

Characterization of a Potexvirus Infecting *Hosta* spp.

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ABSTRACT

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A previously undescribed potexvirus, named *Hosta virus X* (HVX), was found in 17 naturally infected *Hosta* (*Hosta* spp.) cultivars from Minnesota, Indiana, Illinois, Iowa, and Michigan. HVX was readily transmitted mechanically but infected only *Nicotiana benthamiana* and *Hosta* spp., in which symptoms ranged from severe mosaic and leaf necrosis to latency. Particles of HVX averaged 530 nm in length, had a buoyant density in cesium chloride of 1.28 gm/cm³, and contained a single genomic species of ssRNA approximately 3 kb in size. The capsid protein of HVX had a molecular mass of approximately 27 kDa. In indirect enzyme immunoassays, HVX reacted with an antiserum to clover yellow mosaic virus (CIYMV), and less strongly with antiserum to hydrangea ringspot virus (HRSV), but not with antisera to any of 14 other potexviruses tested. However, in reciprocal tests, CIYMV reacted only very weakly with HVX antiserum. HVX did not infect any of three cultivars of pea that were susceptible to infection by CIYMV, and CIYMV did not infect any of three *Hosta* cultivars susceptible to infection by HVX. Spread of HVX infection in *Hosta* appears to occur by vegetative propagation and accidental mechanical transmission, and management of the disease can be achieved by virus indexing and cultural practices that minimize the risk of virus spread to susceptible cultivars.

Additional keywords: *Hosta* potexvirus, *Hosta* virus, virus disease of ornamentals

Hostas (*Hosta* spp.) are hardy, shade-tolerant members of the *Liliaceae* and have become the most popular perennial garden plant in the United States (1). Although the occurrence of viruslike disorders in *Hostas* has occasionally been observed, no viruses have been previously identified in *Hostas* in North America, and no previous reports of virus infection in *Hostas* were found in the literature. In 1993, a viruslike disorder was observed in several *Hosta* cultivars in a commercial nursery in Minnesota. Symptoms consisted of mosaic, mottling, chlorotic spotting, interveinal chlorosis between secondary veins, and leaf desiccation (Fig. 1A to C). Viruslike particles were observed by electron microscopy (EM) in negatively stained, partially purified preparations from symptomatic leaf tissue. This paper demonstrates that the particles are those of a previously unreported potexvirus, which we named *Hosta virus X* (HVX). This study verifies that HVX is the causal agent of the foliar disease observed in *Hosta* and reports the occurrence and the biological, biochemical, biophysical, and serological characteristics of the virus.

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MATERIALS AND METHODS

Virus source and propagation. The virus isolate used in these studies was obtained from the naturally infected *Hosta* cv. Yellow Splash Rim. Because no local-lesion host plants were identified for propagation of single-lesion isolates, the virus was purified from naturally infected *Hosta* as described below. Only potexviruslike particles of a single median length were observed in the purified preparation, which was used to inoculate *Hosta lancifolia* (Thunb.) Engl., in which the virus isolate was maintained. Plants of *H. lancifolia* clonally derived from a single original stock plant and verified to be virus-free by EM examination of partially purified extracts were used as test plants in subsequent experiments.

Mechanical transmission and host range. Mechanical transmission tests were done by grinding systemically infected leaf tissue of *H. lancifolia* in 1% (wt/vol) K₂HPO₄ and 0.5% (wt/vol) Na₂SO₃, and using the extract to mechanically inoculate Carborundum-dusted leaves of at least four each of the following test plants: *Allium schoenoprasum* L. (chive), *Chenopodium quinoa* Willd., *Cucumis sativus* L. cv. National Pickling, *Cucurbita pepo* L. cv. Black Beauty, *Gomphrena globosa* L., *Hosta* cvs. Honeybells and Royal Standard, *H. lancifolia*, *Hosta undulata*, *Iris*, *Pisum sativum* (L.) (pea) Mammoth Melting Sugar and Sugar Daddy, *Narcissus* cv. Paperwhite (daffodil), *Nicotiana benthamiana* Domin., *N. clevelandii* Gray, *N. glutinosa* L., *Ornithogalum* sp., *Phaseolus*

vulgaris L. cv. Red Kidney, *Pisum sativum* L. cv. Dwarf Gray Sugar, *Tulipa gesneriana* L. (tulip), *Vicia faba* L., *Vigna unguiculata* (L.) Walp., and *Zinnia elegans* (Jacq.). After inoculation, plants were kept in the greenhouse at 24 to 28°C for up to 18 months.

Virus purification. *Hosta virus X* was purified from systemically infected leaves of inoculated *H. lancifolia* by a modification of the procedure described for pea seedborne mosaic virus (12). The virus was further purified by isopycnic density gradient centrifugation in CsCl for 30 h at 320,000 × *g* (max). Virus concentration was estimated using an assumed extinction coefficient of 3.0.

Electron microscopy. For EM examination, partially purified or purified virus preparations were mounted on carbon-coated Formvar grids and negatively stained with either 2% sodium phosphotungstate (PTA), pH 6.5, or 2% aqueous uranyl acetate (UA), both containing bacitracin at 100 µg/ml.

Serology. Antiserum against HVX was raised in rabbits by intramuscular injection of virus purified by isopycnic banding on CsCl density gradients. An initial injection of 1 mg of virus emulsified in Hunter's Titermax adjuvant (Sigma Chemical Co., St. Louis, MO) was followed by two biweekly injections each of 0.5 mg of virus emulsified in Freund's incomplete adjuvant. Blood was collected starting at 2 weeks after the final immunization.

Serological tests were done using double antibody sandwich enzyme immunoassay (DAS-EIA) (2) or indirect EIA (9). Antisera against 16 previously described potexviruses were obtained from the following sources: cactus virus X, cassava common mosaic virus, Cymbidium mosaic virus, clover yellow mosaic virus, *Dioscorea* latent virus, Argentine Plantago mottle virus, white clover mosaic virus, (American Type Culture Collection, Bethesda, MD); narcissus mosaic virus, nerine virus X, hydrangea ringspot virus (D. Z. Maat, I.P.O., Wageningen, Netherlands); bamboo mosaic virus, khaki weed potexvirus (J. E. Thomas, Department of Primary Industries, Queensland, Australia); tulip virus X (A. T. Jones, Scottish Crop., Research Institute, Dundee, Scotland); foxtail mosaic virus (A. Q. Paulsen, Kansas State University, Manhattan); viola mottle virus (V. Lisa, Istituto di Fitovirologia applicata, Torino, Italy); and potato virus X (E. E. Bantari, University of Minnesota, St. Paul).

Characterization of HVX virion capsid protein and genomic nucleic acid.

The molecular mass of HVX capsid protein was estimated by discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (6) using a 4% stacking gel and a 12% separating gel. Electrophoresis was performed at 150 V for 45 min, and polypeptide bands were visualized by Coomassie blue staining. Low molecular weight range markers (Bio Rad, Richmond, CA) and capsid proteins of brome mosaic (BMV), maize chlorotic mottle (MCMV), and tobacco mosaic (TMV) viruses were used as standards for estimating the size of HVX capsid protein.

Genomic nucleic acid was extracted from purified HVX by SDS-phenol-chloroform treatment followed by ethanol precipitation. The nature of HVX genomic nucleic acid was determined by digestion with RQ1 RNase-free DNase (Promega, Madison, WI) and S-1 nuclease (Promega) as described (8). For use as controls in these experiments, genomic ssRNA was extracted from a purified preparation of TMV by SDS-phenol-chloroform treatment followed by ethanol precipitation, and dsRNAs of BMV were prepared from infected brome grass (*Bromus inermis*) using a previously described procedure (10). Results of nuclease digestion were assessed by electrophoresis in 1.2% agarose gels in Tris-borate-EDTA (TBE). The size of HVX genomic nucleic acid was estimated by agarose gel electrophoresis under both nondenaturing and denaturing conditions. Genomic RNAs of HVX and TMV were denatured by glyoxal treatment and electrophoresed at 60 V for 2 h in 1.5% agarose gels in 10 mM sodium phosphate, pH 7.0, as described (11), except that RNA bands were visualized by ethidium bromide rather than silver staining.

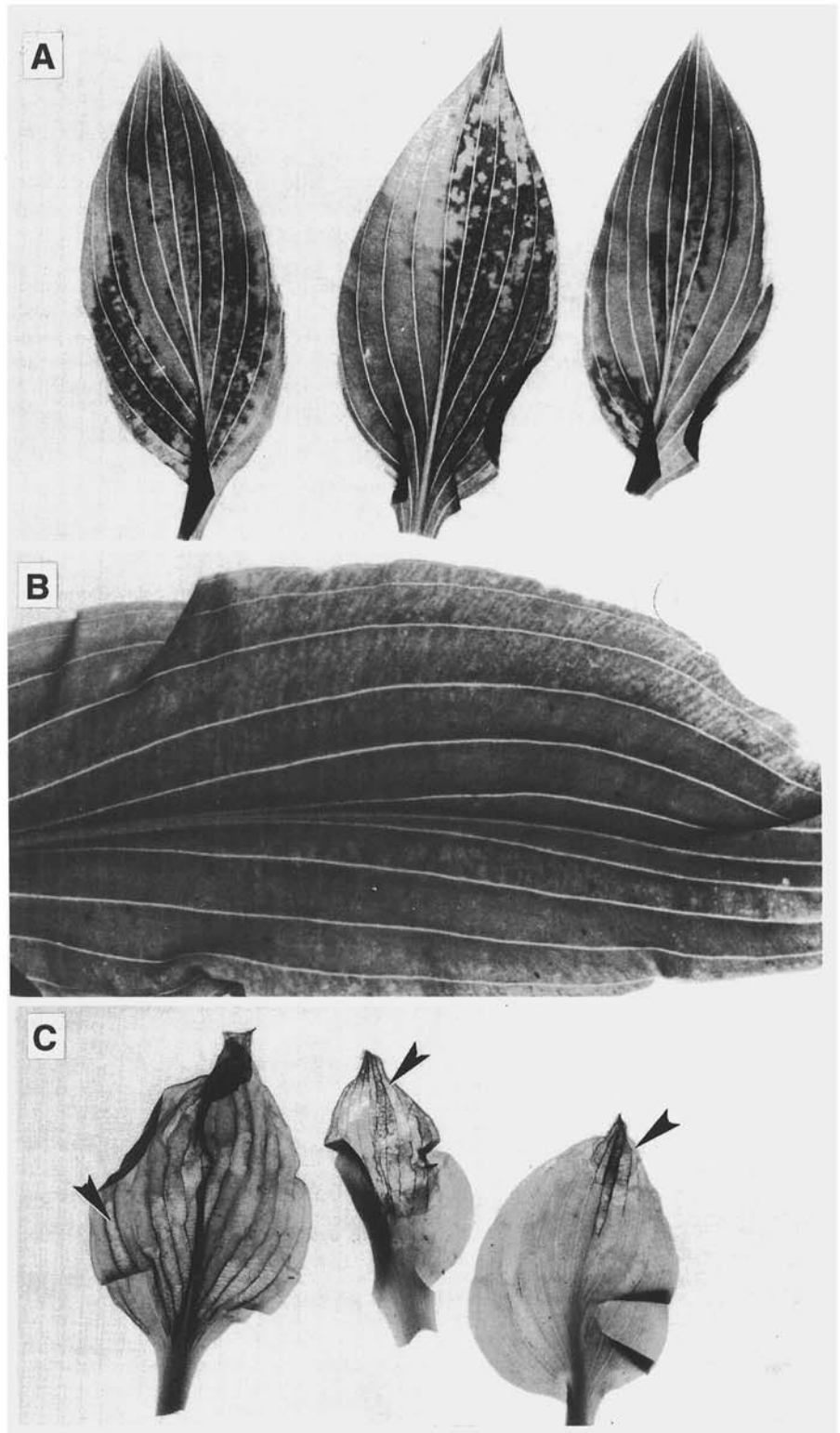
RESULTS

Natural occurrence and symptomatology of HVX infection in hostas. Natural infection by HVX was confirmed by EM and DAS-EIA indexing in the following hosta cultivars: Hydon Sunset, Janet, Richland Gold, Golden Prayers, Gold Edger, Kara, Lunacy, Harvest Glow, Moonlight Sport, Louisa, Birchwood Parky's Gold, Rim Rock, Yellow Splash Rim, Montana Chirifu, Opipara (Benedict), *Hosta clausa normalis* and *H. decorata*. Symptoms in these cultivars varied from severe mosaic leading to leaf desiccation (e.g., Hydon Sunset, Yellow Splash Rim, Golden Prayers) (Fig. 1A) to chlorotic spotting (Moonlight Sport) (Fig. 1B) or interveinal chlorosis between secondary veins (e.g., Green Fountain, Kara, Opipara [Benedict]) (Fig. 1C). No other viruslike particles were observed in these plants. In both naturally and experimentally infected blue-flowered hosta cultivars, color-breaking was frequently observed under greenhouse conditions. No experiments

were done nor observations made to determine whether HVX infection affected other aspects of plant growth.

Mechanical transmission and experimental host range of HVX. Purified HVX was transmitted to *H. lancifolia* by mechanical inoculation and produced sys-

temic symptoms consisting of mosaic, chlorosis, leaf deformation, and desiccation (Fig. 2). Color-breaking was also observed in flowers of infected plants. Similar symptoms were observed in plants of *H. lancifolia* inoculated with sap extracts. *H. undulata* and hosta cv. Honeybells were



FIGS. 1. Symptoms of hosta virus X (HVX) infection in naturally infected hostas. (A) Mosaic and chlorotic spotting in hosta cv. Moonlight Sport. (B) Mottle and interveinal chlorosis between secondary veins in hosta cv. Green Fountain. (C) Leaf desiccation in hosta cv. Golden Prayers. Arrows indicate necrotic leaf tissue.

also infected by HVX and developed mosaic symptoms without leaf desiccation. No local symptoms were observed in any of these three hostas. Systemic symptoms appeared in new growth 4 to 5 weeks after inoculation and persisted in plants observed for more than 1 year. The presence of HVX in symptomatic hostas was verified by EM and DAS-EIA. The hosta cv. Royal Standard was not infected by HVX in these tests. No symptoms developed in this hosta cultivar, and no HVX was detected by EM or DAS-EIA.

Hosta virus X produced no symptoms and could not be detected by DAS-EIA in any of the herbaceous indicator plants listed above, except for *N. benthamiana*, which was infected systemically and showed mild mosaic and vein-clearing symptoms. Presence of HVX in systemically infected *N. benthamiana* leaves was confirmed by EM and DAS-EIA. None of the six indicator plants in the families *Liliaceae*, *Amaryllidaceae*, and *Iridaceae* were infected by HVX, as determined by DAS-EIA and EM examination of partially purified preparations.

Virus purification and particle characterization. The purification method described above (12) yielded preparations containing relatively unaggregated virions (Fig. 3). The lengths of 224 particles measured were distributed normally about a mean of 530 nm. Purified HVX had a A_{260}/A_{280} ratio of 1.24 (uncorrected for light scattering) and a buoyant density of 1.286 in CsCl. From SDS-PAGE analysis (Fig. 4), the molecular mass of the capsid

protein of HVX was estimated at 27 kDa. A second, less abundant polypeptide of slightly lower molecular weight (Fig. 4) was also observed. Hosta virus X genomic nucleic acid comigrated with that of TMV under nondenaturing and denaturing conditions (Fig. 5), indicating that the size of HVX genomic nucleic acid is similar to that of TMV, which has been reported to be 3 kb (3). The genomic nucleic acids of both HVX and TMV were completely digested by RNase and S1 nuclease but were unaffected by DNase, while the dsRNAs of BMV were unaffected by S1 nuclease (data not shown). Particle morphology, capsid protein size, and genomic nucleic acid properties of HVX were found to be consistent with those of previously described members of the Potexvirus group (5).

Serology and serological relationships. The polyclonal rabbit antiserum raised against HVX could reliably detect the virus by DAS-EIA in both symptomatic and asymptomatic hostas infected with HVX. Using standard conditions of 1 $\mu\text{g}/\text{ml}$ coating IgG and a 1/1,000 dilution of IgG-alkaline phosphatase conjugate, HVX could be detected in infected *H. lancifolia* at sap dilutions of 1/10,000. Under these conditions, the A_{405} values for infected *H. lancifolia* averaged 0.12, compared to 0.01 for healthy *H. lancifolia* controls. In one-way indirect EIA, HVX antigen reacted well with antiserum to clover yellow mosaic virus (CIYMV) and weakly with antiserum to hydrangea ringspot virus (HyRSV), but did not react with antisera to

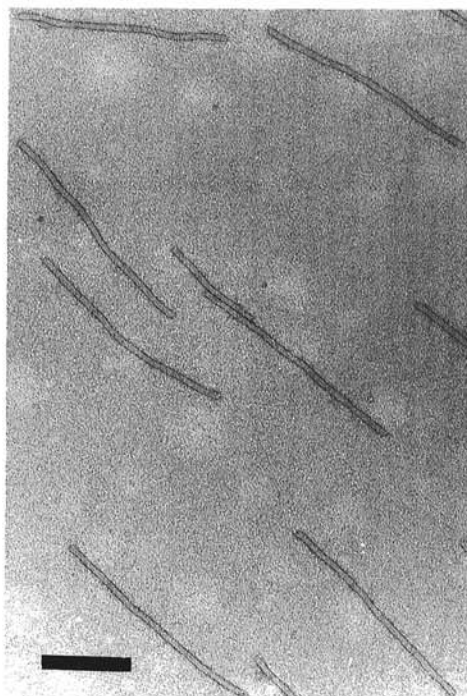


Fig. 3. Virions of hosta virus X (HVX) purified from hosta cv. Rim Rock. The preparation was negatively stained with 2% aqueous uranyl acetate containing bacitracin at 100 $\mu\text{g}/\text{ml}$. Scale bar represents 200 nm.

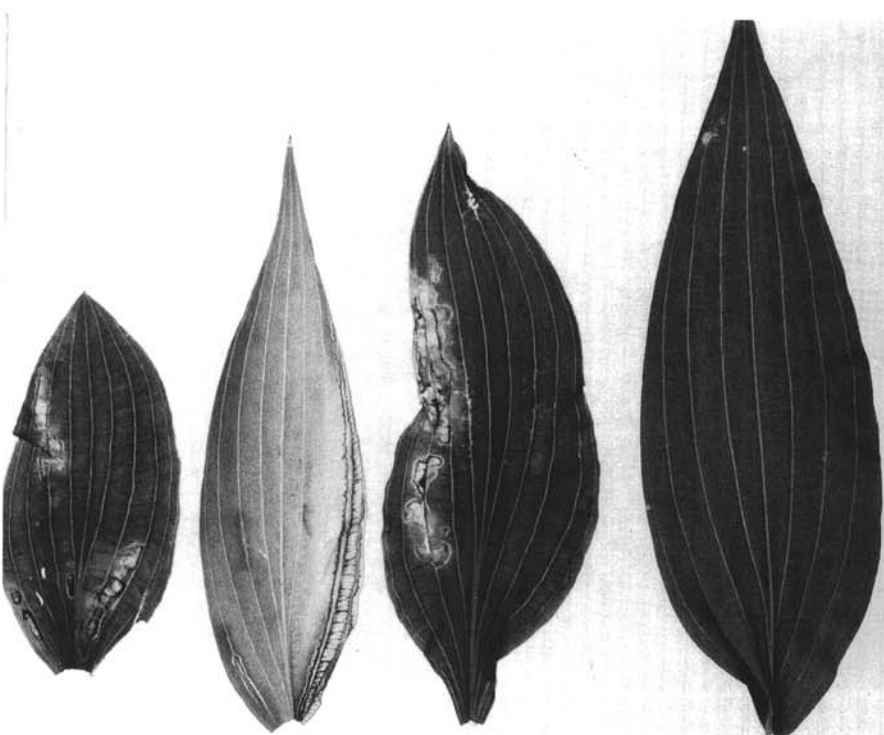


Fig. 2. Mosaic, chlorosis, leaf deformation, and necrosis in *Hosta lancifolia* resulting from mechanical inoculation with purified hosta virus X (HVX).

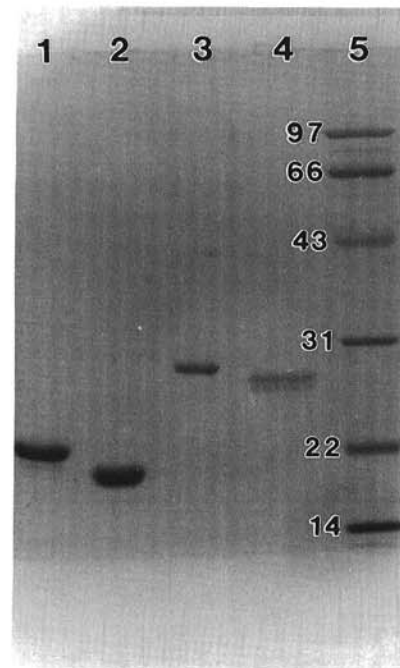


Fig. 4. Estimation of the molecular mass of hosta virus X (HVX) capsid protein by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Electrophoresis conditions are described in the text. Viral capsid proteins used as molecular weight markers and their reported molecular weights are as follows: lane 1: bromo mosaic virus, 20.3 kDa (7); lane 2: tobacco mosaic virus, 17.5 kDa (3); lane 3: maize chlorotic mottle virus, 24 to 27 kDa (4); lane 4: hosta virus X; and lane 5: Bio-Rad low molecular weight range markers with sizes indicated in kDa.

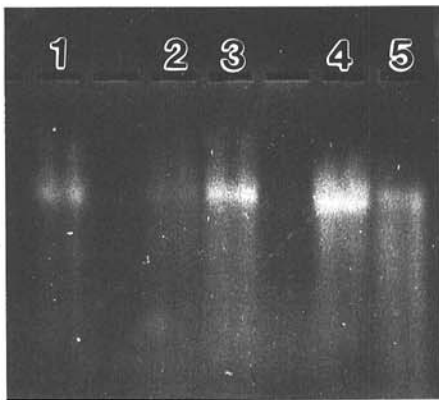


Fig. 5. Estimation of the size of hosta virus X (HVX) genomic RNA by agarose gel electrophoresis using tobacco mosaic virus (TMV) genomic RNA as a size marker. Denaturation and electrophoresis conditions are described in the text. Lanes 1 and 5: TMV RNA; lanes 2 to 4: increasing quantities of HVX RNA.

any of the other 14 potexviruses tested. However, in reciprocal tests, a Minnesota isolate of CIYMV reacted only very weakly with HVX antiserum in indirect EIA using sap sample dilutions of 1/25. In these tests, HVX gave average A_{405} readings of 1.24 and 0.82 with HVX and CIYMV antisera, respectively, while corresponding A_{405} values for the same sap dilution of CIYMV antigen were 0.06 and 0.34, respectively. In both cases, A_{405} values for healthy pea and hosta controls were 0.03 and 0.01, respectively.

DISCUSSION

On the basis of particle morphology, genome, and serological properties, it was concluded that HVX is a previously unde-

scribed species in the genus Potexvirus (5). Although HVX reacted well with an antiserum to CIYMV, it did not infect any of three pea cultivars that were all readily susceptible to infection by a Minnesota isolate of CIYMV. Conversely, this CIYMV isolate did not react well with HVX antiserum and did not infect *H. lancifolia*, *H. undulata*, and hosta cv. Honey Bells, all of which were readily infected by HVX. On susceptible hostas, HVX infection can produce a serious and sometimes destructive disease. Like other potexviruses (5), HVX probably does not have an arthropod or other vector, and virus spread most likely occurs through vegetative propagation and mechanical contact (e.g., lawnmower injury), and patterns of HVX infection under natural conditions support the latter assumption. Some hosta cultivars that are susceptible to infection by HVX manifest few or no readily apparent symptoms and may act as symptomless carriers of the virus. Management of the disease caused by HVX infection would therefore seem to be predicated on minimizing the possibility of mechanical transmission of HVX between susceptible cultivars and avoiding propagation of virus-infected hostas. The sensitivity of HVX detection by DAS-EIA provides a reliable method for indexing of potential sources of infection.

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