Economically Important Orchid Viruses and How to Produce Clean Orchid Plantlets
By Chin-An Chang, Ph.D.

Most of the 30 reported orchid viruses are actually not orchid-infecting ones. They were sporadically found in special cases without economical significance. Our survey in Taiwan also shows the Cymbidium mosaic virus (CymMV) and Odontoglossum ringspot virus (ORSV) being the two most widely distributed and economically important.

Both viruses are characterized by their unusual stability in vitro and their ease of mechanical transmission. Injuries caused by a contaminated tool or even only a close contact between a healthy orchid plant and an infected one can result in a successful transmission of the viruses. After contacting the sap from an infected plant, the surface of tools, pots, containers, benches, laminar flows hoods and, possibly, clothing and hands of nursery workers are contaminated by living virus particles. These particles stay alive and infective for long periods of time. At present, there is no direct evidence showing insect vectors that can specifically transmit ORSV and CymMV. However, scientists have suspected that any insects which can make mechanical injuries on orchids will possibly transmit these two viruses through their contaminated mouthparts. Nevertheless, the most efficient way for the spread of ORSV and CymMV is by mass propagation of orchid plantlets from the infected mother stock through the meristeming (cloning) process. As a result of intensive international trading, such infected plantlets or tissue culture flasks are easily transmitted to a wide range of orchid genera and exported to many countries or regions.

Typical symptoms induced by CymMV is recognized by elongated yellow streaking on the infected leaves (Fig. 1), whereas ORSV includes mosaic, mottling, and chlorotic or yellow patches (Fig. 2).

Infection by ORSV alone usually does not develop necrotic symptoms unless the orchid is dually infected by CymMV (Fig. 2). However, CymMV has a tendency of inducing necrotic symptoms on a wide range of orchid species even infected alone, especially at a late stage. Sometimes, necrosis only occurs on the lower side but not on the upper side of the infected leaves on Dendrobium, Oncidium, Phalaenopsis, and Vanda (Fig. 3). Necrosis can appear as spots, strips or areas on leaves or flowers (Fig. 4) of the infected orchids. Color break on flowers is often a result of viral infection.
recently a research team in Taiwan has shown that it is actually caused by a fairly new virus species named *Capsicum chlorosis virus* (CaCV).

The virus belongs to the Genera of *Tospovirus*, having enveloped isometric particles and transmitted persistently by thrips. CaCV was first reported in Australia and Thailand causing chlorosis disease on peppers. The vector thrips species that transmits CaCV among peppers in Thailand has been identified. However, the species to vector *Phalaenopsis* CaCV remained to be identified. Specific antisera and PCR primers have been developed for the detection of CaCV in Taiwan. Interestingly, it was found that CaCV can only be detected in the leaf tissue within the ringed area but not outside the rings or in other symptomless leaves of the same plant. Furthermore, the plant does not develop additional symptoms once the diseased leaf has been removed. This implicates that CaCV may only induce localized infection on some *Phalaenopsis* cultivars, instead of being translocated in tissues to result in systemic infection of the entire plant. Another evidence for CaCV’s inability to induce systemic infection is that none of the spikes from symptomatic *Phalaenopsis* plants have been tested positively for CaCV infection. All together, I feel that CaCV is not as economically important as ORSV and CymMV for its causes only localized infection in *Phalaenopsis*. Therefore, control of this virus may be approached by eliminating the population of thrips in the growing environment.

**Production of clean orchid plantlets Screening for non-infected plants.** Apply techniques that are at least equivalent to or more sensitive than ELISA as preliminary screening test. Using techniques with lower sensitivity usually result in virus-infected orchids escaping from being detected. Techniques with higher detection sensitivities, such as RT-PCR or biochip hybridization, are suggested to be used in the secondary confirmation test on the screened plants that were obtained from the first round of testing. Very importantly, screened plants from each testing should be kept well separated to prevent from possible re-infection by mechanical contacts among nearby plants. At least two consecutive monthly tests should be performed on target plants before recognizing them as clean stocks. This precaution is taken to prevent possible missed detection during the initial screening. Nevertheless, it should be emphasized that there is no guarantees of 100% detection for any virus detection techniques. Therefore, re-testing and alternating different techniques are the solutions to minimizing the risks of missed detection.

**Maintaining clean mother stocks.** The clean mother stocks, once obtained from screening tests, should be kept in a growing house that guarantees no viral re-infection. Standard sanitation procedures to clean the growing environment should be adopted into routine management protocol. For example, tools, clothing, benches and even hands of workers should be disinfected appropriately to prevent from carrying virus particles. Using flame to burn or dry heat (200 °C for 2 hours) to sterilize metal tools can easily eliminate contaminated virus particles. For benches, clothes and other non-metal materials, 0.5% (W/V) of sodium hypochlorite solution can be used for sterilization but at least 1 minute of treating period is required for a complete disinfection. As for nursery workers, the easiest way to prevent their hands being contaminated is by washing thoroughly with soap. Nevertheless, minimizing contacts of orchid plants with bare hands or fingers is strongly recommended. To prevent those screened clean stocks from infection...
by insect-transmitted viruses such as CaCV, precautions should be taken to minimize intrusion and colonization of insects in growing houses. If unfortunately virus-like symptoms do appear on the stocks, suspicious ones should be removed and virus testing performed immediately. Ideally, all clean stocks should be retested at least once a year to make sure they are virus-free. Furthermore, virus checks should be conducted on the stock plants before or after spike induction for mericlone. Special precaution should be taken to prevent possible virus infection whenever the clean stocks are moved to other houses, for example, spike induction rooms.

Executing sanitation procedures. If clean mother stocks were consistently used as starting materials during mericloneing, the tissue-cultured plantlets in flasks should be reasonably clean. However, sanitation procedures are still necessary to make sure such plantlets maintain their virus cleanliness after transplanting from tissue culture flasks. For example, at the stage of removing the plantlets from flasks, close contacts between plantlets and with workers’ hands or tools are so frequent that mechanical injuries are easily resulted. Contamination by virus-infected plant sap on the tools or hands will certainly facilitate virus infection of the plantlets. Therefore, cleaning of growing environment, tools, clothing and worker’s hands appropriately from contaminated viruses should be adopted as standard operation procedures in order to produce clean orchids. In conclusion, minimizing mechanical contacts during cultivation is the key to producing clean orchids.

Concluding remarks
Cultivation of virus-free orchids is a win-win situation for propagators and providers of finished plants. It is therefore necessary for all of the partners in the orchid production chain to implement serious sanitation and strict production procedures for growing virus-free plants. However, it generally takes at least 3 years to propagate from a clean mother stock to marketable flowering orchids. It is a big challenge for nurseries to guarantee absolutely clean flowering orchids considering the high possibilities of virus infection during such a lengthy production period. The smaller the plants that a propagator/supplier provides, the less chance they may be re-infected by viruses. As plants have been grown longer in greenhouses, they have an increasing chance of being infested with viruses. We believe it is a common goal for all nurseries to provide guaranteed virus-free mature and flowering plants, but in reality it is difficult, expensive and time consuming task to pursue. My opinion is that orchid consumers or downstream growers should compromise and accept the reality that certain percentage of virus infection in the finished flowering plants would not affect the mutual benefits of consumers and providers. This understanding and compromise will certainly help the harmony of international orchid trading. It should be emphasized again that, in the long run, screening of virus-free stocks before mass propagation is a must for all orchid propagators. No companies can overlook this common demand and still maintain their competitiveness in the orchid industry around the world. ICOGO

Chin-An Chang, Ph.D., is the immediate former head of the Plant Pathology Division, Taiwan Agricultural Research Institute, Wufeng, Taichung. Dr. Chang conducted extensive research on orchid viruses. He is now a professor at Chaoyang Technology University, Wufeng, Taichung 413, Taiwan. Dr. Chang may be reached at cachang543@gmail.com. Tel. 886-4-2332-3000 Ext. 5105.