Alfalfa Mosaic Virus in *Pachysandra terminalis*

DONALD E. HERSHMAN and EUGENE H. VARNEY, Department of Plant Pathology, Cook College, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick 08903

ABSTRACT

Hershman, D. E., and Varney, E. H. 1982. Alfalfa mosaic virus in *Pachysandra terminalis*. Plant Disease 66:1195-1196.

A disease of pachysandra, characterized by line pattern, ring spot, and mosaic symptoms, was observed at several locations in central New Jersey. A virus consistently associated with infected plants was readily transmitted by sap inoculation to selected herbaceous plants; symptoms induced were similar to those caused by alfalfa mosaic virus (AMV). In vitro properties of the pachysandra virus were within limits for those reported for AMV. The pachysandra virus reacted with AMV antiserum. Electron microscopy of the purified virus revealed bacilliform to spherical particles characteristic of AMV. This virus, like AMV, was transmitted in a nonpersistent manner by the green peach aphid, *Myzus persicae*. This is believed to be the first report of a virus infecting *Pachysandra* and the first report of AMV infecting a member of the Buxaceae.

Japanese pachysandra (*Pachysandra terminalis* Sieb. & Zucc.), introduced into the United States from Japan in 1881, is used extensively as a ground cover, especially in shaded areas. A line pattern and ring spot disease of pachysandra (Fig. 1A) was first observed by S. H. Davis, Jr., at Dennisville, NJ, in 1970. The disease now is known to occur in several locations in central New Jersey. This paper characterizes the virus associated with the disease.

MATERIALS AND METHODS

Infected pachysandra plants were collected at the Rutgers University Horticultural Farm, New Brunswick, NJ, and a virus was mechanically transmitted from pachysandra leaves to *Nicotiana sylvestris* Speg. & Comes. The virus isolate selected for study was obtained by single-lesion transfers and maintained in *N. sylvestris*.

New Jersey Agricultural Experiment Station, Publication No. D-11190-1-82, supported by State and U.S. Hatch Act Funds.

Accepted for publication 12 July 1982.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0191-2917/82/12119502/$03.00/0

Host-range studies were conducted using standard procedures (1). Leaves of infected *N. sylvestris* were ground in a mortar in 0.02 M phosphate buffer, pH 7.0, containing 0.02 M 2-mercaptoethanol. Herbaceous plants, representing 34 species or cultivars in seven families, were dusted with 500-mesh Carborundum and mechanically inoculated. Symptoms were observed for 3–4 wk. Sap from symptomless plants was back-inoculated to susceptible indicator plants to detect symptomless carriers.

In vitro properties of the virus were determined using methods modified from those described by Bos et al (1). *Vigna unguiculata* (L.) Walp. 'California Black eye' was used as the local lesion assay host (2).

Insect transmission tests were done using the green peach aphid (*Myzus persicae* Sulzer) cultured on disease-free pepper plants. Wingless adults were removed from culture plants and starved for 2 hr in plastic petri dishes. Infected leaves of *N. sylvestris* were placed in dishes containing 10 aphids that were allowed an acquisition access of 2 min. Five aphids were then placed on each of 10 healthy tobacco plants and allowed to feed overnight. Plants were treated with insecticide and retained for observation.

The method used to purify the pachysandra virus was adopted from Gillaspie and Bancroft (4). Purified preparations were tested for infectivity on cowpea.

The Ouchterlony immunodiffusion method (6) was used for serological testing. Crude sap from *N. sylvestris* systemically infected with either pachysandra virus or alfalfa mosaic virus (AMV, ATCC PV-92) was used as the source of antigen. Crude sap from healthy *N. sylvestris* was used as the control. Outer wells cut in 1% agarose gel in tris-buffered (normal) saline, pH 7.2, were charged with antigens of either the pachysandra virus or AMV. The center well was charged with AMV antiserum. Plates were incubated at room temperature and observed after 24 hr.

For electron microscopy, Formvar-coated, 200-mesh copper grids were floated for 2 min on a drop of purified virus suspended in phosphate buffer. Adsorbed virus particles were fixed in a 2% formalin solution, pH 7.0, for 10 min and stained with 2% phosphotungstic acid, pH 7.0, for 60 sec. Prepared grids were observed at ×38,000 with a Siemens I electron microscope.

RESULTS AND DISCUSSION

Host range and symptomatology. In general, the hosts infected with the pachysandra virus and their symptomatology coincided with those reported as diagnostic for AMV (2,7,8). Of the 26 species or cultivars tested and reported to be susceptible to certain strains of AMV (5), only *Gomphrena globosa* L., *Cucumis melo* L. 'B63,-3,' and *Cucurbita pepo* L. failed to become infected. Eight additional species not known to be susceptible to AMV were also negative.

The pachysandra virus induced spreading, necrotic local lesions in *Phaseolus vulgaris* L. 'Tendercrop.' *Chenopodium amaranticolor* Coste & Reyn. developed chlorotic local lesions in inoculated leaves and a systemic chlorotic flecking associated with the veins.

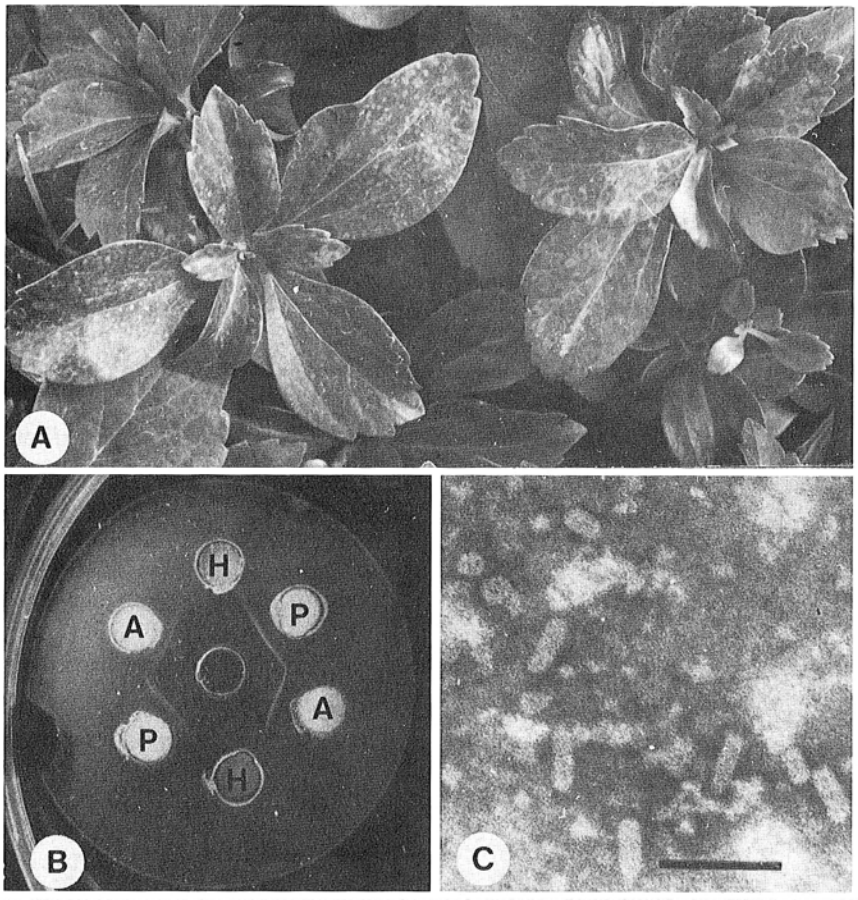

Fig. 1. (A) Symptoms in naturally infected *Pachysandra terminalis*. **(B)** Immunodiffusion test: center well was charged with alfalfa mosaic virus (AMV) antiserum; peripheral wells were charged with the pachysandra virus (P), AMV (A), or virus-free sap of *Nicotiana sylvestris* (H). **(C)** Purified preparation from *N. sylvestris* fixed in 2% Formalin and stained with 2% phosphotungstate (bar = 100 nm).

Inoculated leaves of *Vigna unguiculata* 'California Black Eye' and *Vicia faba* L. formed necrotic local lesions. In addition, *V. faba* developed necrosis of the stem and a mild systemic mottle. Most species or cultivars of tobacco that were tested formed chlorotic local lesions in inoculated leaves, followed by systemic symptoms ranging from a mild mottle to bright veinbanding, line patterns, and ring spots. Plants frequently recovered, especially at high temperatures often encountered in greenhouses during the summer months.

The in vitro properties for the pachysandra virus were well within limits reported for AMV (2). The virus in partially clarified sap of *N. sylvestris* was viable for 3–4 days at room temperature, remained infectious to a dilution of 10^{-3}, and was inactivated between 55–60 C.

The green peach aphid transmitted the pachysandra virus from infected *N. sylvestris* to 90% of the healthy plants tested. Transmission occurred after a short acquisition-feeding period, indicating that the virus is transmitted in a nonpersistent fashion as reported for AMV (2).

In immunodiffusion plates, both pachysandra virus and AMV antigens produced identical immunoprecipitin bands in response to AMV antiserum (Fig. 1B).

Electron micrographs (Fig. 1C) of the purified pachysandra virus revealed bacilliform and spherical particles characteristic of AMV. The necessity to stabilize particles of the pachysandra virus in formalin before negatively staining with PTA was consistent with that reported for AMV (3). The purified virus preparation was highly infectious on cowpea.

Disease symptoms in pachysandra are visible throughout the year and seem to occupy small, restricted areas in larger established plantings. In a survey of pachysandra beds in New Jersey, disease symptoms were observed in only three of 65 plantings. There is no evidence of rapid spread of the virus by aphids from either wild or cultivated hosts to pachysandra or within pachysandra plantings.

Since pachysandra is vegetatively propagated, eradication of any infected plant material that might be used in propagation by nurserymen is probably the easiest and most effective way of preventing introduction of the virus into plantings where AMV is not currently a problem.

This is believed to be the first report of a virus infecting *Pachysandra* and the first report of AMV infecting a member of the family Buxaceae.

LITERATURE CITED

1. Bos, L., Hagedorn, D. J., and Quantz, L. 1960. Suggested procedures for international identification of legume viruses. Tijdschr. Plantenziekten 66:328-343.
2. Bos, L., and Jaspars, E. M. J. 1971. Alfalfa mosaic virus. No. 46. Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 4 pp.
3. Gibbs, A. J., Nixon, H. L., and Woods, R. D. 1963. Properties of purified preparations of Lucerne mosaic virus. Virology 19:441-449.
4. Gillaspie, A. G., Jr., and Bancroft, J. B. 1965. Properties of ribonucleic acid from alfalfa mosaic virus and related components. Virology 27:391-397.
5. Hull, R. 1969. Alfalfa mosaic virus. Adv. Virus Res. 15:365-433.
6. Ouchterlony, O. 1960. Interpretation of comparative immune patterns obtained by diffusion-in-gel techniques. Pages 5-19 in: Immunochemical Approaches to Problems in Microbiology. M. Heidelberger, O. J. Plescia, and R. A. Day, eds. Rutgers University Press, New Brunswick, NJ.
7. Provvidenti, R. 1979. Alfalfa mosaic virus infection in *Valerianella olitoria*. Plant Dis. Rep. 63:820-821.
8. Schroeder, W. T., and Provvidenti, R. 1972. Alfalfa mosaic virus in *Ajuga reptans*. Plant Dis. Rep. 56:285.