

**Failure of Aphids to Transmit the
Odontoglossum Ringspot and Cymbidium
Mosaic Viruses to Orchid Plantlets
Derived from Meristem Cultures**

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ABSTRACT

The use of plantlets derived from "protocormlike" bodies in orchid meristem culture as test plants for aphid transmission of orchid viruses was studied. *Myzus persicae* and *Cerataphis orchidearum* readily probed into the test plantlets, indicating feasibility of their use; however, the aphids failed to transmit *Odontoglossum* ringspot and *Cymbidium* mosaic viruses to them. *Phytopathology* 61:582-583.

The difficulty of obtaining large numbers of uniform orchid test plants is one of the limiting factors preventing extensive studies of insect transmission of orchid viruses. Clonal cuttings, the usual means of asexual propagation, produce only a few plants per clone per year for such orchids as *Cattleya*, some *Vanda*, and *Dendrobium*. Such cuttings are of relatively large size, and require sizable pots which take up considerable greenhouse space. Although propagation by seeds produce numerous plants, seedlings of our cultivated orchid hybrids are highly heterozygous. Moreover, it takes a long period of time for plants to grow from seeds to a size suitable for transmission tests with insects. Orchid meristem culture provides a means by which numerous homogenous plants can be obtained from a single clone in a relatively short period of time (5, 6, 7, 8, 9, 10).

Orchid meristem culture consists of explanting apical meristems in culture medium. The meristems *in vitro* produce numerous "bulblets" which are similar to the protocorms of embryogeny. These protocormlike bodies, when left undisturbed, develop shoots first, then roots. They eventually develop into normal plants. We refer to the shoot stage as "plantlets." Ishii et al. used these plantlets as test plants for mechanical and aphid transmission of the *Cymbidium* mosaic (CMV) and *Odontoglossum* ringspot (ORSV) viruses; this paper is an expanded report of the aphid-transmission portion (2). We also include a report of transmission studies conducted with the fringed-orchid aphid, *Cerataphis orchidearum* (Westwood), which is a pest of orchids in Hawaii.

MATERIALS AND METHODS.—The test plantlets used in the study were propagated from an apical meristem explant of a *Cattleya* hybrid. The protocormlike bodies

produced by the meristem explant were imbedded shallowly in agar culture medium contained in covered 23-ml glass vials, 1 body/vial. They were left undisturbed in an air-conditioned room under two 40 w fluorescent (Westinghouse Plant-Gro 40W F40/Gro) light tubes held about 15 cm above the vials. In about 5 weeks, the bodies developed into plantlets about 7-8 mm in length which were considered to be of usable size for transmission studies with aphids. The original explant and its subcultures continuously produced protocormlike bodies in large quantities. Aseptic conditions prevailed during the production of the test plantlets and during the transmission tests.

ORSV and CMV were used in this study. The source for ORSV was a mature *Cattleya* plant. The sources for CMV were small *Cattleya* plants about 8 cm tall, and plantlets from meristem culture growing in agar culture medium under aseptic conditions in 23-ml vials. In one series, a mature *Cattleya* hybrid plant infected with both ORSV and CMV was used as the source plant.

Two species of aphids were used in the transmission tests: (i) late instar apterae of the green peach aphid, *Myzus persicae* (Sulzer); and (ii) first- and second-instar nymphs and alates of the fringed-orchid aphid, *Cerataphis orchidearum*. The first instar of the fringed-orchid aphid is mobile, but the other instars of the apterae are immobile and scalelike in appearance. The green peach aphids were the progeny of a single female, and they were colonized in cages on mustard cabbage, *Brassica juncea* (L.) Cosson. The fringed-orchid aphids were from a colony reared on a mature *Cattleya* plant.

The aphids were fasted for about 30 min in 4-ml glass vials prior to access to the virus source. The green peach aphids were allowed a virus-acquisition access period of 2 min, while the fringed-orchid aphids were allowed a 5-min period because their movements were slower and they were slower to initiate feeding probes into the virus source plant. The aphids were transferred to the test plantlets immediately after the acquisition period. Ten green peach aphids and five fringed-orchid aphids were placed on each test plantlet. The aphids were allowed a test-feeding access period of over 30 min. A camel's-hair brush was used for transferring the aphids.

In one series of tests, the fringed-orchid aphids were colonized on a CMV-diseased *Cattleya* plant. Those in feeding position were dislodged and placed immediately on the test plantlets.

In order to minimize the possibility of contamination of the agar culture medium by microorganisms, test feedings were conducted outside the aseptic vials with the plantlets on moist filter paper in petri dishes. After a test-feeding period of over 30 min, the aphids were removed. The plantlets were washed in 0.26% NaClO (5% Clorox) for about 3 min, rinsed in sterile distilled water, then returned to their sterile vials. Forceps were used for manipulation of the test plantlets. They were sterilized in 0.53% NaClO (10% Clorox) before each use. No contamination occurred with this method.

TABLE 1. Attempts to transmit the Cymbidium mosaic (CMV) and Odontoglossum ringspot viruses (ORSV) with the green peach and fringed-orchid aphids, using plantlets from meristem culture of Cattleya orchid as test plants

Virus	Virus-source plant	Insect species	Insect stage	No. test plantlets
CMV	Mature plant	<i>Myzus persicae</i>	Apterae	24
CMV	Mature plant	<i>Cerataphis orchidearum</i>	Nymph	10
CMV	Mature plant	<i>C. orchidearum</i>	Alate	4
CMV	Young plant	<i>C. orchidearum</i>	Nymph	8
CMV	Young plant	<i>C. orchidearum</i>	Nymph reared on virus-source plant	6
CMV	Young plant	<i>C. orchidearum</i>	Alate	1
CMV	Young plant	<i>C. orchidearum</i>	Alate reared on virus-source plant	3
CMV	Plantlet from meristem culture	<i>C. orchidearum</i>	Nymph	3
CMV	Plantlet from meristem culture	<i>C. orchidearum</i>	Alate	1
CMV + ORSV	Mature plant	<i>M. persicae</i>	Apterae	27
ORSV	Mature plant	<i>M. persicae</i>	Apterae	12
ORSV	Mature plant	<i>C. orchidearum</i>	Nymph	21

The plantlets were kept in the vials for about 5 weeks. Symptoms are not perceptible on the plantlets at this stage, so the plantlets were assayed on local lesion hosts (2) to determine if virus was present. Whole plantlets were triturated and assayed on *Cassia occidentalis* L. for CMV and *Gomphrena globosa* L. for ORSV (4).

RESULTS AND DISCUSSION.—Aphids of both species readily assumed a feeding position when placed on the test plantlets. Most of them appeared to make brief probes initially, then settled into a prolonged feeding position. When these aphids were dislodged at the end of the test-feeding period, the stylets of many were exposed to a great extent, indicating that the aphids were definitely probing into the tissues of the plantlets. Thus, insofar as aphid probing is concerned, the plantlets from meristem culture appear usable for aphid transmission studies of plant viruses.

That the plantlets can be infected with orchid viruses has been established by the routine transmission of CMV and ORSV to the plantlets by means of sap inoculation (2). There has been, however, no report of these viruses being transmitted by arthropods. If they are transmitted by insects, they would most likely be of the nonpersistent or stylet-borne type because of the ease with which they can be sap-inoculated. The method used in this study, prefasting, short acquisition probes, and immediate test feedings, which is used routinely for the aphid transmission of nonpersistent viruses, should achieve transmission; however, none resulted in 120 attempts. It is likely that the aphids used were not vectors of the CMV and ORSV. Perhaps these viruses are transmitted only by mechanical means, as suggested by Brandes & Bercks (1) in their classification of elongated plant viruses. Both fit into groups of elongated viruses which are less than 580 nm in length and are usually transmitted by mechanical

means only. Elongated viruses which are known to be aphid-transmissible belong in groups which have longer lengths. The summary of attempts to transmit CMV and ORSV with the green peach aphid and the fringed-orchid aphid is presented in Table 1.

A mosaic virus of Cattleya orchid, the identity of which is not yet established, was reported to be transmissible by the green peach aphid (3). Thus, there is at least one orchid virus that can be transmitted by aphids and with which the use of meristem culture plantlets for the study of vector-virus relationships might be fruitful.

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