Experimental and Applied Acarology

Orchid fleck virus: *Brevipalpus californicus* mite transmission, biological properties and genome structure

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ABSTRACT

Orchid fleck virus (OFV) causes necrotic or chlorotic ring spots and fleck symptoms in many orchid species world-wide. The virus has non-enveloped, bacilliform particles about 32-40 x 100-150 nm and is sap-transmissible to several plant species. OFV is transmitted by the mite *Brevipalpus californicus* (Banks) in a persistent manner and efficiently transmitted by both adults and nymphs, but not by larvae. Viruliferous mites retain their infectivity for 3 weeks on a virus-immune host. The genome of OFV consists of two molecules of 6431 (RNA1) and 6001 nucleotides (RNA2). The RNAs have conserved and complementary terminal sequences. RNA1 contains five open reading frames (ORF), and RNA2 encodes a single ORF. Although some of the encoded proteins of OFV have sequences similar to those of proteins of plant rhabdoviruses, OFV differs from viruses in the family *Rhabdoviridae* in having a bipartite genome.

Key words: *Brevipalpus californicus*; mite transmission; orchids; *Orchid fleck virus*; plant virus; rhabdovirus; virus genome structure.

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INTRODUCTION

Orchid Fleck Virus

Orchid fleck virus (OFV) was first found in Japan in *Cymbidium* plants with chlorotic or necrotic fleck symptoms (Doi *et al.,* 1977). It has subsequently been reported in Australia, Brazil, Denmark, Germany Korea and USA (Lesemann and Doraiswamy, 1975; Doi *et al.*, 1977; Chang *et al*., 1991; Kitajima *et al.*, 1974, 2001), causing chlorotic or necrotic spots and rings (Fig. 1) in many genera of the Orchidaceae (Gibbs *et al.*, 2000). Recently OFV has been shown to be transmitted by the false spider mite *Brevipalpus californicus* (Banks) (Acari: Tenuipalpdae) (Maeda *et al.*, 1998). It can also be transmitted by mechanical inoculation both to orchids and some non-orchid plant species. OFV virions are non-enveloped and bacilliform or bullet-shaped, 100-150 nm long and 32-40 nm in diameter and show a helical structure with a pitch of 4.5 nm (Chang *et al*., 1976). The particle morphology resembles that of plant rhabdoviruses with unenveloped particles. In cells of infected orchids, OFV induces a characteristic, intranuclear, electron-lucent viroplasm. Virus particles are visible in both the nuclei and cytoplasm; enveloped virions are occasionally seen in the cytoplasm (Lesemann and Begtrup, 1971; Chang *et al.*, 1976; Kitajima *et al.*, 1974, 2001). The genome of a Japanese isolate of OFV was found to be a bipartite, single-stranded RNA, and some of the encoded-proteins are similar to those of the plant rhabdoviruses (Kondo *et al.*, 1998). In this review, we describe the results of mite transmission, biological properties and the genome structure of OFV.

Mite transmission

Natural infection of OFV in some orchid plants in Japan has suggested that the virus might be vector transmitted. Attempts to transmit this virus using aphids (*Myzus persicae* Sulz. and *Aulacorthum solani* Kaltenbach), a whitefly (*Bemisia argentifolii* Bellows & Perring) and a mealybug (*Diaspis boisduvallii* Signoret) were unsuccessful (Doi *et al*., 1977; Maeda *et al.,* 1998).

Transmission tests were carried out using two species of mites, *Tetranychus urticae* Koch and *B. californicus*. The mites were reared separately on OFV-infected *Cymbidium* plants in an insect-free cage and then transferred to leaves of New Zealand spinach (*Tetragonia expansa* Murray) and bean (*Phaseolus vulgaris* L.) seedlings for 3 days. After 2 to 3 weeks, several yellow spots (Fig. 2) appeared on leaves of both test plants by inoculation of *B. californicus* (Fig. 3), but not by inoculation of *T. urticae*. These yellow spots were the same as symptoms produced by mechanical inoculation with sap. OFV particles were also found in extracts of yellow lesions after negative staining by E.M.

To test the transmission ability of *B. californicus*, viruliferous adult mites reared on OFV-infected *Cymbidium* leaves were placed singly on separate bean leaves for 3 days. Twenty-four of 35 mites caused yellow lesions on inoculated leaves. Individual mites produced one to 14 lesions per leaf. In other tests, lesion numbers increased with increasing numbers of mites. An inoculation access period of viruliferous mites was tested by placing groups of 5 viruliferous mites on leaves of *T. expansa* for 5 min, 10 min, 30 min, 1 h, 6 h and 24 h. The results showed that the mites were able to transmit the virus after 30 min, but not after 10 min. The number of yellow spots increased by increasing the length of the inoculation access period. Thus, transmission ability of mites was estimated by the number of lesions appearing on inoculated leaves (Fig. 2).

Larval, nymph and adult mites from infected plants were placed separately on leaves of *T. expansa* for 3 days. OFV was efficiently transmitted by the nymphs (mixture of proto- and deuto-nymphs) and adults, but not by the larvae. In addition, the mites were able to transmit the virus after molting. The transmission ability of *B. californicus* was retained after the viruliferous mites had been maintained for 3 weeks on a tea (*Camellia sinensis* (L.) O. Kuntze) plant that was immune to OFV. Immunoelectron microscopy revealed OFV particles in extracts of mites 3 weeks after removal from an infected plant. These results indicate that the transmission of OFV by *B. californicus* is persistent.

Hosts and symptomatology

OFV or OFV-like particles has been detected in the following orchid genera: *Angraecum, Aspasia, Baptistonia, Bifrenaria, Brassia, Bulbophyllum, Calanthe, Cattleya, Coelogyne, Colmanara, Cymbidium, Dendrobium, Diplocaulobium, Dockrillia, Encyclia, Flickingeria, Hormidium, Liparis, Masdevallia, Maxillaria, Miltonia, Odontoglossum, Oncidium, Phaius, Paphiopedilum, Pescatorea, Phalaenopsis, Polstachya, Renanthera, Stanhopea, Stenia, Trigonidium, Vanda* and *Zygopetalum* (Gibbs *et al.*, 2000; Kitajima *et al.*, 2001).

Chang *et al.* (1976) reported that OFV was transmitted by sap inoculation to *Dendrobium, Cymbidium, Nicotiana tabacum* L., *N. glutinosa* L., *Petunia hybrida* Vilm. (Solanaceae), *Chenopodium amaranticolor* Coste & Reyn., and *C. quinoa* Willd. (Chenopodiaceae). These inoculations were only successful when the temperature was higher than 30°C. Chlorotic or necrotic local lesions appeared on the inoculated leaves after 2-3 weeks. Systemic symptoms then developed on *Dendrobium* and *Cymbidium* species, but not on other plant species. Kondo *et al.* (1995) showed that non-orchid plants

such as *C. quinoa*, *C. murale* L. and *Beta vulgaris* L. (Chenopodiaceae) were systemically infected with OFV by manual inoculation of sap.

Furthermore, Maeda *et al.* (1998) found that 17 plant species in 12 families were infected with OFV by inoculation of viruliferous mites. All of these inoculations were from OFV-infected *Cymbidium* plants. Most of inoculated plants showed chlorotic or necrotic local lesion symptoms, but some plants produced systemic symptoms. Orchid plants such as *Cymbidium, Calanthe, Dendrobium*, and *Phalaenopsis* produced systemic symptoms of chlorotic or necrotic flecks about 1 to 2 months after inoculation. A non-orchid plant, *B. vulgaris* was also systemically infected, showing clear yellow vein symptoms. The following species produced local lesion symptoms: *T. expansa* (Aizoaceae), *Amaranthus lividus* L. (Amaranthaceae), *Vinca major* L. (Apocynaceae), *Lactuca laciniata* Makino (Compositae), *Pharbitis nil* (L.) Choisy (Convolvulaceae), *Acalypha australis* L. (Euphorbiaceae), *Cassia tora* L., *P. vulgaris* (Leguminosae), *Hibiscus manihot* L., *H. syriacus* L. (Malvaceae), *Citrus hassaku* Hort.ex Tanaka (Rutaceae), and *Datura stramonium* L. (Solanaceae).

Cytopathology

OFV particles are bacilliform or bullet-shaped, 32-40 nm in diameter and 100-150 nm long in purified preparations (Fig. 4A) or in dip preparations stained with uranyl acetate. In sections of virus-infected cells, OFV induces a characteristic, intranuclear, electron-lucent viroplasm (Fig. 4B). Virions can be seen scattered throughout the viroplasm, sometimes arranged side-by-side (Fig. 4B); and they are often found attached and perpendicular to the inner membrane of the nuclear envelope. Virions are also found in the cytoplasm arranged perpendicular to the endoplasmic reticulum. The

virion-membrane complexes on the nuclear envelope or endoplasmic reticulum frequently form tubular or cylindrical structures so that, in sections, the virions appear to be radially arranged as a "spoked wheel" (Figs. 4C, D) (Lesemann and Begtrup, 1971; Chang et al., 1976, 1991; Kitajima *et al.,* 1974, 2001). Enveloped virions are very rarely found in the endoplasmic reticulum (Lesemann and Doraiswamy, 1975). It was shown that intranuclear viroplasms of OFV result from the accumulation of OFV structural proteins and may be the site of virion assembly (Kitajima *et al.*, 2001).

Genome structure

Northern hybridization suggests that the genome of OFV is a negative stranded RNA divided into two parts (Kondo *et al.*, 1998). The complete sequence of the OFV genome was determined by producing a cDNA library using random primers (Kondo *et al.*, 1998). The 3' and 5' terminal regions were analyzed as described by Wetzel *et al.* (1994). The genome consists of two single-stranded RNA molecules of 6413 nucleotides (RNA 1) and 6001 nucleotides (RNA 2) (Fig. 5). The 3' and 5' terminal sequences of the two RNAs are conserved and complementary. RNA1 includes five open reading frames (ORFs) which encode 49 kDa, 26 kDa, 38 kDa, 20 kDa and 61 kDa proteins (Fig. 5). The 49 kDa and 61 kDa proteins have slight sequence similarities to the nucleoprotein (N) and glycoprotein (G) of plant rhabdoviruses such as *Sonchus yellow net virus* and *Rice yellow stunt virus*. RNA 2 has a single long ORF encoding 212 kDa protein (Fig. 5), which is similar to the polymerase protein (L) of viruses of the family *Rhabdoviridae*. A phylogenic tree derived from the L protein alignments suggests that OFV is more closely related to plant rhabdoviruses than to animal rhabdoviruses. The 3' end sequences of the six mRNA species corresponding to each ORF were detected in total RNA extracted from

infected leaves. The potential transcription termination and polyadenylation sites of each mRNA have highly conserved, consensus sequences. These results indicate that OFV is similar to rhabdoviruses but that it differs in having a bipartite genome (Kondo *et al.*, 1998).

CONCLUSIONS

OFV has been found to cause chlorotic and necrotic fleck disease in many genera of the Orchidaceae in the world (Gibbs *et al.*, 2000). OFV isolates collected from several countries were serologically undistinguished (Kitajima *et al.*, 2001). Nucleocapsid gene sequences of some of these isolates showed three distinct clusters of OFV: two are represented by single isolates, one from Japan and the other from Germany, and the third included all the other isolates collected in Australia, Germany and Brazil (Gibbs *et al.*, 2000).

We observed that OFV has rapidly spread in some orchid plants (*Cymbidium* and *Calanthe*) in greenhouses and in fields in Japan. *Brevipalpus californicus* is an important and potential vector of OFV. Indeed, about 70% of *B. californicus* were tested singly and transmitted the virus from diseased *Cymbidiu*m plants to healthy plants. The mites retained infectivity for a long period, but it is not clear whether the virus replicates in the mite vector. Although natural infections are limited to orchid genera, experimental host range tests using viruliferous mites included many plant species in at least 12 families. Most of the inoculated plants showed local necrotic lesions. Little information about *B. californicus* is available in Japan, and ecological studies of *B. californicus* and its role in OFV infection to orchids or to non-orchid plants are needed.

The bacilliform or bullet-shaped particles of OFV resemble those of rhabdoviruses with unenveloped virions. OFV-infected cells have an electron-lucent viroplasm with unenveloped rod-shaped virions in the nucleus and cytoplasm. In particle morphology and cytopathic effects, OFV closely resembles citrus leprosis (Kitajima *et al.*,1972; Colariccio *et al.,* 1995; Rodrigues *et al*., 2003). *Coffee ringspot virus* (Kitajima and Costa, 1972; Chagas, 1980), *Viola ringspot virus* (Gowanlock *et al.*, 1995), *Clerodendron chlorotic spot virus* (Kitajima and Moraes, 2000) and *Hibiscus chlorotic spot virus*, all of which are transmitted or associated with the mite *Brevipapus phoenicis* (Geijskes) (Kitajima *et al.*, 2001).

The genome structure of OFV is very similar to that of plant rhabdoviruses, except that it is divided (Kondo *et al.,* 1998). Plant rhabdoviruses have a monopartite genome of 11 to 13 kb nucleotides, in which six proteins are encoded (Jackson et al., 1999). Some of the encoded proteins of OFV also have similarities to those of the rhabdoviruses (Kondo *et al.*, 1998). So far, two genera of plant rhabdoviruses have been established, and most of the viruses are transmitted by aphids, leafhoppers or planthoppers (Jackson *et al.,* 1999). Some of these viruses have been shown to replicate in arthropod vectors. Based on particle morphology, genome structure and vector transmission, OFV and other allied viruses, probably *Brevipalpus* mite-borne viruses, should be classified as a new genus in the family *Rhabdoviridae*.

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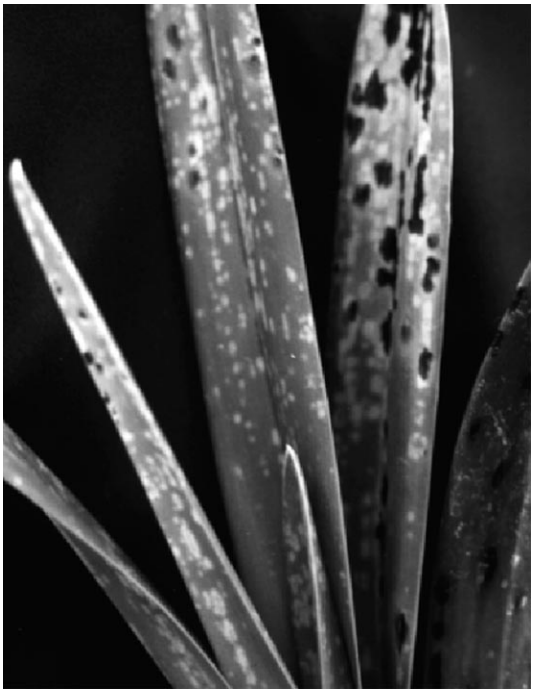

Fig. 1. Chlorotic and necrotic fleck symptoms in shoots of *Cymbidium* infected with Orchid fleck virus.

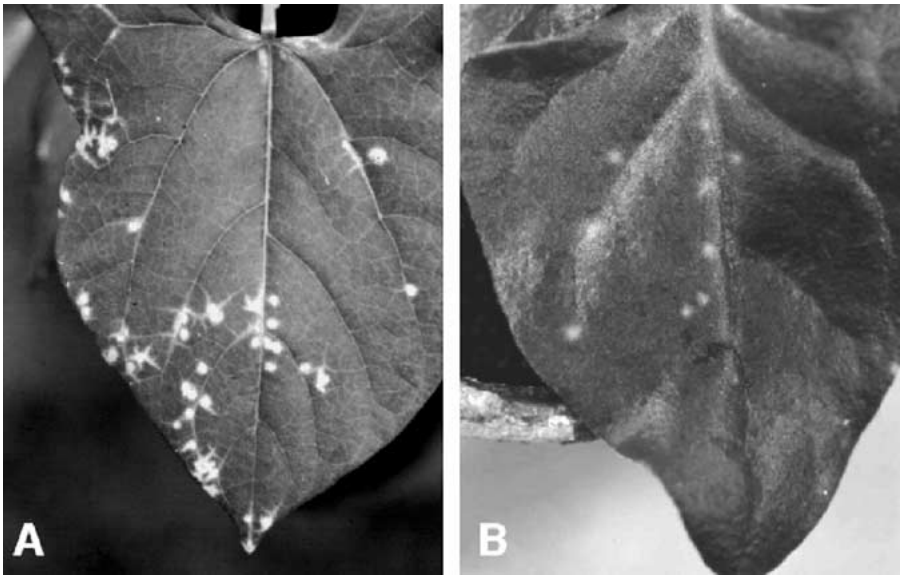

Fig. 2. Yellow spots in a leaf of *Phaseolus vulgaris* (A) and *Tetragonia expansa* (B) inoculated by *Brevipalpus californicus*.

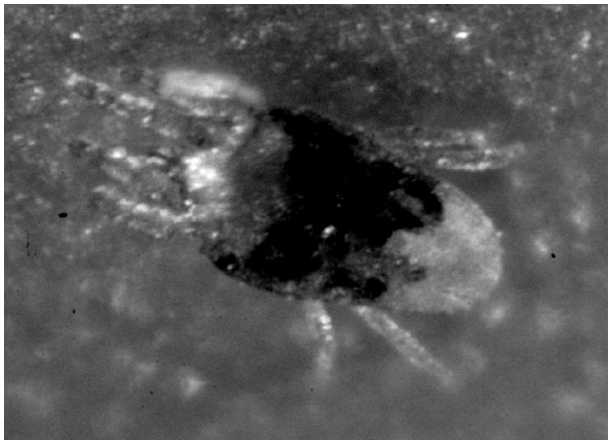

Fig. 3. The adult of *Brevipalpus californicus.*

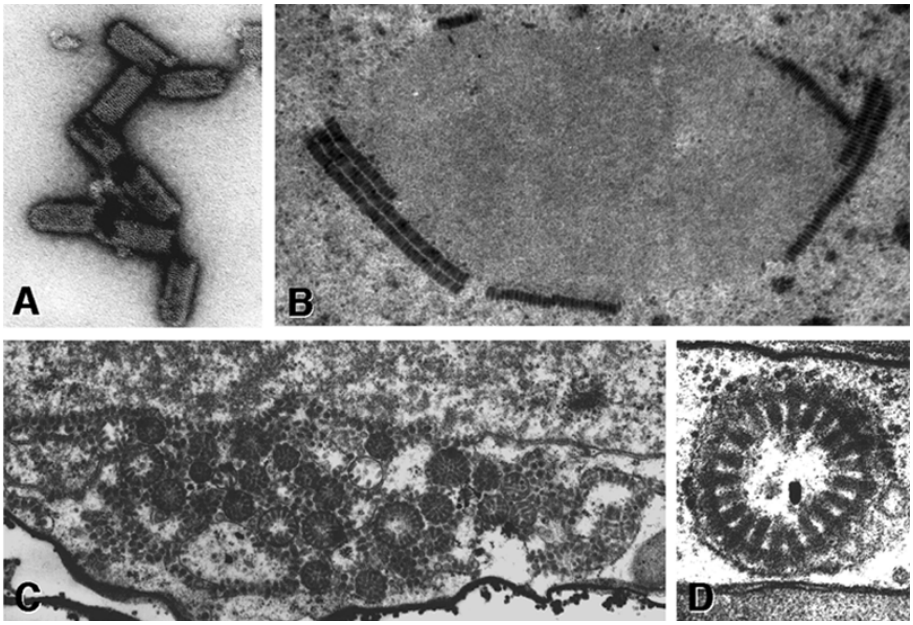

Fig. 4. Electron micrographs of virus particles (A) of orchid fleck virus and sections (B, C and D) *of Tetragonia expansa* leaves infected with orchid fleck virus. A) Purified virus particles stained with uranyl acetate; B) Side-by-side arrangements of virus particles around the viroplasm in the nucleus; C) Virus particles forming "spoked wheel" structures, associated with the inner nuclear membrane in perinuclear area; D) A "spoked wheel" structure in the cytoplasm.

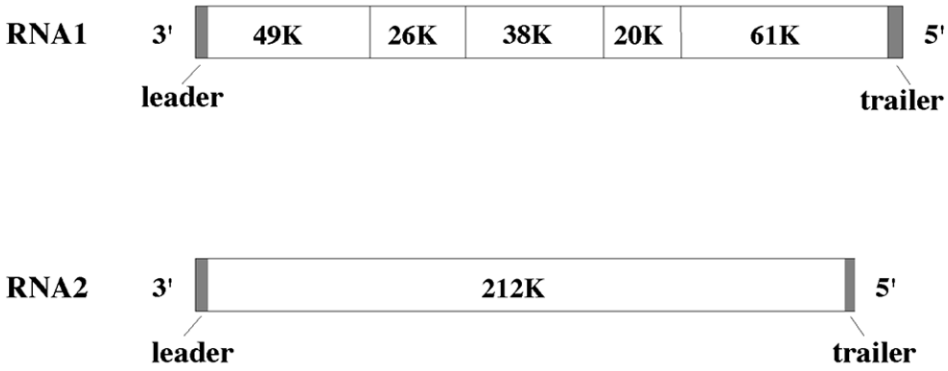

Fig. 5. Map of the genome organization of Orchid fleck virus. The 6413 nucleotides (RNA1) and 6001 nucleotides (RNA2) encode five proteins and one protein, respectively, in a negative-sense orientation. Each RNA contains the leader and trailer sequences.