VIRUS DISEASE OF CYMBIDIUM AND CATTLEYA CAUSED BY CYMBIDIUM MOSAIC VIRUS

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In virus diseases reported from many genera in the *Orchidaceae*, the two most commonly known viruses are Cymbidium mosaic virus (CyMV) (3, 12, 13, 15, 17) and Odontoglossum ringspot virus (ORSV) (10, 16, 18). CyMV, reported first by Jensen (11, 12), has already been found to be infectious to many plant species in 11 genera of orchids (2, 15, 18, 20, 21). In the previous paper, the author showed that a disease of *Cymbidium* caused by ORSV was encountered very commonly in many nurseries in Japan (8, 10). In the present paper the author identified the virus causing chlorotic areas and necrotic streaks in the leaves of *Cymbidium* as CyMV, on the basis of the investigations of symptoms, host range, physical properties, transmission, serology and electron microscopy. CyMV was also isolated from *Cattleya* exhibiting leaf necrosis.

MATERIALS AND METHODS

The virus used in the studies was isolated from the plants of *Cymbidium* and *Cattleya* collected from many commercial orchid nuseries in the western part of Japan, during the period of 1960 to 1963. Diseased plants collected were maintained in a greenhouse for the inoculum source.

Datura stramonium was used as an indicator plant for many of the experiments. To confirm the virus infection, back inoculation was made on the indicator plants, and electron microscopic observation was also made to detect virus particles in the inoculated plants. Preparation for electron microscopy was made by means of dip method according to Brandes (1). Unless otherwise stated, mechanical inoculation was conducted in the usual manner in the experiments of host range, physical properties and others.

RESULTS

1. *Symptoms in naturally infected plants*

(a) *Cymbidium* Symptoms in young leaves are characterized by chlorotic patches and conspicuous, elongated chlorotic areas, about 1 to 10 *mm* in length (Fig. 1. F, G). Some additional necrosis are seen on the young mottled leaves of some of the diseased plants. Symptoms in older leaves are characterized by elongated yellowish green areas, and sunken necrotic spots and irregularly shaped streaks (Fig. 1, A-E). The necrosis is severe on the lower surface of leaves (Fig. 1. C, E) compared with that on the upper surface (Fig. 1. B, D). In some instances, necrotic symptoms appeared to be necrotic ring patterns, about 2 *mm* in

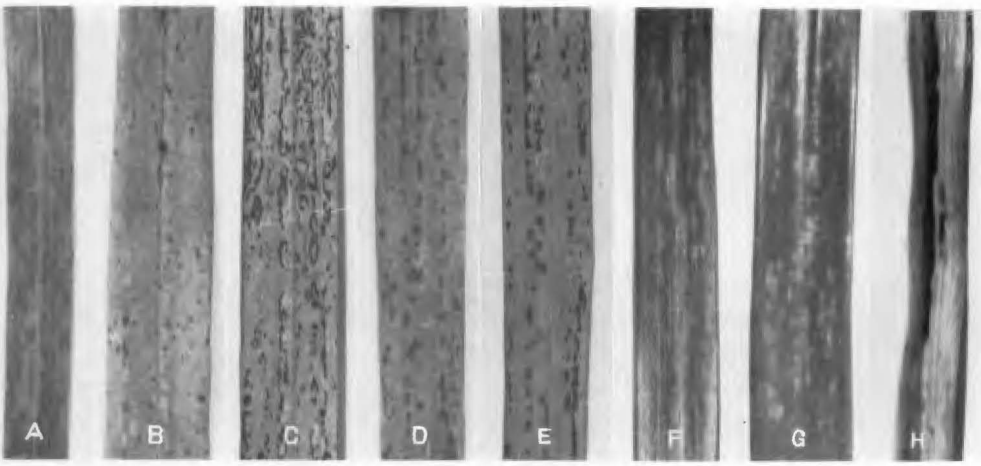

Fig. 1. Symptoms of Cymbidium mosaic virus in ***Cymbidium*** leaves.
(B, D, F, G) upper surface, (A, C, E) lower surface, (H) Chlorotic mottle and necrotic streaks on younger leaf of artificially infected plant.

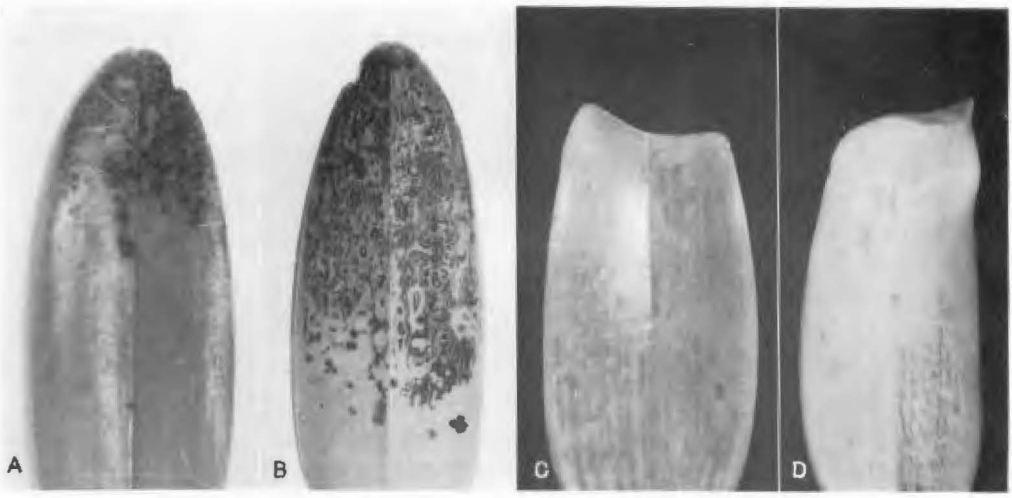

Fig. 2. Symptoms of Cymbidium mosaic virus in ***Cattleya*** leaves.
(A, C) upper surface, (B, D) lower surface.

width and 5 to 50 *mm* in length (Fig. 1. C). The necrosis appeared more frequently on the basal portions of the leaves than the upper parts. Double infection of CyMV and ORSV was also noticed in ***Cymbidium*** plant showing chlorotic areas, necrosis and necrotic rings (Fig. 3. B, C).

(b) ***Cattleya*** On younger leaves, light brown necrotic spots and streaks are formed in internal tissue, and sunken, reddish brown necrotic streaks are also produced on the lower surface of the leaves (Fig. 2. C, D). On older leaves, sunken dark-red or brownish purple patches are formed on the top part of the upper leaf (Fig. 2. A) and distinct concentric necrotic ring patterns are produced on the

lower surface of the leaves. These patterns are characterized by concentric necrotic rings enclosing normal tissue or necrotic spots, and becoming larger compound patterns overlapping with each other (Fig. 2. B). Many flowers of diseased *Cattleya* plants are observed symptomless. However, light color removing break of flowers was observed in a variety, Lc. Corisande (Fig. 4. A), and light color adding break in Bc. Cliftonville and Lc. Aphrodite (Fig. 4. B). The presence of virus in these flowers was demonstrated by bioassay and electron microscopy. Double infection of GyMV and ORSV was also noticed in *Cattleya* plants showing light reddish-purple patches and sunken necrotic streaks (Fig. 3. A).

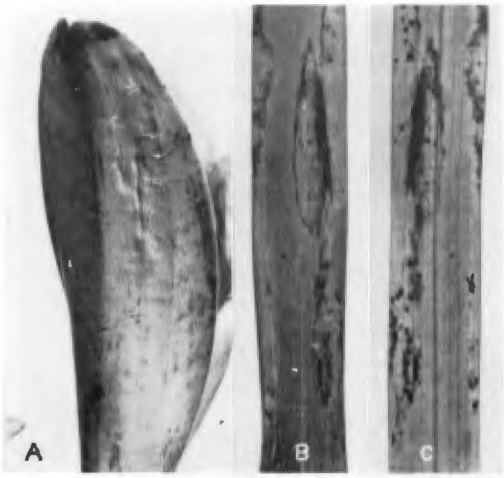

Fig. 3. Symptoms in *Cattleya* and *Cymbidium* caused by both of Cymbidium mosaic virus and Odontoglossum ringspot virus.
(A) *Cattleya*, (B) *Cymbidium*; Upper surface, (C) lower surface of leaf 'B'.

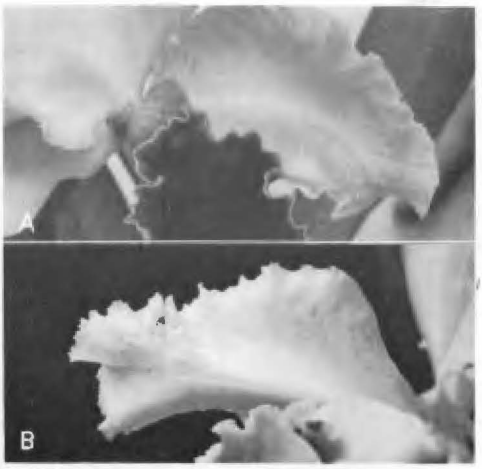

Fig. 4. Light color breaking in the flowers of Cattleya.
(A) Lc. Corisande, (B) Lc. Aphrodite.

2. *Transmission*

Causal virus is easily transmitted to healthy plants of *Cymbidium*, *Cattleya* and *Dendrobium* with diseased juice containing carborandum as an abrasive. Most of the *Cymbidium* seedlings become diseased after about 1-3 months from the inoculation, but after 19 days in one case. In *Cattleya* most of incubation periods are observed to be approximately 1-2 months, but 12 days in shorter case. These orchid plants above mentioned are found to be highly susceptible when inoculation is applied on their younger leaves and roots. The virus is easily transmitted by alternate cuttings of the leaves of healthy and diseased plants with a razor blade. All the attempts to transmit the virus by means of green peach aphid to *Cymbidium* failed.

3. *Host range and symptoms*

Symptoms in susceptible plants are as follows:

(a) *Cymbidium* Symptoms appear first on the basal portion of the younger

inoculated leaf. Chlorotic areas appear systemically first on the leaf of the new growth. After a few day, the areas become more sharply marked and develop into elongated, broad chlorotic streak (Fig. 1. H). In 2 weeks to 2 months after the first appearance of the symptoms, necrotic spots and streaks appear on the diseaesd leaves. The necrosis appears first on the lower surface of the leaves, and later extends to the upper surface. The growth of the affected plants is extremely retarded.

(b) *Cattleya* Brown patches appear first in internal tissue of the young inoculated leaves (Fig. 5. C). The discoloration is observable through the cuticle of the leaves. These patches become in coalescence with one another, darkening the entire leaf, and extending into the pseudobulb. Leaves developing severely necrotic in whole surface drop prematurely. Sunken necrotic streaks are also formed (Fig. 5. A, B). On matured inoculated leaves, sunken brown necrotic streaks are formed slowly expanding along the veins. In some of the inoculated plants, only brown patches appear on the inoculated leaf, and all tissues of newly developed shoot become necrotic and dies (Fig. 5. D). Plants were severely affected when the seedlings and leaves were inoculated at their younger stage of growths.

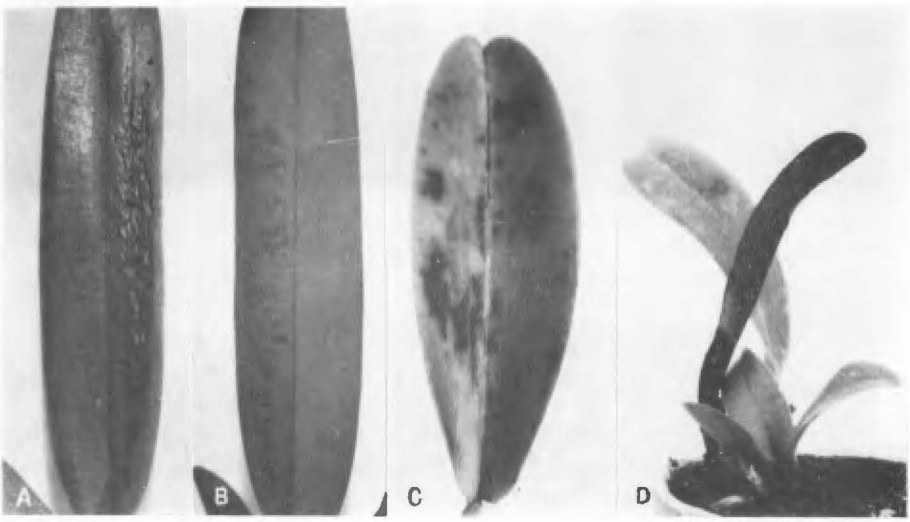

Fig. 5. Symptoms of Cymbidium mosaic virus in artificially infected *Cattleya* hybrids.

(c) *Dendrobium* Chlorotic patches and faint mottling appear on the inoculated leaves. Chlorotic areas ranging from small spots to large mottled patches appear as the systemic symptoms. Some necrosis are also formed in the mottled leaves.

(d) *Epidendrum* Light brownish-red discoloration developed on the leaves. On the lower surface of the leaves, sunken necrotic spots were formed.

(e) *Miltonia* Brown patches in the forms of spindle or ring appear first on the inoculated leaves (Fig. 6. A). These patches soon extend to the entire leaves

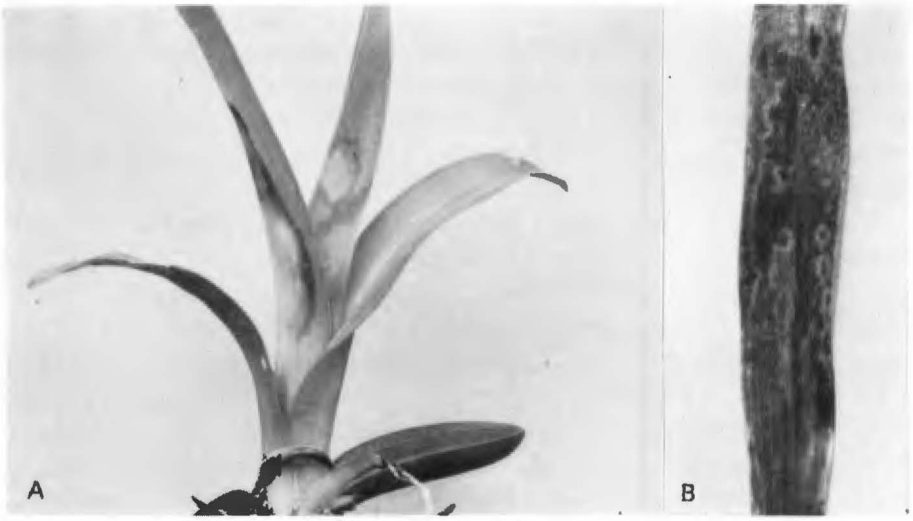

Fig. 6. (A) Symptoms in the inoculated leaves of ***Miltonia*** infected with Cymbidium mosaic virus. (B) Mosaic in ***Dendrobium*** caused by Cymbidium mosaic virus.

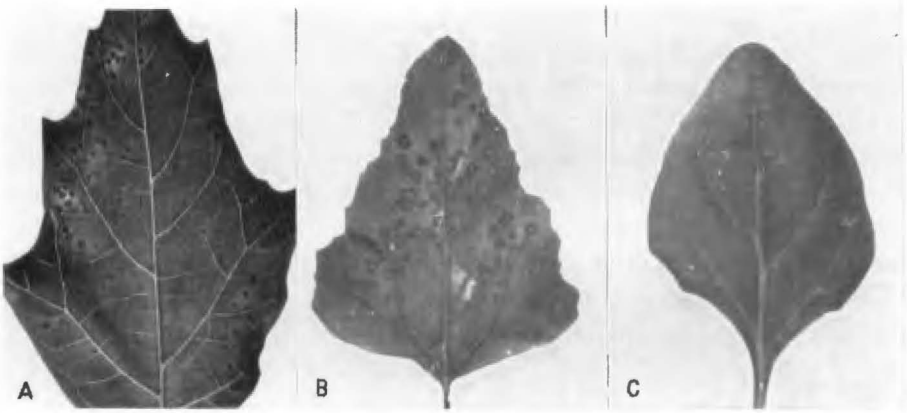

Fig. 7. Symptoms of Cymbidium mosaic virus in several host plants. Local lesions on (A) ***Datura stramonium***, (B) ***Chenopodium amaranticolor***, (C) ***Tetragonia expansa***.

and pseudobulb. Later, all tissues of the plant became brown and died. *Miltonia* plants are found to be highly sensitive to the infection of the virus.

(f) ***Datura stramonium*** Necrotic local lesions are formed on the inoculated leaves, but no systemic infection is noted (Fig. 7. A). The lesions appear after the incubation period of about 10 days in older leaves, and about 20 to 25 days or more in younger ones. The lesions in this plant induced by the virus resembled somewhat with those caused by TMV, although there are differences of incubation period.

(g) ***Cassia occidentalis and C. tora*** Small black necrotic spots are formed on the inoculated leaves, 4–6 days after inoculation, but no systemic infection is

noted.

(h) *Chenopodium amaranticolor and Tetragonia expansa* In *Chenopodium amranticolor*, local green ring spots are formed when the inoculated leaves begin to turn yellow (Fig. 7. B). In *Tetragonia expansa*, faint small chlorotic spots are formed locally on the inoculated leaves, 15–30 days after inoculation (Fig. 7. C).

The following plant species were found to be insusceptible to CyMV. *Nicotiana tabacum* L., variety Blight Yellow, Samsun, *N. rustica* L., *N. glutinosa* L., *Petunia hybrida* Vilm., *Lycopersicon esculentum* Mill., *Solanum Melongena* L., *Beta vulgaris* var. *cicla* L., *Gomphrena globosa* L., *Zinnia elegans* Jacq., *Cucumis sativus* L., *Cucurbita moschata* Duch., *Pisum sativum* L., *Vicia faba* L., *Phaseolus vulgaris* L., *P. aureus* Roxb., *Vigna catiang* Walp., variety Daruma, *Trifolium incarnatum* L., *Sesamum indicum* L., *Phytolacca americana* L., *Zea mays* L., *Lilium formosanum* Stapf, *Brassica rapa* L. var. *Komatsuna* Hara, *Raphanus sativus* L., var. *acanthiflormis* Maikino.

4. *Physical properties*

The physical properties of the virus isolated in Japan were examined to compare with those reported in the literature for CyMV. The virus is infective at the dilution of 5×10^{-4}, but not 10^{-5}. However, some isolate is still infective at dilution of 10^{-5}. The virus is infective at 65 °C for 10 minutes exposure, but is inactivated at 70 °C. The results agree with those reported by Jensen (12), Murakishi (21), Corbett (3) and white et al (24). In aging tests, the virus remains infective in expressed juice after the storage of over one month at 18 °C. Dried residue of diseased leaf juice is infective after the storage of 8 days, but not after 10 days at 20°C. Tolerance to aging of the virus in this paper is somewhat higher than those reported by Jensen (12), Murakishi (21) and Corbett (3).

5. *Serology*

Partially purified virus for immunity was obtained according to the following produces: Leaves (70 g) of artificially diseased *Cattleya* were ground in a grind-bowl with 2.5 v/w of 0.1 M phosphate buffer, pH 7.0, and the juice was ex-

TABLE 1

The reactions in microagglutination tests of Cymbidium mosaic virus antiserum with CyMV

Antigen (×10 dilution)		Anti-CyMV serum dilution											
		8	16	32	64	128	256	512	1024	2048	4096	8192	saline
CyMV	Cy-10	⁺⁺⁺	⁺⁺⁺	⁺⁺⁺	⁺⁺⁺	⁺⁺⁺	⁺⁺	⁺	+	+	−	−	−
	Cy-16	⁺⁺⁺	⁺⁺⁺	⁺⁺⁺	⁺⁺⁺	⁺⁺⁺	⁺⁺	⁺⁺	⁺	+	−	−	−
Healthy orchid		−	−	−	−	−	−	−	−	−	−	−	−

⁺⁺⁺∼+……signs indicate positive reactions,
− ……indicates no reaction.

pressed through cheescloth. The expressed sap was centrifugated for 10 min. at 1,500 *g*. The supernatant was shaken with 1/5 volume of chloroform for 3 min, and clarified by a low-speed centrifugation. The supernatant fluid was further centrifugated for 15 min. at 9,000 *g*. After three cycles of high- and low-speed centrifugations (70,000 *g* for 1 hr. and 1,500 *g* for 10 min.), partially purified virus suspension was obtained.

Antiserum to CyMV was prepared by giving a rabit 4 intramuscular injections by the use of Freund's adjuvant and 5 intravenous injections, with partially

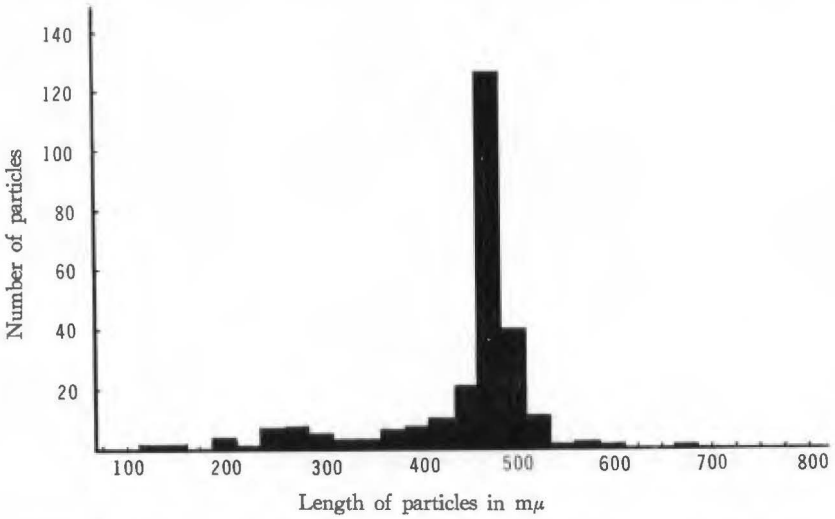

Fig. 8. Distribution of particle length of Cymbidium mosaic virus isolated from diseased *Cymbidium*.

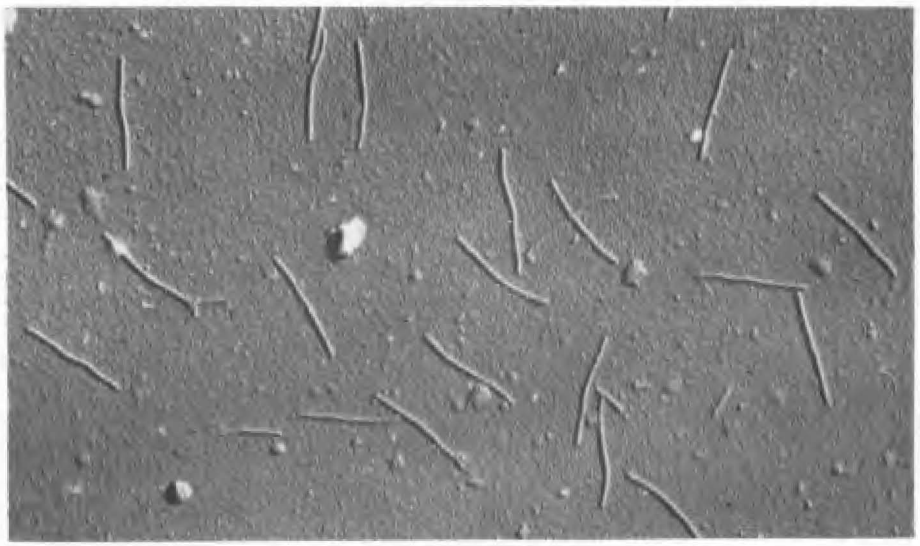

Fig. 9. Electron micrograph of Cymbidium mosaic virus from a leaf-dip preparation of Cattleya. (× 30,000)

purified virus suspension. precipitin test was performed by microagglutination technique. Plant juice for antigen in test was diluted to 1/10 with 0.85 % saline.

The results of the precipitin tests are shown in table 1. The specific titer of the antiserum was found to be 1 : 2048. The antiserum did not react with the juice of healthy plants.

6. *Electron microscopy*

A drop of virus preparation was placed on the colodium-coated grid and air-dried specimen was shadowcasted with cromium. The grids were examined under the electron microscope. Fig. 8 shows the distribution of particle lengths.

Preparations from diseased orchid plants and artificially infected plants contained sinuous particles similar in shape and size to those described for CyMV by Gold and Jensen (6, 7). The particles lengths ranged from about 125 to 700 mμ, and the most common length appeared to be 475 mμ (Fig. 8, 9). The width of the virus particles in dip-preparation stained with phosphotungstic acid for electron microscopy appeared to be 13 mμ. No rod-shaped particles were observed in the specimens from healthy plants.

DISCUSSION

The results show that the causal virus of the foliar necrosis disease of ***Cymbidium*** and ***Cattleya*** plants is Cymbidium mosaic virus. The disease of those plants is characterized by elongated chlorotic areas and necrotic streaks on the leaves of ***Cymbidium***, and reddish-brown necrotic streaks and necrotic ring patterns on the leaves of ***Cattleya***. These symptoms appeared very similar to the figures of CyMV infected plants presented by other workers (11, 12, 13, 14, 15, 17, 18, 19). Many flowers of ***Cymbidium*** and ***Cattleya*** infected with CyMV are found to be symptomless as shown by Jensen (15) and Kado (18). However, on some ***Cattleya*** hybrids, very faint discoloration of flowers is noticed. As the symptoms are faint color breaking, which many growers may have usually failed to notice. The virus produces local lesions on inoculated leaves of ***Datura stramonium***, ***Cassia occidentalis*** and ***Chenopodium amaranticolor*** after the incubation periods of 10–25, 4–6 and 15–25 days, respectively. The lesions in these plants were similar to those reported for CyMV (17, 18, 19, 24). TMV also produces local lesions in ***Datura stramonium*** and ***Ch. amaranticolor***. However, CyMV is easily distinguished from TMV by its long incubation period for the development of local lesions. Namely, those lesions on ***Datura*** caused with TMV can be detected within 2–3 days after inoculation, while those for CyMV required 10–25 days for develoment and appear first in the older leaves. It has already been described that the local lesion reactions of ***D. stramonium***, ***Ch. amaranticolor*** and ***C. occidentalis*** have been a reliable means for identification of CyMV (5, 18, 24). Particles of CyMV reported in literatures as sinuous rods are found to be 475–480 mμ in length and the diameter was usually 18 mμ (3, 6, 7, 20, 22, 23). However, Franki (5) reported that the size of CyMV was 475×13 mμ, in phosphotungstic

acid-stained preparations. He described that the discrepancy of this width was probably due to the fact that previous measurments had all been made on metal-shadowed preparations. Particles of causal virus in the present paper are sinuous rods 475 mμ in length and about 13 mμ in diameter, and the width agrees with those reported by Franki.

Antiserum reacted strongly against all of the juice of diseased plants containing the particles of about 475 mμ in length. The result shows that serological methods are useful in detecting and distinguishing viruses in orchid plants, as described by Zaitlin et al (25).

The disease casued by CyMV is observed to be widespread in *Cattleya*, *Cymbidium* and other orchid plants in Japan, especially in the older commercial *Cymbidium*. The virus disease will probably spread from plant to plant by mechanical means. CyMV was also isolated from plants of *Calanthe*, *Dendrobium*, *Epidendrum*, *Miltonia*, *Oncidium*, *Peristeria*, *Phalenopsis*, *Vanda*, and *Zygopetalum* in Japan.

SUMMARY

A disease characterized by chlorotic areas and sunken, necrotic streaks on the leaves of *Cymbidium* proved to be caused by CyMV from the results of experiments on host range, physical properties, and morphology of virus particles. The virus was also isolated from *Cattleya* exhibiting sunken, reddish-brown necrosis and necrotic ring patterns on the leaves. Many flowers of *Cymbidium* and *Cattleya* infected by CyMV are commonly symptomless, although faint discoloration is observed on some varities of *Cattleya*. Causal virus is easily transmitted by diseased plant juice. It is also transmitted by artificial alternate cuttings of leaves or roots of diseased and healthy plants. The virus is transmitted systemically to *Cymbidium*, *Cattleya*, *Epidendrum*, *Dendrobium*, *Miltonia*, and *Zygopetalum*. Local lesions are formed on *Datura stramonium*, *Cassia occidentalis*, *Chenopodium amaranticolor* and *Tetragonia expansa*, in 10–25, 4–6, 15–25 and 15–30 days after inoculation, respectively. Among other plants tested, 22 species in 11 families, are found to be insusceptible to the virus.

The virus in diseased plants juice is inactivated at temperatures of 65—70 °C for 10 minutes exposure. It withstood dilution of 5×10^{-4} or 10^{-5} but not 10^{-6}, and aging *in vitro* for one month at 18 °C. Dried residue of diseased plant juice is infective after the storage of 8 days, but not after 10 days at 20 °C.

Particles of the virus appear under the electron microscope as sinuous rods, about 475 mμ in length and 13 mμ in diameter. Antiserum with titer of 1 : 2048 in microagglutination test was obtained from a rabbit being injected with partially purified virus intramuscularly and intravenously.

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