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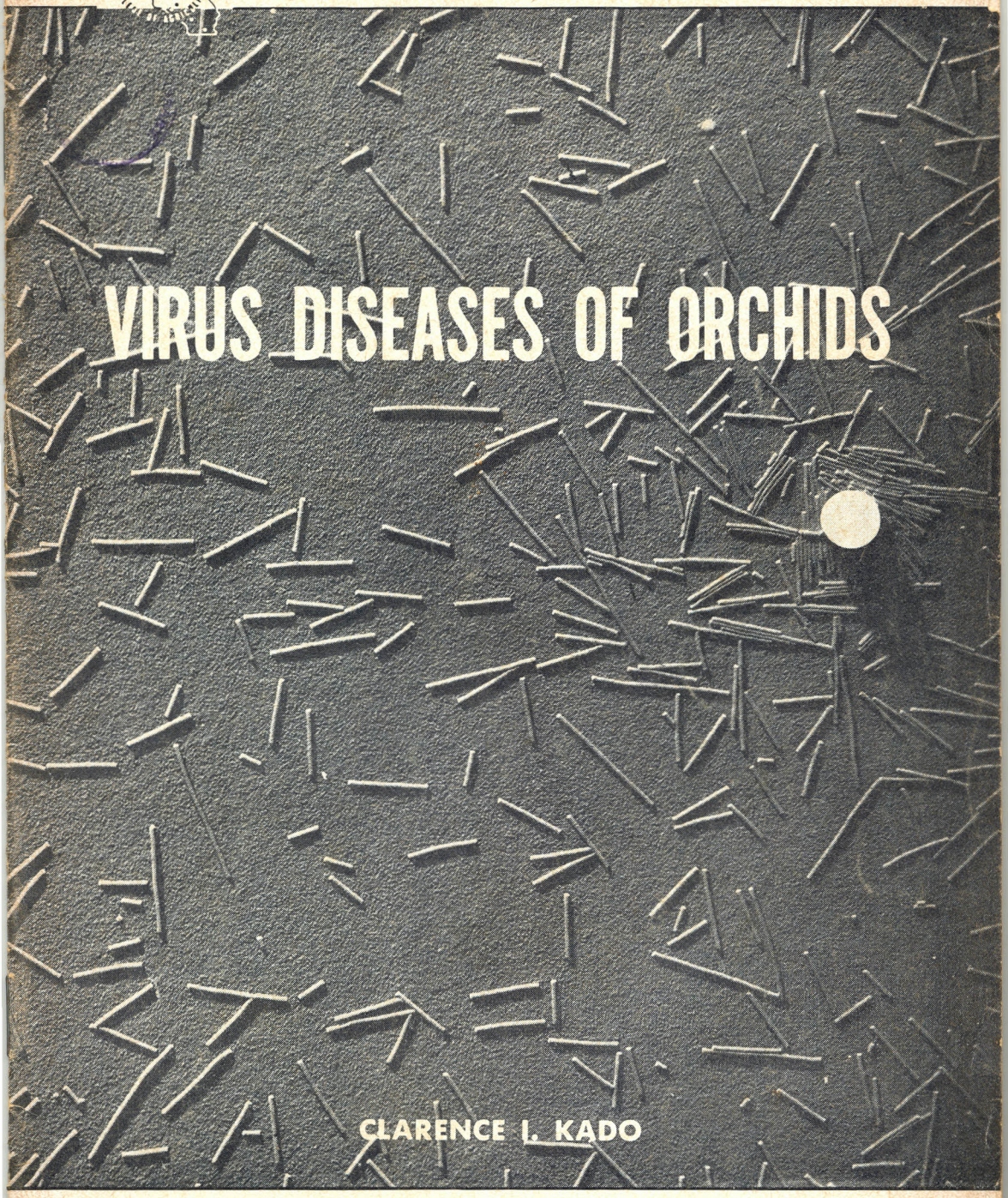

VIRUS DISEASES OF ORCHIDS

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Virus diseases are probably responsible for the mosaic and necrotic symptoms in orchids that are often blamed on other disorders. This circular discusses the four viruses of orchids that have been identified thus far: cymbidium mosaic, orchid tobacco mosaic, vanda mosaic, and cymbidium ringspot. Symptoms in several orchid genera are described, and methods are suggested for disease control. It also tells how to test for these orchid virus diseases on non-orchidaceous, herbaceous plants.

COVER PHOTO: Particles of orchid tobacco mosaic virus (strain 03) as seen in the electron microscope and magnified ×35,000. Various lengths of these particles appear, but only those measuring 300 nanometers are infectious. The white sphere is a polystyrene latex particle measuring 260 nanometers which was used as an internal calibration standard.

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VIRUS DISEASES OF ORCHIDS

MOSAIC AND necrotic symptoms in orchids were once thought to be caused by nutritional or genetic disorders—or by chemical and mechanical injuries. These assumptions were based on the frequent disappearance of symptoms when growing conditions were altered, or plants were repotted, or with subsequent new growth. Today, viruses—probably a large number or many strains of given viruses—are recognized to be the main causes of these maladies. To identify true pathogens, however, careful laboratory analyses are usually required.

Virus diseases occur in most of the common genera of orchids grown in the United States. The incidence in orchid nurseries has varied from a few infected plants among hundreds of healthy ones to a few healthy plants among hundreds of diseased ones. In any case, rapid transportation and increased inter- and intracontinental trade have helped to spread "old" and introduce "new" viruses.

Although many disease symptoms have been listed and assumed to be caused by different viruses in orchids, only four have been isolated, morphologically distinguished, and characterized by their chemical properties. They are: cymbidium mosaic and orchid tobacco mosaic (both widespread); vanda mosaic (limited, so far as is known, to species of *Vanda* and *Aranda*); and cymbidium ringspot (reported only in England). Some of these virus diseases are serologically and chemically related to virus diseases in other plants. Those known thus far are listed in table 1.

Based on continuing and limited experiments, this circular describes these and at least a few of the other viruses as they affect some orchids commonly grown in commercial nurseries. Methods for control are also suggested.

TABLE 1

SOME ORCHID VIRUSES AND THEIR RELATIVES IN OTHER PLANTS

Orchid viruses	Related viruses*
orchid tobacco mosaic strains	common tobacco mosaic Holme's ribgrass strain tomato mosaic strains tomato aucuba mosaic Sammon's opuntia virus cucumber viruses 3 and 4 cowpea or bean strain
cymbidium mosaic	potato aucuba mosaic white clover mosaic cactus virus X clover yellow mosaic potato virus X hydrangea ringspot
vanda mosaic	unknown
cymbidium ringspot†	undescribed virus from white clover

* As identified by chemical and serological tests. These related viruses have not been found in orchids, and it is not known if they occur in orchids naturally.

† Discovered in England; not reported elsewhere.

Diseases and Symptoms

Symptoms expressed by a given virus disease may differ considerably in degree of severity and pattern among orchid genera, among species and hybrids of a given genus, and even among varieties. Some plants infected by two different viruses may not survive. Leaves may senesce prematurely and break at their abscission zone. *Cattleya* leaves and pseudobulbs, for instance, may shrivel as though they were drained of their fluid.

On the other hand, diseased orchids may remain symptomless or may develop new shoots that are devoid of typical symptoms displayed by older leaves. Mere inspection, therefore, may not be adequate. For this reason, it is suggested that the tests discussed in the section "Diagnosis" be used to detect the presence of virus disease.

Cymbidium mosaic

Also called black streak, orchid mosaic, leaf necrosis, and chlorotic ringspot, this virus or a closely related strain affects more than 20 genera of the Orchidaceae. Tests showed that cymbidium mosaic is highly infectious and relatively stable. Virus in the sap from infected *Cattleya* leaves can be diluted as much as 100,000 times before infectivity is lost. The purified virus was destroyed when heated in buffer solution for 10 minutes at about 70°C. Transmission by an insect was not demonstrated.

In *Cattleya*, the most common symptoms in the older leaves are brownish, sunken, necrotic, and irregular streaks (fig. 1A). In severe cases, the epidermis of the leaf collapses under the necrotic regions; dry conditions cause infected leaves to be irreversibly dehydrated (fig. 1B). Species of *Cattleya* and their related hybrids developed early symptoms in one to eight weeks after inoculation. Initial symptoms in *Laeliocattleya shoshone* appeared four to six weeks after inoculation with sap from diseased *Cattleya*.

In *Cymbidium*, the leaves usually show scattered mosaic, chlorotic, and necrotic symptoms (figs. 1C and 1D). Light, chlorotic areas in the new shoots appear first, followed by distinct mosaic and chlorotic flecking patterns in the mature leaves. Necrotic or black stripes generally develop along the veins as the leaves grow older. After experimental inoculation, *Cymbidium* seedlings developed symptoms in one to 12 weeks; in older leaves, symptoms occurred in 12 to 16 weeks, if at all.

In *Vanda*, chlorotic flecks or spots developed in the leaves eight to 12 weeks following inoculation with infectious sap.

In *Phalaenopsis*, symptoms appeared in 15 to 26 days following inoculation with freshly expressed juice from a diseased *Phalaenopsis* leaf. Initial symptoms were varied: light-green to chlorotic spots, green, ring-like patterns (which later became necrotic) on young leaves, and irregular, green, chlorotic, or necrotic areas on inoculated older leaves. In *naturally* infected *Phalaenopsis*, symptoms ranged from mosaic patterns to tissue collapse (fig. 1E)—that is, broad, chlorotic, furrowed, and water-soaked areas. Tissue collapse was particularly marked on the under-surface of leaves. This disease is not restricted to the orchids described but it is found in most other genera commonly grown in nurseries (figs. 1F and 1G).

Orchid tobacco mosaic

The virus causing this disease induces ringspot symptoms on *Odontoglossum grande* and has been known as odontoglossum ringspot virus. Recent evidence indicates that it is related to the common strain of tobacco mosaic virus and is one of several tobacco mosaic virus strains that infect orchids. In tests, the virus was still infective even when sap was diluted

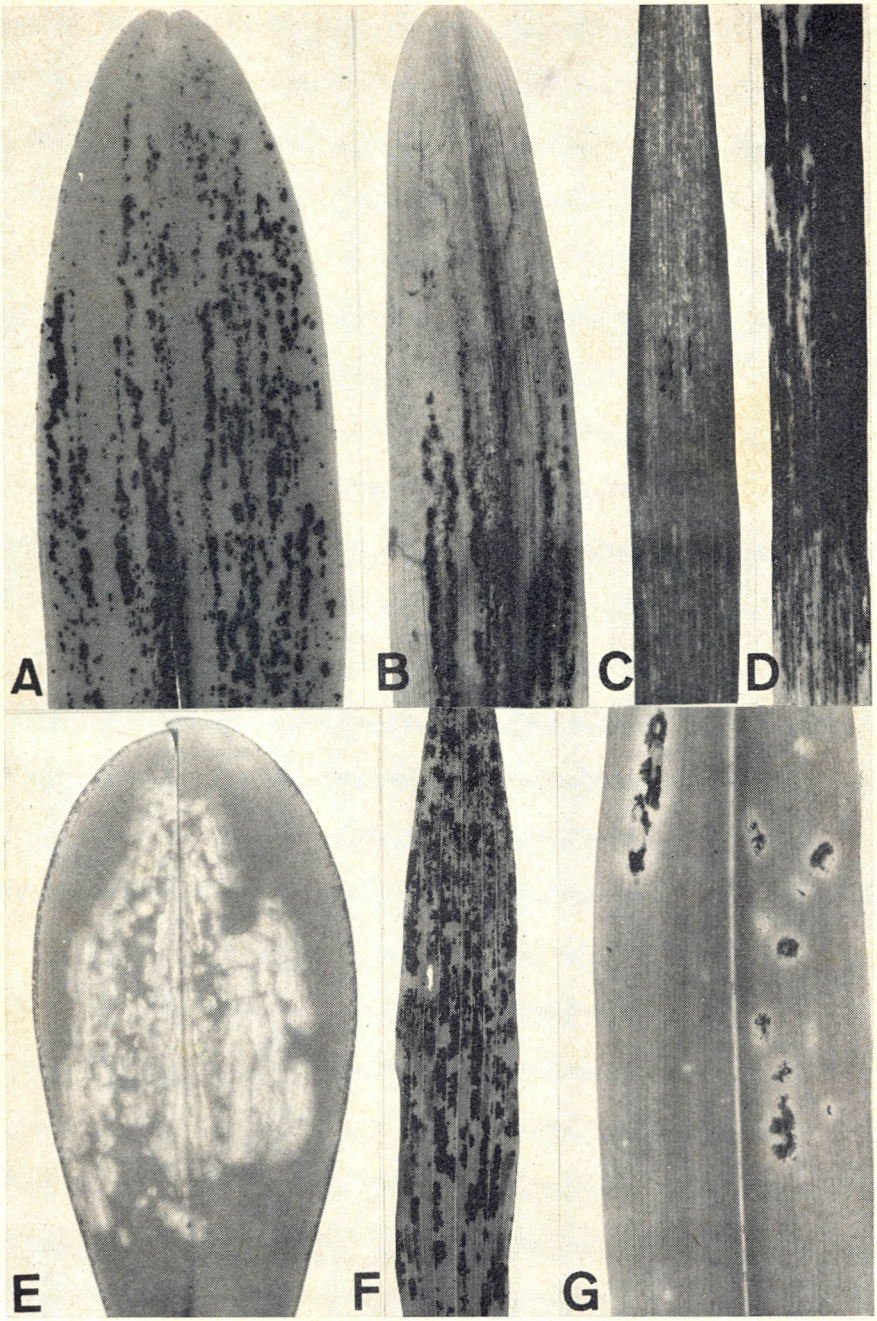

Fig. 1. Symptoms caused by cymbidium mosaic virus. **A.** Veinal necrosis of a Cattleya leaf. **B.**Veinal necrosis, streaking and severe dehydration. **C.** Chlorotic speckling and necrosis of a Cymbidium leaf. **D.** Severe necrosis and blackening of an entire leaf of Cymbidium. **E.** Chlorotic, water-soaked, veinal grooves or furrowing of a Phalaenopsis leaf. **F.** Interveinal necrosis and black patches of a Zygopetallum leaf. **G.** Water-soaked spots and necrosis of a Phragmipedium leaf.

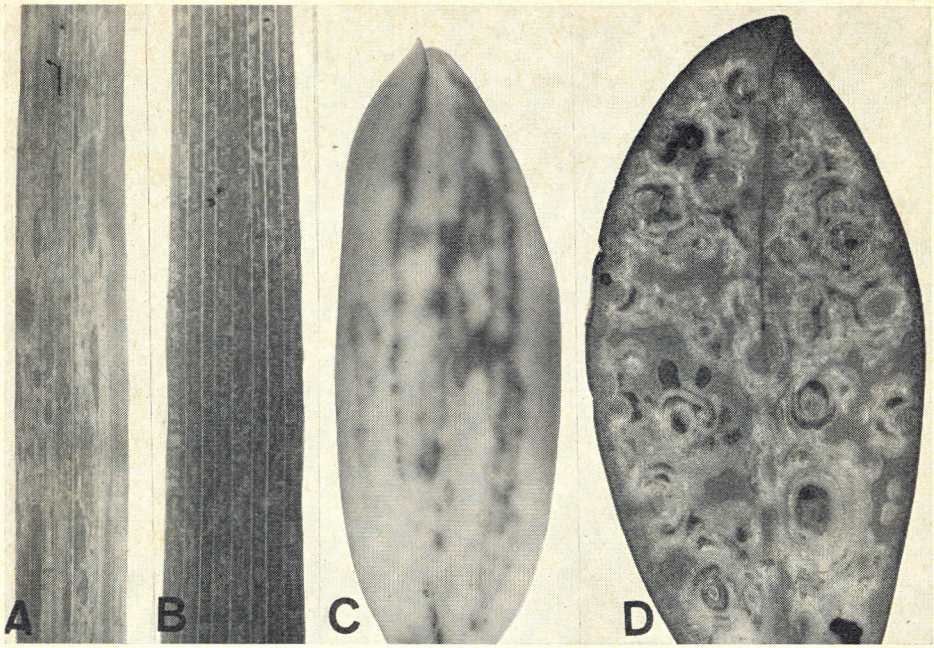

Fig. 2. Symptoms of a dual infection by orchid tobacco mosaic and cymbidium mosaic virus. **A.** Diamond-mottle, chloratic speckling of a Cymbidium leaf. **B.** Ring-like mosaic patterns of a Cymbidium leaf. **C.** Dark-green ring patterns of a young Cattleya leaf infected by tobacco mosaic virus strain 03. **D.** Deep, grooved, chlorotic ring patterns in a Phalaenopsis leaf infected by tobacco mosaic virus strain 06 and cymbidium mosaic virus.

in buffer solution 100,000 times; it remained stable and active when infectious sap was heated at 90°C for ten minutes. Transmission of this virus by insects to orchids has not been demonstrated.

In *Cymbidium*, the disease is characterized by elongated, chlorotic streaks and diamond-shaped, light-colored patches on the leaves. Under severe conditions, the streaks and patches become necrotic and turn brown or black. *Cymbidium* was once thought to be nonsusceptible to this virus—probably because in early studies symptoms did not appear until a year after inoculation.

In *Cattleya*, symptoms were very mild or absent when infected by the TMV-03 strain of orchid tobacco mosaic virus. At times, ring patterns formed on young leaves.

Multiple virus infections

A limited survey of orchid viruses in California disclosed that cymbidium mosaic virus and two strains (TMV-01 and TMV-03) of orchid tobacco mosaic virus were simultaneously infecting about 10 per cent of the *Cattleya* and *Cymbidium* plants tested. Doubly infected *Cymbidium* plants showed a mixture of mosaic, bar, and diamond-shaped patterns (figs. 2A and 2B), as well as necrosis.

Cattleya plants which may have been infected by more than one virus displayed chlorotic patches, sunken necrotic spots, and light-green zones in older leaves (fig. 2C). In *Phalaenopsis*, the presence of both viruses resulted in deeply furrowed ring patterns in the underside of older leaves (fig. 2D). Younger leaves apparently do not show such symptoms. These

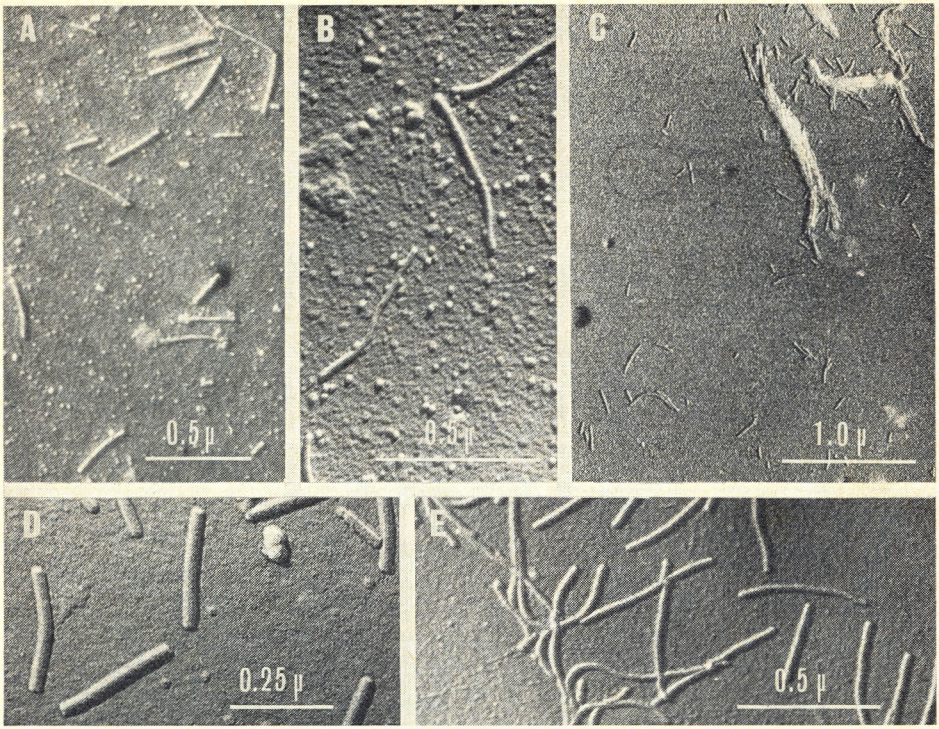

Fig. 3. Electronmicrographs of some common viruses of orchids. Viruses were shadowed with uranium and carbonized. **A.** Orchid tobacco mosaic virus in sap obtained from a contaminated knife blade. **B.** Cymbidium mosaic virus in sap obtained from a contaminated shear. **C.** Vanda mosaic virus cross-absorbed with heterologous antiserum of cymbidium mosaic virus (photo courtesy of Dr. Harry H. Murakishi). **D.** Orchid tobacco mosaic virus purified from diseased Cymbidium. **E.** Cymbidium mosaic virus purified from diseased Phalaenopsis. Some of these viruses are of different magnifications and should be judged according to the scales given.

ring symptoms are unlike those described for *Phalaenopsis* infected with cymbidium mosaic virus alone.

Cymbidium ringspot

The virus causing this disease has been transmitted by sap to various herbaceous hosts. It has been found in England only, but because of its highly stable properties (it requires 10 minutes at temperatures of 85°C or higher for inactivation), cymbidium ringspot probably occurs in the United States as well. Unlike the other three rod-shaped viruses shown in figure 3, it is a spherical particle. The disease causes conspicuous leaf mottling and the brown, necrotic flecks that are commonly associated with cymbidium mosaic. In fact, both cymbidium ringspot virus and cymbidium mosaic virus have been found in the same plant.

Vanda mosaic

Color-break and malformation of the flowers of *Vanda 'Miss Joaquin'* are caused by vanda mosaic virus which can be transmitted readily by juice inoculation. The symptoms appear after 16 to 30 days. The virus is different from both the orchid tobacco mosaic virus and the unidentified aphid-transmitted color-break virus of *Cattleya*. Streaks may develop in any portion of the affected flower, but the most striking color-break occurs in the dorsal sepal and petals. Sometimes, necrotic, brown streaks develop at the

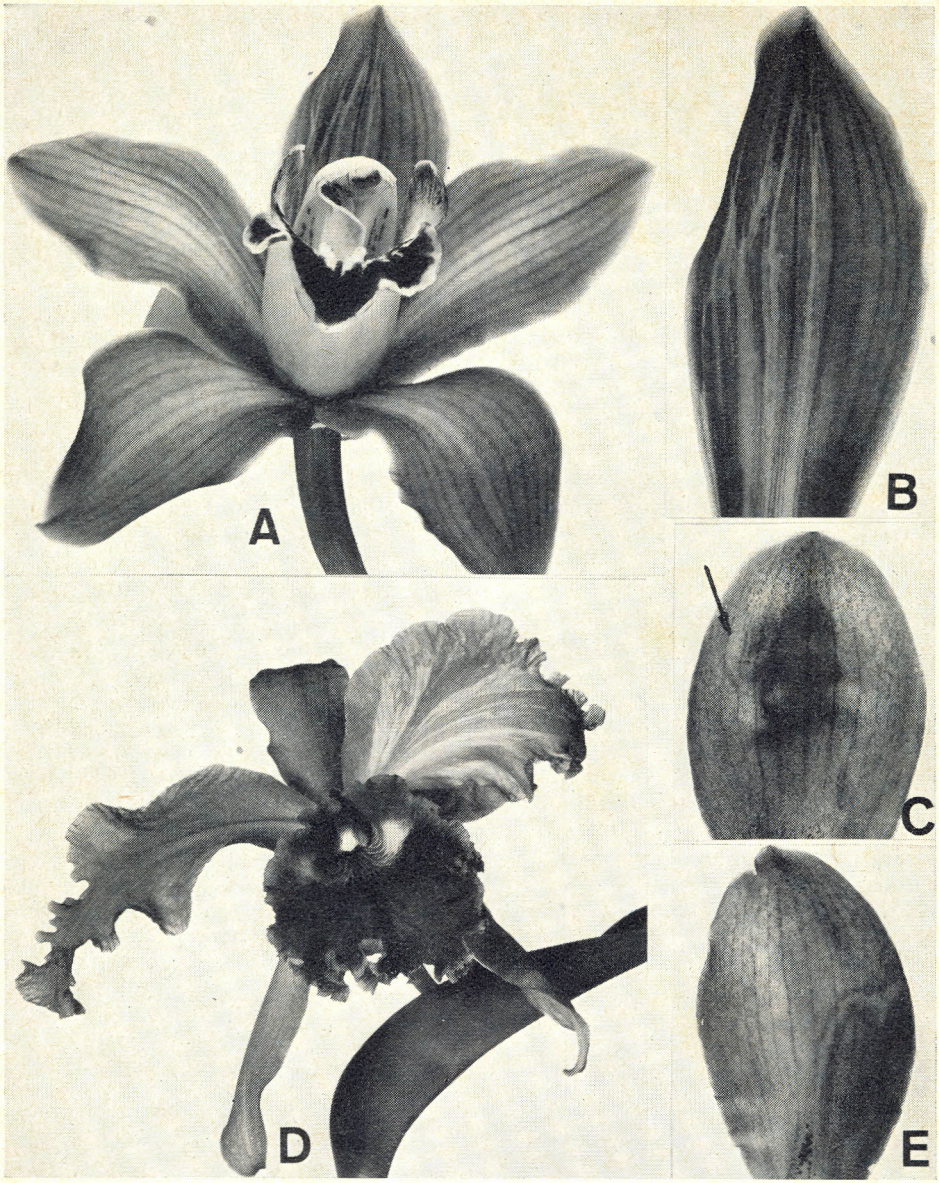

Fig. 4. Color-break symptoms caused by a strain of orchid tobacco mosaic virus. **A.** Color-break symptoms in the upper sepal and left petal and lip distortion of a Cymbidium flower. **B.** Close view of the upper sepal in flower A. **C.** Ringspot in the sepal of a Cymbidium flower (arrow). **D.** Color-break of a petal and distorted symmetry of a Cattleya flower. **E.** Line break pattern in a petal of a Cymbidium flower.

edges of the petals, sepals and labellum. Another juice-transmitted virus that causes leaf mosaic in *Dendrobium superbum* was associated with color-break in both sepals and petals.

Color-break

Unsightly patches of discoloration on

flowers occur in various orchid genera and may be caused by one or more viruses. *Cattleya* plants show the most distinctive markings of this disease. As far as is known, the virus cannot be transmitted by juice, but it does appear to be conveyed by the green peach aphid, *Myzus persicae*. Although characters of the virus have not been fully ascertained, it was observed that the time between infection of the flower buds and occurrence of color-break was 12 to 19 days after the flower matured.

A second form of pigment distortion in *Cattleya* flowers (fig. 4D) appears to be caused by rod-shaped viruses with measurements similar to cymbidium mosaic virus and tobacco mosaic virus. Studies using antiserum confirmed these observations; however, other tests in which highly purified viruses were used showed that color-break was *not* caused by cymbidum mosaic. Nevertheless, a strain of cymbidium mosaic virus may produce the brown, necrotic streak in *Cattleya*. Cymbidium flowers infected by one of the orchid tobacco mosaic strains also show pigment distortions (fig. 4A). In these instances, the pastel color of the *Cymbidium* flower is disrupted by dark-green or white line patterns (fig. 4B). In dark-colored *Cymbidium* flowers, the symptom is more marked. Sometimes, ringspot patterns appear on the petals or sepals (figs. 4C and 4E). In tests, orchid tobacco mosaic virus strains were also recovered from individual plants displaying the above symptoms.

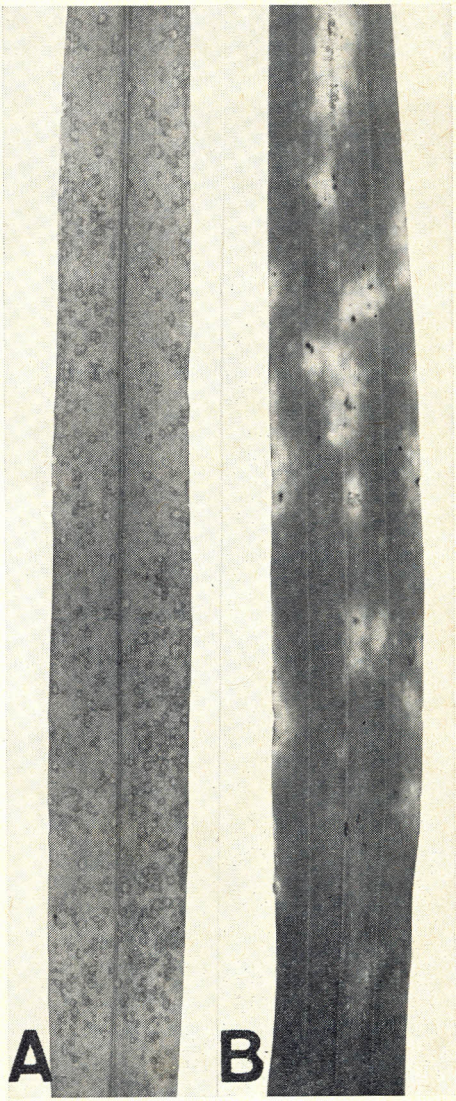

Fig. 5. Pseudo-virus symptoms in Cymbidium leaves. **A.** Enlarged, water-soaked spots originally created by spider mite damage. **B.** Chlorotic patches caused by the feeding of scales.

Virus-like Symptoms

Nutritional or chemical disorders or insect damage can produce various virus-like symptoms. Sodium in the water supply, for instance, has been blamed for certain virus-like disorders. Also, symptoms resembling color-break in *Vanda* flowers were reported to be caused by thrips, and distorted forms of *Cymbidium* flowers and discolored spots have been attributed to aphid-feeding injuries on immature flower buds.

The hard, tough tissues of orchid leaves seem to be more resistant to insect feeding damage, but some damage actually can be caused by red spider mites on the undersides of leaves and certain egg-laying insects, such as the katydid. On *Cymbidium* plants, the feeding areas of the mites resemble tiny dots that later become water-soaked under hot, humid conditions. (fig. 5A).

Other virus-like symptoms are caused by scales feeding on *Cymbidium* leaves. Usually a yellow area surrounds the point of feeding (fig. 5B). When many scales are present, the areas become confluent, and the entire leaf appears chlorotic.

Besides insects—smog, fungi, bacteria, and improperly used insecticides, miticides, fungicides, and the like, can cause symptoms that may resemble those of different viruses.

How Virus Diseases Spread

The most commonly encountered viruses are transferred by hands and tools from infected plants into wounds or cuts made in healthy plants during pruning, dividing, and harvesting operations (fig. 6). Otherwise, the only other vector of virus disease appears to be the green peach aphid, *Myzus persicae,* which transmits the unknown virus that causes severe color-break in *Cattleya.* (The common orchid aphid, *Ceratophis lataniae,* does not transmit this virus.)

Spider mites, scales, slugs, snails, ants, mealy bugs, and flies have been seen on orchids, but they have not been carefully tested as possible vectors of orchid viruses. Factors that do not appear to be likely vectors are: splashing water, root grafts, and contact with other intact plants, leaves, infected debris, contaminated pots, benches, compost, and stakes. However, compost should never be reused for potting. It has been demonstrated that seedlings can be infected when grown in sphagnum moss obtained from pots in which virus-infected plants were grown.

Although weeds outside the greenhouse probably do not serve as the source of virus, they may be breeding hosts of possible insect vectors. More likely, newly purchased infected plants are the potential primary sources, since viruses can spread through the sap released from such plants during dividing of clones and during harvesting of blossoms.

Since cymbidium mosaic virus and orchid tobacco mosaic virus strains are highly infectious, and since these viruses occur in pollen, the chance of transmission during artificial pollination is probably high. On the other hand, infection of progeny seedlings from seeds derived from such pollination seems less likely. More research is required on this possible means of transmission.

Virus symptoms in orchids have been seen in their native habitat of the Central American jungles, but they have not been investigated. Also, the severe color-break disease, which is known to be aphid-

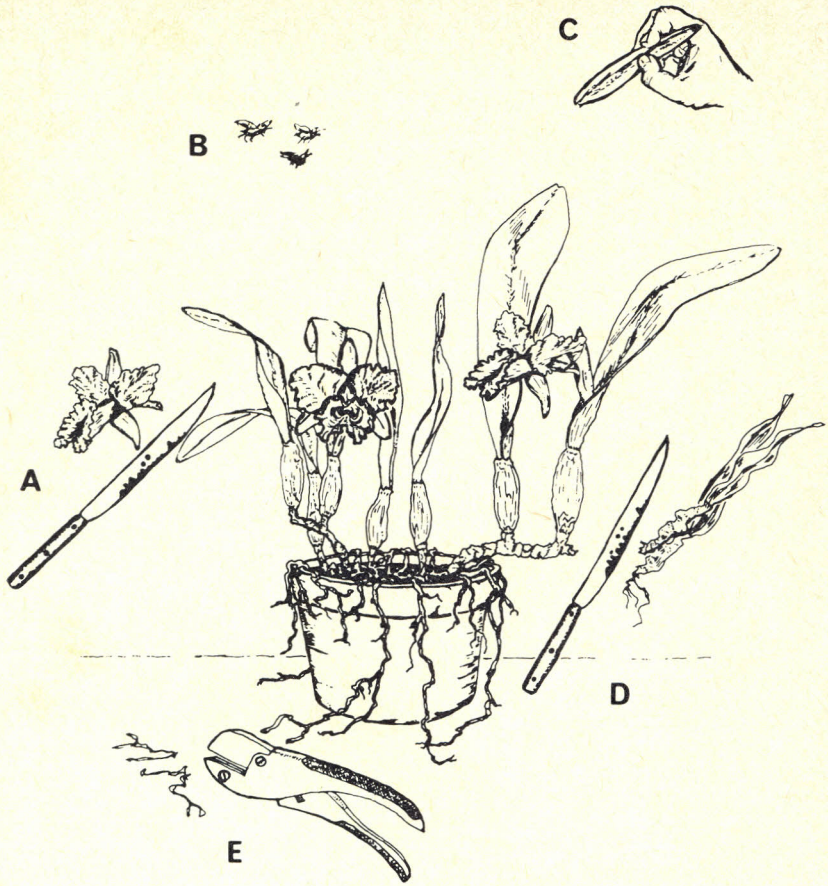

Fig. 6. Spreading infectious virus: **A.** During the harvest of flowers. **B.** By aphid or other insect vectors. **C.** By hands. **D.** During dividing and cleaning operations. **E.** During the trimming of excess roots.

transmitted, has been observed in *Cattleya* imported from tropical America.

Transmission by seed

Although several other plant viruses are transmitted via the seed, this vector is an unlikely means for transmission of the orchid virus diseases described here. The writer has personally sown many *Cattleya* and *Cymbidium* seeds and performed assays on test-plant extracts from seedlings that were developing in sterilized medium. Many of the seedlings were crosses made between parents that were infected by cymbidium mosaic virus. Of all the seedlings tested, none showed signs of virus disease—visible or otherwise. Nevertheless, further research is necessary on this aspect of virus transmission. Perhaps seeds from certain orchid crosses can carry virus; at least, the carpels housing the seeds can contain virus.

TABLE 2

NON-ORCHIDACEOUS PLANTS USED TO TEST FOR SOME COMMON ORCHID VIRUS DISEASES

Orchid viruses*	Non-orchidaceous test plant†	Lesions after inoculation		General, local-lesion character and symptoms on test plant leaves
		Time of appearance	Approx. size	
		days	*mm.*	
Orchid tobacco mosaic virus strains:	*Capsicum frutescens* cv. 'California Wonder' (bell pepper)	8-9	2	Chlorotic. Disease becomes invasive, systemic.
	Chenopodium amaranticolor (pigweed, lamb's-quarters)	5-6	1	Small, white, necrotic.
	Chenopodium quinoa	7-10	5	Chlorotic.
	Datura stramonium (Jimsonweed)	6-7	1-2	White, necrotic.
	Gomphrena globosa (globe-amaranth)	8-10	2-3	White, necrotic, red-bordered.
	Nicotiana glutinosa (tobacco)	3-4	1-2	White, necrotic, may spread circumferentially into ring patterns.
	Nicotiana debneyi (tobacco)	4-6	3-4	Chlorotic, later develops into ringspot patterns.
	Nicotiana tabacum cv.'Kentucky Burley' (tobacco)	3-4	4-5	Necrotic lesions with reddish borders.
	Tetragonia expansa (New Zealand spinach)	7-8	3-4	Chlorotic, sometimes develops into ring patterns.
Cymbidium mosaic virus strains:	*Cassia occidentalis* (senna)	3-4	1-2	Dark, necrotic.
	Cassia tora	3-4	1-2	Dark, necrotic.
	Chenopodium amaranticolor (pigweed, lamb's-quarters)	14-18	2-3	Dark-green, occasionally develops into rings.
	Datura stramonium L. (white flower Jimsonweed)	8-12	3-4	Dark-green, remains green after leaves turn yellow.
	Tetragonia expansa (New Zealand spinach)	8-22	1-2	White.
Vanda mosaic strains‡	Non-orchid test plants unknown, but the weed orchid *Spathoglottis* can be used	14-21	1-2	Necrotic, later develops into ringspots and mosaic.
Cymbidium ringspot strains§	*Emilia sagittata* (tassel flower)	4-7	3-4	Chlorotic spots and rings; large, ringed etchings develop later.
	Chenopodium amaranticolor (pigweed, lamb's-quarters)	7-10	2-3	Necrotic lesions.

* These viruses are not restricted to one orchid genus, but can infect orchids of many different genera, as well as non-orchid plants as listed in this table.
† Many other herbaceous plants have been used, but those listed are the most reliable.
‡ Described only in Hawaii but may occur elsewhere.
§ Described only in England, but may occur elsewhere.

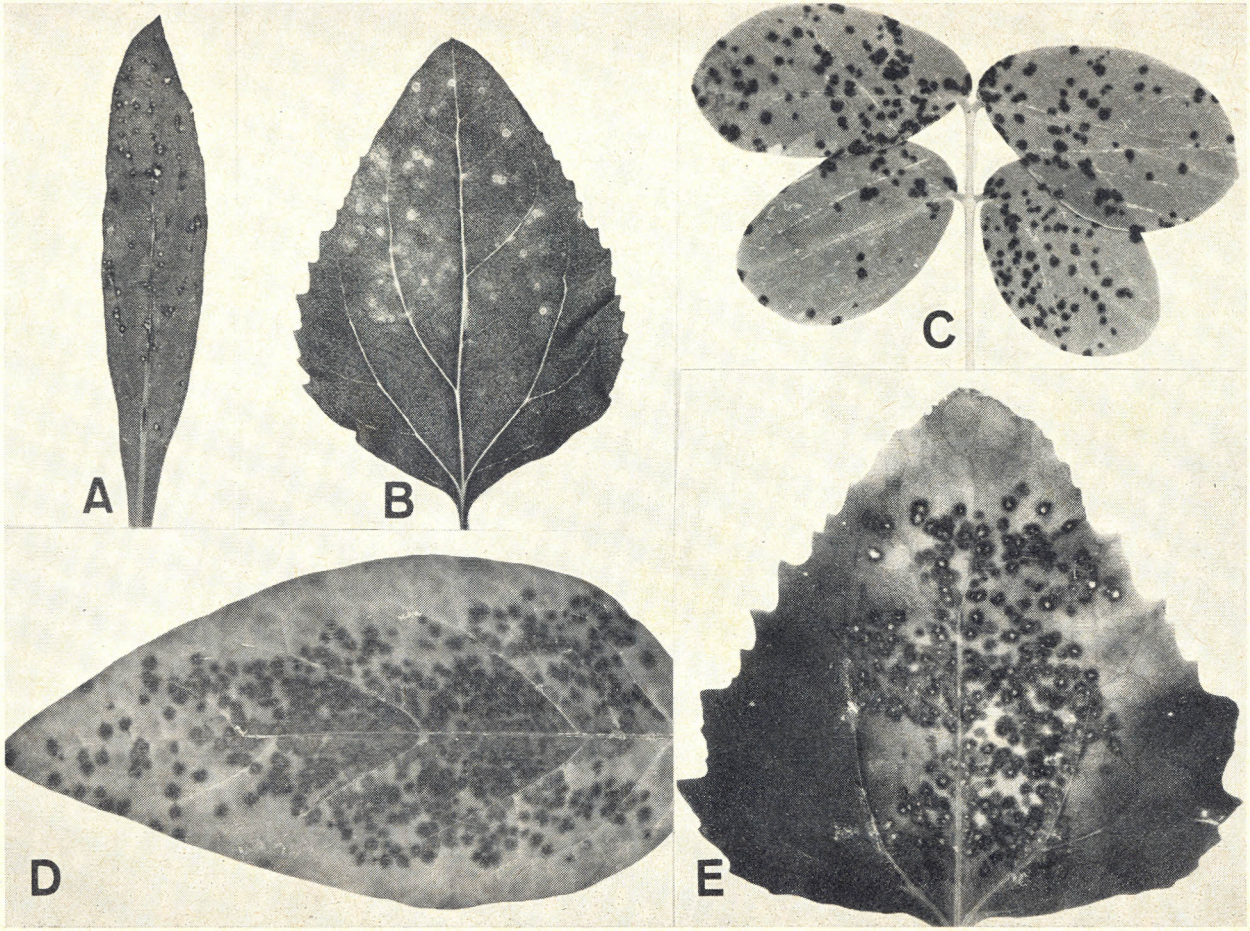

Fig. 7. Local lesions on leaves of non-orchidaceous test plants inoculated with orchid viruses. **A.** Red-bordered, necrotic lesions from orchid tobacco mosaic virus on Gomphrena globosa L. **B.** Chlorotic lesions from orchid tobacco mosaic virus on Chenopodium amaranticolor Coste and Reyn. **C.** Red, necrotic lesions from cymbidium mosaic virus on Cassia occidentalis L. **D.** Dark-green lesions from cymbidium mosaic virus on Datura stramonium L. **E.** Green-bordered lesions from cymbidium mosaic virus on C. amaranticolor.

Diagnosis

Non-orchidaceous plants known to be highly susceptible to some of the virus diseases discussed in this circular are listed in table 2 as indicator test plants for diagnosis. Note that *Chenopodium amaranticolor* (sometimes called lamb's-quarters, goose-foot, or pigweed) can be used for three of the four virus diseases.

To test for virus disease using indicator test plants, a small piece of leaf tissue from the suspect plant is usually ground in a sterile mortar or pulverized with a hammer between layers of waxed paper. Test plants are then inoculated by gently swabbing the homogenized material—mixed with a little water or a 1 per cent solution of potassium phosphate (K_2HPO_4)—with a cotton swab stick onto the upper surface of the test plant's leaves. To facilitate infection, 500 to 600 mesh carborundum (an abrasive also known as silicon carbide) is usually sprinkled until barely perceptible on the leaves before inoculation. It is helpful to use a folded piece of paper towel to support the leaves during inoculation. After inoculation, the leaves may be washed with tap water and the plant appropriately tagged. The paper towel, cotton swab, and folded piece of paper should be placed in a disposable container and burned when convenient. Hands should be thoroughly washed after each inoculation.

If the orchid plant tested is diseased, the test plants should respond by producing localized, dark spots called local lesions. Juice from orchids containing cymbidium mosaic virus, orchid tobacco mosaic virus, or both, will cause local lesions to appear on the inoculated leaves of corresponding test plants (see fig. 7).

Other diagnostic methods, such as electron microscopy and serological tests (in which specific antisera are prepared for specific viruses) require sophisticated techniques and elaborate equipment. Certainly, they are not practical for the grower; and even under laboratory conditions, they are less sensitive than the local lesion tests described here.

Control

Exclusion and sanitary measures are the only known methods to control virus diseases of orchids. Virus-resistant orchid varieties have not yet been developed (although this could be the cheapest and easiest means of control); and no one yet knows how to destroy viruses in living plants without killing the plants themselves.

Exclusion and Eradication

Many old plants are probably diseased since they have had more chances to become infected through repeated harvesting, cleaning, and repotting. Some of them appear symptomless; nevertheless, they can serve as common sources of virus.

All obviously diseased plants should be isolated from healthy plants—particularly from young seedlings. Diseased plants of outdated crosses should be burned or buried. Newly traded or purchased plants should be examined carefully for virus symptoms and separated from healthy plants until they are known to be free of disease. Seedlings should be isolated from older plants.

Sanitation

All cutting tools should be sterilized, and strict sanitary measures should be used during repotting, harvesting, cleaning, and, especially, dividing operations of plants. Experiments have shown that a single cut on a diseased plant with a knife

or pruning shears was sufficient to initiate a 60 per cent infection in healthy *Vanda* plants. Merely washing such tools with water or detergent is insufficient.

To sterilize effectively, do *one* of the following:

(1) Wash tools vigorously in a strong disinfectant such as sodium hypochlorite (household laundry bleach), followed by rinsing in water.

(2) Boil tools in water for 30 to 60 minutes.

(3) Bake tools in dry oven for two hours at 350°F.

(4) Autoclave the tools (or use a pressure cooker) for 20 minutes at 15 pounds pressure per square inch.

Single-edge stainless steel razor blades can be used for cutting operations—and re-used if placed in boiling water for 30 to 60 minutes. "Flaming" with portable gas torches also can be an effective decontaminant.

Hands should be carefully washed with soap and hot water after handling diseased material and tools. Although they may not be likely sources of infection, dirty pots, stakes, and wire hangers should be cleaned and sterilized if possible. Orchid viruses can be transmitted by used sphagnum moss, but not by compost. Pasteurization of the compost, however, helps to control phytopathogenic bacteria, fungi, and weed seeds. Also, areas surrounding the greenhouse should be cleared of all weeds, since they may harbor potential insect vectors.

Spraying

The timely application of proper insecticides can protect healthy plants from infection that might be carried by virus-carrying insects, but no sprays will eliminate orchid virus diseases without inflicting serious damage to the living plant.

Doubtful Means of Control

Methods *unsuited* for curing virus-diseased plants include soaking bulbs in acid, copper sulfate, milk, or detergent. Exposure of bulbs and plants to direct sunlight and adding oxidizing agents such as potassium permanganate, hydrogen peroxide, or adding iodine to the compost are also ineffective, as are fungicides and bactericides.

Orchid viruses are unusually stable; therefore, extremely high or low temperatures affect the plant rather than the infecting virus. Limited experiments with high-temperature air and water treatment of virus-infected *Cattleya* plants were unsuccessful. Viruses isolated in test tubes were vulnerable to 60 to 90°C (140 to 194°F) temperatures, but living plants are usually killed at such temperatures. Limited experiments have also shown that some virus diseases are inactivated when infected plants are exposed to somewhat lower temperatures (30 to 35°C) for several days; this possibility should be further investigated.

Recently, a successful attempt was made to remove and culture virus-free, so-called "meristematic" tissue[1] from diseased plants. However, this operation requires skilled technicians, adequate facilities, and time to obtain mature, virus-free plants. Furthermore, the danger of reinfecting these cured plants is always present.

[1] "Meristematic tissue" usually refers to the apical region of the plant where only a few cells are truly meristematic. True meristem cultures have not been made because meristematic cells cannot be delineated precisely for excision. It is more accurate (if not common) to say "shoot-apex" or "shoot-tip" culture.

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