BEGINNER'S SERIES

Orchid Culture — 17 — Diseases, Part 3 — Victims of Virus

STEPHEN R. BATCHELOR

ARE WE ALL victims of virus? I wonder as I write, sniffling and sniffing, coughing and sneezing, suffering from a frequent viral complaint! Is there no cure for the common cold? Are there no remedies for orchid virus? Unfortunately not! "Isolate or destroy all virus-infected plants" is the typical, rather harsh, indeed chilling recommendation. (Just imagine if this applied to virused humans as well!) So little seems known about these only quasi-living proteinaceous particles. Viruses are now suspected of being responsible for many serious maladies affecting mankind — including some cancers. For plants, viruses have been implicated in over 600 diseases identified by an array of disturbing symptoms: leaf mottling or streaking, flower color-break, plant yellowing or stunting (Roberts, 1972).

INCIDENCE OF VIRUS IN ORCHIDS

Sadly, orchids arc not immune to viruses! Quite the contrary, it can be said with considerable justification that virus is a problem of epidemic proportions in many orchid collections today. A sampling of orchid collections in Florida yielded incidences of virus as high as 69% and as low as 18%. *Epidendrum* and *Cattleya* species in cultivation were found to be infected at the rate of 20% and 48% respectively, while the same species sampled in nature showed zero incidence of virus (Bodnaruk, *el al.,* 1979). A direct correlation has also been uncovered between the length of time an orchid has been in cultivation and the probability of it being virused. Freshly collected orchids and young seedlings (virus is not transmitted in seed, as far as we know) are unlikely to be virused, while clones which have been in cultivation for many years stand a greater chance of being infected. The longer the time a clone is in cultivation, the greater likelihood that it will be virused (Sheehan, 1981). Could we as orchid growers be responsible for this virus epidemic?

VIRUS TRANSMISSION

Viruses are essentially lifeless outside of living tissue. But once inside, like dictators they take over the controls at the cellular level and subvert them to their own designs. Virus particles have been found on the surfaces of orchid leaves by researchers (Bertsch, 1982), but here they are inactive and do no harm. Unlike the bacterial and fungal diseases discussed in the previous two articles of this series, environmental conditions, such as the presence of water, do not activate them. Only when the virus particles come into direct contact with cellular sap do they become active and infectious. In orchid culture, when is this likely to happen?

There is little doubt among those who have done research on orchid viruses that such viruses are transmitted predominately by cutting tools. "The most important method of transmitting the commonly

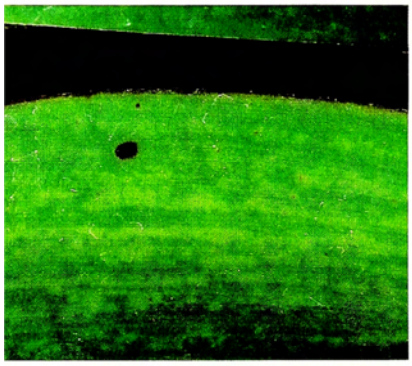
occurring orchid viruses is in sap on cutting tools when plants are divided or when flowers are harvested." (Lawson and Ali, 1975, page 78) Aphids and other sap-suckers are also thought to be responsible for some virus spread in orchids, as they are with other plants. Nevertheless, we cannot realistically blame the bugs. The real virus "vectors" (agents of dispersal and infection) are those who wield the cutting tools — you and I. Assuming some incidence of virus in our orchid collections, every time we go among our plants removing old, decaying leaves and flowers, every time we divide or propagate an orchid — and do not use a sterile cutting tool — we are taking a dangerous risk. If that tool has been used previously to cut a virused plant, and has not been sterilized, it will be carrying countless virus particles and will infect any healthy tissue subsequently cut. We can claim ignorance about virus, and innocence about our active role in its spread, but the end result is the same: an appalling rate of virus in many orchid collections today.

TYPES AND SYMPTOMS OF ORCHID VIRUS

If only we could easily identify those members of our collections which arc virused and get rid of them! Then we could be assured of a collection free from the threat of virus, whatever our sanitary or propagation practices. Two types of virus regularly infect orchids: Cymbidium Mosaic Virus (CyMV) and Tobacco Mosaic Virus — Orchid Strain (TMV-O), also known as Odontoglossum Ringspot Virus and Cattleya Color-Break Virus.

Vegetative symptoms — Infection by either of these two viruses results in some form of necrosis on the leaves of the orchid plant infected. The actual pattern of this necrosis, however, can vary tremendously from plant to plant. Viral symptoms usually begin as somewhat elongate formations of chlorotic (yellow) tissue often running parallel to the veins in the leaves of new growths, though, again, the shape and prominence of these areas of chlorosis differ greatly for the plant involved (Bertsch, 1982) (FIGURES 1 and 2). With leaf maturation and age, these streaks, spots, rings or patches, whatever they may be, either darken and become necrotic or disappear altogether. In either case, it is common for older leaves to show necrotic symptoms (Figures 3 and 4). Necrotic areas caused by virus can be cither raised or depressed, though with time the tissue usually "collapses" and becomes sunken. "Because of this variation in symptoms expression in a plant infected with one or more viruses it is often impossible to determine if a plant is virus infected by the appearance of the foliage." (Lawson and Ali, 1975, page 70) The plant in Figure 5 further underscores this point.

Unfortunately, too, the dark, necrotic vegetative symptoms of virus infection arc not sufficiently distinct from those of the leaf-spotting fungi discussed in the previous article for the series ("Orchid Culture — 16 — Diseases, Part 2 — The Flagrant Fungi", *Amer. Orchid Soc. Bull.* **51** (6): 593-600) to make for positive identification. Leaf-spotting fungal infections can cause similar, elongated, necrotic patches (FIGURE 6). Other factors enter into the expression of viral infection, including the



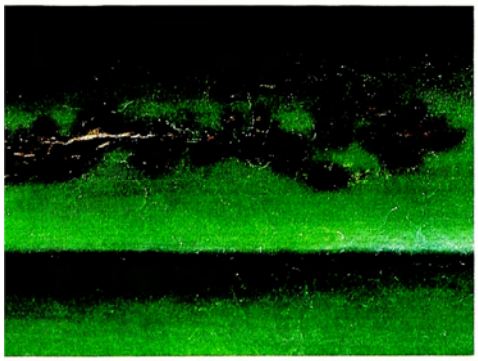
Credits, Figures 1, 2, 4 and 7:
Photography: Stephen Morgan
Electron Microscopy: Dr. Dennis Meyhew
Serology: Dr. Walter Bertsch

Left, Figure 1 — A pattern of chlorosis appears on a one-year-old leaf of a *Cattleya* hybrid infected with Cymbidium Mosaic Virus.

Below, Figure 2 — Symptoms of Tobacco Mosaic Virus — "O" strain in infected *Cymbidium* leaves of varying ages. *Left*, an immature leaf; *center*, a one-year-old leaf; *right*, a leaf three to four years old.

Right, Figure 3 — This laeliocattleya, which tested positive for Cymbidium Mosaic Virus, shows heavy necrotic spotting and a general lack of vigor.

Below, Figure 4 — A three-year-old leaf from an infected brassocattleya exhibits well-developed necrotic spots, typical symptoms of Tobacco Mosaic Virus — "O" strain.



Photography: Stephen R. Batchelor

health and genetics of the host plant, and the conditions under which it is grown. Some virused orchids show no symptoms whatsoever. Plants infected with both Cymbidium Mosaic Virus and Tobacco Mosaic Virus— "O" strain, however, can exhibit a severe reduction in plant size and flowering. Ultimately, though, all virused orchids should show some decline in growth and flowering. This decline is often not noticed, or is attributed to factors in culture.

Floral Symptoms — Cymbidium Mosaic Virus and Tobacco Mosaic Virus — "O" strain can cause more distinct symptoms in the flowers

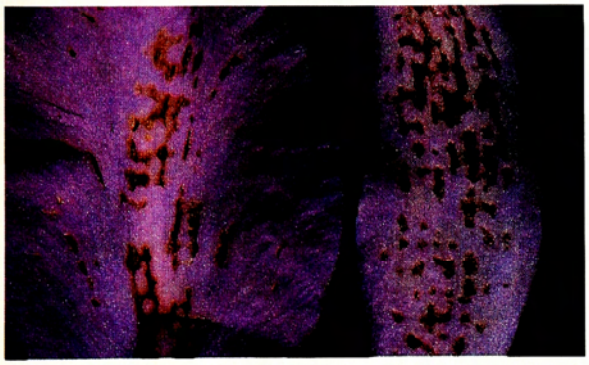
produced by the plants they infect. Cymbidium Mosaic Virus infection occasionally expresses itself as "Blossom Brown Necrotic Streak", the development of brown lesions on flowers which have aged prematurely because of the virus (Figure 7). Tobacco Mosaic Virus — "O" strain has been called "Cattleya Color-Break Virus" because it is most conspicuous in Cattleya-type flowers. Such flowers often manifest the presence of TMV-O with an irregular dispersal of color pigment, leading to blotching and streaking (Figure 8).

These floral symptoms, when present, more conclusively suggest viral infection than foliar symptoms because little else could cause them. Orchid growers, when presented with such conspicuous symptoms, are

Above, Figure 5 — Though this laeliocattleya has what many would consider viral symptoms, samples sent to be tested on three separate occasions yielded negative results.

Photography: Stephen R. Batchelor

Right, Figure 6 — These symptoms on a *Cochleanthes* leaf, resulting from a fungal infection of *Phyllostictina pyriformis*, could be mistaken for virus.



Above, Figure 7 — "Brown Necrotic Streak" on *Cattleya* flower segments, caused by Cymbidium Mosaic Virus.

Right, Figure 8 "Cattleya Color Break" on a plant infected with Tobacco Mosaic Virus — "O" strain.

Photography: courtesy of Harry C. Burnett

more inclined to suspect virus and give the hapless plant "the heave-ho". In a few cases, however, flower streaking can be caused by "genetic abnormalities" of a hybrid (Lawson and Ali, 1975).

Besides Cymbidium Mosaic Virus and Tobacco Mosaic Virus — "O" strain, other diseases in orchids are thought to be possibly viral in nature. One such disease is called "Vanda Ringspot" which, as the name suggests, is characterized by circular, yellow rings and spots on the leaves of Vandaceous orchids (Figure 9). Because this disease has not been transmitted experimentally, it has not yet been classified as a virus (Burnett, 1974).

Photographs: Stephen R. Batchelor

FIGURE 9 — Symptoms of "Vanda Ringspot" disease on an aeridovanda.

TESTING FOR VIRUS

"The most important point to consider is that a virus-diseased orchid may produce normal foliage and that the only valid procedure that can be used to determine the health of such a plant is to test it for the presence of virus." (Lawson and Ali, 1975, page 74) Three kinds of tests are used to determine the presence of virus in orchid tissue, and these are described and pictured in detail in an article on the subject by Lawson and Ali in the A.O.S. *Handbook on Orchid Pests and Diseases,* from which the above quote was taken. Bioassay, or plant test, is one such procedure. This test involves the use of indicator plants, the leaves of which react with highly visible, necrotic spots when treated with an extract of a virused orchid. Another type of virus test comes under the heading of "serological". Such tests employ an antiserum, animal in origin, containing antibodies which will also visibly react in the presence of virus. An extract from the plant in question is brought into contact with the antiserum in the laboratory and observed for any reaction. A visible reaction in this or the previous type of test means that the orchid from which the extract was made has tested "positive" for virus. Unlike the prior two tests which through indirect methods indicate the presence of virus, the third type of virus test used today allows the observer to directly sec the virus in plant sap. This is no minor feat, since virus particles are so minute that terrific magnification is necessary. For example, the CyMV particles pictured

on page 93 of the A.O.S. *Handbook on Orchid Pests and Diseases* are shown at a magnification of 53,000X. Such magnification is only possible through the use of an electron microscope.

Logically, only the third test discussed conclusively proves the presence of virus in any plant sample, because the virus particles can actually be seen. Yet even with an electron microscope the presence of virus can be missed, especially if the infection is a low-level one (Bertsch, 1982). A virus particle here and there in a sample can be literally passed by. So while a positive test using electron microscopy is conclusive, a negative one does not necessarily mean no virus is present in the plant (though it could then be thought unlikely). The bioassay, or plant test has been found to be generally reliable and certainly more economical (Lawson and Ali, 1975) — if the indicator plants can be grown healthy and free of foliar lesions to begin with. Even under controlled conditions this can present a problem (Bertsch, 1982). Serological tests are now available at reasonable cost and are not difficult to learn to use.

VIRAL PARANOIA

We see to be today in the middle of what could be called a "viral paranoia." In some neighborhoods of the greater orchid community just mentioning the word "virus" with an incriminating look in one's eyes can cause bursts of hysteria quickly followed by rampaging suspicion. (I fret over whether this article itself will result in massive, midnight "orchid burnings" across the nation!) In such a climate, one person can state with conviction in his voice that firm "X" sells virused orchids and those around him will experience a pang of anxiety about the plants they have purchased from the firm, along with a nagging doubt as to whether they should continue buying from the now "suspect" source. In such an environment of uncertainty, one can reduce an aristocrat in the orchid-growing community to a pariah merely by sniffing, in the right quarters, "I know for a fact her plants are all virused." Few things are said regarding orchids and orchid growers that are any more libelous.

One can attempt to quell this "viral paranoia" by asking for some proof that an orchid is virused. The usual response from the accuser is that the plant under suspicion is obviously virused because it has unmistakable symptoms. This is "insufficient evidence", for reasons explained earlier. Under the circumstances, one should see to it that a green leaf of the plant is sent to be laboratory-tested. A positive serological test for Cymbidium Mosaic Virus or Tobacco Mosaic Virus — "O" strain is pretty good proof, in combination with foliar/floral symptoms, that a plant is indeed virused. Once an orchid has tested positive, it can be assumed that the infection is systemic, and that virtually all tissue in the plant is virused. Any vegetative propagation of the virused plant, in turn, will be virused — and that includes meristems of most common genera. (This is why it is so vital that plants to be meristemmed are proven, as much as humanly possible, free of virus.) This being the case, in addition to the very important fact that the presence of a virused orchid is a continual threat in any collection, an

orchid adequately proven through testing to have virus should be destroyed at once.

Note here that we are talking about one plant. Just because one plant is implicated as virused does not naturally mean that all orchids in the accompanying collection are likewise. This, of course, depends on the sanitation practices of the grower. Each plant needs to be tested individually to establish whether the other plants arc virused as well.

Orchid hobbyists have a tendency to question whether "Such and Such" laboratory is "reliable", particularly after they have sent in a leaf sample of an orchid they were "absolutely convinced" was virused, only to have the test come out negative. No one can be absolutely certain of the presence of virus particles unless they "see them with their own two eyes" with an electron microscope — not a commonly available item! One has to accept that those who perform the tests, and the tests themselves, are, for the most part, reliable. This is, in many ways, a matter of trust, something that the subject of viruses does not encourage! One can understand the sense of frustration a grower feels when a plant in his congested collection exhibits viral symptoms. He now perceives it as an infectious, growing enemy right there among "the children". The first impulse is to destroy, the second, to test. He yearns for some justification to kill, to remove the threat — and he feels cheated or betrayed when the test comes back negative. Under the circumstances, his only recourse is to have the plant retested by sending in yet another leaf sample. Plants have been known, for whatever reasons, to test negative for virus the first time around, only to test positive the second (and the converse is also true – false positive tests are not at all uncommon). Of course, the grower could have been mistaken in assuming virus to begin with.

VIRUS PREVENTION

If virus is indeed widespread in orchid collections today, and we as orchid growers are collectively responsible, while at the same time faced with frustration in attempting to identify the plants actually virused, then what can we do, short of destroying all our plants and starting afresh with jungle-collected plants or seedlings right out of flask? We can fight back by diligently applying sterile procedures in our daily activities of sanitation, repotting and propagation. While it is a little unrealistic to expect the typical orchid grower to don a surgical mask and sterile gloves every time he approaches his collection, it is not unreasonable to expect the conscientious individual to use a sterile cutting blade with every orchid plant to be cut in some way.

A cutting tool never used before to cut plant tissue can be considered free of plant viruses. But after it is used just once to cut any plant tissue, there are no assurances that it has remained so. How, then, to clean a presumably contaminated, used cutting tool? A number of chemicals have been found to "inactivate" viruses (called such, I suppose, because one cannot kill something not quite living!). These include the readily available and inexpensive household bleaches, such as Clorox (Lawson and Ali, 1975). Recommended concentrations of bleach for the purpose of inactivating both Cymbidium Mosaic Virus and

Tobacco Mosaic Virus — "O" strain (TMV-O being the more resistant of the two) vary from full-strength (Lawson, 1967) to 20%, or one part Clorox to four parts water (Bertsch, 1982). The lengths of time recommended for soaking contaminated tools in these solutions vary from 30-60 seconds to ten minutes, respectively. All sources, however, emphasize that "inactivating power" of such solutions is reduced with every addition of plant sap. For this reason, and for the reason that virus particles can "hide out" in fragments of plant tissue on cutting surfaces, all cutting tools should be wiped before insertion into these solutions. Such inactivating solutions cannot be "stored" from one day to the next, and must be made up fresh on a frequent basis when in use — certainly before they take on a greenish-yellow cast after repeated soakings.

Other than virus-inactivating solutions, heat is another effective means of sterilizing cutting tools. "The safest and most effective method of sterilizing tools is with heat." (Lawson and Ali, 1975, page 78) Heat can be applied to cutting tools as an open flame, or in an oven. As in the case of sterilizing solutions, it is wise to wipe the cutting surface free of debris before applying the heat. Tobacco Mosaic Virus — "O" strain has been found to be inactivated after ten minutes of temperatures of 205—212°F (Lawson and Ali, 1975). Therefore, heat must be applied to the cutting surface until such temperatures are reached, or overcome. But for how long? Does the flame from an ordinary disposable lighter, the type I have recommended in previous articles for this series, apply sufficient heat in the short time one's patience (and thumb!) sustains the flame to inactivate all virus particles on a cutting surface? Who knows? Some virus researchers recommend flaming a cutting tool until it nearly "glows" in order to inactivate any virus particles embedded in sap accumulations (Bertsch, 1982).

All these sterilizing procedures sound good on paper, but in practice they are messy and awkward (not to mention what they do to a decent pair of cutters!). This may be one good reason why many normally conscientious growers just don't bother, or are "lax in their procedures". Who has the patience to wait a minimum time for sterilizing in a soaking solution or flame between cutting off decayed flowers and leaves from one plant to the next in a sizable collection? If one is using just one cutting tool, the delay involved would be most aggravating. Having a large number of cutting tools sterilized and ready for use at any one time is an answer, but what about the expense?

Ingenious orchid growers maneuver around this problem of sterilized cutting tools very easily simply by using disposable, single-edged razor blades, one per plant. This is what is heartily recommended by at least one researcher (Bertsch, 1982), and would be, I imagine, by the rest. Single-edged razor blades are commonly available at hardware stores in boxes containing multiples of a hundred. In these quantities, single-edged razor blades cost between three and five cents each. At that price one blade can be used for one plant and then thrown away — thus avoiding the time and effort — and risk — involved in re-sterilizing. These blades can be used hand-held on the non-blade side for easy-to-cut leaves and

flower stems but extreme caution should be exercised if trying to cut tough rhizomes with a razor blade. For those who find this alternative too simple, or perhaps a bit wasteful, the used blades can be collected, wiped, and soaked in an inactivating solution, or baked at 350-400°F for one to three hours. The higher the temperature, the longer the duration, the greater likelihood of complete sterilization. Just the same, one has the greatest assurance that a blade is free of virus if it has never before been used for cutting plants. (— or handled by contaminated hands. It goes without saying that the "old-fashioned" method of clipping off flowers and leaves with the thumb, finger and nail is far from sterile, or advised.)

Used pots are also suspect for transmitting virus and really should be sterilized if reused. Unfortunately, few conventional pots — plastic or clay — are inexpensive enough to be blithely thrown away after "one-time use". Neither can one place plastic pots in a 400-degree oven and expect satisfying results! A deactivating solution is the only reasonable solution (sorry!) for sterilizing pots, and a ten-minute soaking in a 1:4 solution of Clorox should do the trick — provided the pots are thoroughly scrubbed of media and roots beforehand! The addition of a wetting agent to this solution can enhance its effectiveness. It would be best, after a soak in a deactivating solution, to soak clay pots in fresh water to avoid fumes and possible plant injury.

CONCLUSIONS

Using sterile cutting tools can go far in halting the further spread of orchid viruses. Yet is there any hope of not just limiting this "viral disorder", but of causing its remission? Some research into virus control and remission is being undertaken but much work is yet to be done. Perhaps someday, with long-term research adequately funded by the orchid community, we may even see "a cure for the common orchid virus"! — *84 Sherman Street, Cambridge, Massachusetts 02140.*

ACKNOWLEDGMENTS

I would like to thank Dr. Walter Bertsch of Pomona College, Claremont, California for his valuable help in the preparation of this article.

REFERENCES

Bertsch, Dr. Walter. 1982. ***personal communication.***

Bodnaruk, W.H., Hennen, G.R., Zettler, F.W. and TJ. Sheehan. 1979. **Incidence of Cymbidium Mosaic and Odontoglossum Ringspot Viruses in Wild and Cultivated Orchids of the Cattleya Alliance Surveyed in Florida.** *Amer. Orchid Soc. Bull.* 48(1): 26-27.

Burnett, Harry C. 1974. **Orchid Diseases.** Bulletin 10 of the Florida Department of Agriculture and Consumer Services, Tallahassee, Florida.

Lawson, Roger H. 1967. **Chemical Inactivation of Cymbidium Mosaic and Odontoglossum Ring-spot Viruses.** *Amer. Orchid Soc. Bull.* 36(11): 998-1001.

Lawson, R.H. **Orchid and Their Control.** In the *Handbook on Orchid Pests and Diseases,* 2008 Revised Edition. American Orchid Society, Inc., Delray Beach, Florida.

Northen, Rebecca Tyson. 1973. **Orchid Ailments: Part II, Diseases.** *Amer. Orchid Soc. Bull.* 42(9): 816-821.

Roberts, Daniel A. and Carl W. Boothroyd. 1972. **Fundamentals of Plant Pathology.** W.H. Freeman and Company, San Francisco.