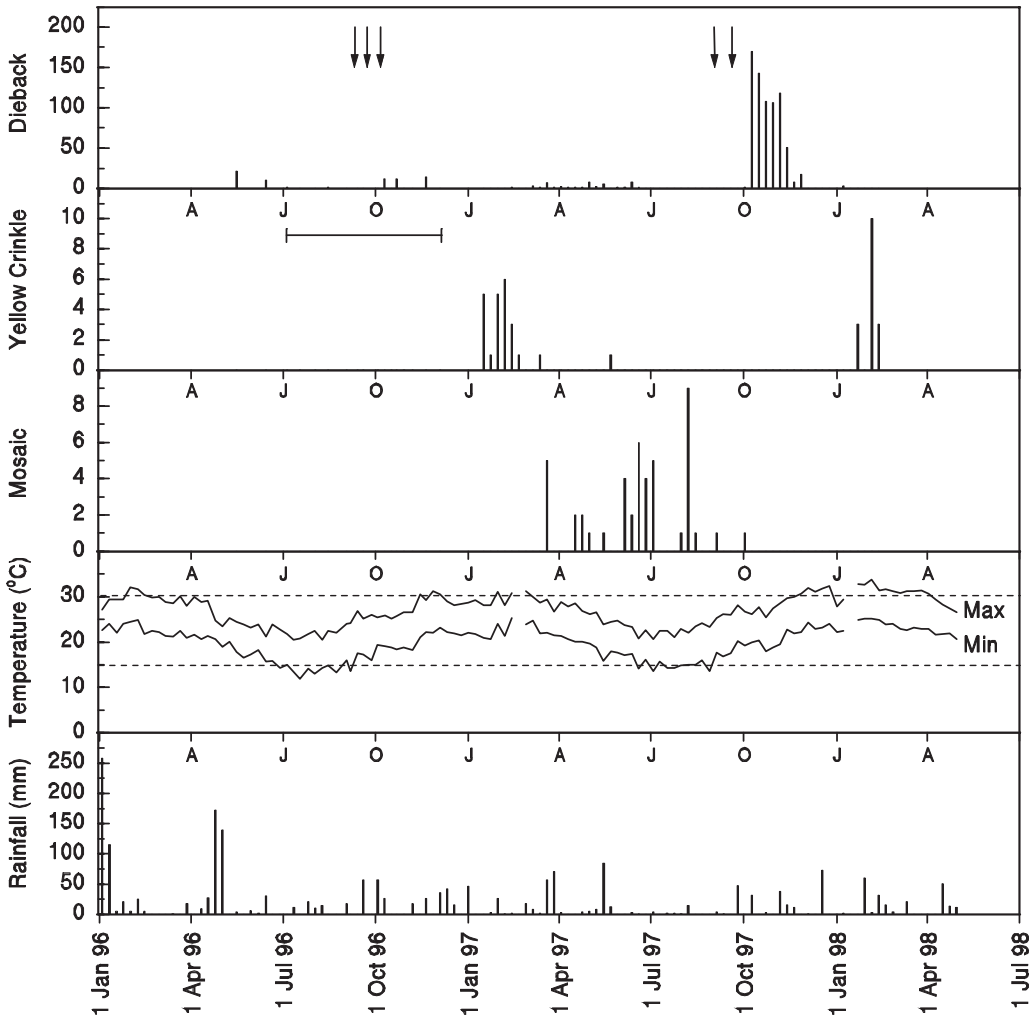

Fig. 4. Weekly incidence of plants exhibiting dieback, yellow crinkle and mosaic disease symptoms at Site 2 and mean weekly maximum and minimum temperatures and weekly rainfall at Gladstone, the closest site with complete climatic data. The vertical arrows indicate the presence and time of troughs that produce air flow down the eastern coast of Queensland. The horizontal bar in the yellow crinkle graph indicates the vacuum sampling periods for insects. Note that the scales for each disease are different and represent the number of affected plants.

moved out of the crop or possibly moved some distance from the first plant.

Knowledge of the feeding sites of papaya hoppers may indicate candidate vector species. A literature survey failed to identify the feeding sites of any of the hopper species that we collected from papaya (Tables 2 and 3). However, several species closely related to papaya hoppers are known to feed on phloem. Most hopper species collected from papaya were either in the planthopper family, Delphacidae or the leafhopper subfamily, Deltocephalinae. All eight species of these two taxa whose feeding sites have been recorded in the literature feed on phloem, although some also feed on xylem (Table 4). Xylem only was recorded as the feeding site for one species of *Oliarus*, a genus in the family Cixiidae that we also collected. This contrasts with the finding that *Oliarus atkinsoni* is the vector of *Phormium* yellow leaf phytoplasma (Liefting *et al.* 1997) and presumably feeds on phloem. For two of the five investigated typhlocybine species, phloem as well as parenchyma were recorded as feeding sites, whereas only parenchyma was fed on by the remaining three species. The feeding sites of species from the remaining families and subfamilies listed in Tables 2 and 3 are unknown. Based on the available feeding site information, all of the papaya hopper species that we collected are potential vectors of papaya phytoplasmas.

The incidence of plants with dieback symptoms was greater than yellow crinkle and mosaic. The vector of dieback was presumably greater in number, stayed feeding

Table 2. Total monthly collections of planthopper and leafhopper species from papaya at Site 1 (Yarwun) sampled weekly between 26 September 1995 and 20 June 1996. The individual sample collections for September and October are in bold. The total number of each species and collection frequencies (the number of weekly samples in which a species was collected) are also shown. The number of specimens that were PCR-tested for phytoplasma using primers P1/P7 and fU5/AGY2 is shown

	26 Sept. 1995	**04 Oct. 1995**	**12 Oct. 1995**	**26 Oct. 1995**	Nov. 1995	Jan. 1996	Feb. 1996	Mar. 1996	Apr. 1996	May 1996	June 1996	Total insects	Collection freq.	No. insects tested for phtoplasma[A]
Superfam. Fulgoroidea (Planthoppers)														
Fam. Cixiidae														
Oliarus sp. *lubra* group			**2**									2	1	1
Fam. Delphacidae														
Cemus koebelei (Kirkaldy)												0	0	0
Corbulo sp.								4				4	2	2
Sardia rostrata Melichar								1				1	1	0
Sogatella longifurcifera (Esaki & Ishihara)[B]							4	4				8	3	5
Toya dryope (Kirkaldy)[B]				**1**	1		2	1	1		1	7	6	3
Fam. Flatidae														
Dascalina aegrota Melichar												0	0	0
Not able to be identified					1		1					2	2	0
Superfam. Cicadelloidea (Leafhoppers)														
Fam. Cicadellidae														
Subfam. Agalliinae														
A*ustroagallia torrida* (Evans)												0	0	0
Subfam. Deltocephalinae														
*Balcluth*a*y* spp.							5	13	3			21	4	6
B. sp.1								1				1	1	0
B. sp.2								1				1	1	0
B. sp.3									1			1	1	0
B. sp.4									1			1	1	0
Cicadulina bimaculata (Evans)			**1**									1	1	0
Hishimonus sp.							1					1	1	0
Orosius spp.[C]	**2**		**3**									5	2	3
Subfam. Typhlocybinae														
Austroasca alfalfae (Evans)	**3**	**1**	**1**					4	5			14	5	8
Zygina honiloa (Kirkaldy)	**23**	**20**			2	1						46	5	8
Subfam. Xestocephalinae														
Xestocephalus tasmaniensis (Evans)												0	0	0
Not able to be identified	**1**		**1**		2		2	2	1			9	7	0

[A]Specimens were tested by PCR; specimens tested include those collected during, as well as some outside, the survey period.
[B]Female *Sogatella* sp. and *Toya* sp. are very difficult to identify to species. Because the *Sogatella* and *Toya* females were associated with *S. longifurcifera* and *T. dryope* males, respectively, it was assumed that the females were the same species as the males.
[C]Two *Orosius* specimens were collected on 26 September, one was confirmed to be *O. canberrensis* and the other, *O. argentatus* by M. Fletcher. The three specimens collected on 12 October were identified to genus only, before being PCR-tested for phytoplasma.

longer in the crop and/or was a more efficient vector than the vectors of phytoplasmas associated with the other two diseases. The difference in occurrence times of the three diseases indicates that there are probably different vectors for each disease even when the time from infection (i.e. first detected by the PCR test) to the expression of disease symptoms is considered. The time from infection to expression of symptoms has been shown to be 1–2 weeks for dieback, 9–13 weeks for yellow crinkle and up to 11 weeks for mosaic (Greber 1966; Guthrie *et al*. 1998). The likely vector of yellow crinkle, *O. argentatus* (Hill 1943) was among the five *Orosius* specimens collected in September/October 1995 at Site 1. It was collected during the 9–13 week period before yellow crinkle symptoms were

Table 3. Total monthly collections of planthopper and leafhopper species from papaya at Site 2 (Yarwun) sampled weekly between 5 July and 4 December 1996. The individual sample collections for September and October are in bold. The total number of each species and collection frequencies (the number of weekly samples in which a species was collected) are also shown. The number of specimens that were PCR-tested for phytoplasma using primers P1/P7 and fU5/AGY2 is shown

	July 1996	Aug. 1996	**02 Sept. 1996**	**13 Sept. 1996**	**19 Sept. 1996**	**27 Sept. 1996**	**10 Oct. 1996**	**17 Oct. 1996**	Nov. 1996	Dec. 1996	Total insects	Collection freq.	No. insects tested for phtoplasma by PCR
Superfam. Fulgoroidea (Planthoppers)													
Fam. Cixiidae													
Oliarus sp. *lubra* group											0	0	0
Fam. Delphacidae													
Cemus koebelei (Kirkaldy)			**1**								1	1	2
Corbulo sp.						**1**					0	0	0
Sardia rostrata Melichar											0	0	
Sogatella longifurcifera (Esaki & Ishihara)[A]			1								1	1	9
Toya dryope (Kirkaldy) [A]											0	0	0
Fam. Flatidae													
Dascalina aegrota Melichar										1	1	1	0
Superfam. Cicadelloidea (Leafhoppers)													
Fam. Cicadellidae													
Subfam. Agalliinae													
A*ustroagallia torrida* (Evans)						**1**		**1**			2	2	0
Subfam. Deltocephalinae													
Balclutha spp.	1	2									3	2	4
B. sp.1											0	0	0
B. sp.2											0	0	0
B. sp.3											0	0	0
B. sp.4											0	0	0
Cicadulina bimaculata (Evans)		1					**1**				2	2	2
Hishimonus sp.											0	0	2
Orosius spp.											0	0	0
Subfam. Typhlocybinae													
Austroasca alfalfae (Evans)	7	5				**2**					14	5	17
Zygina honiloa (Kirkaldy)			**1**	**2**	**2**	**11**			2		18	6	17
Subfam. Xestocephalinae													
Xestocephalus tasmaniensis (Evans)						**1**					1	1	0

[A]Female *Sogatella* sp. and *Toya* sp. are very difficult to identify to species. Because the *Sogatella* and *Toya* females were associated with *S. longifurcifera* and *T. dryope* males, respectively, it was assumed that the females were the same species as the males.

observed in plants during December 1995 and January 1996 (Fig. 3). However, *Orosius* spp. were not collected prior to other yellow crinkle outbreaks.

Because low numbers of hopper species were collected and PCR-tested for phytoplasmas, the chances of detecting a vector were presumably small. The insects had been stored frozen without a protective medium for at least 1 year prior to testing. This procedure may have been a reason why phytoplasma was not detected in the insects. However, researchers working on the *Phormium* yellow leaf phytoplasma (closely related to papaya dieback; White *et al*. 1998) still detected phytoplasma a year or more after infected insects had been placed live in plastic tubes and subsequently frozen (L. Liefting, personal communication).

Table 4. Literature references[A] to feeding sites of planthoppers and leafhoppers (see Tables 2 and 3 for genera/species collected from Yarwun papaya)

Subfamily, family, species	Feeding sites[B]	Reference
	FULGOROIDEA	
Cixiidae		
Oliarus sp.	X, endoderm	*Aust. J. Entomol.* (1996) **35**, 115–118
Delphacidae		
Nilaparvata lugens	P	RAE 72, 7813; RAE 74, 5082
Nilaparvata lugens	X, P	RAE 81, 7890
Perkinsiella saccharicida	P	RAE 67, 171
P. vitiensis	P	RAE 67, 171
Sogatella furcifera	P	RAE 72, 7813; RAE 82, 11067
	CICADELLIDAE	
Deltocephalinae		
Cicadulina mbila	P	RAE 84, 3585
Nephotettix virescens	P, X	RAE 79, 489; RPP 70, 2018; RAE 72, 7813; RAE 74, 1314; RAE 80, 3480; RAE 73, 1425; RAE 76, 3233; RAE 76, 2553
N. nigropictus and N. malayanus	P	RAE 80, 3480
Typhlocybinae		
Empoasca fabae	P, mesophyll	RAE 78, 8541
E. decipiens	Stem & palisade	RAE 69, 4657
Eupteryx atropunctata	Parenchyma, P	RAE 69, 4657
Ribautiana ulmi	Palisade mesophyll	RAE 74, 2057
Typhlocyba pomaria	Palisade cells	RAE 84, 8719

[A]Originals not seen, data sourced from CAB Abstracts; RAE = Review of Applied Entomology; RPP = Review of Plant Pathology. Reference given as volume and abstract number.
[B]P, phloem; X, xylem.

It is possible that only a small number of insects in a population actually become infected with the phytoplasma.

Disease incidence was negligible in July 1996 to January 1997. This was also the year when the early spring rainfall was the greatest. In contrast, outbreak years occurred when spring rainfall was low (Figs 3 and 4). The papaya plants were irrigated and grew slowly in winter and actively in spring so they were green every year. Meanwhile, in the dry years, the surrounding countryside, other than the savanna woodland trees and scattered shrubs, was mainly dry brown herbs and grasses. It is proposed that larger outbreaks occur in drier years when the surrounding vegetation is dry and the relatively large, green papaya plantations become attractive to leafhoppers and planthoppers. It follows that, if the surrounding vegetation is dry, it is more likely that leafhoppers and planthoppers arrive in the papaya plantations after flying from more distant wetter areas where the unknown hosts harbour dieback, yellow crinkle and mosaic phytoplasmas. Pasture/herb growth models could be used to relate growth of surrounding vegetation to rainfall as these models make use of soil type, plant species and transpiration data to estimate seasonal growth (Hunter and Elder 1999). Pasture growth models may provide more insight than rainfall alone since the same rainfall amount in winter will provide plant growth for longer than in summer.

Troughs/fronts offer the disturbed weather conditions that move insects into the upper atmosphere where they can be transported large distances (Thresh 1983; Elder 1997). Troughs in which there was a north-south air movement occurred in September/October in the 4 years of the study (Figs 3 and 4). These air movements could move leafhoppers and planthoppers from, for example, the wet tropics in the Mackay district (21°S, 149°E) into the Yarwun district, approximately 350 km to the south, and explain the observed north-south progression of phytoplasma outbreaks over a period of about 30 days. However, the diseases did not occur with every front, for example September 1996 (Figs 3 and 4). Presumably disease symptoms will appear after the passage of a trough only if insect vector/s carrying the appropriate phytoplasma are available and the surrounding area is dry.

Insect vector/s and alternative plant hosts need to be identified. The insect sampling technique used in this study was time consuming because samples from each tree were kept separately. To improve catch rates and number of vectors collected, any further studies should vacuum-sample as many trees as possible. This could be done by bulk sampling 50 trees for 1 min each. In addition, vacuum sampling could be more efficient for some hopper species than others. For example, species confined to young leaves within the tree crown may not be easily picked up using this

method. A combination of in-crop methods such as vacuum sampling, light traps and sticky traps may more effectively sample the papaya hopper fauna.

Temporal disease incidence

Despite extensive PCR testing within, and close to, papaya plantations alternative plant hosts for dieback have not been found (Guthrie *et al*. 1998). The papaya dieback phytoplasma is closely related to the *Phormium* yellow leaf and Australian grapevine yellow phytoplasmas and the three have been grouped in the taxon '*Candidatus* Phytoplasma australiense' (White *et al.* 1998). A strain of phytoplasma included within this group has been found in strawberry in Queensland and in garden bean in Western Australia (Padovan *et al*. 1998, 2000; Schneider *et al.* 1999). It has not been determined if this strain is the same as the one causing dieback. The sudden outbreaks of dieback in papaya plantations after periods free of dieback symptoms and the short time (1–2 weeks) between when phytoplasma DNA can be detected and when symptoms appear, suggest that the disease organism is delivered from areas some distance from papaya crops. Dieback outbreaks are thought to start in north Queensland (Mareeba to Innisfail) and progressively appear within 1–2 weeks in central Queensland (Mackay to Yarwun) and finally in south-eastern Queensland (Redland Bay). This was observed in October/November 1997 when the dieback outbreak occurred at Sites 1 and 2 (Yarwun; Figs 3 and 4) and then a few weeks later at Site 3 (Redland Bay).

Dieback incidence also varied from north to south. Outbreaks are uncommon in north Queensland (17°S, 146°E) and occur mainly in the Kurramine Beach district. During the 4-year period of this study, significant dieback outbreaks occurred in 3 years out of 4 in October to December and once in 4 years in March to May at the two central Queensland study sites. This is more frequent than previously observed from occasional records for central Queensland which indicated that there were major outbreaks in 1922, 1972 and 1995 (Drew and Considine 1995). In some areas of southern Queensland [e.g. Bundaberg (25°S, 152°E) and Redland Bay] dieback affects most plants in a crop each year (Drew and Considine 1995).

Plant losses

Under conditions where there are initially three to five plants at each plant position and where plants with dieback symptoms are cut back to 30–75 cm high stumps as soon as the symptoms are apparent, 37% of plants can be affected, and the crop will still yield satisfactorily (Elder *et al.* 2000). The cutting back of plants results in a loss of about 6 months production (Elder *et al*., 2002) but regrowth does not show symptoms. This suggests that the plant is a poor host of the phytoplasma, as reinfection of new growth does not occur (Guthrie *et al*. 1998). Although plant losses due to yellow crinkle and mosaic are generally low (< 5%), they can be quite considerable as observed at Site 1 (27% and 8%, respectively). Guthrie *et al*. (1998) observed that regrowth material of ratooned yellow crinkle and mosaic plants exhibited symptoms and were positive for phytoplasma DNA by PCR. This indicates that plants showing symptoms of these diseases do not recover even if cut back.

Conclusions

It is hypothesised that the phytoplasmas causing dieback and yellow crinkle diseases are transmitted by leafhoppers or planthoppers. The insects are transported into the district by weather troughs/fronts that involve a north-to-south air movement generally occurring in spring (September–November). Dieback and yellow crinkle outbreaks occur in years with dry conditions in late winter and early spring, as the insects are attracted to the green papaya plantations surrounded by unattractive dry, brown vegetation. The insects do not remain on the papaya plants for long as papaya is not a favoured host.

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