Isolation of a Potyvirus from Declining Clones of *Populus*

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ABSTRACT

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A virus was transmitted mechanically to herbaceous indicator plants from six declining *Populus* × *euramericana* clones, two declining *Populus tremuloides* clones, and two declining *Populus grandidentata* clones. The virus induced local lesions on inoculated *Chenopodium quinoa* and both local lesions and systemic symptoms on *Phaseolus vulgaris* and *Vigna unguiculata* (cowpea). Usually less than 10% of the herbaceous indicator plants inoculated with leaf sap from declining poplars became infected. When inoculated with leaf sap of infected cowpea, *P. tremuloides* seedlings developed chlorotic and necrotic leaf spot symptoms similar to those observed on declining poplars. The virus was transmitted back to cowpea from inoculated symptomatic but not asymptomatic seedlings of *P. tremuloides*. Electron microscopy of four representative isolates revealed flexuous rod-shaped virus particles (with a modal length of 800–810 nm) in sap from cowpea leaves and associated potyvirus-induced inclusions in thin sections of cowpea leaf tissue. All four isolates had a thermal inactivation point between 58 and 60 C, a dilution end point of 10^{-4} in cowpea leaf sap, a longevity in vitro of 24 hr at 22 C, and identical host ranges. This virus is a member of subdivision I of the potyvirus group. It can be differentiated from other viruses in that group by particle length, host range, and symptomatology.

Additional key words: aspen decline.

An unexplained deterioration of aspen (*Populus* spp.) stands has been reported, both in the Rocky Mountain region (14,16,19) and in the Lake states (11,13). Stand deterioration has usually been attributed to site quality, climate, genotype, and fire history (13). However, various pathogens may also be involved in aspen deterioration (11,13,14,16,19).

In Wisconsin, decline symptoms in aspen were first observed in 1973. Declining clones of large-toothed aspen (*Populus grandidenta* Michx.) have shown a leaf bronzing in the lower crown during July and August. The bronzed leaves later became necrotic, and branches with bronzed leaves often were dead the next year. In successive years, the leaf bronzing developed in the upper crown and eventually the affected trees died. Clones of declining quaking aspen (*Populus tremuloides* Michx.) developed chlorotic and necrotic leaf spot symptoms early in the growing season and some developed bronzing symptoms, scattered throughout the crown, in late July and August. These decline symptoms have been observed

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in both aspen species throughout Wisconsin. Decline has also appeared in a Wisconsin collection of *Populus* × *euramericana* (Dode) Guinier clones. In these clones, decline symptoms appeared as chlorotic and necrotic leaf spots, branchlet mortality, and decreased growth followed by death of successively larger branches.

Decline problems in many other vegetatively propagated crops have been attributed to single or multiple viral infections (15). There are only a few reports of virus diseases of *Populus* spp. In Europe, poplar mosaic virus (PMV) causes leaf spotting, petiole necrosis, and stem lesions of poplar (1). Although found in Canada, PMV has not yet been reported in the USA (18). Tobacco necrosis virus (TNV) has been reported from quaking aspen (14) and from *Populus* hybrids (5), but the significance of TNV in *Populus* spp. has not been determined. Viruslike particles also have been found in leaves of a graft- and aphid-transmissible leaf spotting disease of aspen (2,3).

In a survey of declining clones of *P.* × *euramericana* and native aspen in Wisconsin, a flexuous rod-shaped virus was recovered from 10 declining poplar clones. In this paper, we describe the host range, symptomatology, physical properties, size, and associated inclusions of a potyvirus isolated from these poplar clones.

MATERIALS AND METHODS

Source of materials. Eleven poplar clones were selected for virus transmission trials to herbaceous host plants. Clones W2, W5, and W136 had been selected as native Wisconsin trees (*Populus deltoides* Bartr.), but showed morphological evidence of hybridization with European black poplar (*Populus nigra* L.). Clone W401 was acquired as *P.* × *euramericana* 'Gelrica,' W450 as *P.* × *euramericana* 'IH30A,' and W87 as *P.* × *euramericana* 'Charkowiensis' from C. C. Heimburger, Ontario, Canada. Clone NE244 was received from the USDA Northeast Forest Experiment Station as *P. deltoides* 'Virginiana' × *P. deltoides* 'Angulata.' Clones W2, W5, W136, W401, W450, and W87 exhibited decline symptoms, but clone NE244 showed none. Two native declining clones each of *P. tremuloides* and *P. grandidentata* were selected from natural stands in Wisconsin.

Host range. Test plants were grown from seed in 10-cm-diameter pots containing steam-sterilized soil in a greenhouse maintained at 22–26 C with supplemental light provided by fluorescent and incandescent bulbs to give a 14-hr daylength. Leaves from infected cowpea plants were used for preparing inocula for host-range studies. Leaves were triturated 1:5 (w/v) in 0.03 M phosphate buffer (pH 7.2) containing 4% polyvinylpyrrolidone with a molecular weight of 10,000 (PVP-10) and 0.01 M 2-mercaptoethanol (2-ME). Inocula were applied with a cheesecloth pad to carborundum-dusted (22-μm [600-mesh]) leaves of test plants.

Symptoms and host range of isolates were compared on 25 plant species representing eight families. Plant species used in host range studies were: Apocynaceae—*Vinca rosea* L.; Chenopodiaceae—*Chenopodium amaranticolor* Coste and Reyn., *Chenopodium quinoa* Willd.; Compositae—*Lactuca sativa* L., *Zinnia elegans*, Jacq.; Cruciferae—*Raphanus sativus* L.; Cucurbitaceae—*Curcubita peop* L., *Cucumis sativus* L. 'Improved Chicago Pickling'; Leguminosae—*Medicago sativa* L. 'Vernal', *Phaseolus limensis* Macf. (lima bean), *Phaseolus vulgaris* L. (bean) 'Bountiful,' *Pisum sativum* L., *Vigna unguiculata* (L.) Walp. (cowpea) 'Blackeye'; Solanaceae—*Capsicum frutescens* L. 'Yolo Wonder,' *Lycopersicum esculentum* Mill., *Nicotiana megalosiphon* Huerch and Muell., *Nicotiana glutinosa* L., *Nicotiana rustica* L., *Nicotiana tabacum* L. 'Xanthi,' *N. tabacum* L. 'Havana 38,' *N. tabacum* L. 'Havana 425,' *Nicotiana clevelandii* Gray, *N. clevelandii* × *N. glutinosa, Petunia hybrida* Hort. Vilm. & Andr., and *Physalis floridana* Rydb. Each species was inoculated mechanically with the poplar virus isolates. An equal number of check plants was treated with buffer. Plants were observed daily for 4 wk for symptom expression. After 4 wk, all test plants were assayed by test inoculations onto cowpea and *C. quinoa.*

Physical properties. For infectivity dilution end point (DEP) and thermal inactivation point (TIP) determinations, infected cowpea leaves were triturated 1:5 (w/v) in 0.03 M phosphate buffer (pH 7.2) containing 4% PVP-10 and 0.01 M 2-ME. For DEP determinations, 10-fold dilutions of leaf sap from 10^{-1} to 10^{-7} were prepared from infected cowpea leaves at 3-day intervals after inoculations. The TIP was determined by heating leaf sap in capillary tubes in a thermostatically controlled water bath for 10 min and then immediately immersing them in an ice bath. After each treatment, primary leaves of 10 cowpea plants were inoculated with treated sap and 10 primary leaves were rubbed with buffer to serve as controls. Each experiment was repeated twice.

Electron microscopy. Leaf-dip preparations were prepared by dipping freshly cut edges of infected leaves in a drop of 1% neutral sodium phosphotungstate on a collodion fronted carbon-coated grid. After the grids were dried, they were examined with a JEM 7 electron microscope. Magnification was calibrated with a carbon replica grating containing 54,900 lines per inch (E. F. Fullam, Inc., Schenectady, NY 12301). Virus particles were measured and grouped in classes of 10-nm increments. Infected leaf tissue was prepared for examination according to de Zoeten and Gaard (7) except that tissue was embedded in Spurr's medium (20). Ultra-thin sections were cut with a diamond knife on a Reichert OM-U3 ultramicrotome. Sections were stained with lead citrate and examined with a JEM 7 microscope.

RESULTS

Transmission. Cowpea and bean developed conspicuous symptoms when inoculated with extracts from six declining clones of *P.* × *euramericana* (Fig. 1A) and four declining clones of aspen (Fig. 2A). No symptoms were induced by extracts of healthy *P. deltoides.* Transmission efficiency varied from 0 to 30% with generally less than 10% of the inoculated plants becoming infected. Therefore, 60 cowpea or bean plants were inoculated in subsequent transmission trials with 30 additional plants serving as uninoculated controls.

In transmission trials attempted from newly emerged leaves, we were unable to transmit the virus either in samples from fresh flushes of leaves from excised branches kept in a greenhouse or from newly emerging leaves of field trees during early May. Only one of 240 cowpea plants became infected in eight trials during late July and August. Best results (mean transmission rate of 8%) were achieved in late May to early June when poplar leaves were between three-fourths and just fully expanded.

Chenopodium quinoa, P. limensis, N. Clevelandii, bean, and cowpea developed symptoms when inoculated with leaf sap of virus-infected cowpea. Infectivity assays on *C. quinoa* and cowpea indicated that the remaining 20 herbaceous plant species tested were not infected. Symptoms produced by four representative isolates on the susceptible herbaceous plants were identical. Chlorotic local lesions (3–4 mm in diameter) developed on leaves of *C. quinoa* (Fig. 1F) 14–18 days after inoculation, but systemic symptoms were not observed. Primary leaves of *P. limensis* and bean developed small chlorotic lesions (1–2 mm in diameter) 10–12 days after inoculation (Fig. 1E) and trifoliate leaves developed larger chlorotic lesions (0.5–1.0 cm in diameter) 18–22 days after inoculation; some vein necrosis developed 28 days after inoculation. A systemic mild mottle developed in *N. clevelandii* 16–20 days after inoculation. Small red lesions (2–4 mm in diameter) on the primary leaves (Fig. 1C) and stem necrosis (Fig. 1B) developed on cowpea plants 8–10 days after inoculation. Necrotic lesions developed along veins of the primary leaves (Fig. 1C), and mottling and vein clearing developed on trifoliolate leaves of cowpea 18–23 days after inoculation (Fig. 1D).

Physical properties. The DEP experiments were conducted at 3-day intervals to determine the time required to achieve maximum virus concentration in cowpea and to compare virus concentration in cowpea, bean, and *P. limensis.* In cowpea trifoliolate leaves, the maximum virus concentration was obtained 18 days after inoculation, when a dilution of 10^{-4} produced a mean of 0.75 lesions per inoculated cowpea leaf; no lesions developed at higher dilutions. The DEP 18 days after inoculation was 10^{-2} in bean and

10^{-3} in trifoliolate leaves of *P. limensis*.

Initial experiments done at intervals of 10 C from 40 to 80 C showed that the TIP was between 50 and 60 C. Lesions developed on cowpea leaves inoculated with sap heated at 58 C, but not on leaves inoculated with sap heated at 60 C. Thus, the TIP in cowpea leaf sap was between 58 and 60 C.

Longevity in vitro (LIV) was determined by infectivity assays of cowpea leaf sap stored at 22 C for increasing lengths of time. Lesions developed on cowpea plants inoculated with infected cowpea leaf sap that had been stored for up to 25 hr, but not on plants treated with sap stored for 28 hr or longer.

Electron microscopy. Negatively stained leaf dip preparations from infected cowpea leaves demonstrated that the four isolates contain flexuous particles with a modal length of 800–810 nm. Of 100 particles measured for a representative isolate, 88% were between 775 and 835 nm in length and had a mean width of 12.6 nm (Figs. 3A and 4). Flexuous rod-shaped particles were not observed in leaf dip preparations from poplar leaves.

Ultrathin sections of infected cowpea leaf tissue revealed cells with cylindrical inclusion bodies. The inclusions consisted only of pinwheels (Fig. 3B) and scrolls (Fig. 3C); laminated inclusions were not observed. Viruslike particles were also observed in ultrathin sections. Ultrathin sections from uninoculated cowpea plants contained neither pinwheel nor scroll inclusions nor any viruslike particles.

Proof of pathogenicity. Twenty quaking aspen (*P. tremuloides*) seedlings were inoculated mechanically with sap from infected cowpea when 2, 4, 6, or 10–12 true leaves were present. An equal number of aspen seedlings served as uninoculated controls. Foliar symptoms on inoculated aspen seedlings first appeared 3–4 wk after inoculation as chlorotic lesions that later became necrotic (Fig. 2B and C). These leaf symptoms were identical to those observed on the original source trees (Fig. 2A). All control seedlings remained symptomless. About 50% of the seedlings inoculated at the two-, four-, or six-leaf stage developed symptoms, while only 20% of the seedlings inoculated at the 10- to 12-leaf stage developed symptoms. Transmissions back to cowpea plants were attempted from both the inoculated seedlings and the controls to correlate symptom expression with infection. The virus was transmitted back to cowpea from 18% of the inoculated aspen seedlings showing symptoms. The presence of the original virus in cowpea plants that became infected was confirmed by electron

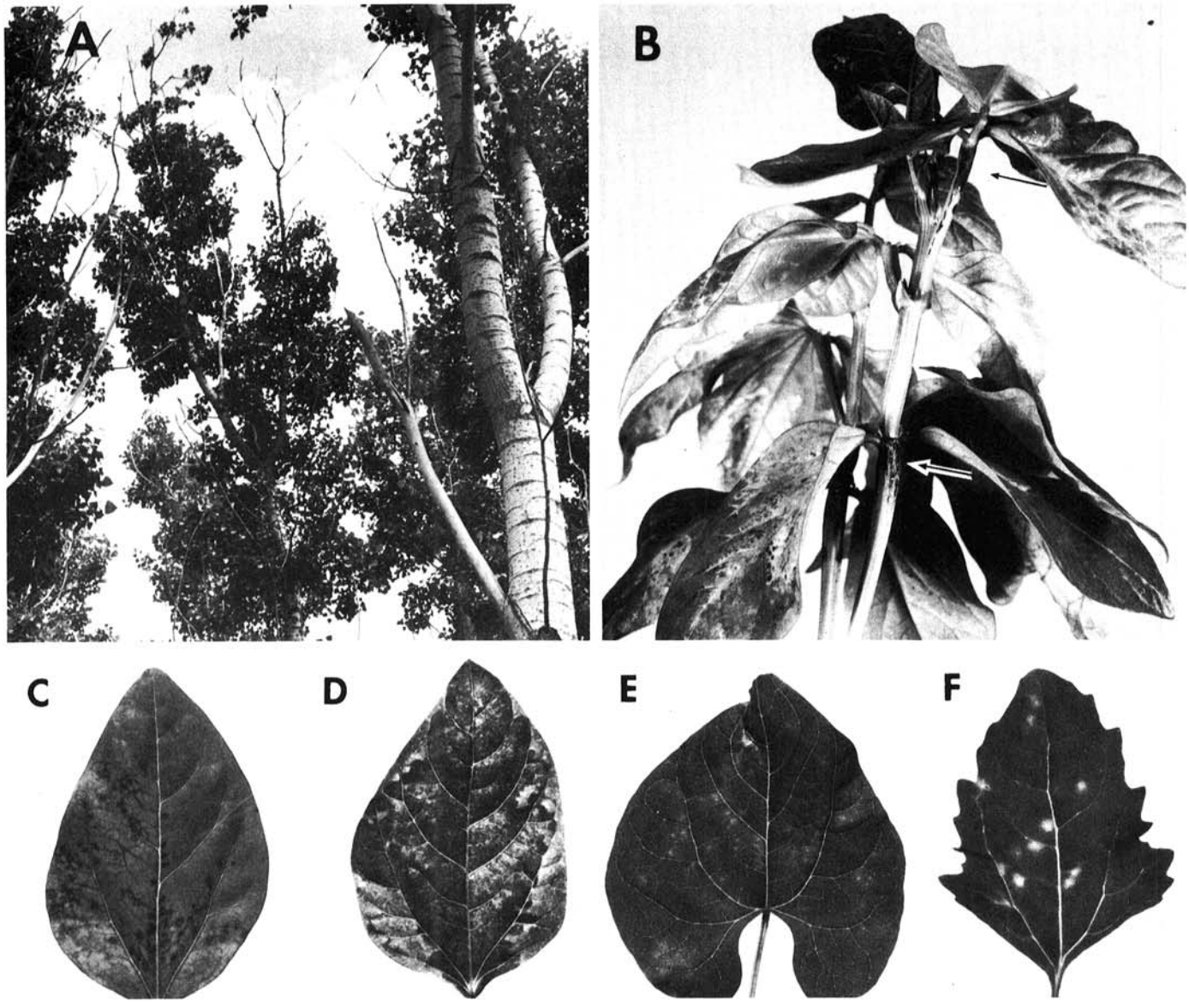

Fig. 1. A, Declining *Populus* × *euramericana* hybrid poplar clone W136. **(B–F)** Symptoms on mechanically inoculated herbaceous hosts produced by the potyvirus from poplar. **(B–D)** Symptoms on *Vigna unguiculata*. **B,** Stem necrosis (arrows) and systemic symptoms; **C,** leaf spots and vein necrosis on primary leaf; **D,** mottling symptoms on a trifoliolate leaflet; **E,** chlorotic lesions on a primary leaf of *Phaseolus vulgaris*; and **F,** local lesions on a leaf of *Chenopodium quinoa*.

microscopy of leaf dip preparations. No transmissions to cowpea were obtained from inoculated symptomless aspen seedlings or from uninoculated control seedlings.

DISCUSSION

Particle morphology and induction of cylindrical and scroll inclusions place the poplar virus isolates in the potyvirus group (10). The structure of cylindrical inclusion bodies induced by members of the potyvirus group has been used to subdivide this group of viruses into three subdivisions (8). The virus from poplars is a member of subdivision I of the potyvirus group sensu Edwardson (9). Host range, symptomatology, and particle size distinguish this virus from other members of subdivision I of the potyvirus group (9).

The potyvirus was isolated from six declining clones of *P.* × *euramericana* and from four declining native aspen clones, but not from a symptomless clone of *P. deltoides*. The four representative isolates examined produced the same symptoms on bean, cowpea, *C. quinoa, N. clevelandii*, and *P. limensis*. The morphology, associated inclusions in cowpea, and physical properties of these four isolates also were identical.

As with other woody plant viruses (12), the frequency of transmission of the potyvirus from poplar to susceptible herbaceous host plants was low. Transmission attempts made with newly emerged leaves or after mid-July generally failed. Transmission to herbaceous hosts was most successful early in the growing season after the poplar leaves were three-fourths expanded. A similar phenomenon has been reported for cassava brown streak virus, in which case the virus could be transmitted from mature, but not from immature cassava leaves (17).

In inoculation trials, only about 50% of the inoculated *P. tremuloides* seedlings developed symptoms. These leaf symptoms were similar to those that appeared on declining trees. Fewer seedlings inoculated at the 10- to 12-leaf stage developed symptoms than those inoculated at the two- to six-leaf stage. Although difficulty was encountered in transmitting the virus from cowpea back to poplar, seedlings were considered infected if leaf spot symptoms developed after inoculation, even though we were only able to transmit the virus back to cowpea from 18% of the seedlings that showed symptoms. No transmissions were achieved from symptomless aspen seedlings to cowpea plants. This low rate of transmission back to cowpea might be expected because the

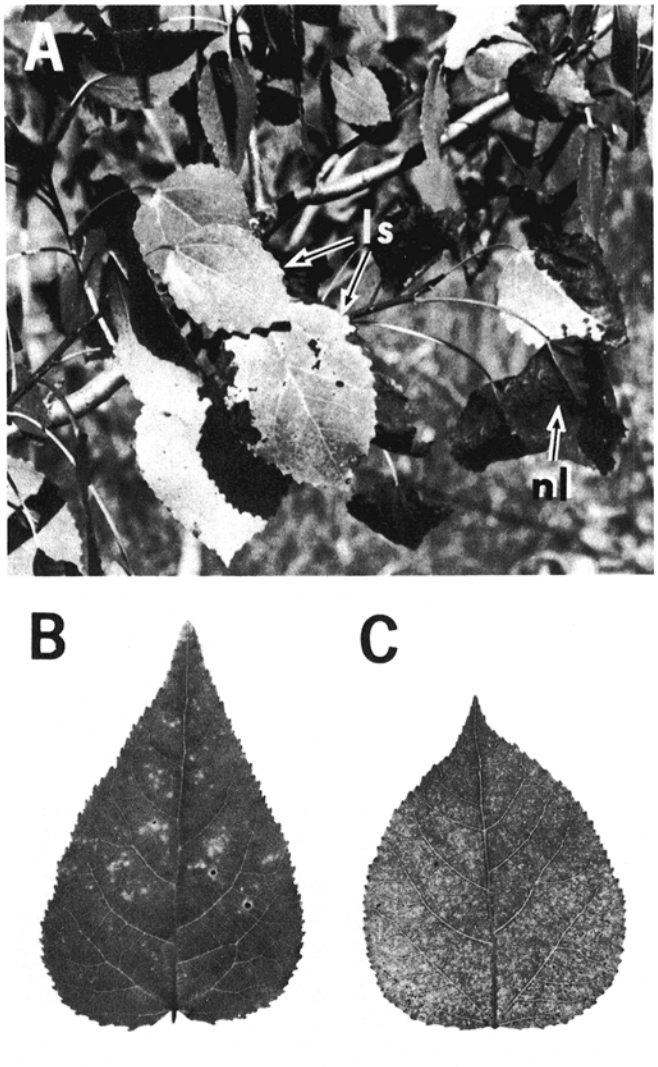

Fig. 2. A, Branch of declining *Populus tremuloides* tree showing necrotic leaves (nl) and chlorotic and necrotic leaf spot (ls) symptoms on leaves not yet bronzed. (**B and C**) Symptoms on leaves of inoculated *P. tremuloides* seedlings. B, Chlorotic leaf spots on aspen seedling 12 wk after inoculation with the poplar potyvirus; and **C,** chlorotic and necrotic leaf spot symptoms on an aspen seedling 18 mo after inoculation.

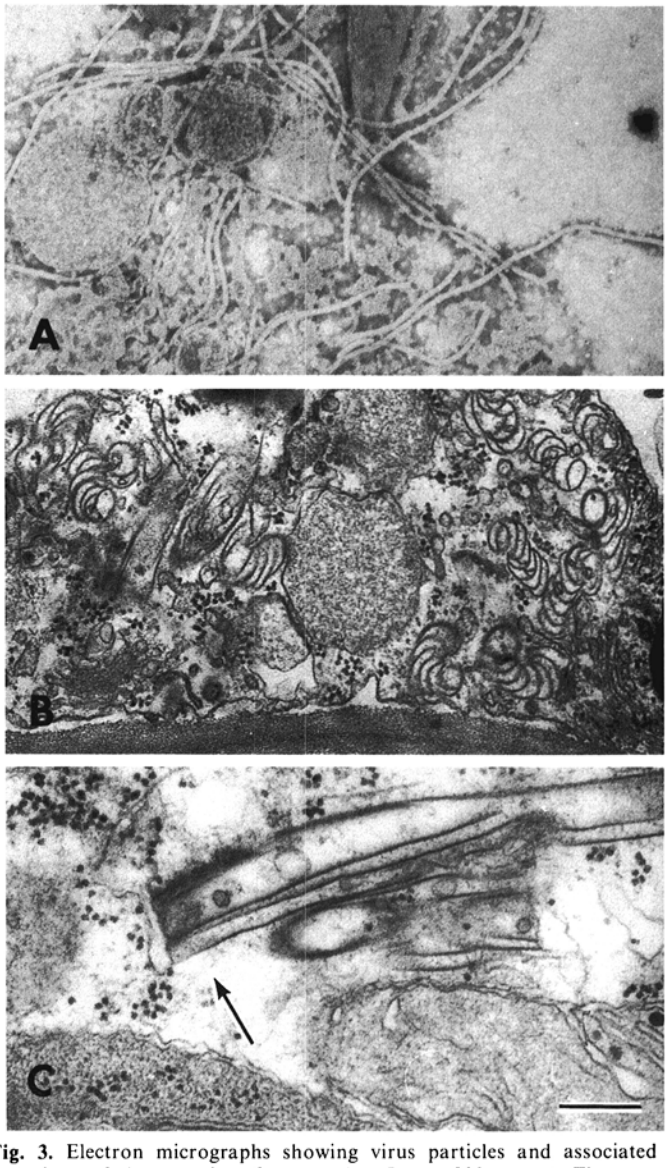

Fig. 3. Electron micrographs showing virus particles and associated inclusions of the potyvirus from poplar. Bar = 200 nm. **A,** Flexuous rod-shaped virus particles in a leaf dip preparation from *Vigna unguiculata* stained with 1% phosphotungstic acid (pH 7.0). Portions of potyvirus-type inclusions are evident at the top of the picture. **B,** Pinwheel inclusions; and **C,** other potyvirus-induced inclusions in ultrathin sections of an infected leaf of *V. unguiculata*.

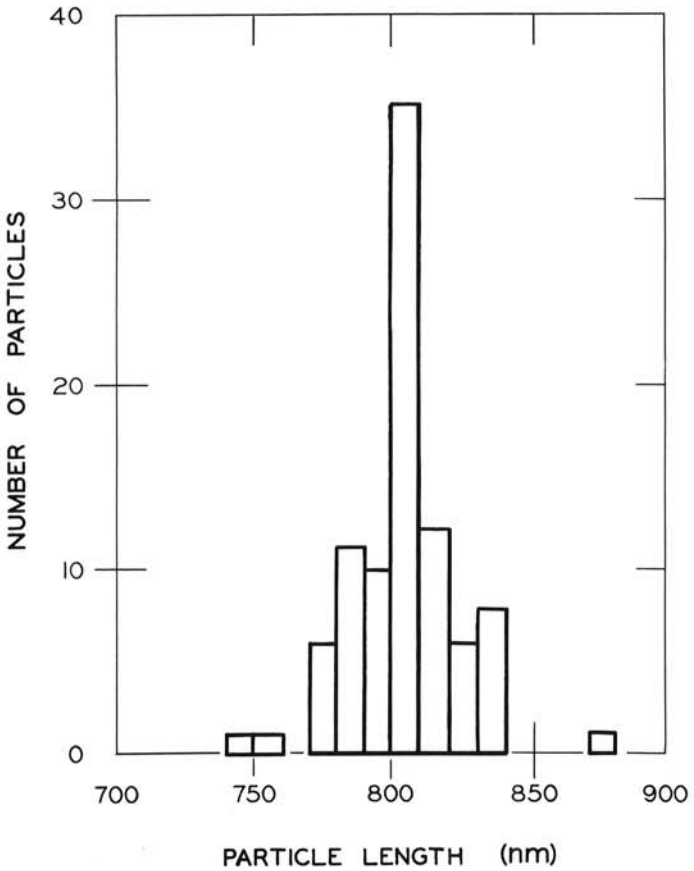

Fig. 4. Histogram showing length distribution of particles of the potyvirus from poplars in leaf dip preparations from infected cowpea leaves.

original transmissions from *Populus* spp. were difficult and similar cases have been reported for other woody hosts (12). The difficulty in transmitting this virus from poplars precludes the use of infectivity assays to determine its presence and distribution in poplar trees. A serological method will be needed to detect this virus consistently in poplar leaf sap.

The potyvirus isolated from the declining poplar clones differs from other viruses reported from poplars. Tobacco necrosis virus isolated from aspen (14), *Populus* hybrids (5), and *P.* × *euramericana* (6), as well as tomato black ring virus and arabis mosaic virus reported from *P.* × *euramericana* (6), are polyhedral viruses. Tobacco rattle virus, reported from *P.* × *euramericana* (6) is a member of the tobravirus group and PMV is a member of the carlavirus group (4). This is the first report of a potyvirus isolated from *Populus* spp.

Although the role of this virus in poplar decline remains unknown, our transmission trials have established that it is a pathogen of poplars.

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