Leaf-Curl of Impatiens Caused by Tobacco Streak Virus Infection

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ABSTRACT

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Plants of impatiens (*Impatiens holstii*) with symptoms of leaf deformation and stunting from a commercial greenhouse in Minnesota were infected with tobacco streak virus (TSV). The virus was similar in biologic and physical properties to previously described strains of TSV and reacted positively with antisera to the TSV-HF, TSV-NC, and TSV-R strains of TSV.

During indexing of impatiens (*Impatiens holstii*) from commercial greenhouses in Minnesota for tobacco ringspot virus (TRSV) (6), plants from one source with foliar symptoms distinct from the mosaic typical of TRSV were found to contain a mechanically transmissible virus that was serologically and biologically distinct from TRSV. Infected plants were stunted and had small, twisted, curled leaves. The virus causing this disease was subsequently identified as a strain of tobacco streak virus (TSV).

MATERIALS AND METHODS

Virus culture, mechanical transmission, and purification. The virus was obtained from young infected leaves of *I. holstii* 'Fancifrills.' The leaf tissue was ground in cold 0.01 M phosphate buffer (pH 7.2) containing 0.1% 2-mercaptoethanol and sufficient Polyclar AT (GAF Corporation, New York) to make a slurry. Carborundum (600 mesh) was added to the slurry, and the mixture was used to inoculate *Nicotiana clevelandii*. From this source a single lesion isolate was obtained from inoculated leaves of *Phaseolus vulgaris* 'Bountiful' and propagated in *N. clevelandii* and *N. glutinosa*. Mechanical inoculations were done with crude extracts of systemically infected *N. clevelandii* or *N. glutinosa* leaves (7–10 days after inoculation) ground in cold phosphate buffer containing 0.1% 2-mercaptoethanol. *Chenopodium quinoa* was used as the test plant in back-inoculation to assay for virus in host range studies.

Virus was purified by the method described by Lister and Saksena (5) for tomato aspermy virus.

Properties in crude sap. Sap from systemically infected *N. clevelandii* diluted 1:3 with 0.01 M phosphate buffer (pH 7.2) was used in thermal inactivation point (TIP) determinations, and the same buffer was used for dilution end point (DEP) determination. Five *C. quinoa* plants were used for each treatment.

Serology. Immunodiffusion tests were done in 0.8% agarose gels prepared in distilled water. The TSV-HF strain (ATCC PV 49) and homologous antiserum (ATCC PVAS 49) were obtained from the American Type Culture Collection. The North Carolina (NC) strain of TSV (4) was obtained from G. V. Gooding, Jr., and its homologous antiserum, from R. W. Fulton. The black raspberry strain (TSV-R) (1) and its homologous antiserum were supplied by R. H. Converse. All serologic tests were done with undiluted sap from *N. clevelandii* systemically infected (7–10 days after inoculation) with these viruses and the impatiens isolate.

RESULTS

Host range and symptoms. In naturally infected impatiens, symptoms consisted of leaf twisting, deformation, and stunting. Identical symptoms were produced in indexed, virusfree impatiens inoculated mechanically with the single-lesion virus isolate (Fig. 1). Infected plants set fewer, smaller flowers than did healthy plants, but no flower deformation was observed. The host range of the impatiens virus was, with minor exceptions, essentially similar to that reported for other TSV strains and isolates (3). The virus produced necrotic or chlorotic local lesions without systemic infection in *P. vulgaris* 'Bountiful' and 'Red Kidney,' *Cucumis sativus* 'National Pickling,' *Cucurbita pepo* 'Fordhook Zucchini,' and *Beta patellaris*. Local and systemic symptoms were produced in *C. quinoa, Petunia hybrida, Gomphrena globosa, Glycine max, Datura stramonium, Ocimum basilicum, Lactuca sativa, Lycopersicon esculentum* 'Sheyenne,'

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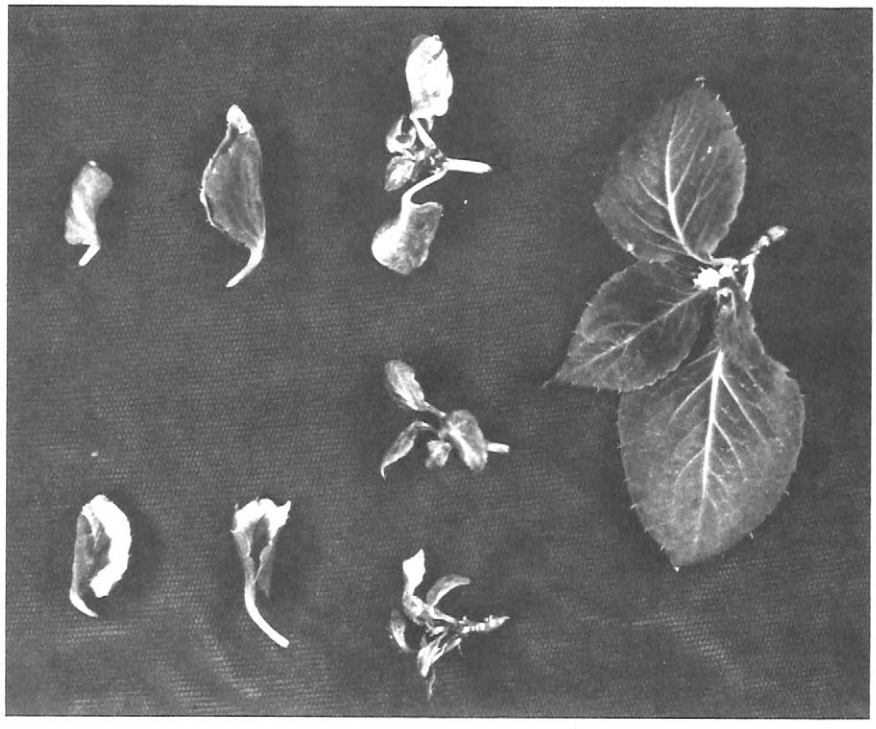

Fig. 1. Symptoms of tobacco streak virus infection in impatiens (*Impatiens holstii* 'Fancifrills'). Infected leaves (three rows at right) are twisted, deformed, and smaller than normal leaves (left).

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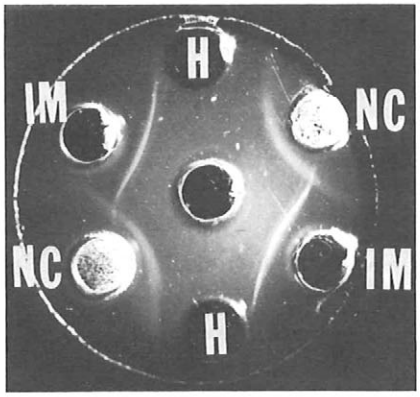

Fig. 2. Serologic reaction among impatiens tobacco streak virus (IM), North Carolina strain of tobacco streak virus (NC), and antiserum to TSV-NC (center well). Viruses are in undiluted sap from infected *Nicotiana clevelandii*. H = sap from healthy *N. clevelandii*.

Vigna unguiculata, Zinnia elegans, N. clevelandii, N. glutinosa, and *N. tabacum*. Flower deformation typical of TSV infection (2) was produced in all three tobacco spp., and systemically infected "recovered" leaves of *N. tabacum* had characteristically dentate margins (2,3). Local symptomless infection without systemic invasion occurred in *Trifolium repens,* and no infection occurred in *C. amaranticolor* or any of three cultivars of *Pisum sativum*.

Properties in crude sap. In crude sap from systemically infected leaves of *N. clevelandii*, the impatiens virus had a TIP of 50–55 C and a DEP of 10^{-3}–10^{-4}.

Virus purification. The virus was readily purified from *N. clevelandii* or *N. glutinosa*. In rate-zonal density gradient centrifugation in 10–40% sucrose gradients in 0.05 M sodium acetate buffer (pH 5.0), the virus sedimented as three components. No other ultraviolet-absorbing material was present at the meniscus or elsewhere in the gradients. A sample of the bottom (rapidly sedimenting) component produced typical symptoms on *N. clevelandii* and impatiens and reacted positively with antiserum to the HF strain.

Serology. In immunodiffusion tests using crude *N. clevelandii* sap, the impatiens virus reacted positively with antisera to TSV-HF, TSV-NC, and TSV-R. A positive homologous reaction was also observed in each case. The precipitin lines of the impatiens TSV and TSV-HF fused completely when tested against TSV-HF antiserum. When tested alongside TSV-NC against its homologous antiserum, the impatiens TSV gave precipitin lines that spurred with those of the homologous antigen (Fig. 2). Similar results were obtained with TSV-R and its homologous antiserum. No reaction was obtained with antisera to a number of other isometric plant viruses, including tomato ringspot, tobacco ringspot, cucumber mosaic, Tulare apple mosaic, rose mosaic, broad bean wilt, cowpea mosaic, prunus necrotic ringspot, arabis mosaic, and bean pod mottle viruses.

DISCUSSION

Based on its biologic, physical, and serologic properties, the virus causing a disease in impatiens is identified as TSV. The host range of the virus shows some minor differences from those reported for other strains (1–3). From the serologic tests it can be concluded that some serologic differences exist between the impatiens TSV and TSV-NC and TSV-R. No further tests were done to determine the degree of serologic relatedness between the impatiens TSV and the other TSV strains, however.

Because of the effect produced by TSV infection in impatiens, the disease is of potential economic importance in commercial greenhouses. Although no vector of TSV has been found, vegetative propagation of infected impatiens can lead to wider distribution of the virus.

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