

Identification of Potato Yellow Dwarf Virus Occurring Naturally in California

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ABSTRACT

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Potato yellow dwarf virus (PYDV) was isolated from naturally infected periwinkle (*Vinca rosea*) in California. Electron microscopy of infected tissues and of purified virus preparations from infected plants showed virus particles typical of plant rhabdoviruses. California PYDV reacted strongly with antiserum to the New York isolate of PYDV. This is the first report of a PYDV in the western United States.

Potato yellow dwarf virus (PYDV) is reported to be limited in geographical distribution to eastern North America (2). In the 1920s through the 1940s, it was responsible for serious disease outbreaks, especially in dry years when agallian leafhoppers migrated from native vegetation into potato fields (2). The disease caused by PYDV has been more difficult to find recently (3).

Two vector-specific isolates of PYDV are known. The New York isolate (SYDV) is vectored specifically by *Aceratagallia sanguinolenta*, and the New Jersey isolate (CYDV) is vectored by *Agallia constricta*. At least three species of the leafhopper genus *Aceratagallia* are common in California and have transmitted SYDV but not CYDV in controlled experiments (1). We report the discovery and identification of an isolate of PYDV occurring naturally in California. A preliminary report has been published (5).

MATERIALS AND METHODS

Virus source. In 1977 and 1978, diseased periwinkle plants (*Vinca rosea* L.) with yellowing and apical necrosis of stems were collected from locations in Riverside County, California, by G. Oldfield, Boyden Entomological Laboratory. Infected plants were brought to the greenhouse and propagated by

grafting to healthy periwinkle plants.

Transmission and host range studies. Attempts were made to transmit a virus from diseased to healthy *V. rosea* plants by grafting and with dodder (*Cuscuta subinclusa*). Attempts were made to transmit the virus mechanically from infected *V. rosea* to indicator species by grinding infected leaves in a mortar and pestle with 0.03 M KPO₄, pH 7.0, and applying the inoculum to Carborundum-dusted leaves using a cotton swab. Inoculated plants were placed in the greenhouse and observed for 1-2 mo for symptom development.

Electron microscopy. Infected and healthy leaf tissues of *V. rosea* and *Nicotiana debneyi* were prepared for electron microscopy by standard procedures. Small tissue pieces were fixed

by vacuum infiltration of 5% glutaraldehyde in 0.01 M phosphate buffer, pH 7.0, for 10 min, followed by postfixation in 1% osmium tetroxide. Tissues were then dehydrated through an acetone series and embedded in Spurr's embedding medium (11). Ultrathin sections were cut using a Porter-Blum MT-2 ultramicrotome and diamond knife. Sections were stained with aqueous saturated uranyl acetate, counterstained with lead citrate (10), and examined using an Hitachi HU-12 electron microscope.

Purified virus preparations were also examined by placing a drop of purified virus suspension on carbon-formvar-coated electron microscope grids. Samples were either fixed in 2.5% glutaraldehyde in 0.05 M phosphate buffer, pH 7.0, and stained with 2% aqueous uranyl acetate or 2%



Fig. 1. Leaves of *Vinca rosea* with typical symptoms of infection by California potato yellow dwarf virus. Healthy leaf is on the left.

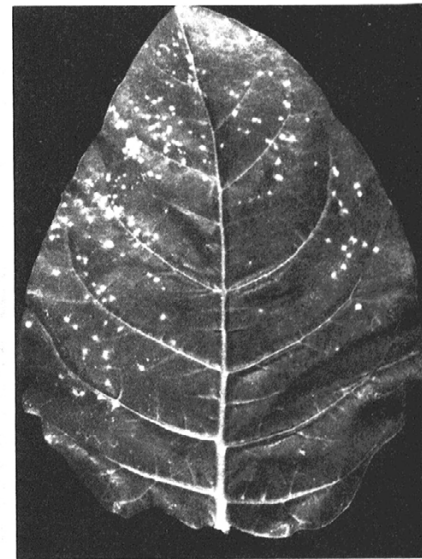


Fig. 2. Leaf of *Nicotiana debneyi* with yellow chlorotic lesions on mechanically inoculated leaves.

phosphotungstic acid, pH 7.0, or stained without glutaraldehyde fixation.

Serology. Antisera were provided as follows: American wheat striate mosaic virus, R. C. Sinha; broccoli necrotic yellows virus, R. N. Campbell; SYDV and CYDV isolates of PYDV, Hei-ti-Hsu; sonchus yellow net virus, A. O. Jackson. Agar diffusion tests were done in 0.6% ionagar and 0.02% sodium azide in water. Freshly expressed sap from

healthy and infected *N. debneyi* was sonicated for 15 sec using a Biosonik sonicator and probe and placed in wells 4 mm from the test antiserum. Plates were observed for 1 wk.

Virion purification. Three methods of purification previously used for rhabdoviruses were initially attempted. All were essentially performed as described previously.

Hsu and Black's method (6) for PYDV

consisted of blending infected leaves in 0.1 M glycine and 0.01 M $MgCl_2$, pH 8.4, followed by low-speed centrifugation, polyethylene glycol (PEG) 6000 precipitation, and sucrose density gradient centrifugation.

By the method of Lastra and Acosta (9) for maize mosaic virus, infected tissue was diced with a razor blade and vacuum-infiltrated with 0.1 M glycine, 0.01 $MgCl_2$ and 0.001 M DIECA, pH 8.0. The suspension was filtered first through cheesecloth and then through a celite pad. The sample was then subjected to discontinuous and then rate-zonal sucrose density gradient centrifugation in sucrose dissolved in 0.1 M glycine, 0.01 $MgCl_2$, pH 7.0.

The third method followed that of Jackson and Christie (7) for sonchus yellow net virus. Infected tissues were blended in solution A (0.1 M Tris, 0.01 M $MgCl_2$, 0.04 M Na_2SO_3 , pH 8.4), filtered through cheesecloth and subjected to low-speed centrifugation (8000 rpm for 10 min in the SS-34 rotor). The supernatant was layered on discontinuous sucrose gradients of 6 ml of 60% sucrose and 4 ml of 30% sucrose in solution B (0.1 M Tris, 0.01 M $MgCl_2$, 0.04 M Na_2SO_3 , pH 7.5) and centrifuged in the SW27 rotor for 1 hr at 17,000 rpm. The green interface between the 30% and 60% sucrose layers was removed, diluted with solution B, and vacuum-filtered through a 5–10 mm celite pad. The filtrate was centrifuged in the Type 30 rotor for 30 min at 25,000 rpm to pellet the virus. The pellet was resuspended in solution B and subjected to rate-zonal sucrose density gradient centrifugation on linear sucrose 10–40% gradients prepared in solution B for 30 min at 35,000 rpm in the SW41 rotor.

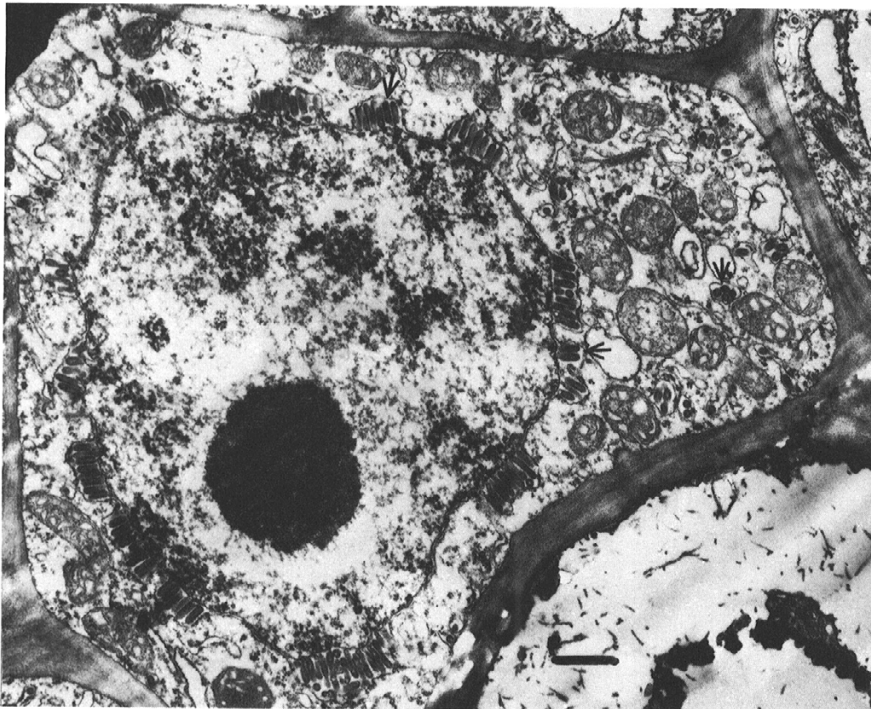


Fig. 3. Ultrathin section of *Nicotiana debneyi* infected with California potato yellow dwarf virus. Arrows indicate virions surrounding nucleus in perinuclear space and small groups of virions in the cytoplasm. Bar represents 500 nm.

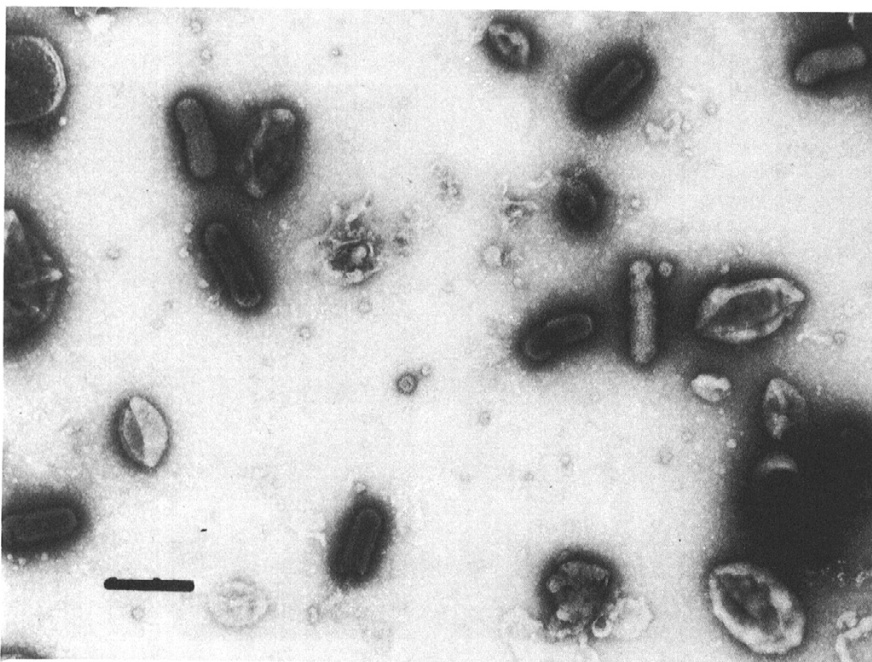


Fig. 4. Electron micrograph of purified California potato yellow dwarf virus stained only with uranyl acetate. Bar represents 200 nm.

RESULTS

Transmission and host range studies.

Symptoms of naturally infected *V. rosea* are shown in Fig. 1. Plants were typically chlorotic overall, with a mosaic pattern in older leaves and twisting and necrosis of very young leaves. These symptoms were reproduced in uninfected *V. rosea* by grafting with wedge grafts or leaf patches. Transmissions were also successful from virus-infected to healthy *V. rosea* and infected *N. debneyi* to healthy *V. rosea* using *C. subinclusa*.

The virus was mechanically transmissible to seven species of tobacco. *N. debneyi* and *N. rustica* were the best hosts for symptoms and virus maintenance. Bright yellow local lesions developed on inoculated leaves 10–15 days after inoculation (Fig. 2), followed by a systemic mosaic in the upper leaves, bright yellowing of the veins, and eventual yellowing of entire leaves. For best symptom development, plants were decapitated above the last inoculated leaf to force axillary growth as with the eastern isolates of PYDV (6).

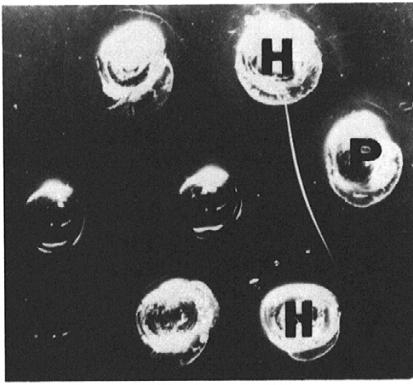


Fig. 5. Reaction of sap from healthy *Nicotiana debneyi* (H) and sap from *N. debneyi* infected with California potato yellow dwarf virus (P) on agar diffusion plate. Antiserum to the New York isolate of potato yellow dwarf virus is in the center well.

Plants of *N. tabacum*, *N. tabacum* var. nc, *N. glutinosa*, *N. clevelandii*, and *N. clevelandii* × *glutinosa* also showed primary local lesions followed by systemic mosaic and vein yellowing.

Chenopodium amaranticolor, *C. quinoa*, *Capsella bursa-pastoris*, *Cucumis sativus*, *Phaseolus vulgaris*, *Vigna unguiculata*, *Gomphrena globosa*, *Saponaria vaccaria*, and *Physalis floridana* were not hosts for the virus.

Electron microscopy. Abundant viruslike particles were seen in tissues of infected *V. rosea* and *N. debneyi* (Fig. 3). Particles were approximately 185 × 75 nm and are typical of the Rhabdoviridae (4). Virions appeared to be budding from the nuclear membrane into the perinuclear space. Small groups of virions surrounded by a membrane were also seen in the cell cytoplasm.

Purified preparations of the virus from infected *N. debneyi* contained similar virions (Fig. 4). Fixation of the purified virions with glutaraldehyde resulted in preparations with more intact virions than preparations with no fixation. Purified virions were approximately 230 × 87 nm, which differs slightly from virions in imbedded tissues. Size differences between purified virions and virions in embedded tissues have been noted before for rhabdoviruses (8). Both sizes reported here are well within earlier size estimates for PYDV (8).

Serology. Precipitin lines were sharp in agar diffusion plates when antiserum to the SYDV isolate of PYDV was tested

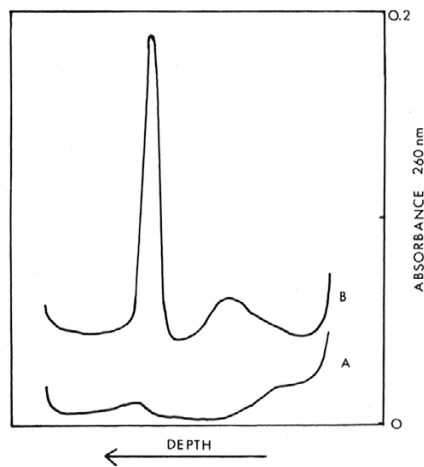


Fig. 6. Absorbance profiles (254 nm) of sucrose density gradients of potato yellow dwarf virus purified from 100 g of infected *Nicotiana debneyi*: Methods of (A) Lastra and Acosta (9) and (B) Jackson and Christie (7).

against the California isolate from sap of infected *N. debneyi* (Fig. 5). Only a faint reaction, closely surrounding the antigen well, occurred with CYDV antiserum. None of the other rhabdovirus antisera reacted with California PYDV.

Virion purification. All three purification methods yielded infectious preparations. Methods used for maize mosaic virus and sonchus yellow net virus gave much cleaner preparations when analyzed by rate-zonal sucrose density gradient centrifugation than did the method of Hsu and Black (6), using PEG to precipitate the virus. PEG precipitation gave preparations that were slightly green and often did not allow resolution of a typical virus peak as the gradients were fractionated.

Density gradient profiles are shown in Fig. 6. The method for sonchus yellow net virus yielded considerably more virus than the method for maize mosaic virus. Typical virions were found when the peaks were collected and samples examined by electron microscopy (Fig. 4).

DISCUSSION

Particle morphology, host range, and serologic characteristics clearly indicate that the virus isolated from naturally infected *V. rosea* is an isolate of PYDV. Although the virus occurs naturally in eastern North America, this is the first

report of a PYDV in the western United States.

The stronger serologic reaction between California PYDV and antiserum to SYDV indicates that California PYDV is more closely related to SYDV than CYDV. This is significant in light of the fact that three species of *Aceratagallia* reported by Black (1) to vector SYDV have been known to occur in California for at least 40 yr. An unidentified *Aceratagallia* sp. in southern California is now known to vector California PYDV (B. W. Falk and R. Yokomi, unpublished).

The origin and distribution of California PYDV are unknown, even though the virus and vector have probably been in California for a number of years. It is an enigma why PYDV has not been discovered in California previously. Black reported that Eastern isolates of PYDV are no longer commonly found in nature (3). A similar situation may exist in California. Conditions that favor migration of *Aceratagallia* spp. and virus spread from reservoirs into economically important crops such as potatoes, where PYDV disease could occur, may not be common in California.

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