Filamentous Viruses Infecting Dasheen and other Araceous Plants

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ABSTRACT

Araceae of the genera *Aglaonema, Caladium, Colocasia, Dieffenbachia, Xanthosoma,* and *Zantedeschia* were found to be naturally infected with one or more flexuous-rod viruses which proved mechanically transmissible to seedlings of the aroid *Philodendron selloum*. The isolate from dasheen (*Colocasia esculenta*) is tentatively designated as dasheen mosaic virus (DMV). This isolate infected seedlings of the two aroid species tested, *P. selloum* and *Z. elliottiana*. No seedlings of the nonaraceous species mechanically inoculated with DMV developed symptoms. DMV has characteristics in common with other viruses assigned to the "potato virus Y" group of Brandes and Bercks in (i) being aphid-transmitted in a stylet-borne manner; (ii) having a mean particle length of 750 mμ; and (iii) inducing characteristic cylindrical inclusions. Phytopathology 60: 983-987.

This study reports mechanically transmissible flexuous-rod viruses infecting plants of the family Araceae. Despite the economic importance and worldwide distribution of these plants, little is known about the virus diseases that infect them. This is of particular significance when it is considered that the aroids are usually propagated vegetatively and are commercially distributed on an international scale.

The only virus known to be consistently associated with any araceous plant is tomato spotted wilt, a virus of spheroid morphology, which was reported to infect calla lily (*Zantedeschia* spp.) by several workers, including Tompkins & Gardner (11), Gardner & Whipple (7), and Tompkins & Severin (12). Raychaudhuri & Ganguly (9) reported the aroid *Acorus calamus* as a host for the chirke disease of cardamon. Verplancke (13) reported a mechanically transmissible "filterable virus" infecting aroids of the genera *Anthurium, Monstera, Philodendron,* and *Zantedeschia;* he also reported the susceptibility of the nonaroid *Datura stramonium* to this virus.

MATERIALS AND METHODS.—Araceous plants were collected from various locations throughout Florida and tested as virus sources. With the exception of calla lily (*Zantedeschia elliottiana* [Knight ex Watson] Engl.), all collections were from Florida-grown stock. The calla lily sample was obtained from bulbs imported from The Netherlands.

Prior to mechanical inoculations, extracts from pieces of each of the leaves to be used as virus sources were prepared according to the "dip" method of Brandes (2) for detecting virus particles. All leaf extracts were negatively stained in 1% potassium phosphotungstate at a pH of 7.75. This technique was shown by Sampson & Taylor (10) to be a sensitive means for detecting the presence of rod-shaped viruses in plant tissues. Mechanical inoculations were made from sap expressed from these same leaves and diluted in tap-water. Carborundum (600 mesh) was dusted onto the leaves of test plants as an abrasive. In all these transmission trials, the plant species inoculated was *Philodendron selloum* C. Koch. In an attempt to avoid inoculating test plants already virus-infected through vegetative propagations, only plants grown directly from seed were used. Noninoculated seedlings were included in all trials as controls; none of a total of 40 control plants developed symptoms. Two to 3 weeks after inoculation, leaf extracts obtained from affected and symptomless test plant leaves that developed after inoculation were examined for the presence of virus particles.

A detailed study was made of the virus obtained from affected leaves of dasheen, *Colocasia esculenta* (L.) Schott. In a host range study, seedlings of 20 different species of plants representing 10 plant families were mechanically inoculated as described above.

Aphids were tested as vectors of the virus from dasheen. The aphids used were *Aphis craccivora* Koch and *Myzus persicae* (Sulzer) which were reared on virus-free plants of *Vicia faba* L. 'Longpod' and *Nicotiana tabacum* L. 'Samsun NN', respectively. Aphids were starved 1-6 hr and transferred either singly or in groups of 20-25 to the virus source. On the virus source, aphids were allowed virus-acquisition probes < 1.5 min in duration or access periods < 5 min. All aphids were killed 12-24 hr after being transferred to test plants with a liquid formulation containing malathion. In all aphid transmission trials, affected leaves of dasheen were used as virus sources and *P. selloum* seedlings were used as test plants.

Thin sections were made from the affected leaves of dasheen. Tissue pieces were fixed in 6.25% glutaraldehyde and postfixed in 1% OsO_4; both fixatives were buffered with 0.1 M sodium phosphate at pH 6.8. The fixed material was dehydrated in a graded acetone series, embedded in Maraglas-Cardolite plastic, and sectioned with a diamond knife. Prior to examination with the electron microscope, the sections were stained in uranyl acetate and lead citrate.

All size determinations from electron micrographs were made by comparing projected micrographs of leaf extracts or sectioned material with projected micrographs of a 54,864 line/inch diffraction grating.

TABLE 1. Araceae naturally infected with flexuous-rod viruses mechanically transmissible to seedlings of *Philodendron selloum* C. Koch

Virus source plants[a]	Mechanical transmissibility
Aglaonema, *Aglaonema commutatum* Schott 'Pseudo-bracteatum'	5/5[b,c]
Caladium, *Caladium hortulanum* Birdsey 'Candidum'	5/5
	5/5
'Mrs. W. B. Halderman'	5/5
	3/5
'White Christmas'	5/5
'Red Ensign'	5/5
Dasheen, *Colocasia esculenta* (L.) Schott	14/35
Dieffenbachia, *Dieffenbachia picta* Schott 'Exotica'	5/5
Dieffenbachia, *Dieffenbachia picta* Schott	3/5
Malanga, *Xanthosoma sagittaefolium* (L.) Schott	1/5
	10/15
Violet stemmed yautia, *Xanthosoma violaceum* Schott	5/5
Calla lily, *Zantedeschia elliottiana* (Knight ex Watson) Engl.	5/5

[a] Leaf dips were made of each of the virus sources before inoculation; flexuous-rod particles were detected in every instance.

[b] Numerator is number of infected *P. selloum* seedlings; denominator is total number inoculated.

[c] In each trial, a leaf dip was made from a seedling that developed symptoms after inoculation; flexuous-rod particles were always recovered from these plants. Similarly, leaf dips were made from inoculated or control seedling that did not develop symptoms after inoculation; flexuous-rod particles were never recovered from these plants.

RESULTS.—Species of six different genera of Araceae were found to be naturally infected with viruses mechanically transmissible to seedlings of *P. selloum* (Table 1). In every instance where transmission was effected, flexuous-rod particles were present in leaves used as virus sources (Fig. 2-D). Flexuous-rod particles were also found in extracts of symptomatic test plant leaves. Such particles were never detected, however, in extracts of leaves from test plants that did not develop symptoms after inoculation. That the observed flexuous rods were indeed virus particles was indicated by their constant association with affected *P. selloum* test seedlings and their absence in symptomless seedlings used in the same trials.

Systemic symptoms varied considerably among the different araceous species tested as virus sources. Foliar symptoms were indistinct and scarcely distinguishable from natural leaf variegations in caladium, whereas in calla lily, symptoms were more readily apparent and consisted of a foliar mosaic accompanied by a pronounced leaf distortion (Fig. 1-A). Symptoms of infected dasheen and malanga (*Xanthosoma sagittaefolium* [L.] Schott) plants varied as new leaves developed. Many leaves were without apparent symptoms, whereas other leaves on the same plant displayed various mosaic patterns (Fig. 2-A, B). Symptoms exhibited by inoculated seedlings of *P. selloum*, similar for all inoculations, were always pronounced and consisted of leaf streaking accompanied by foliar distortion and a marked reduction in growth (Fig. 1-B).

The isolate from dasheen, tentatively designated as dasheen mosaic virus (DMV), infected seedlings of both aroid species tested in the host range study; 14 of 35 inoculated *P. selloum* seedlings and 6 of 10 inoculated *Z. elliottiana* seedlings developed symptoms. As described previously for *P. selloum*, symptomatic and symptomless seedlings of *Z. elliottiana* were checked for virus particles 2-3 weeks after inoculation. Flexuous-rod particles were always found in extracts from seedlings exhibiting symptoms, but never from symptomless seedlings. None of the following plant species, including known suscepts of tomato spotted wilt, developed symptoms after inoculation with DMV (the numbers indicate the total number of test plants inoculated): *Apium graveolens rapaceum* DC. 'Giant

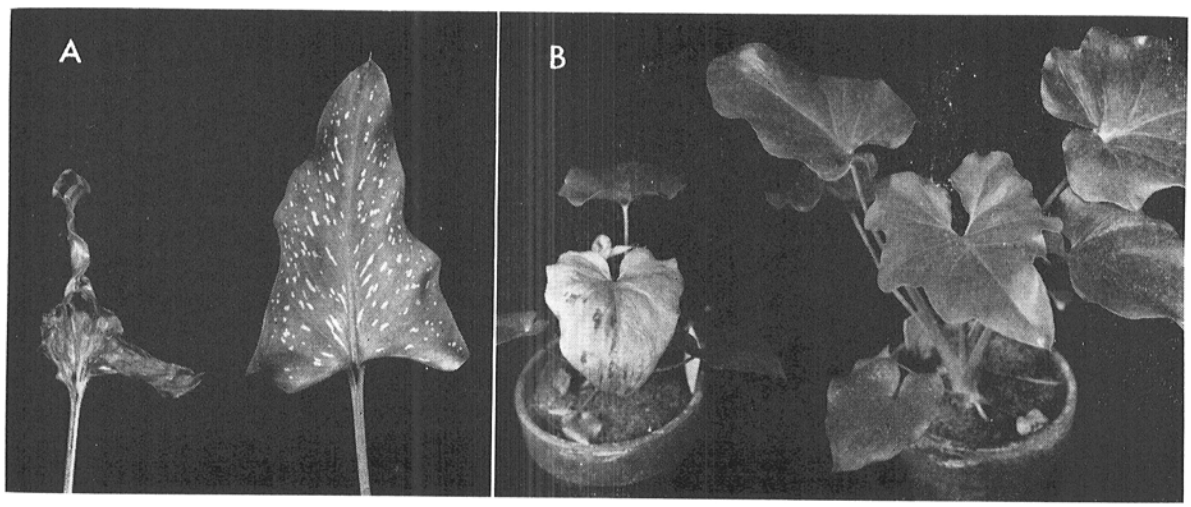

Fig. 1. A) Leaves of *Zantedeschia elliottiana* showing symptoms (left) and no symptoms (right); the former typifies leaves from which flexuous-rod particles were recovered from leaf extracts and from which inoculum was obtained to infect seedlings of *Philodendron selloum*. **B)** (left) Infected and (right) noninoculated seedlings of *P. selloum*; the former was inoculated from symptomatic leaves of dieffenbachia, *Dieffenbachia picta*.

Prague', 5; *Capsicum annuum* L. 'California Wonder', 5; *C. annuum* 'Tabasco', 5; *Carica papaya* L., 5; *Cassia occidentalis* L., 5; *Chenopodium amaranticolor* Coste & Reyn., 15; *Crotalaria spectabilis* Roth, 4; *Cucurbita pepo* L. 'Small Sugar', 14; *Datura stramonium* L., 10; *Gomphrena globosa* L., 5; *Lupinus angustifolius* L., 11; *Nicotiana tabacum* L. 'Samsun NN', 5; *N. tabacum* 'Samsun Turkish', 5; *Phaseolus vulgaris* L. 'Red Kidney', 14; *Pisum sativum* L. 'Alaska', 14; *Sorghum vulgare* Pers. 'E-57', 5; *Tropaeolum majus*

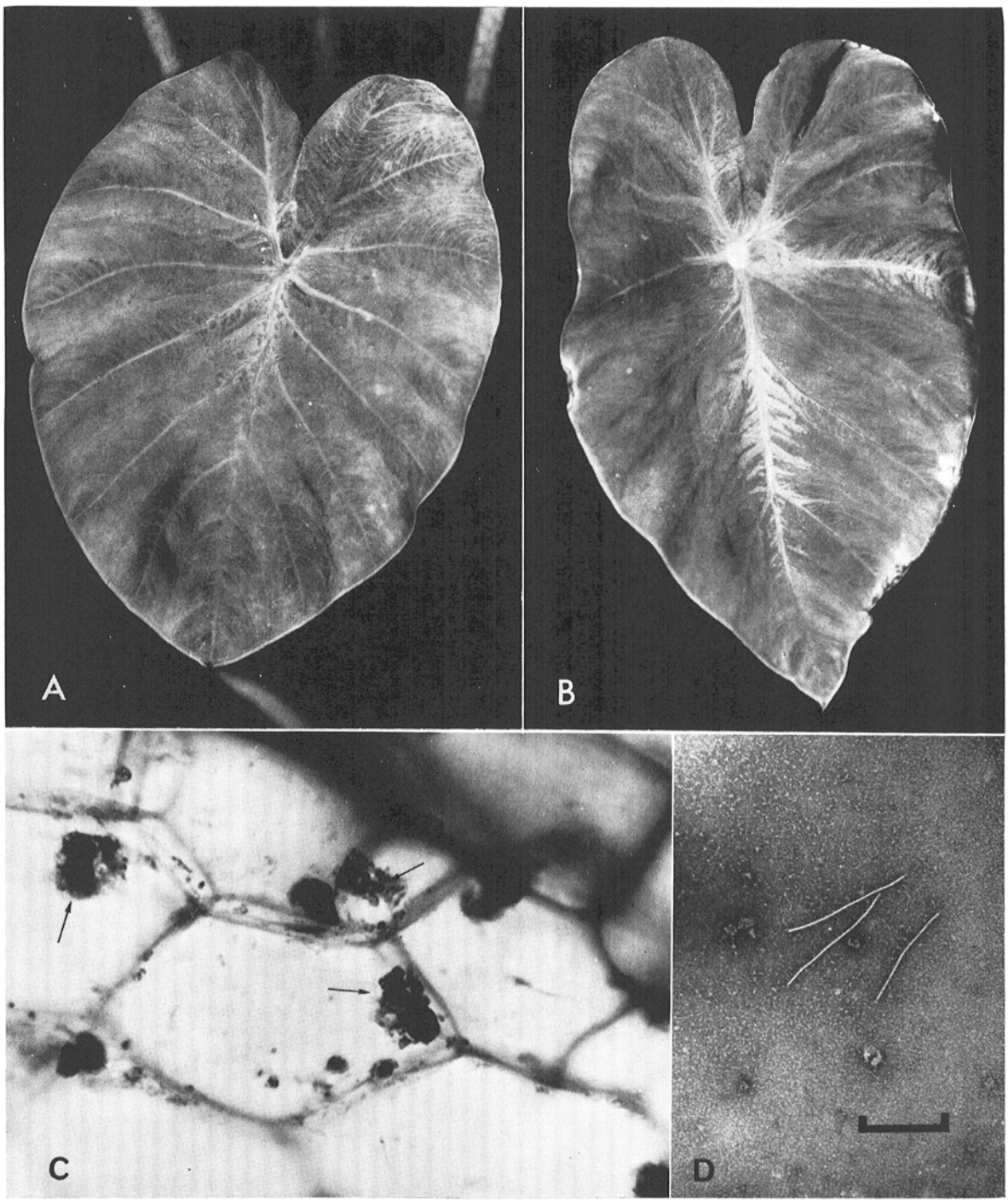

Fig. 2. A, B) Leaves of dasheen (*Colocasia esculenta*) showing dispersed (A) and veinal (B) mosaic patterns. **C)** Stained epidermal cells removed from affected leaves of dasheen showing amorphous cytoplasmic inclusions (arrows) as resolved with the light microscope (×970). **D)** Negatively stained flexuous-rod particles extracted from affected leaves of dasheen; scale line is 0.5 μ.

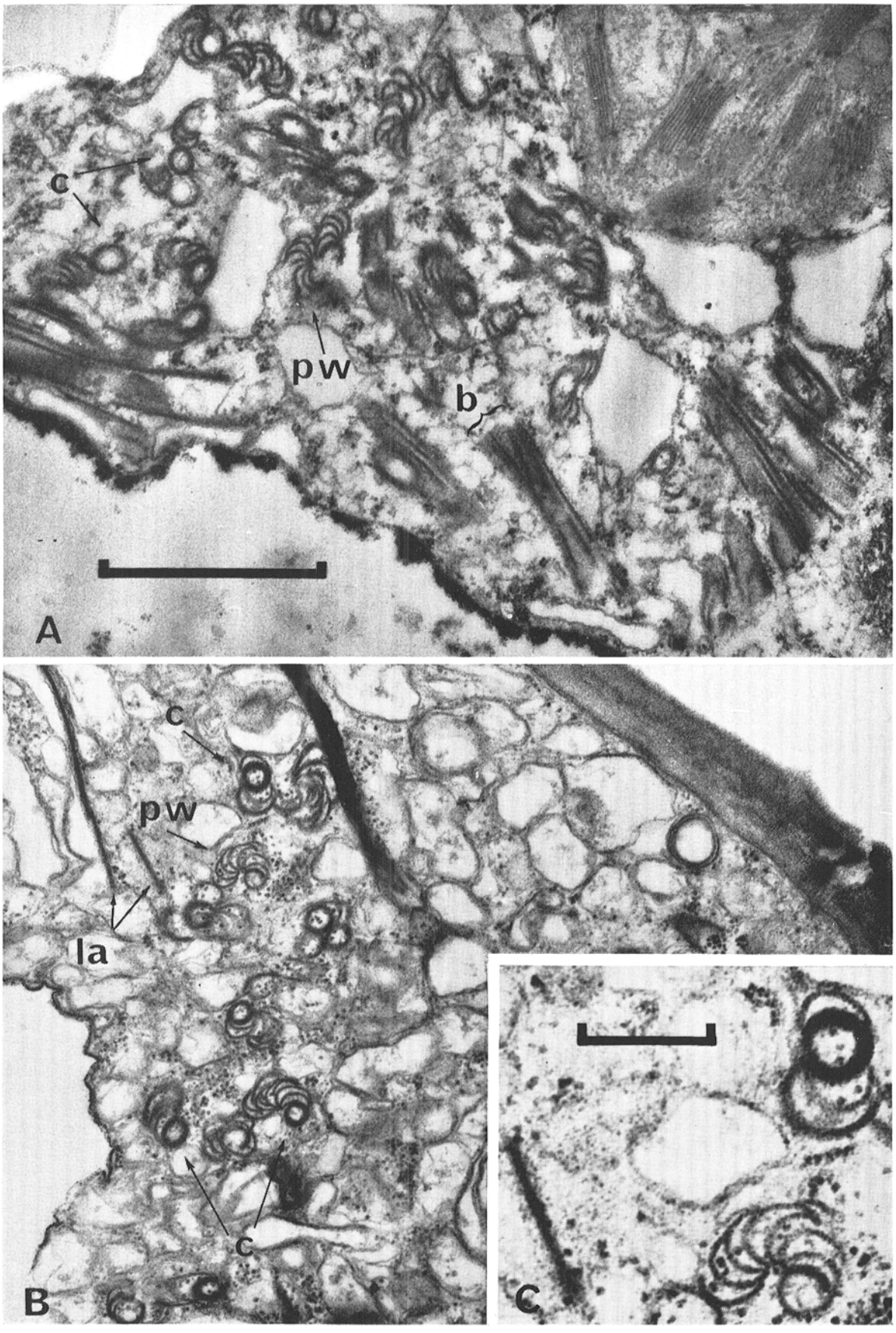
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Fig. 3. A, B) Thin sections of affected dasheen leaf tissue showing pinwheel (pw), circular (c), bundle (b), and laminated aggregate (la) inclusions; scale line is 1.0 μ. **C)** Inset of B showing magnification of pinwheel and circular inclusions; scale line is 0.25 μ.

←

L., 3; *Vicia faba* L. 'Longpod', 5; *Vigna sinensis* (L.) Endl. 'Black Local', 15; *Zea mays* L. 'Golden Cross Bantam', 6; *Z. mays* 'Ioana', 11.

DMV was transmitted from dasheen leaves showing symptoms to seedlings of *P. selloum* by *A. craccivora* and *M. persicae*. In one test, individuals of *A. craccivora* which were allowed single-timed virus acquisition probes (< 90 sec duration) and then transferred (10 aphids/plant) to test seedlings infected three of five inoculated plants. That DMV was transmitted in a stylet-borne manner is indicated by the brevity with which virus was acquired. In another test, groups of 20-25 aphids were permitted virus access periods of 5 min, then transferred to test seedlings. With this technique, transmission was effected to 2 of 10 plants using *M. persicae* and 1 of 10 plants using *A. craccivora*. None of the 10 noninoculated seedlings of *P. selloum* used as controls in these aphid transmission trials developed symptoms.

Of 178 flexuous-rod particles seen in crude extracts from affected dasheen leaves, 91% measured 700-800 mμ in length; the arithmetic mean length of these DMV particles was 750 mμ.

Amorphous cytoplasmic inclusions (Fig. 2-C) were seen with a light microscope in epidermal strips removed from affected dasheen leaves and stained in calcomine orange and "luxol" brilliant green (4). Thin sections from such tissue revealed the presence of inclusion bodies of the types described by Edwardson et al. (6) as pinwheels (pw), circular inclusions (c), laminated aggregates (la), and bundles (b) (Fig. 3-A, B, C). Such inclusions are assumed to be two-dimensional aspects of three-dimensional cylindrical and tubular inclusions characteristically associated with certain stylet-borne viruses of the 700- to 800-mμ length group (5, 6).

DISCUSSION.—This study shows the susceptibility of araceous plants to filamentous viruses and the apparent widespread nature of infections. All the virus isolates encountered in this study are mechanically transmissible, are flexuous rods, and induce common symptoms in inoculated seedlings of *P. selloum*. The exact relationship of each of these virus isolates to one another and to other previously described viral diseases of aroids remains to be seen.

The isolate from dasheen is typical of other known viruses of the "potato virus Y" group of Brandes & Bercks (3) in (i) being stylet-borne by aphids; (ii) having a mean particle length of 700-800 mμ; and (iii) inducing characteristic cylindrical inclusions (3, 5, 6).

Virus diseases may prove to be of particular significance to members of the Araceae since most cultivated aroids flower infrequently or may require special techniques to effect their fertilization (1, 8). Thus, the possibility of eliminating virus in stock plantings through seed production would prove impractical for most cultivated aroids. For example, the various commercially available caladium varieties are F_1 hybrids. These varieties are therefore not homozygous. Thus, the elimination of virus in a uniformly infected variety through seed is contingent upon the re-establishment of that variety by rehybridization, an almost impossible task.

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