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Presence of a Clostero-like Virus and a Bacilliform Virus in Pineapple Plants in Australia

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Abstract

When sap of asymptomatic or mealybug wilt–affected pineapple plants of the Smooth Cayenne group was negatively stained and examined in an electron microscope, clostero–like virus particles were occasionally seen. However, numerous clostero–like virus particles and occasionally some bacilliform particles were seen in partially purified preparations from both asymptomatic and wilted pineapple leaves. An antiserum, made by injecting partially purified preparations of clostero–like particles into a rabbit, trapped and decorated the clostero–like particles. Using this antiserum, the clostero–like particles (*c.* 1700 –1900×12 nm) were found in almost all plants tested of Smooth Cayenne selections C10, C13, C30 and F–180, the hybrid cv. 53–116 and a selection of the rough leaf Queen group. The particles were more readily trapped from extracts of roots of hybrid cv. 53–116 and Smooth Cayenne selection C10 than from leaves, crowns and fruits. They were not detected in seedlings of a cross between a Queen selection and the Smooth Cayenne selection C10. The clostero–like particles are similar to those reported to occur in pineapple plants in Hawaii and South Africa. This is the first report of their occurrence in Australia. Trapping and decoration tests of particles in pineapples in quarantine from Brazil, France, Malaysia and Taiwan indicated that a similar clostero–like virus occurs in all these countries.

The bacilliform particles measured about 133×33 nm. They were trapped and decorated by the Queensland pineapple virus antiserum and also by an antiserum to sugarcane bacilliform badnavirus. They were detected occasionally in various smooth leaf and rough leaf pineapples in north and south Queensland and northern New South Wales. However, in one commercial planting of Smooth Cayenne selection C10 in south Queensland, bacilliform particles were trapped from 29/47 plants. This is the first report of a small bacilliform virus, probably belonging to the badnavirus group, occurring in pineapple plants. The relationship of the clostero–like and bacilliform viruses to yield loss and mealybug wilt in pineapples is unknown.

Keywords: pineapple mealybug wilt, closterovirus, badnavirus, antiserum, immune electron microscopy.

Introduction

Pineapple (*Ananas comosus* (L.) Merr.) is a vegetatively propagated crop which originated in Central/South America and is now widely grown throughout tropical and subtropical regions of the world. Although seedlings can be produced, propagation is normally vegetative by means of the crowns of the fruit, or side branches taken from the stem (Collins 1960).

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Viruses acquired by a vegetatively propagated crop are likely to be retained indefinitely. However, only a few viruses have been reported to infect pineapple. Tomato spotted wilt virus (TSWV) has caused a severe necrotic disease of pineapple in Hawaii (Collins 1960; Py *et al.* 1987), and a rhabdovirus has been associated with a chlorotic leaf streak of pineapple in Brazil (Kitajima *et al.* 1975). In Hawaii, Carter (1951) showed that certain pineapple plants were a source of the factor causing mealybug wilt disease. He first considered that the disease was caused by a mealybug toxin (Carter 1933, 1939), but later speculated that a virus might somehow be involved (Carter 1963).

Recently, a long, flexuous, rod–shaped virus with morphology, size of coat protein and size of genomic RNA and dsRNAs typical of a closterovirus type II has been detected in cv. Smooth Cayenne pineapple plants with mealybug wilt in Hawaii (Gunasinghe and German 1989). Serological tests have shown that this virus is also widely distributed in asymptomatic cultivated pineapple plants in the Hawaiian islands (Ullman *et al.* 1989). Further, heat treatment of infected pineapple crowns in hot water at 40° or 50°C for 30, 60 or 120 min and 60°C for 30 or 60 min resulted in a proportion of plants which were apparently virus–free when examined by ELISA (Ullman *et al.* 1991). The early work on mealybug wilt and this recent work done in Hawaii have been reviewed (German *et al.* 1992).

Mealybug wilt is currently one of the most important field diseases of pineapple in Australia. Outbreaks are controlled by applying insecticidal sprays to kill the mealybugs, but reinfestation, presumably by wind–borne and ant–borne crawler mealybugs, is common (C. Scott, pers. comm.). The association of virus(es) with the disease in Australia has not been reported. This paper gives details of an investigation to determine the virus(es) present in Australian commercial pineapple selections.

Methods

Plant Materials and Maintenance

Twelve asymptomatic commercial pineapple plants and plants with the characteristic mealybug wilt symptoms (German *et al.* 1992) were collected from a pineapple farm near Beerwah, south-east Queensland. Most of their older leaves were removed for virus purification experiments. The plants with remaining young leaves were grown in glasshouses for other studies. Five selections were collected, i.e. Smooth Cayenne selections C10, C13, C30, and F–180 and the hybrid 53–116. Additionally, some crowns from 53–116 and F–180 were collected and also grown in the glasshouses. Other leaf samples (various) were obtained from commercial farms at Moggill, Townsville and Ballina.

Seedling pineapple plants resulting from a cross of a Queen group selection (a rough leaf type) and Smooth Cayenne selection C10 were received from G. Sanewski, Horticulture Division, Department of Primary Industries, and were grown in pots in glasshouses free of other pineapple plants.

Field samples from a germplasm collection and tissue-cultured Smooth Cayenne selection F–180 and the Mordilona group selection Perolera were obtained from the Maroochy Horticultural Research Station. Imported pineapple plants from Brazil, France, Malaysia, Taiwan and USA, growing in post–entry quarantine in Brisbane, were sampled and the leaves were stored in a freezer at −40°C before examination. Sixteen ornamental bromeliads belonging to the genera *Aechmea*, *Billbergia*, *Guzmania* and *Neoregelia* were obtained from Brisbane gardens.

Virus Purification

The purification method most used was a modification of that devised by Gunasinghe and German (1989). Leaves of pineapple plants were cut or pulled from the stem and then were

sliced transversely into 1–2 mm strips. The sliced leaves were either stored at −40°C for up to several months or used directly. For virus purification, the leaf material was put in a plastic container, liquid nitrogen was added and the frozen tissue was immediately placed into a metal blender bowl and blended into powder. Sixty grams of the leaf powder were thawed in 120 mL extraction buffer (0·5 M Tris–HCl, pH 8·4, containing 4% (v/v) Triton X-100 and 0·5% (w/v) Na_2SO_3) and the mixture stirred for 1 h at 4°C. The extract was then filtered through one layer of terylene. The fibre remaining on the terylene was put in a mortar, 15 g acid-washed sand and 120 mL of the extraction buffer added, and the fibre further crushed with a pestle. The second extract was also filtered through the terylene, both filtrates were combined and the extract clarified by centrifugation at 8000 *g* for 10 min. The supernatant was retained and layered over a 5 mL cushion of 20% (w/v) sucrose in extraction buffer and centrifuged at 30 000 rpm for 3 h in a Beckman 30 rotor. The supernatant was decanted and the inside of the tube was rinsed with distilled water and wiped with tissue paper. The pellet was resuspended in 1 mL TM buffer (0·1 M Tris–HCl, pH 8·5, containing 0·01 M $MgCl_2$) for each of 12 tubes. The suspension was stirred overnight at 4°C, then clarified by centrifugation at 8 000 *g* for 10 min and the supernatant was retained. The supernatant was layered over a Cs_2SO_4 step gradient in TM buffer (2 mL of 0·5 g/mL solution over 2 mL of 1 g/mL solution) and was centrifuged at 34 500 rpm for 20 h in a Beckman SW 41 Ti rotor. The gradients were fractionated using a syringe and needle or an ISCO fractionator, and the fractions examined by electron microscopy for virus particles. Virus–containing fractions were diluted with TM buffer and virus collected by ultracentrifugation. The pellet was resuspended in 300 μL TM buffer and clarified by centrifugation at 8000g for 5 min in a microfuge.

Antiserum Production and Testing

The purified virus suspensions were emulsified with an equal volume of incomplete Freund's adjuvant and the mixtures were injected intramuscularly into the back legs of a rabbit using about 0·5 mL of the mixture each time. A total of 19 injections of preparations from six different cv. 53–116 plants and one F–180 plant from Beerwah was given over 23 months. The rabbit was bled eight times at 1 week after the 3rd, 4th, 6th, 10th and 12th injections, 1 and 5 weeks after the 18th and 2 weeks after the 19th injections. The blood was processed according to the method of Ball (1990) and the antisera mixed with an equal volume of glycerol and stored at −40°C.

The ability of the antisera to react was tested in microprecipitin, trapping and decoration tests (Ball 1990). In the microprecipitin tests, serial two-fold dilutions from 1/10 to 1/320 of both the antiserum and the purified virus preparations were prepared and cross mixed in 10 μL volumes. The ability of the antisera to trap virus particles was tested using four-fold dilutions from 1/250 to 1/16 000. The ability to decorate particles was tested using 1/30 and 1/300 dilutions and five-fold dilutions from 1/200 to 1/25 000.

Electron Microscopy and Immune Electron Microscopy (IEM)

Formvar-coated grids (200 mesh) were used to observe the virus particles following negative staining. A drop of the virus preparation was placed on the grid for 1 min, and then touched dry with a filter paper wick. The grid was then washed with 300 μg/mL bacitracin solution and stained with 2% (w/v) phosphotungstic acid (PTA), pH 6·7, containing 250 μg/mL bacitracin solution (Gunasinghe and German 1989). Leaf extracts were prepared by grinding 0·1 g sliced basal leaf tissue in a mortar with liquid nitrogen, thawing the powder in 0·5 mL of the extraction buffer, and microfuging at 8000*g* for 5 min. Formvar-coated grids were also used for observing virus particles decorated with antiserum by the method of Yanagida and Ahmad-Zadeh (1970).

For routine trapping of virus particles by IEM, a method similar to that described by Lockhart (1986) for banana streak badnavirus was used. Antisera to Queensland pineapple virus particles (7th bleed) and to sugarcane bacilliform virus (ScBV-4MX, B.E.L. Lockhart, University of Minnesota) were each used at a dilution of 1/1000 to treat carbon-stabilized collodion-coated grids by floating the grids upside-down on 10 μL drops of the diluted antiserum on parafilm for 30 min at 37°C. The grids were washed 2×10 min in 0·5 mL drops of 0·06 M phosphate buffer, pH 6·8. After touch drying with filter paper, the grids were floated on

10 μL drops of the plant extract for either 3 h at room temperature or overnight at 4°C. The grids were washed with 20 drops of distilled water and stained with 2% PTA, pH 6·7 for 1 min, then drained with filter paper. For routine decorating of the particles, the grids were floated on 10 μL drops of 1/500 antiserum before being stained. All incubations were done on a rocking platform.

Particles were counted in a standard way. The magnification of the electron microscope was adjusted to 30 000 times. Starting from a corner of a grid square and following a clockwise direction around the border, the virus particles observed were counted. One to three grid squares were counted for each grid. All the pineapple samples collected were tested for viruses by IEM, and distribution of the virus in pineapple plants was also determined. For measuring, particles were trapped by IEM from crude sap using Queensland antiserum from the seventh bleed and stained with PTA as described above. Particle dimensions were determined from EM micrographs using the instrument magnification.

IEM Test of Quarantine Samples

Ten quarantined pineapple plants (Table 2) were tested for virus particles by IEM trapping and trapped particles identified by IEM decoration using the Queensland pineapple virus antiserum.

Virus Distribution in Pineapple Plants

Numbers of virus particles in different parts (crowns, green fruits, leaves and roots) of pineapple plants grown in a glasshouse were compared by IEM trapping. Three plants of Smooth Cayenne selection C10 and one plant of cv. 53-116 were each sampled three times. The particles trapped by the Queensland pineapple virus antiserum were counted by the standard method described above.

Results

Presence of Virus-like Particles in Crude and Purified Extracts of Pineapple Plants

When leaf extracts of Smooth Cayenne group pineapple selections were negatively stained without trapping and examined in an electron microscope, flexuous rod-shaped particles resembling those of closteroviruses were occasionally seen (Fig. 1*a*). The particles were observed from plants which had mealybug wilt symptoms (11/18 plants with particles) and also from plants which were asymptomatic (8/16 plants with particles).

Using a modification of the purification method of Gunasinghe and German (1989), flexuous rod-shaped particles *c.* 1200–1600×12 nm were isolated consistently (57 times out of 57 attempts) from the leaves of 17 pineapple plants. The particles were obtained from a total of 11 plants of cv. 53-116, two plants each of C30 and F-180 and one plant each of C10 and C13. The particles were similar in appearance to those of pineapple clostero-like virus (PCV) found in Hawaii (Gunasinghe and German 1989). Particle preparations invariably contained some host contaminants.

On two occasions, bacilliform virus particles 100 to 158 nm long (modal length 133 nm) and 27 to 42 nm wide (modal width 33 nm) from a total of 153 particles were recovered along with PCV-like particles (Fig. 1*b*). These bacilliform particles were found in wilted plants of cv. 53-116 from Beerwah and Smooth Cayenne selection C10 from Moggill.

Production and Testing of Antisera

The antisera produced by injecting a rabbit with up to 19 purified virus preparations varied in their ability to clump purified flexuous rod-shaped particles

in microprecipitin tests. Positive reactions were given only by antisera obtained after six injections. Titres were 1/80 for the antisera and 1/80 for the virus. The antisera did not give a positive reaction when tested with crude sap of pineapple plants at 1/10 to 1/320 dilution.

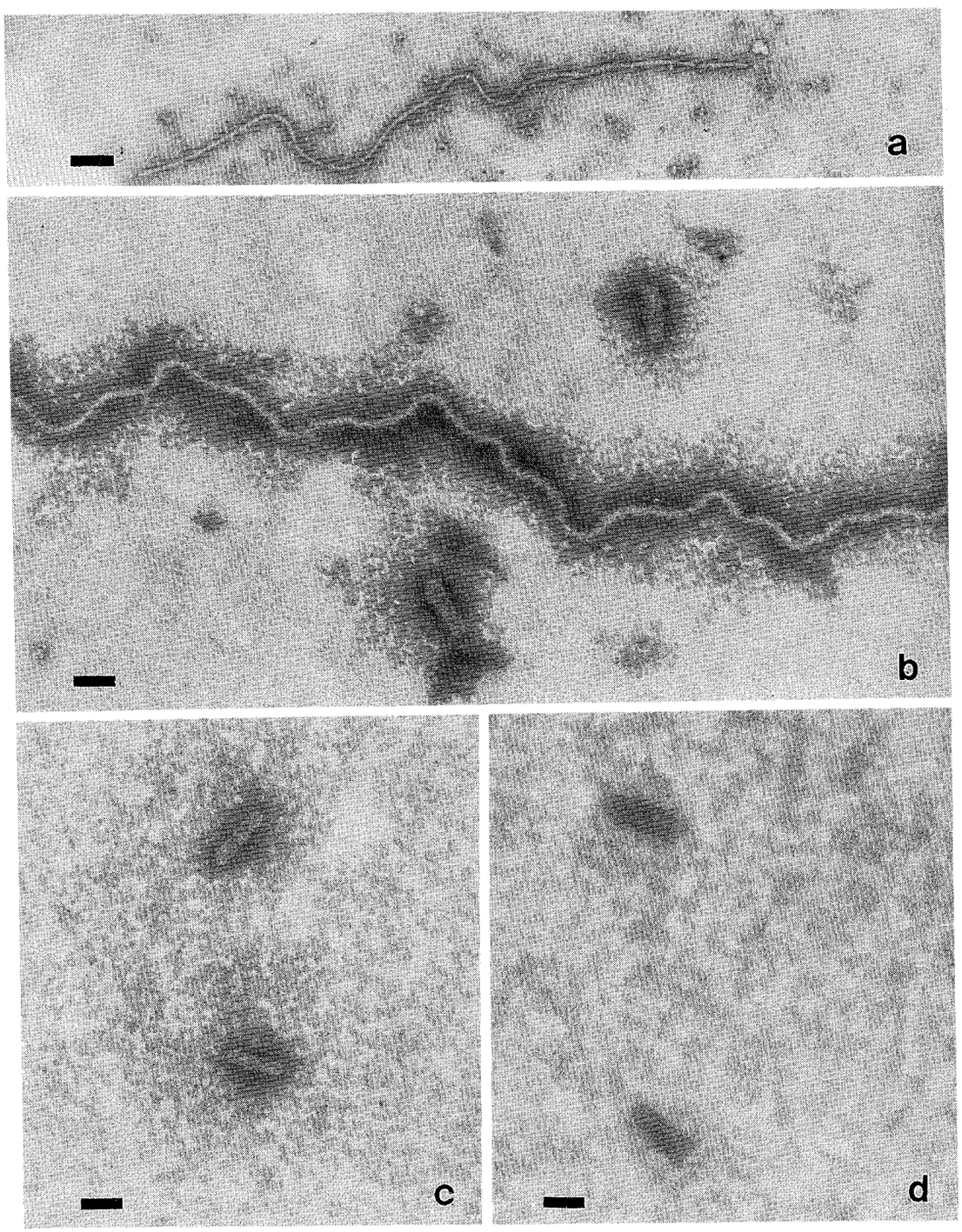

Fig. 1. Clostero-like virus and bacilliform virus particles from Queensland pineapple: (*a*) not decorated; (*b*) decorated by 1:500 dilution of pineapple clostero-like virus antiserum; (*c*) undecorated; (*d*) decorated by 1:500 dilution of sugarcane bacilliform virus (4MX) antiserum. Bar = 100 nm.

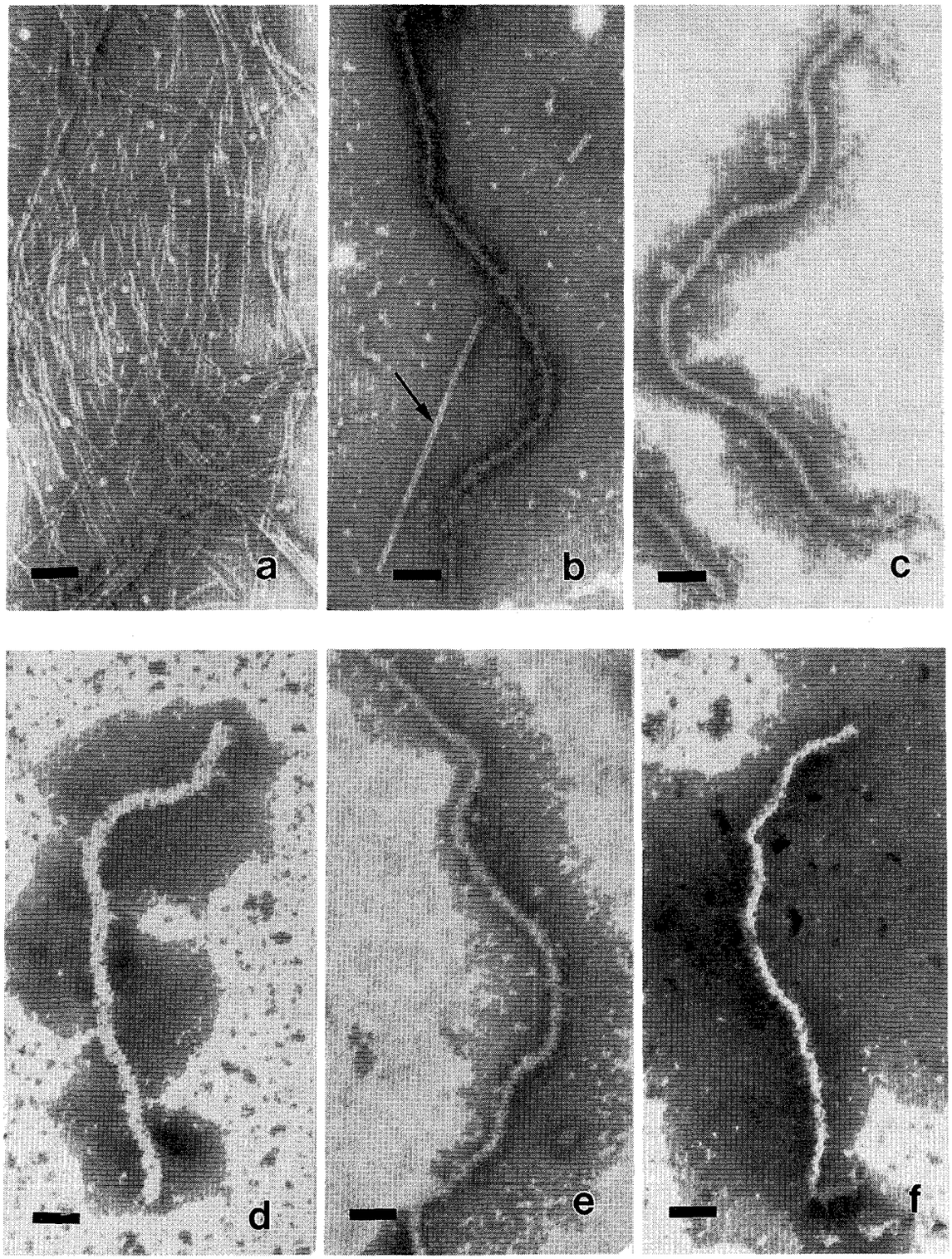

Fig. 2. Clostero-like virus particles from pineapple from five countries: (*a*) purified preparation from Queensland, hybrid cv. 53–116; (*b*) particle from hybrid cv. 53–116 from Queensland with undecorated particle of tobacco mosaic virus (arrow); (*c*) particle from cv. JK-3 (Malaysia); (*d*) particle from cv. Perolera (France); (*e*) particle from cv. F.R.F.-19 (Brazil); (*f*) particle from cv. Tainung 4 (Taiwan). (*b*)–(*f*) decorated with 1:250 dilution of Queensland pineapple virus antiserum. Bar = 100 nm.

Detection of Virus Particles in Pineapple Plants from Queensland using Immune Electron Microscopy

Using clarified crude sap of pineapple leaves, up to 40 times more PCV-like particles were found on Queensland antiserum-coated grids than on untreated grids. These particles were decorated using dilutions of the antiserum from the third bleed of up to 1/5000 and the optimum dilution for decoration was between 1/250 and 1/500 (Figs 1*b*, 2*b*). Flexuous particles were decorated by a 1:30 dilution of antiserum from all bleeds tested (2nd to 8th) and by a 1:300 dilution of the 3rd to 8th bleeds.

Flexuous particles were observed by IEM from pineapple plants of six of the seven selections tested of the Smooth Cayenne group (Table 1). The particles were also detected from most of the other field-grown pineapple clones tested. Of 53 particles measured, 29 had lengths in the range of 1700–1900 nm (Fig. 3). Whether or not they showed mealybug wilt symptoms did not appear to affect the number of particles found. However, no particles were detected by IEM in extracts of 13 seedling pineapple plants of Smooth Cayenne selection C13×Queen which had remained continuously in a glasshouse (Table 1). Similarly, no particles were detected in extracts of five tissue culture plants of Smooth Cayenne selection F-180 and two of Mordilona selection Perolera (Table 1).

Bacilliform virus particles in pineapple were trapped (Fig. 1*c*) and decorated with ScBV-4MX antiserum (Fig. 1*d*) and less strongly with Queensland pineapple virus antiserum (7th bleed, Fig. 1*b*). They were readily detected by IEM in pineapple plants Smooth Cayenne selection C10 collected from Moggill (26/40 crowns and 3/7 leaves of different plants). However, they were detected only occasionally in leaves of other pineapple plants from Townsville, north Queensland (2/2 unidentified smooth leaf, 1/8 Queen), Ballina, NSW (2/2 unidentified smooth leaf), and south Queensland (1/10 cv. 53-116 and 2/17 Queen) (Table 1). In all cases, except for one leaf sample of selection C10 at Moggill, flexuous particles were found with the bacilliform particles.

Distribution of PCV-like Particles in Mature Pineapple Plants

When the numbers of flexuous particles detected by IEM trapping from roots, leaves, fruits and crowns from four glasshouse-grown pineapple plants of Smooth Cayenne C10 or cv. 53–116 were compared (Fig. 4), the roots gave many more virus particles than the leaves, crowns and fruit. The number of particles obtained from the roots was significantly greater (1% F-test) than the number obtained from the other plant parts.

Presence of PCV-like Particles in Pineapple Plants in Quarantine and in Ornamental Bromeliads

Two pineapple plants each from five countries (Brazil, France, USA, Malaysia and Taiwan) growing in a post-entry quarantine glasshouse in Queensland were tested for virus by IEM trapping and decoration tests using the Queensland pineapple virus antiserum. Flexuous virus particles were trapped from 7 of 10 samples (Table 2) and these particles were decorated in IEM (Fig. 2*c–f*). No particles were detected in extracts of the two plants of Spanish Jewel from the USA (Hawaii) or from one of the Tapiricanga plants from France, despite testing three times each.

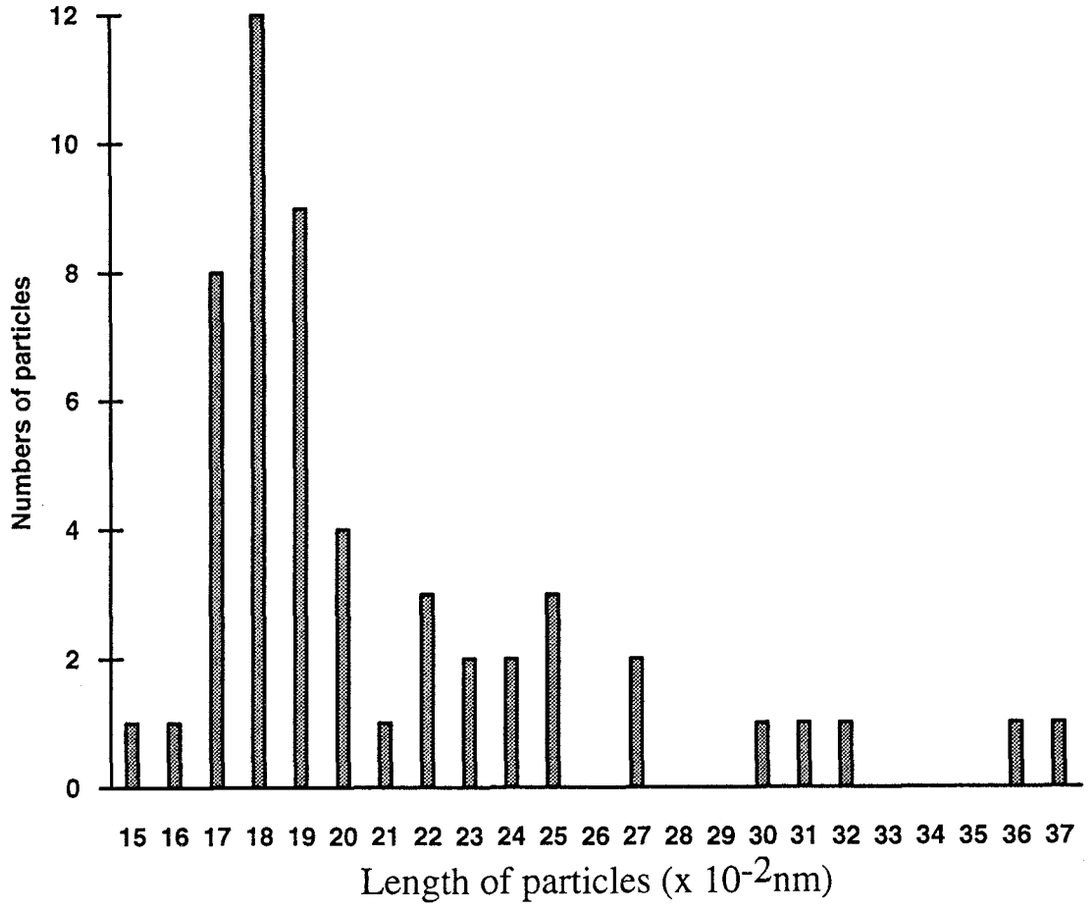

Fig. 3. Histogram showing the lengths of 53 clostero-like virus particles, trapped from crude sap of cv. 53–116 and C10 pineapple by Queensland pineapple virus antiserum.

Sixteen different ornamental bromeliad plants from the genera *Aechmea*, *Billbergia*, *Guzmania* and *Neoregelia* were tested for virus particles by the IEM trapping method. All were negative.

Discussion

The ability to detect PCV-like particles in commercial pineapples of Smooth Cayenne and Queen groups depended on the method employed. Detection was difficult using negative-stain electron microscopy of crude sap extracts. However, the rate of detection was 100% when virus was concentrated from field pineapple plants by a suitable purification procedure. Similarly, flexuous particles were trapped by IEM from about 60% of field plants which were tested once, and from the majority of those initially negative plants which were retested. Whether or not mealybug wilt symptoms were present did not affect the results. We conclude that most if not all commercial pineapples in Queensland are infected with a flexuous PCV-like virus. The failure to detect this virus in some plants

Table 1. Immunoelectron microscopy tests of pineapple and bromeliad samples from Queensland and New South Wales

Plant sampled Selection	Group	No. of samples tested	No. of samples with clostero-like particles	No. of samples with bacilliform particles	Origin of sample[B]
C10	Smooth Cayenne	47	46	29	Moggill
C13	Smooth Cayenne	3	3	0	Beerwah
C30	Smooth Cayenne	2	2	0	Beerwah
C33	Smooth Cayenne	1	1	0	Maroochy
F-7	Smooth Cayenne	1	1	0	Maroochy
F-180	Smooth Cayenne	4	4	0	Maroochy
F-180	Smooth Cayenne	9	8	0	Moggill
F-180 tissue culture	Smooth Cayenne	5	0	0	Maroochy
Philippines unident.	Smooth Cayenne	1	0		Maroochy
Unidentified	Smooth Cayenne	2	2	2	Townsville (NQ)
Unidentified	Smooth Cayenne	3	3	3	Ballina (NSW)
Alexander A	Queen	1	1	0	Maroochy
Alexander B	Queen	1	1	0	Maroochy
Mauritius	Queen	1	1	0	Maroochy
Pace	Queen	2	2	0	Maroochy
Queen	Queen	17	14	2	Maroochy
Queen	Queen	8	8	1	Townsville (NQ)
Perolera	Mordilona	1	1	0	Maroochy
Perolera tissue cult.	Mordilona	2	0		Maroochy
Masmerah	Spanish	1	1	0	Maroochy
Hawaiian 53–116	Intergroup hybrids	10	10	1	Beerwah
Hawaiian 59–656	Intergroup hybrids	1	1	0	Maroochy
Hawaiian 73–50	Intergroup hybrids	1	1	0	Maroochy
C10×Collard	Intergroup hybrids	1	0	0	Maroochy
C13×Alexander	Intergroup hybrids	2	2	0	Maroochy
C13×McGregor	Intergroup hybrids	1	0	0	Maroochy
[A]C13×Queen	Intergroup hybrids	13	0		Maroochy
Alexander×F-180	Intergroup hybrids	1			Maroochy
Alexander×F-200	Intergroup hybrids	1	1	0	Maroochy
Philippiines 72–194	Intergroup hybrids	1	1	0	Maroochy
Bromeliads		16	0	0	Brisbane
Total		160	115	38	

[A] C13×Queen plants were seedlings which had been grown in isolation only under glasshouse conditions.
[B] All samples were from south-east Queensland except NQ, North Queensland; and NSW, New South Wales.

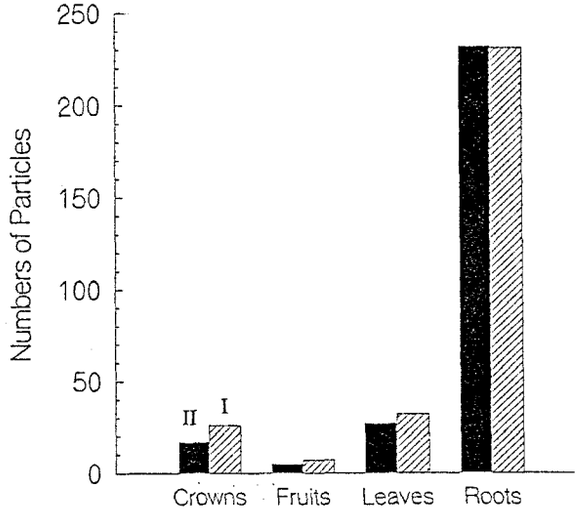

Fig. 4. Histogram showing the number of clostero-like virus particles detected in pineapple plant parts by IEM trapping with Queensland pineapple virus antiserum. Three plants of hybrid cv. 53–116 (I) and one plant of Smooth Cayenne C10 (II) were tested, with samples from each part replicated three times.

may have arisen because of the relatively low concentration of virus particles in pineapples and because the IEM test lacked sufficient sensitivity. It is also likely that the distribution of the virus throughout the plant is uneven and that sampling procedures are inefficient.

Table 2. Detection of clostero-like virus particles in quarantine pineapple plants by immunoelectron microscopy trapping and decoration with antiserum to clostero-like virus from Queensland

Pineapple cultivar/clone	Country of origin	Classification	No. of trapped particles	Decoration reaction
F.R.F.-19	Brazil	*Ananas bracteatus*	5	+
F.R.F.-414	Brazil	*A. bracteatus*	3	+
Perolera	France	*A. comosus*	4	+
Tapiricanga	France	*A. comosus*	0	
JK-2	Malaysia	*A. comosus* hybrid	4	+
JK-3	Malaysia	*A. comosus* hybrid	3	+
Spanish Jewel	USA (Hawaii)	*A. comosus* hybrid	0	
Spanish Jewel	USA (Hawaii)	*A. comosus* hybrid	0	
Tainung-4	Taiwan	*A. comosus* hybrid	4	+
Tainung-6	Taiwan	*A. comosus* hybrid	4	+

The failure to detect PCV-like particles in 13 seedling pineapple plants of the cross Smooth Cayenne selection C10×Queen could indicate that the virus is not seed-borne. Closteroviruses are not known to be seed-borne (Bar-Joseph and Murant 1982). Further tests with seedlings derived from known infected plants are being carried out to substantiate this observation.

The fact that our Queensland pineapple virus antiserum has decorated flexuous rod-shaped virus particles extracted from pineapple plants in South Africa (G. Pietersen, pers. comm.), and growing in quarantine from Brazil, France, Malaysia and Taiwan (Table 2), indicates that this virus is of worldwide geographic distribution. It is likely that the clostero-like virus infecting pineapples in Hawaii described by Gunasinghe and German (1989) is the same as that occurring in Queensland. Since many introductions of vegetative planting material of pineapples from Hawaii and other countries to Australia have been made, the introduction of any latent viruses infecting pineapples in Hawaii and elsewhere is highly likely. Virions of the Hawaiian clostero-like virus are reported to have a modal length of *c.* 1200 nm in purified preparations (German *et al.* 1992). This is within the range of Australian PCV-like particles from purified preparations (1200–1600 nm), but substantially shorter than those trapped directly from sap (modal length 1700–1900 nm). It is likely that the latter estimate is more accurate and that some particle breakage has occurred during the purification procedures. A modal length of 1700–1900 nm would place the clostero-like virus in the closterovirus group C, rather than the capillovirus group (Brunt and Gunasinghe 1990), the closterovirus group A (Coffin and Coutts 1993) or the trichovirus group (Martelli *et al.* 1994).

The discovery of a small bacilliform virus, hereafter called pineapple bacilliform virus (PBV) in pineapple Smooth Cayenne selection C10, the hybrid cv. 53–116 and Queen is of particular interest. The morphology and dimensions of the

particles, approximately 133×33 nm, suggest that it may be a member of the badnavirus group. Previous efforts to detect bacilliform viruses in pineapples in Hawaii have been unsuccessful (German *et al.* 1992). In Queensland, PBV infects the two major commercial pineapple groups Smooth Cayenne and Queen, and is present in at least four districts, Townsville, Beerwah, Maroochy and Moggill. The extent of its distribution in Queensland is unknown, awaiting the application of more sensitive diagnostic tests that are currently being developed. This virus was also found in an unidentified clone in NSW. The ability of ScBV antiserum to trap and decorate PBV particles raises the possibility that PBV is a member of the ScBV-banana streak virus complex of badnaviruses (Lockhart and Autrey 1988).

How PCV, PBV and mealybug toxins may be involved in the pineapple mealybug wilt syndrome is unclear. It is known that wilt follows feeding of the mealybugs, *Dysmicoccus brevipes* (Cockerel), *D. neobrevipes* (Beardsley) and *Pseudococcus longispinus* (Targioni-Tozzetti) (German *et al.* 1992). PCV from Hawaii has been detected using cDNA probes in mealybugs from wilt-affected pineapple but not in those maintained on *Agave* sp. (German *et al.* 1992). *Pseudococcus longispinus* is known to transmit the closterovirus, grapevine virus A (Rosciglione *et al.* 1983). Also, at least seven members of the badnavirus group are mealybug-transmitted (B. E. L. Lockhart, pers. comm.). Badnaviruses are frequently found in nature as a component of mixed infections, for example ScBV and sugarcane mild mosaic closterovirus (Lockhart *et al.* 1992), rice tungro spherical and bacilliform viruses (Hibino *et al.* 1979) and taro large and small bacilliform viruses (Gollifer *et al.* 1978). The question arises whether the presence of both PCV and PBV in pineapple is required for mealybug transmission of the viruses and/or mealybug wilt disease expression. Direct evidence of the involvement of viruses in the etiology of pineapple mealybug wilt remains to be established. However, the significantly higher number of PCV particles detected in extracts of roots than of tops of two pineapple clones may be significant. The possibility that this and other viruses contribute to root damage and to development of wilt symptoms should be investigated.

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