Virus Infections of *Vanilla* and Other Orchids in French Polynesia

G. C. WISLER, Biological Scientist III, Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Bureau of Plant Pathology, Gainesville 32602; F. W. ZETTLER, Professor, Department of Plant Pathology, University of Florida, Gainesville 32611; and L. MU, Plant Pathologist, Ministère de l'Agriculture, Recherche Agronomique, Papeete, Tahiti, French Polynesia

**ABSTRACT**


Four of 66 *Vanilla tahitiensis* samples collected from Moorea, Raiatea, Tahaa, and Tahiti, French Polynesia, and assayed by SVB virus, had either cymbidium mosaic virus (CyMV) or odontoglossum ringspot virus (ORSV). Neither virus was found in any of the 51 wild plants of the same native orchid, *Spathoglottis plicata*, growing in or near *Vanilla* plantations in French Polynesia. Much higher amounts of infection were noted in ornamental orchids; 40% of 74 samples had CyMV and 20% had ORSV. Four of the nine experimental *Vanilla* hybrids assayed tested positively for CyMV. A mosaic-inducing virus distinct from CyMV and ORSV was detected in *V. tahitiensis* plantations in Huahine, Raiatea, Tahaa, and Tahiti but not in Moorea. The virus was determined to be a potyvirus with a mean particle length of 767 nm, transmitted in a tymborin manner by aphids, induced cylindrical inclinations, and was serologically related to other potyviruses.

Additional key words: Arachnis, bean yellow mosaic virus, Cattleya, clover yellow vein virus, sheen mosaic virus, *Dendrobium, Myzus persicae*, soybean mosaic virus, immunofluorescence serology, subdivision III potyviruses, *Vanda, Vanilla fragrans, V. planifolia*

*Vanilla* is one of only two export crops in French Polynesia. Unlike other important vanilla-producing countries such as Madagascar and Tonga, where *Vanilla planifolia* G.Jacks. in Andrews (V. fragrans Ames) is grown, *V. tahitiensis* J. W. Moore is cultivated exclusively in French Polynesia. *Vanilla* was first introduced in 1848, and from 1899 to 1966, French Polynesia exported an average of 158 t of cured beans annually. In 1967, however, production levels declined rapidly to 0.6 t in 1981. In response to a subsequent increase in vanilla prices, the government of French Polynesia initiated a program to increase production to its former levels by 1990 through the nationwide release of propagating stock to growers (5). The possible dissemination of viruses in this release program prompted this investigation.

This study was undertaken in part to determine whether cultivated *V. tahitiensis* orchids in French Polynesia were infected with cymbidium mosaic virus (CyMV) or odontoglossum ringspot virus (ORSV). Elsewhere, high incidences of these viruses in ornamental orchids were detected (21,22). This study also describes a mosaic-inducing potyvirus of *V. tahitiensis* that was first detected in the islands of Huahine, Raiatea, Tahaa, and Tahiti by the third author (19). Although not previously reported on *Vanilla*, potyviruses have been described for the following ornamental orchid genera: Calanthe (8,9), Cyripedium (14), Dendrobium (7), Masdevallia (11,13), and Orchis (14).

**MATERIALS AND METHODS**

**Surveys.** Surveys were conducted 12-25 April 1986 in the islands of Moorea, Raiatea, Tahaa, and Tahiti, where most of the vanilla in French Polynesia is produced. To test for CyMV and ORSV, at least 20 leaf samples were collected, regardless of symptoms, from each of seven, eight, nine, and six plantings of *V. tahitiensis* in Moorea, Raiatea, Tahaa, and Tahiti, respectively. Specimens of a wild terrestrial orchid, *Spathoglottis plicata* Bl., found in or near *Vanilla* plantations were collected from one, four, and five locations in Moorea, Raiatea, and Tahaa, respectively. Privately maintained ornamental orchids (primarily Arachnis, Cattleya, Dendrobium, Epipedium, and *Vanda*) growing near *Vanilla* plantings were collected from two locations in Tahiti and from single locations in Moorea, Raiatea, and Tahaa. The collection of a commercial orchid grower near Papeete, Tahiti, was also sampled. The plants of *V. pompona* Schiede, *V. planifolia*, and/or *Vanilla* hybrids sampled were from single locations, each in Raiatea and Tahiti. In addition, 43 *Vanilla* samples from Tahiti collected in 1984, 16 samples from Tonga, and five from Puerto Rico were assayed serologically for CyMV and ORSV.

**Serology.** Immunodiffusion tests were conducted in Tahiti and Gainesville, FL, with CyMV and ORSV antisera as previously described (20). The medium contained either 0.8% Noble agar, 0.5% sodium dodecyl sulfate (SDS), and 1% sodium azide (17,20) or 0.8% Noble agar, 0.5% SDS, 1% NaCl, 0.6% Trizma base (Sigma), and 0.1% citric acid. Normal serum, CyMV and ORSV homologous reference antigens, and extracts from healthy orchids were routinely used as controls.

The antisera to CyMV and ORSV were prepared as described previously (20). Antisera to the following potyviruses were used in SDS immunodiffusion tests with extracts of *V. tahitiensis* and *V. pompona* with mosaic and blistering symptoms: red clover and gladiolus isolates of bean yellow mosaic virus (16), the pea mosaic isolate of bean yellow mosaic virus (2), clover yellow vein virus (16), sheen mosaic virus (1), and bidens mottle, tobacco etch, potato Y, watermelon mosaic 2, papaya ringspot type W, peanut stripe, and peanut mottle viruses supplied by D. E. Purcell (Department of Plant Pathology, University of Florida, Gainesville). In addition, antisera to the capsid, 49K and 54K nuclear inclusion, and 69K cylindrical inclusion proteins induced by bean yellow mosaic virus and provided by C.-A. Chang (2) were tested. Homologous reference antigens, normal sera, and healthy plant extracts were used as controls in all tests.

**Electron microscopy.** Leaves of *V. tahitiensis* with conspicuous mosaic and blistering symptoms (Fig. 1A) were examined for virus particles and/or virus-induced inclusions by light and electron microscopy. For virus particles, extracts were negatively stained in 2% uranyl acetate and examined with a Hitachi 600 electron microscope. Particle lengths were measured by comparing projected micrographs with a diffraction grating containing 2,160 lines per millimeter. Tissues were prepared for thin sectioning by fixing tissues in 5% glutaraldehyde, postfixing in 2% OsO₄, and staining with 1% uranyl acetate and 1% lead citrate.
and embedding in medium grade LR White resin (10). Sections were made with a glass knife mounted on a Sorval Porter-Blum MT 2-B Ultramicrotome.

Light microscopy. Tissues were sliced paradermally with a razor blade. Sections were then cleared in 2-methoxyethanol, treated in 5% Triton X-100, and stained in calconcine orange/Luxol brilliant green (3,4,11).

Transmission trials. *V. tahitensis* cuttings with mosaic and distortion symptoms were shipped to Gainesville by USDA permit for transmission experiments. Healthy *V. pompona* plants used in transmission trials were derived from plants maintained in Gainesville in isolation from diseased *V. tahitensis* plants.

Manual inoculations were made after dusting test plants with 600-mesh Carborundum. Inocula were prepared by triturating symptomatic leaf tissue in 0.01 M sodium phosphate buffer, pH 7.5. Inoculated plants were rinsed with water and maintained in a greenhouse at 20–30 C for symptom development.

The green peach aphid (*Myzus persicae* (Sulzer)) was tested as a vector. Specimens were reared on *Capsicum annuum* L., starved 1–3 hr, allowed <=1 min acquisition probes on young, symptomatic *V. tahitensis* leaf tissue, and transferred in groups of 10 or 25 to vigorous healthy *V. pompona* test plants. Test plants were maintained in a greenhouse and observed for symptoms until at least three leaves developed after inoculation and attained maturity.

RESULTS

Incidence of CyMV and ORSV was very low or absent in *V. tahitensis* plantings at all locations surveyed in French Polynesia (Table 1). Of 663 samples from Moorea, Raiatea, Tahaa, and Tahiti that were assayed serologically, only four were positive for either virus. Foliar mosaic and distortion were not evident on any of the four infected plants. Neither virus was found in any of the 51 wild specimens of *S. plicata* collected from a total of 10 *Vanilla* fields in Moorea, Raiatea, and Tahaa. Similarly, these viruses were found in only one of the 14 *V. planifolia* samples from French Polynesia and in none of the 15 *V. planifolia* samples from Tonga, five *V. planifolia* samples from Puerto Rico, or three *V. pompona* samples from French Polynesia. In contrast, very high CyMV and ORSV incidences were noted for the ornamental orchids sampled in French Polynesia. Of 68 plants assayed serologically, 54% were infected. Only two of the nine infected samples of *Vanilla* from French Polynesia were from privately owned plantations, whereas the remaining seven were from experimental plantings maintained by the French Polynesian government. Three of the four CyMV-infected experimental *Vanilla* hybrids were imported from Madagascar (Table 1).

"Vanilla* tahitensis* plants with conspicuous foliar mosaic and distortion were observed in nine of the 30 fields surveyed in French Polynesia (Figs. 1A and 2). With the exception of an experimental planting in Tahiti, however, low incidences of the mosaic disease in *Vanilla* were noted (Table 2). The affected plants did not appear to be appreciably stunted in comparison to their symptomless counterparts. Neither CyMV nor ORSV was detected in any of the 26 samples tested serologically for these viruses.

Flexuous rod-shaped virus particles were consistently associated with *V. tahitensis* plants with mosaic symptoms. Of 100 particles measured from one sample, 80 were 767 nm long. Cytoplasmic
inclusions typical of potyviruses (4) were seen in calcomine orange/Luxol brilliant green-stained mesophyll cells of leaves with mosaic symptoms (Fig. 1C). In thin sections, pinwheels, laminated aggregates, and scrolls characteristic of subdivision III potyviruses (6) were observed (Fig. 3).


M. persicae transmitted the Vanilla potyvirus from V. tahitiensis to V. pompona in a styleborne manner. One of the five V. pompona plants, each exposed to 10 aphids allowed ≤1-min acquisition probes, developed mosaic symptoms. All 10 of the plants exposed to 25 aphids each became infected with the potyvirus. Initial symptoms included a dieback of the shoot tip followed by distinctive foliar mosaic and blistering 3–8 mo after inoculation. The mosaic and distortion closely resembled those observed in V. tahitiensis (Fig. 1B). As previously noted for V. tahitiensis, flexuous rod-shaped virus particles were seen in negatively stained leaf extracts of these plants. Likewise, cylindrical inclusions were seen in leaf cells stained in calcomine orange/Luxol brilliant green.

In immunodiffusion tests, only bidens mottle, dasheen mosaic, and tobacco etch virus capsid antisera reacted with leaf extracts of V. tahitiensis and V. pompona plants infected with the Vanilla potyvirus. Homologous precipitin lines of dasheen mosaic virus fused, without apparent spur formation, with those of the Vanilla potyvirus (Fig. 4). In tests with bidens mottle and tobacco etch viruses, however, homologous precipitin lines spurred over the much weaker heterologous ones of the Vanilla potyvirus.

**DISCUSSION**

French Polynesian plantations of V. tahitiensis appear to be largely free of CyMV and ORSV. In contrast, a high incidence of these viruses is present in cultivated ornamental orchids grown there (21,22).

It is likely that incidences of CyMV and ORSV will increase sharply in Vanilla in the near future unless measures are taken to segregate plantings of V. tahitiensis from infected ornamental orchids. The popularity of imported ornamental orchids in French Polynesia has increased significantly in the last 15 yr, and there is currently no provision for

**Table 2. Incidence of a Vanilla mosaic disease in six plantings of Vanilla tahitiensis in French Polynesia**

<table>
<thead>
<tr>
<th>Location</th>
<th>Rows (no.)</th>
<th>Assessed ratio</th>
<th>Infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tahiti²</td>
<td>5</td>
<td>114/238</td>
<td>47.9</td>
</tr>
<tr>
<td>Taha²</td>
<td>5</td>
<td>1/291</td>
<td>0.3</td>
</tr>
<tr>
<td>Raiatea²</td>
<td>7</td>
<td>6/212</td>
<td>2.8</td>
</tr>
<tr>
<td>Haapiti²</td>
<td>6</td>
<td>12/184</td>
<td>6.5</td>
</tr>
<tr>
<td>Tiva²</td>
<td>8</td>
<td>2/154</td>
<td>1.2</td>
</tr>
<tr>
<td>Papara²</td>
<td>5</td>
<td>1/51</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*Number of support trees with infected plants/total surveyed. Data recorded 14–23 April 1986 and was based on visual detection of plants with characteristic foliar mosaic and distortion symptoms.

¹Data represent two subplots of one experimental plantation near Papara, Tahiti.

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**Fig. 2.** Locations in Society Islands, French Polynesia, that were surveyed in April 1986. Circles show locations where the Vanilla potyvirus (VPV) was detected. The number of Vanilla support trees in thousands (K) is indicated for each island, followed by the number of Vanilla plantations in parentheses.
Because neither CyMV nor ORSV can be reliably detected in *Vanilla* and other orchids solely on the basis of symptoms, alternative methods must be employed for their diagnoses. The relatively simple serological methods used in this study and others (17,20) could be used by personnel in French Polynesia, providing antisera were available. Alternatively, relatively inexpensive light microscopic or bioassay methods (4,11,12) could be used there for a small number of samples.

The mosaic disease of *V. tahitensis* in Huahine, Raiatea, Taha'a, and Tahiti is caused by a potyvirus, based on virus particle lengths, presence of cylindrical inclusions, transmission by aphids in a stelytborne manner, and serological relationships with at least three other potyviruses. The failure to achieve manual transmission to *V. pompona* plants, unlike the aphid transmission results, can be attributed to the unusually thick texture of *Vanilla* leaves. Similar problems in achieving manual transmission to certain monocotyledons plants have been noted by others, such as Louie and Lorbeer (15) for onions.

Whether or not the *Vanilla* potyvirus is related to other orchid-infecting potyviruses (7-9,11,13,14) is unclear, although it appears to be distinct from either bean yellow mosaic or the closely related clover yellow vein virus. The mosaic virus in *Vanilla* failed to react with any of the bean yellow mosaic or clover yellow vein virus antisera tested, and it did not infect cultivars of *P. sativum* and *P. vulgaris* known to be susceptible to these viruses. Moreover, bean yellow mosaic and clover yellow vein viruses induce subdivision II cylindrical inclusions (6), unlike the *Vanilla* potyvirus. Based on immunodiffusion results, it appears that the *Vanilla* potyvirus and dasheen mosaic virus are closely related, if not identical. These results must be interpreted cautiously at this time, however, pending the outcome of additional studies now under way at the University of Florida. In other crops, potyviruses have been described that apparently are serologically identical but have very distinct host ranges (18).

The relatively low incidence of the *Vanilla* potyvirus in French Polynesia is surprising considering that the virus

Fig. 3. Ultrastructure of *Vanilla tahitensis* leaf tissue infected with the *Vanilla* potyvirus (VPV) showing laminated aggregates, pinwheels, and scrolls characteristic of potyvirus subdivision III cylindrical inclusions. (A) Scale bar = about 500 nm and (B) scale bar = about 1,000 nm.

Fig. 4. Serological evidence for a relationship between the *Vanilla* potyvirus (VPV) and dasheen mosaic virus (DMV). Center well contained antisera to an isolate of DMV from Fiji, and the peripheral wells contained leaf extracts of *Colocasia esculenta* infected with DMV (d), *Vanilla pompona* with VPV (p), *V. tahitensis* with VPV (t), and healthy *V. pompona* (v) and *C. esculenta* (c).
appears to be efficiently transmitted by aphids, that *V. tahitensis* is exclusively propagated vegetatively, and that it is widely distributed throughout the islands. Indeed, aphids (*Cerataphis latanai* (Boisdr.), *Aphis craccivora Koch, and A. gossypii Glover, respectively) were collected on *V. tahitensis* and *Vanilla* support trees (*Teocoma stans* (L.) Juss. and *Gliricidia sepium* (Jacq.) Kunth ex Walp.). It may be that aphid vector activity is relatively low in the *Vanilla*-growing areas of French Polynesia. Unlike CyMV and ORSV, the vanilla potyvirus induces conspicuous and persistent symptoms, making it easy to recognize in *Vanilla* fields. Thus, it is possible that this virus can be controlled through a vigorous program of roguing and avoiding the use of cuttings from plantings where the symptoms are present.

**ACKNOWLEDGMENTS**

We wish to thank L. L. Breman, M. S. Elliott, and N. L. Shockey for technical assistance in Gainesville and D. Cheou, R. E. Thu, F. Riveta, J. Larcher, J. L. Reboul, B. Schmidt, F. Hapataha, B. Taurua, J. M. Timiri, T. Tolioler, and The Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement for their help during surveys in Society Islands. We also appreciate the help of S. Sorin, Vanilla Specialist, Department of Agriculture, Nuku’Alofa, Kingdom of Tonga, who accompanied the authors during surveys in French Polynesia and who forwarded us specimens of *Vanilla planifolia* from Tonga. We also acknowledge the help of H. Denmark in identifying the aphids and of T. J. Sheehan, D. W. Hall, R. L. Dressler, and P. Koers in identifying the orchids.

**LITERATURE CITED**


