Winged Bean Mosaic Caused by Clover Yellow Vein Virus

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ABSTRACT

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Winged bean (*Psophocarpus tetragonolobus*) plants from experimental field plots in eastern Maryland were stunted and showed leaf mosaic and rugosity. Electron microscopy of leaf-dip preparations from symptomatic tissue showed flexuous rod viruslike particles about 750 nm long. The virus was mechanically transmissible, and purified preparations induced symptoms in winged beans identical to those in the original diseased plants. Vector relationships, inclusion characteristics, physical properties, and density-gradient analysis indicated the virus was a member of the potyvirus group. Serological gel double-diffusion tests of sodium dodecyl sulfate-treated preparations showed this pathogen was an isolate of clover yellow vein virus.

Winged beans (*Psophocarpus tetragonolobus* (L.) DC.) growing in experimental plots at the University of Maryland Eastern Shore were stunted and showed severe mosaic symptoms indicative of viral infection. The virus was shown by particle morphology, host range, vector relationships, inclusion characteristics, and serological tests to be an isolate of clover yellow vein virus (CYVV). Winged beans are legumes of current interest as a high-protein crop for humid, tropical regions and are reputed to be generally disease resistant (1). Pathological research on winged beans has been limited, yet diseases of viral etiology have been reported in Africa (5,6), Asia (17), Brazil (11), Southeast Asia (16), and in experimental plots in Florida (12). The only prior report of a flexuous rod particle associated with a disease of winged beans is that of Fortuner et al (6), who associated a 650-nm particle, a member of the carlavirus group, with a foliar necrosis and mosaic disease in Africa.

MATERIALS AND METHODS

Severely stunted winged beans showing mosaic symptoms from field plots were transplanted to 15-cm pots of composted soil and maintained in a screened greenhouse. Symptomatic leaves, ground in a mortar with 0.01 M phosphate buffer, pH 7.0 (1:1, w/v), were used to mechanically inoculate, by the Carborundum gauze-pad method, a group of plant virus indicators. Preliminary inoculations indicated a limited host range, and plants of *Chenopodium quinoa* Willd. reacted with local lesions and thus were used as both source and assay host. For aphid transmission, *Myzus persicae* (Sulzer) colonized on caged tobacco plants (*Nicotiana tabacum* L. 'Turkish') were given a preacquisition starving period of 4 hr. Single aphids were allowed a 15-sec acquisition feeding period on symptomatic winged bean leaves followed by an 8-hr test feeding on winged bean plants. The aphids were removed with Resmethrin (Whitmire Research Lab., St. Louis, MO) spray and the test plants returned to the greenhouse. Physical properties of dilution end point, aging in vitro, and thermal inactivation were conducted with locally infected leaves of *C. quinoa* by the methods of Bos et al (3).

Leaf-dip preparations for electron microscopy were chromium-shadowed or negatively stained with 2% ammonium molybdate on carbon-coated Parlodion-covered grids.

Particle size was determined by comparison with polystyrene latex particles (312 ± 3 nm) (Dow Chemical Co., Midland, MI). For electron microscopy of infected tissue, leaf pieces about 1 × 3 mm were fixed in 6% buffered glutaraldehyde, postfixed in 1% buffered osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in a 66/34 mixture of Maraglas-Cardolite (Acme Chemical Company, New Haven, CT; 3M Company, St. Paul, MN) with 2% (w/v) benzyldimethylamine. Ultrathin sections obtained with a diamond knife on a Porter-Blum MT-1 microtome were stained with uranyl acetate and lead citrate and examined in an Hitachi HU-11C-1 electron microscope.

Partially purified viral preparations were obtained from locally infected frozen plant tissue of *C. quinoa*. Tissue was thawed and blended for 5 min in a Waring Blendor with 0.05 M borate buffer (1:2, w/v) containing 0.005 M ethylene diaminetetraacetate (EDTA) and 0.01 M sodium diethyldithiocarbamate (DIECA).

The homogenate was passed through cheesecloth and stirred with *n*-butanol and chloroform (10%, v/v, of each). The emulsion was separated by low-speed centrifugation of 4,100 *g* for 10 min and the aqueous layer removed. Four percent polyethylene glycol (PEG 6000) and 1% NaCl were added and the mixture was stirred in an ice bath for 30 min, given a low-speed centrifugation, and the resulting pellets resuspended in 0.005 M borate buffer, pH 8.0. The preparation was given an additional low-speed centrifugation and the resulting supernatant centrifuged for 2 hr at 35,000 *g*. The pellets were resuspended in 0.005 M borate buffer, pH 8.0. For highly purified preparations, 2 ml of resuspended high-speed pellets were layered on 10–40% sucrose gradients and centrifuged in a swinging bucket rotor for 90 min at 53,000 *g*. The light-scattering zones, associated with infectivity, were removed by syringe, combined and centrifuged at 35,000 *g* for 2 hr, and resuspended in 2 ml of 0.005 M borate buffer, pH 8.0.

Highly purified virus preparations were used for antiserum production by a series of two intramuscular, three subcutaneous, and eight intravenous injections into a male Flemish Giant Chinchilla rabbit over a period of 8 wk. The homologous titer was determined by microprecipitin tests (2), and serological relatedness was determined in 0.6% agar gels (2) containing 0.1% sodium azide and 0.5% sodium dodecyl sulfate (SDS) (15) in neutral phosphate-buffered saline. All antigen preparations were exposed to a final concentration of 1% SDS for 5 min before application to the appropriate wells. Plates were maintained at room temperature in a moist chamber for 48 hr.

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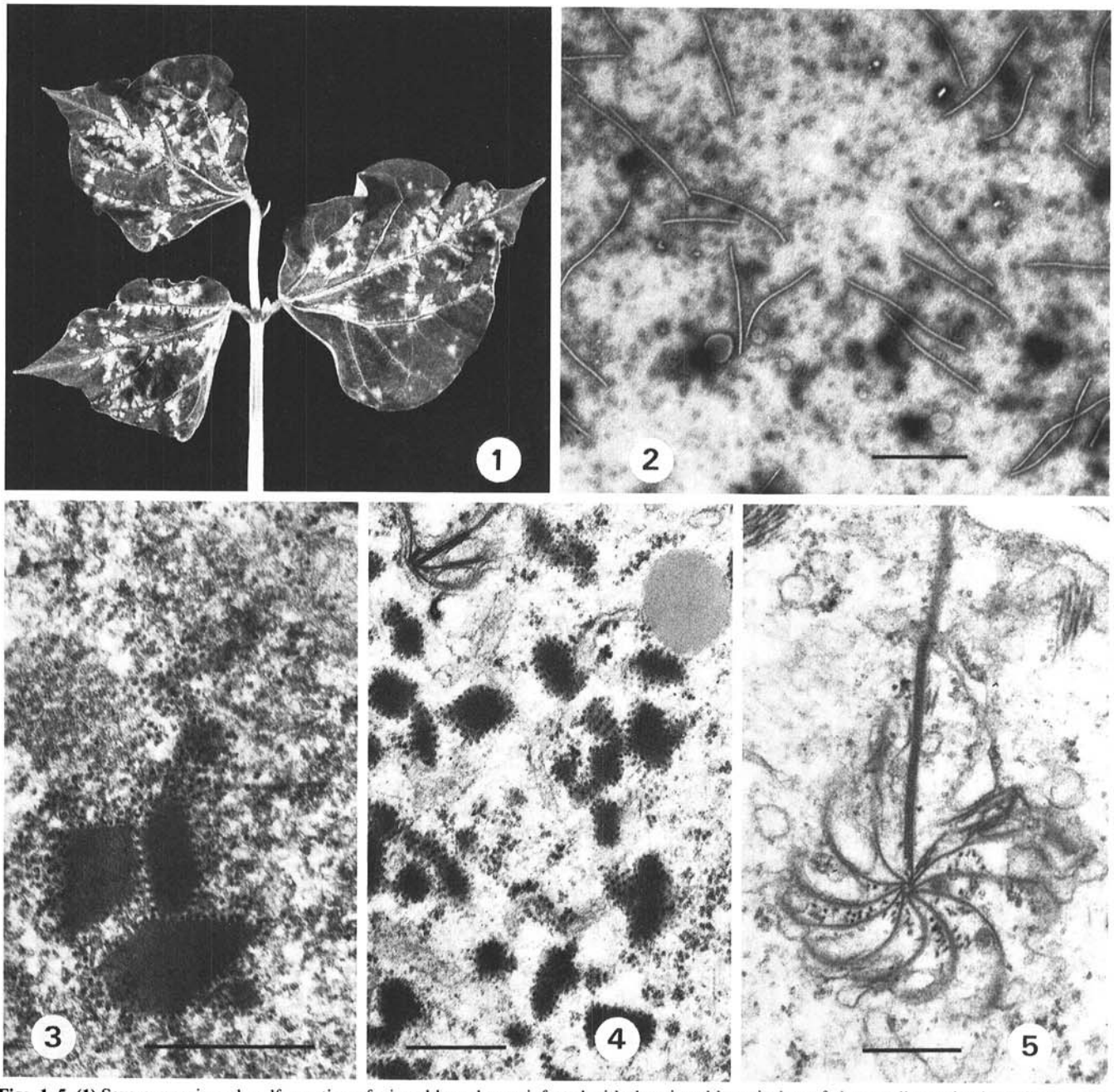
RESULTS AND DISCUSSION

Symptomology and host range. Extracts from diseased winged beans induced diffuse necrotic lesions, veinal necrosis, and epinasty on inoculated primary leaves of winged beans within 7–8 days. Systemic symptoms of tip necrosis occasionally occurred, and plants that survived the initial shock reaction were stunted and developed mosaic symptoms (Fig. 1) similar to those of the original diseased plants. Of the 64 species from 14 families tested, the experimental host range included 14 species from five families. Local lesions on inoculated leaves followed by systemic symptoms occurred in *Phaseolus vulgaris* L. 'Pinto' and 'Bountiful.' None of the systemically infected Pinto plants survived more than 3 wk after inoculation.

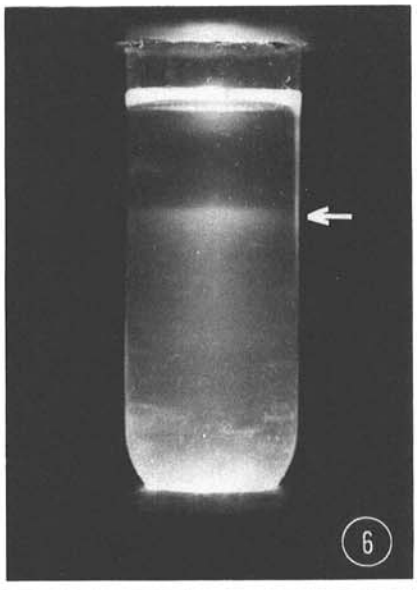
Systemic infection occurred also in inoculated plants of *N. clevelandii* Gray, *Vicia faba* L., *Trifolium incarnatum* L., *Pisum sativum* L. 'Alaska,' and occasionally in *C. quinoa*. Local lesions without systemic infection developed on *Tetragonia expansa* Murr., *Gomphrena globosa* L., *Canavalia ensiformis* DC., *C. gladiata* DC., *Chenopodium quinoa, C. capitatum* L., *C. album* L., *C. murale* L., and *C. amaranticolor* Coste & Reyn. A systemic leaf mosaic and necrosis that developed in *P. sativum* 'Alaska' was similar to that described by Nagel et al (14).

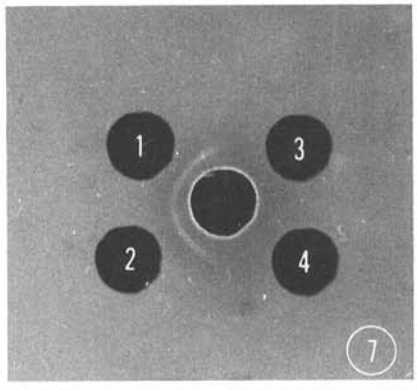
In contrast to the clover isolate of Hollings and Nariani (9), the winged bean isolate did not infect plants of soybean (*Glycine max* (L.) Murr.) or *Trifolium repens* L., locally or systemically. Lisa and Dellavalle (13) also reported no infection on the five different cultivars of *T. repens* inoculated with a strain of CYVV, demonstrating the variability of host reactions to different strains of CYVV (10).

Aphid transmission. Aphid transmission occurred in the nonpersistent manner characteristic of the potyviruses. Two of 25 inoculated winged bean test plants develped symptoms. Electron microscopy



Figs. 1–5. (1) Severe mosaic and malformation of winged bean leaves infected with the winged bean isolate of clover yellow vein virus (CYVV). **(2)** Particles from a partially purified preparation of the winged bean isolate negatively stained with 2% ammonium molybdate. **(3)** Nuclear inclusions from winged bean tissue infected with the winged bean isolate of CYVV. **(4)** Striated cytoplasmic inclusions from winged bean tissue infected with the winged bean isolate of CYVV. **(5)** Pinwheel inclusions and laminated aggregates from winged bean tissue infected with the winged bean isolate of CYVV. Scale bars = 500 nm.





Figs. 6 and 7. (6) Light-scattering zone resulting from centrifugation of partially purified winged bean isolate of clover yellow vein virus (CYVV) in 10–40% sucrose density gradient. **(7)** Gel double diffusion. Center well: antiserum to winged bean isolate; 1 = heterologous CYVV isolate in 1% sodium dodecyl sulfate (SDS), 2 = homologous winged bean isolate in 1% SDS, 3 = 1% SDS, and 4 = preparations from healthy tissue in 1% SDS.

of leaf-dip preparations from these two plants revealed flexuous rod viruslike particles similar to those of the original diseased plants.

Physical properties. Crude sap preparations were infectious after heating for 10 min at 30 C but not at 40 C. Local lesions occurred on plants inoculated with sap diluted 10^{-4} but not 10^{-5} (0.01 M phosphate buffer, pH 7.0) and with preparations exposed to room temperature for 6 days but not 10 days. The dilution end point and aging in vitro values are typical for potyviruses, but the temperature for thermal inactivation is lower than the normal range reported for the group (8), which may reflect the *C. quinoa* source that became viscous when heated.

Electron microscopy. Examination of leaf-dip and partially purified preparations from symptomatic winged bean leaves revealed flexuous rod viruslike particles about 750 nm long (757 ± 21 nm; 220 particles) (Fig. 2). Electron microscopy of ultrathin sections from infected leaf tissues of winged bean, Bountiful bean, and *C. quinoa* showed cytoplasmic and nuclear inclusions characteristic of the potyvirus group (8). Crystalline striated inclusions occurred in both the nucleus (Fig. 3) and the cytoplasm (Fig. 4). Pinwheel inclusions as well as laminated aggregates occurred in the cytoplasm (Fig. 5). The inclusions included pinwheels, bundles, and laminated aggregates but no scrolls or tubes, indicating that this isolate should be included in subdivision II of the potyvirus group as established by Edwardson (4) along with CYVV and a variety of other viruses of legumes.

Density gradients. Centrifugation of partially purified preparations in sucrose density gradients resulted in formation of a single light-scattering zone (Fig. 6) that was infectious. Comparative centrifugation with tobacco mosaic virus (194 S) indicated that the particles in the zone had a sedimentation coefficient of about 150 S, similar to those of potyviruses (8).

Serology. Microprecipitin tests indicated a homologous antiserum titer of 1:512. Reactions were not obtained with preparations from healthy tissue. Gel double-diffusion tests with SDS-treated, purified preparations of the winged bean isolate gave a confluent zone of serological identity between the homologous winged bean isolate and the heterologous CYVV (Stavely) isolate (Fig. 7). No reaction developed between wells containing antiserum and those with SDS alone or SDS-treated preparations from healthy tissue. In reciprocal tests (7), confluent zones of serological identity were obtained with the winged bean and CYVV (Stavely) isolates against CYVV Pratt antiserum.

A variety of viruses in Edwardson's (4) subdivision II of the potyvirus group that infects members of the Leguminosae, such as bean yellow mosaic virus, CYVV, and pea mosaic virus, have no consistent differences in such properties as host range and symptomology (10); and serological relatedness provides the best indicator of identity (10). In this study, gel double-diffusion tests indicated that the winged bean isolate is serologically identical to isolates of CYVV.

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