

VIRUSES OF NATIVE ORCHIDS

By
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Introduction

The aim of this project was to investigate the incidence of rhabdo-like virus and any other viruses that may be infecting native orchids growing in their natural environment.

The disease survey area was initially to include South-East Queensland and Northern New South Wales but this was extended to North Queensland and the coast of New South Wales to Newcastle. This was made possible by an additional grant from Australian Conservation Agency.

Written approaches were made to the various orchid societies along the eastern seaboard for their co-operation in collecting diseased specimens. This method proved a little disappointing, however, I am grateful to several members of native societies who were able to devote time to accompany me to sites or to collect suspect plants.

During the course of the previous project, a number of societies were addressed on the subject of orchid diseases. This education programme has continued but at a much slower rate mainly because of the publication of various pamphlets and the availability of our previous report to growers.

Societies addressed included: Newcastle Native Orchid Society, Orchid Club of South Australia, Bellingen Orchid Society, Toowoomba Native Orchid Society, and Bribie Island Orchid Society.

A virus diagnostic service to growers is still being offered through the University of Queensland, Centre of Microscopy and Microanalysis.

Background

During the course of a previous survey, it was noted that some of the native species growing in cultivation seemed to be infected with either a virus or fungus. Dr. Helen Ogle unsuccessfully cultured these lesions for fungus. On electron microscope examination a small bullet-shaped rhabdovirus was detected. The question then arose, were these plants infected before they were introduced into cultivation or were they already infected when collected from their natural environment.

Published material on the incidence of virus in native orchids is very scant. A search of the literature has found that published papers refer only to the incidence of *Odontoglossum* Ringspot virus and *Cymbidium* mosaic virus having not been found in wild species. No record can be found in the literature search of the incidence of rhabdo-like virus affecting native orchids.

Material and Methods

Source of Material:

Sites visited during this project included: Mackay region (Cape Hillsborough, Eungulla ranges, Crediton State Forest, Cathu State Forest), Blackbutt Ranges, Lamington National Park, Sundowner National Park (incomplete), Bellingen region (Mt. Picket), Border National Park, Barrington Tops, Watagan State Forest, Kangaroo Valley, Brisbane Forest Park, Jimmna State Forest (Galengowan), Krombit Tops (Clutha Creek), Killarney district (Queen Mary Falls and Emu Creek) and Maroota (NSW).

At each site only a small amount of material was collected and only from plants showing symptoms. There was no random sampling of material growing in the natural environment. Limited sampling was also undertaken from orchid houses in Toowoomba, Bellingen, Ballina and Brisbane.

Sap from each sample collected was examined by electron microscopy using the negative stain technique (see previous report for details of technique).

Thin section microscopy was also necessary to gain an understanding of intracellular interactions between virus and the host plant.

Where it was thought another virus other than a rhabdovirus was present further testing was carried out using Reverse Transcriptase Polymerase Chain Reaction technique (RT-PCR). My thanks to Dr. A. Gibbs, Research School of Biological Science, Australian National University (ANU), Canberra, for his co-operation in these techniques. The technique is very sensitive and can detect virus at much lower concentrations than by electron microscopy. The DNA was then sequenced to determine as to its position in the potyvirus family tree.

Results Rhabdovirus incidence:

A number of *Dendrobium speciosum* growing in cultivation seemed to have had their origins at Mt. Binga (Blackbutt Ranges). This was the first area visited to test the theory as to the origins of this virus. The area visited was deep in the range and nowhere near cultivated orchids. Between 60 - 80 plants were inspected, 20% of these showed some sign of infection. While a number of the plants were heavily infected, some however, showed only a few of the leads with symptoms. A few *Dendrobium kingianum* plants growing in the area also showed signs of infection with virus. The symptoms were, however, different from those of *Den. speciosum*.

Electron microscope examination of samples taken in the area revealed the presence of a bulletshaped rhabdovirus in some of the specimens, while in others with obvious symptoms no virus particles were detected. These negative samples were then further examined using thin section electron microscopy. A few virus particles were detected in the cytoplasm attached to membranes. The most conspicuous aspect of these samples was the large nuclear inclusions. During the infection process, the virus appears in the cell nucleus as viroplasm. Electron micrographs of recent infections show large numbers of virions in the nucleus of a phloem companion cell. A few virions can be seen passing through the nuclear membrane while others are membrane in the cytoplasm.

From the data obtained by thin section electron microscopy, it appears that after the initial infection takes place, all the susceptible cells are probably infected fairly quickly (this is possible the acute phase and particles can be detected by electron microscopy negative stain technique). The plant by some mechanism undergoes a period of "recovery" and the virus concentration declines until the plant enters a chronic phase at which stage virus cannot be found by the negative stain technique electron microscopy. One must presume that the virus antigen remains and so the plant still remains infectious to the insect vector. This series of events has been published for other viruses including Sonchus Yellow net virus which is a rhabdovirus possibly belonging to the same family of virus namely nucleorhabdovirus. We have observed that infected plants, especially those in cultivation, quite often only a few leads may show signs of infection. It may be possible to divide the plant, discarding the infected leads and keeping the remainder. Further work would have to be carried out to prove this point before this could be put into practice.

This virus is particularly hard to detect by electron microscopy for several reasons; (1) the virus is very fragile and is normally in very low concentration, (2) the plant may be in the chronic phase of infection. I believe these two factors have led to the virus not being detected in the past and other diagnoses being given, e.g. citrus fungus.

There remains a great deal of work to be carried, out on this virus especially to document the DNA or RNA sequences which hopefully will give an insight into the origin and evolution of rhabdovirus in Australian native orchids.

There was evidence of *Dendrobium* beetle feeding on infected plants. However, electron microscope examination of beetles collected from the Mt. Binga area failed to show rhabdovirus particles to be present. There is an experiment in hand as this report is being written of scale insects feeding on an infected plant. Some of these insects will be processed for electron microscopy in the very near future while attempts will also be made to transfer the remainder to another plant to see if transmission does occur.

Other viruses detected

While no other viruses were detected in the wild, mention must be made of the incidence of potyvirus in cultivated plants as well as in the so called 'Red speciosums' which were the centre of some debate amongst some growers.

In 1994, an outbreak of what appeared to be virus infection was reported in Victorian *Den. kingianum* hybrids. Electron microscope examination revealed the presence of a flexuous rod-shaped virus. In the previous report this virus was reported to be a possible Carlavirus. Further work has been carried out on these specimens to find that the rods measured 680-750nm in length and were most probably a potyvirus. In thin-section electron microscopy what appears to be a pinwheel type structure was also detected. Pinwheels are a diagnostic marker for potyvirus infection. Similar rod-shaped virus particles have been detected in terrestrial orchids from a variety of locations but all have been grown in cultivation. As yet no virus has been detected in terrestrials growing in the wild.

Since that first detection of potyvirus several positives have been recorded. Again all these have been in collections. It is of interest to note that each of the outbreaks of this virus in Victoria, A.C.T., and Queensland has coincided with an aphid infestation.

Of the 'Red speciosum' plants that have been examined most have proved to be positive for potyvirus.

The electron microscope is just one of the tools available for the classification of viruses. It can image a virus particle and from that image one can usually place a virus in its respective family. Within that family there are various subgroups and potyviruses are no exception. Potyviruses previously isolated from orchids include Dendrobium mosaic, Habenaria mosaic, Pecteilis mosaic, vanilla mosaic and vanilla necrosis potyvirus.

Over the last six months, any orchid found by electron microscopy to be infected with what appeared to be a potyvirus was forwarded to Prof. Adrian Gibbs for RT-PCR and subsequent sequence analysis. An analysis of the information gained from these tests has shown that in Australia there exists another potyvirus infecting orchids. This virus has been called Ceratobium mosaic virus (CerMV). The results are to be published in an international journal.

The symptoms are varied and some are shown photographically in this report. The potyvirus detected in Victoria did also exhibit flower colour break. To my knowledge the isolates of this virus found in ACT and Queensland showed no sign of flower colour break.

All the specimens sent to ANU by the author were grown in cultivation but some had been bush collected but not of recent times.

Included in the list of positive (CerMV) were three of the so called 'Red speciosums'. It is hoped some of the hybrids made from these speciosums can be traced and checked for the same virus.

Virus of the family Potyviridae are usually transmitted by aphids in a non-persistent manner or through seed and can also be transmitted experimentally by sap inoculation.

Prof. Gibbs and his team have produced a sensitive diagnostic technique for the identification of orchid potyviruses and it is hoped this test will be used to test out plants without symptoms randomly collected from cultivation and those growing in their natural environment.

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My sincere thanks must go to those growers who gave a considerable amount of their time to travel to sites where a great number of native orchids were to be found. Their efforts are greatly appreciated. A very special thankyou to Prof Adrian Gibbs of ANU and his team for the identification of the potyviruses found during this project.

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