Virus-like Particles Associated with Two Diseases of *Colocasia esculenta* (L.) Schott in the Solomon Islands

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SUMMARY

Bacilliform virus-like particles of two sizes are found associated with the diseases 'Alomae' and 'Bobone' of *Colocasia esculenta* in the Solomon Islands. Neither kind of particle was transmitted mechanically or by aphids. The smaller particles were similar in size and shape to cocoa swollen-shoot virus and measured 125 × 28 to 29 nm in negative stain. In partially purified preparations their sedimentation coefficient was 285 S. In thin sections of diseased *C. esculenta* they were found in the cytoplasm of phloem sieve tubes loosely aggregated with amorphous material, or packed closely, but haphazardly, in bundles. The larger particles were found in sieve tubes, companion cells and mesophyll. They measured 300 to 335 × 50 to 55 nm and in transverse sections showed three electron-dense layers, a central core about 9 nm in diam., an inner annulus approx. 28 nm thick and an outer annulus 3 to 5 nm thick. Large particles were found in the peri-nuclear space but not within the inner nuclear membrane; they were associated with 'viroplasms' and occurred commonly in membrane-bound vesicles. They apparently matured by budding from cytoplasmic membranes.

INTRODUCTION

Gollifer & Brown (1972) described two diseases, 'Alomae' and 'Bobone', of *Colocasia esculenta* (L.) Schott in the Solomon Islands and Kenten & Woods (1973) found two types of bacilliform virus-like particles associated with the diseases. This paper describes some of their properties, particularly their morphology and intracellular localization.

METHODS

Leaves and roots of *Colocasia esculenta*, apparently healthy or with symptoms of 'Alomae' and 'Bobone' diseases, were sent by air from the Solomon Islands to Rothamsted.

Plants of this and other test species were grown in glasshouses at 16 to 25 °C, shaded for 24 to 48 h and dusted with 600-mesh carborundum before being inoculated with extracts or preparations from infected leaves ground in 0·05 M-phosphate, pH 7·5.

Preparations and tissue sections were examined in a Siemens Elmiskop I A. Sap or partially purified preparations were negatively stained with neutral 2 % (w/v) phosphotungstate, 2 % (w/v) ammonium molybdate or 2 % (w/v) uranyl acetate. For sectioning, plant tissue was fixed in 2·5 % (v/v) glutaraldehyde in 0·025 M-phosphate, pH 7, post-fixed in 1 % (w/v) OsO_4 in phosphate at pH 7, dehydrated in an acetone series which incorporated staining with uranyl acetate, and infiltrated and embedded in Epon 812 (Luft, 1961). Thin sections cut with a diamond knife were mounted on uncoated copper grids and further stained with lead citrate for 1 min (Reynolds, 1963).
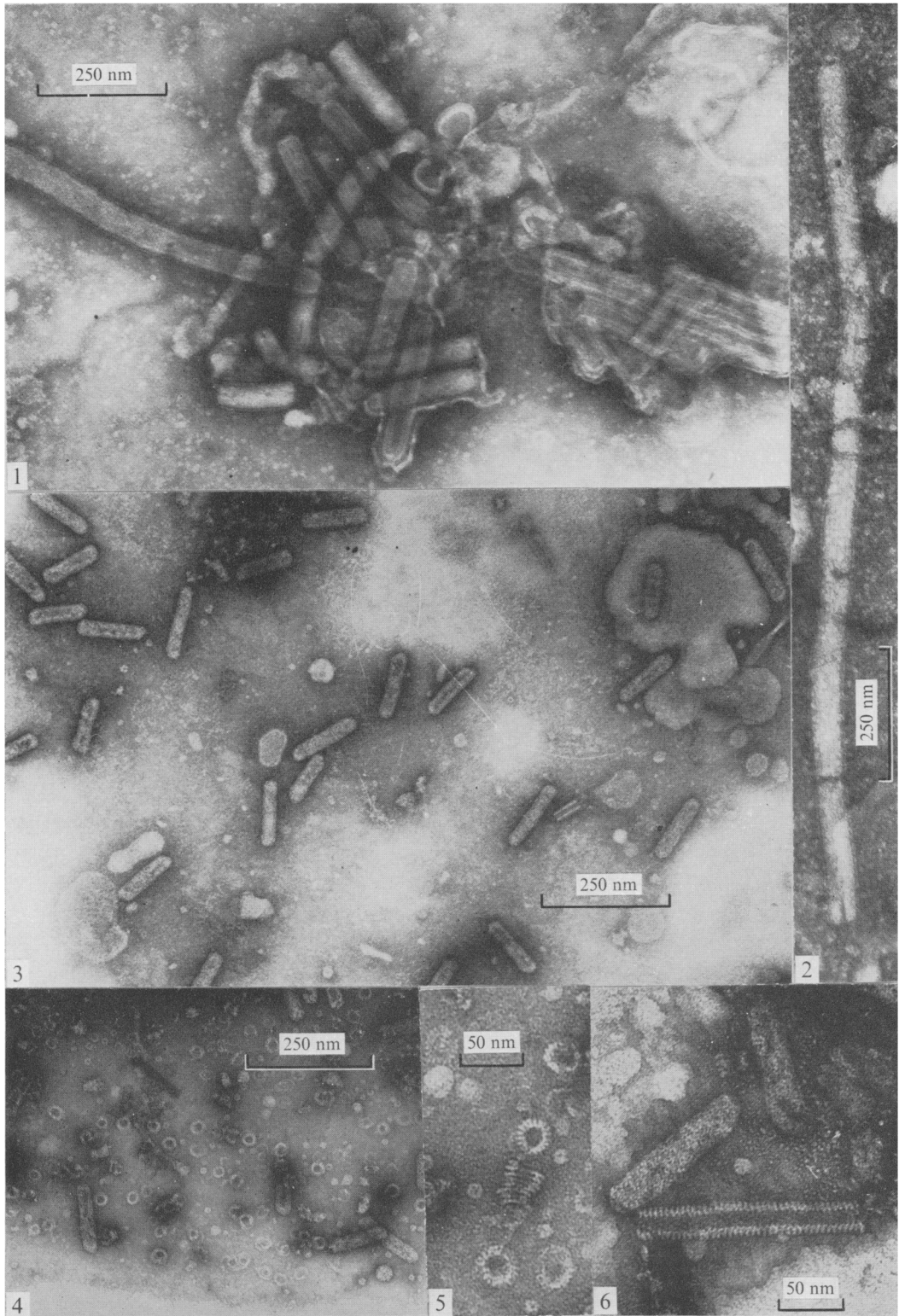
Figs. 1–6. For legends see opposite page.

RESULTS

Transmission

Colocasia esculenta leaves showing 'Bobone' symptoms contained large bacilliform particles and occasionally flexuous thread-like particles similar to those of potato virus Y. Leaves with 'Alomae' symptoms containing, in addition, the smaller bacilliform particles, were used in transmission tests. Inocula were either crude leaf extracts or fractions enriched with large or small bacilliform particles after attempts at purification. Repeated attempts were made to infect apparently healthy *C. esculenta*, and the range of test plants used by Brunt & Kenten (1971 *a*), but only *Tetragonia expansa* Murr. occasionally produced a few chlorotic lesions; only the flexuous thread-like particles were seen when sap from these plants was examined in the electron microscope.

Purification

Colocasia esculenta leaves with symptoms of either 'Alomae' or 'Bobone' disease were used in purification experiments.

Methods which have been successful in purifying morphologically similar viruses, e.g. the infiltration method of Peters & Kitajima (1970) using glycine (0·1 or 0·5 M)–magnesium buffers; the procedure of McLean & Francki (1967) where disodium hydrogen phosphate extracts are clarified with charcoal and Celite; or methods similar to those of Tomlinson Webb & Faithfull (1972) or Herold, Bergold & Weibel (1960) using either phosphate or borate buffers (0·1 to 0·5 M, pH 7·5 to 8), failed to bring more than a small proportion of the large bacilliform particles into suspension and most sedimented when extracts were centrifuged at 5000*g* for 20 min. The sediments in the electron microscope showed clusters of particles (Fig. 1), end-to-end aggregates (Fig. 2) and particles apparently attached to pieces of amorphous material. Most particles appeared to be damaged at one or both ends.

The small bacilliform particles suspended readily in all the extraction media tested, e.g. phosphate, borate and glycine-magnesium buffers (0·05 to 0·5 M) between pH 7 and 8. They were partially purified by three or four cycles of differential centrifuging (20 min at 8000*g* followed by 45 min at 75000*g*) and seemed unchanged after 3 to 4 weeks at 4 °C in 0·05 M-borate at pH 7·5.

In the Spinco Model E analytical ultracentrifuge, at 19160 rev/min using Schlieren or u.v. optics and the An-H rotor, such preparations showed a major fast component of sedimentation coefficient ($s_{20, w}$) 285 ± 15 S.

Fig. 1. Cluster of large particles from 5000 *g* sediment from an extract of 'Bobone' diseased leaves, in sodium phosphotungstate.

Fig. 2. End-to-end aggregate of large particles in sodium phosphotungstate.

Fig. 3. Small bacilliform particles, partially purified from 'Alomae' diseased leaves in sodium phosphotungstate.

Fig. 4. Same preparation as Fig. 3 in uranyl acetate.

Fig. 5. Detail from Fig. 4.

Fig. 6. Stacked disc arrangement from partially purified preparation of small bacilliform particles in uranyl acetate.

Fig. 7. Low-power view of thin section of portion of vascular bundle with virus
particles (arrows) visible in several cells.

Morphology

With negative stain

In negatively stained preparations, the large bacilliform particles had a diam. of about
65 nm. They were so fragile that nearly all had one or both ends broken, so we could not
measure their length (Fig. 1). Particles into which the stain had penetrated deeply showed
a 'helical layer' 45 nm in diam. with a pitch of 4·5 nm, and a central canal approx. 7 nm in
diam. Particles which the stain had not penetrated so deeply showed an amorphous layer,
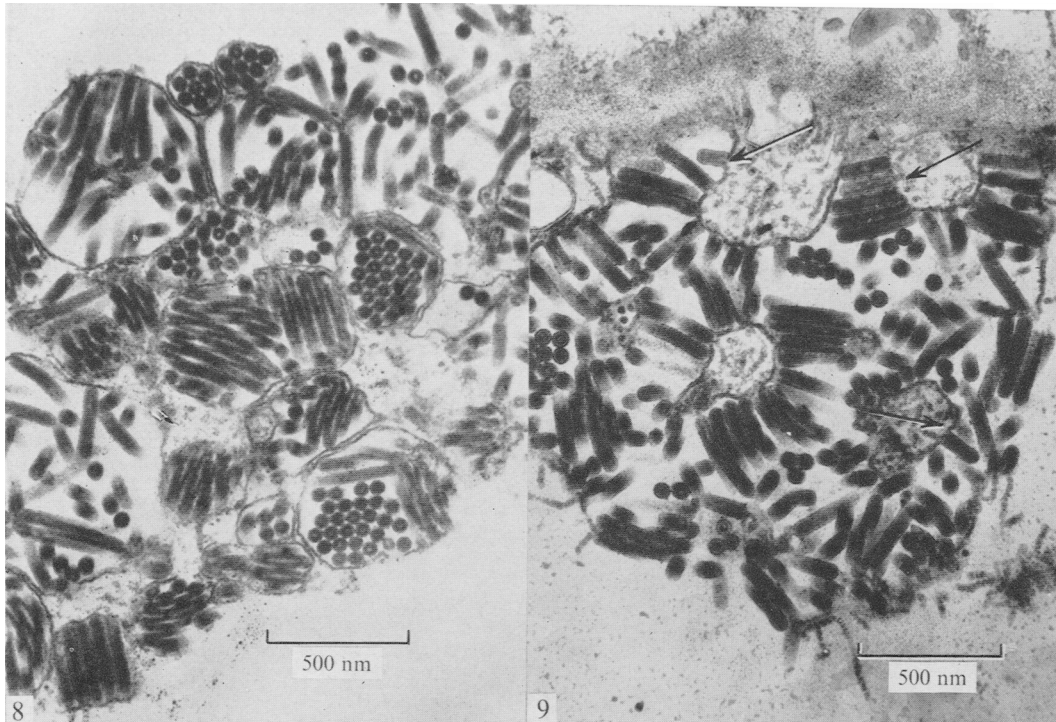possibly a lipoprotein envelope, forming a tube of 52 nm external diam. Outside this there

Fig. 8. Thin section of sieve tube cell containing large bacilliform particles in membrane-bound sacs.

Fig. 9. Thin section of sieve tube cell showing large bacilliform particles apparently budding from the membrane of cytoplasmic cisternae (arrows).

was a thin outer layer which could consist of the tips of surface projections similar to those described on other rhabdoviruses (Howatson, 1970).

The small bacilliform particles were generally uniform in size and appearance, 125 nm long and 28 to 29 nm wide in phosphotungstate (Fig. 3). They were less stable when stained with uranyl acetate; some particles showed a clear central canal and others degraded to broken pieces and discs (Fig. 4), some showing sub-units indicating that there were 18 to 20 subunits per turn (Fig. 5). Some of these discs had apparently aggregated into lengths of stacked disc arrangement (Fig. 6) with a spacing of 4·5 nm.

In thin sections

The larger particles were located in sections from leaves showing either 'Alomae' or 'Bobone' symptoms, whereas the small particles were found only in 'Alomae' leaves.

The large particles were seen in several types of cell (Fig. 7) and large aggregates were visible in the light microscope, in phloem sieve tubes. Particles also occurred in lesser numbers in companion cells and in the mesophyll. Within some sieve tubes the particles seemed haphazardly grouped but in others they occurred within membrane-bound sacs (Fig. 8) or arranged radially around small membrane bound areas of cytoplasm which showed no organized structure. In some sections, the outer coat of the particles appeared to be continuous with the membranes (Fig. 9). Within the cytoplasm of the mesophyll cells, particles often seemed to be arranged around approx. spherical bodies variable in size but similar in appearance to the 'viroplasms' found by Wolanski & Chambers (1971) in leaves
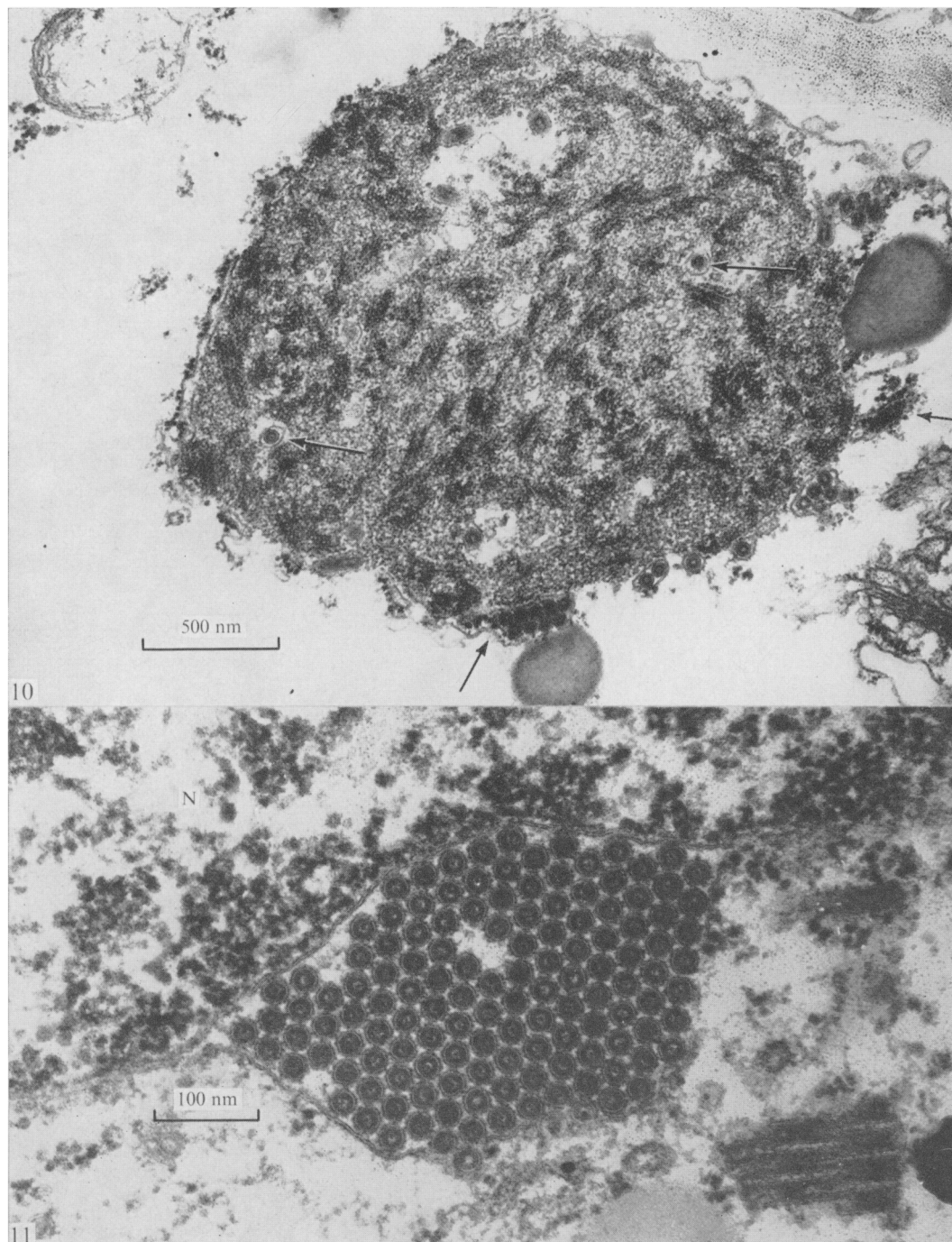
Fig. 10. Thin section of a mesophyll cell showing a 'viroplasm' with large bacilliform particles within the matrix (arrow) and around the periphery (arrow).

Fig. 11. Thin section of mesophyll cell showing perinuclear space with crystalline array of large bacilliform particles. N = nucleus.
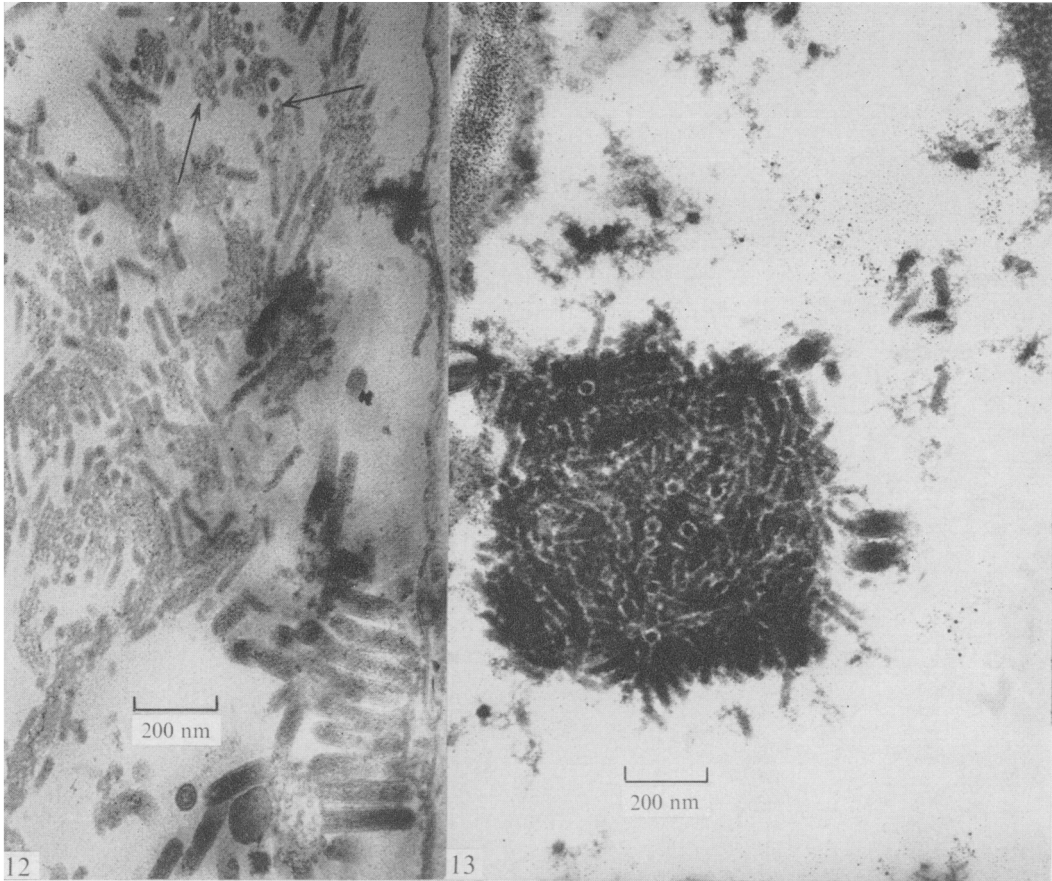
Fig. 12. Thin section of sieve tube cell showing small bacilliform particles loosely packed with amorphous material. Arrow indicates hollow particles in transverse section.

Fig. 13. Thin section of sieve tube cell showing large bundle of small bacilliform particles.

infected with lettuce necrotic yellows virus. Most of the spherical bodies had a ground substance which stained moderately to densely, with virus particles and closely associated ribosomes occurring singly or in small groups around the periphery. Occasionally a few virus particles were visible within the matrix (Fig. 10). The large particles were not found within cell nuclei but crystalline arrays occurred in the perinuclear region (Fig. 11), and small membrane-bound groups appeared nearby in the cytoplasm.

Large particles in thin sections measured 300 to 335 nm long and 50 to 55 nm wide and in transverse section often showed three electron-dense layers: a central core of variable size which was difficult to measure but did not exceed approx. 9 nm diam., a thick annulus of 12 to 18 nm internal and 41 to 45 nm external diam., and an outer annulus 3 to 5 nm thick.

The small bacilliform particles often occurred with the large ones, usually in phloem sieve tubes, but were less numerous. They were loosely packed, with amorphous lightly staining material between them (Fig. 12), or arranged closely but haphazardly in bundles (Fig. 13). The small particles measured 125 to 130 nm long and 25 to 30 nm wide. In transverse section some appeared hollow (Fig. 12) but no other structural details could be seen.

Cells of diseased leaves without bacilliform particles appeared normal, whereas those with either kind of particle showed signs of vacuolated and degenerating cytoplasm and contents. The chloroplasts of infected mesophyll cells contained numerous large starch grains and the sieve tube cells seemed to have lost some of their contents.

DISCUSSION

Although attempts to transmit the diseases by mechanical inoculation or via insects (Kenten & Woods, 1973) failed, the characteristic rhabdovirus morphology of the large bacilliform particle, the similarity of the small particle to cocoa swollen shoot virus and their association with diseased leaves, make it almost certain that the particles are viruses.

The small particles closely resembled those of cocoa swollen-shoot virus (CSSV) in size and shape (Milne & Kenten, 1970; Kenten & Legg, 1971) although their sedimentation coefficient (285 S) was greater than that of CSSV (220 S) (Brunt & Kenten, 1971 b). In thin sections of *Theobroma cacao* L. leaves infected with CSSV, Milne & Kenten (1970) found much necrosis but only a few virus particles in small aggregates in the cytoplasm. Nothing resembling the large bundles of the *Colocasia* small particles was seen in cocoa.

In negative stain, the large bacilliform particle closely resembles broccoli necrotic yellows virus (Lin & Campbell, 1972; Tomlinson *et al.* 1972) but in thin sections it has an electron-dense core suggesting a structure more like maize mosaic, wheat striate mosaic, and melilotis latent viruses (Herold *et al.* 1960; Lee, 1967, 1970; Kitajima, Lauritis & Swift, 1969). The cross-striations presumably representing the virus nucleocapsid are 4·5 nm apart, within the range (4 to 5 nm) found with other rhabdoviruses (Howatson, 1970).

The large particles were associated with cytoplasmic membranes, 'viroplasms' and nuclei. No uncoated particles were seen and although aggregates occurred in the peri-nuclear space there was no evidence that particles budded through the inner nuclear membrane. As with wheat striate mosaic (Lee, 1970), particles apparently budded from cytoplasmic membranes.

The large particles are commonest in large aggregates consisting of many groups of particles in membrane-bound vesicles. Kitajima *et al.* (1969) suggest that with melilotis latent virus these vesicles arise from delamination of the nuclear envelope, but there is no evidence that the *Colocasia esculenta* virus matures by budding through the inner nuclear membrane into the perinuclear space. If the *Colocasia* large particle matures from pools of virus precursor material at cytoplasmic membranes it is not clear how they become encapsulated in groups or enter the perinuclear space.

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