Etiology

Characterization of a Potyvirus Causing a Leaf Distortion Disease of *Tradescantia* and *Zebrina* Species

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ABSTRACT

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Ornamental *Tradescantia* and *Zebrina* spp. in commercial greenhouses in Minnesota frequently were found to be infected with a filamentous virus causing mosaic, leaf distortion, and stunting. The virus was transmissible both mechanically and by two aphids, *Myzus persicae* and *Rhopalosiphum padi*. The experimental host range of the virus was restricted to three genera of the Commelinaceae: *Tradescantia, Zebrina,* and *Rhoeo*. Virus particles isolated in leaf-dip preparations had an average length of 754 nm. Pinwheel and tubular inclusions were observed in ultrathin sections of infected leaf tissue. A purification method for the virus is described. The virus did not react with antisera to any of 13 other potyviruses, and differed from Commelina mosaic virus (CoMV) in not infecting *Commelina diffusa* and in producing distinctly different pinwheel inclusions. In crude extracts from infected leaves, CoMV did not react with an antiserum to the Tradescantia virus. The virus appears to be a new member of the potyvirus group.

Species of the Commelinaceae have been reported to be infected naturally by cucumber mosaic (8) and tobacco mosaic (3) viruses. Recently, Morales and Zettler (5) described a potyvirus, Commelina mosaic virus (CoMV), occurring naturally in *Commelina diffusa* Burm. in Florida and a potyvirus (designated Commelina diffusa virus [CDV]) as well as a bacilliform virus, were reported to occur in *C. diffusa* in Guadeloupe (4). During investigation of a viruslike pathogen causing a disorder of ornamental *Tradescantia* and *Zebrina* spp. in commercial greenhouses in Minnesota, flexuous rods were observed in leaf-dip preparations from diseased, but not from symptomless, plants. A virus was isolated from diseased plants and some of its properties are described.

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MATERIALS AND METHODS

The virus was obtained from a plant of *Tradescantia albiflora* Kunth. 'Albovittata' showing typical leaf deformation and stunting. The virus was transmitted mechanically to healthy *T. albiflora* and maintained in this plant. This virus culture was used in all experiments described herein. Mechanical transmission tests were done with crude sap from infected leaf tissue ground in cold 0.01 M phosphate buffer, pH 7.4. Virus properties in crude sap, as well as back-inoculations, were made by using *Zebrina pendula* Schnizl. as the indicator plant. In aphid transmission tests, nonviruliferous apterous adults of *Myzus persicae* and *Rhopalosiphon padi* were starved for 1–2 hr, allowed acquisition feeding for 2–5 min, and then allowed to feed overnight on healthy *Z. pendula*. Five to 10 aphids were used per test plant.

For electron microscopic examination, leaf-dip samples and

purified virus were stained with 1% potassium phosphotungstate (PTA), pH 7.0. Tobacco mosaic virus (TMV) was used as an internal standard for measurement of virus particle length. Leaf tissue of *T. albiflora* was prepared for ultrathin sectioning by fixation in 2.5% glutaraldehyde in phosphate buffer, pH 7.0, postfixation in 2% osmium textroxide in the same buffer, dehydration in an acetone series, and then embedded in Spurr's medium (7). Sections were stained with uranyl acetate and lead citrate before electron microscopic examination.

The virus was purified from young shoots of systemically infected *T. albiflora*. The tissue was homogenized 1:1 (w/v) in cold 0.2 M sodium citrate buffer, pH 7.2, containing 0.2% of sodium ascorbate and 0.2% sodium sulfite. The extract was filitered through cheesecloth, centrifuged for 30 min at 10,000 *g*, and the supernatant was extracted with 25% (v/v) of a 1:1 ether-carbon tetrachloride mixture. The emulsion was centrifuged for 10 min at 10,000 *g* and the virus was precipitated from the aqueous phase by the addition of 4% polyethylene glycol (PEG) 6000 and collected by centrifugation. The PEG pellet was resuspended in distilled water containing 0.25% Triton X-100. The virus was precipitated by centrifugation at 38,000 *g* for 90 min and further purified by rate-zonal density gradient centrifugation in 10–40% sucrose gradients in distilled water. Virus removed from the sucrose gradients was concentrated by ultracentrifugation and resuspended in distilled water.

Figs. 1 and 2. Symptoms caused in two *Tradescantia* spp. by the Tradescantia potyvirus. **1,** *T. albiflora*: comparison of infected (left) and healthy (right) shoot tips. **2,** *T. blossfeldiana*: systemic mosaic symptoms in leaf.

An antiserum was prepared by using gradient-purified virus antigen. A rabbit was given a single intravenous injection of approximately 0.4 mg of virus, followed 3 wk later by a series of three weekly intramuscular injections of 0.4 mg virus emulsified in Freund's incomplete adjuvant. Serum was collected at weekly intervals starting 2 wk after the last injection. Immunodiffusion tests were done with sodium dodecyl sulfate-treated (SDS) crude sap in agar gels containing SDS and sodium azide (6).

RESULTS

Distribution of virus disease symtoms. Plants of *Tradescantia* and *Zebrina* spp. showing symptoms of infection were found in all of seven commercial greenhouses surveyed. In some greenhouses, 75–80% of these plants showed symptoms. Flexuous viruslike particles were observed in crude leaf-dip preparations from all plants with symptoms, but never from symptomless plants.

Host range. The virus was easily transmissible mechanically from both *Tradescantia* spp. and *Z. pendula* to *T. albiflora*, *Tradescantia blossfeldiana* Mildb., *Tradescantia fluminensis* Vell., *Tradescantia navicularis* Ortg., *Z. pendula* and *Rhoeo discolor* Hance. Symptoms in these plants consisted of varying degrees of mosaic, leaf deformation, and plant stunting (Figs. 1–3). No symptoms were produced in, and no virus was recovered from, three other species in the Commelinaceae: *Setcreasea pallida* Rose, *Chlorophytum comosum* (Thunb.) Jacques, and a local selection of *Commelina elegans* HBK. No symptoms were produced on a Florida selection of *C. diffusa* (F. W. Zettler, *personal com-*

Fig. 3. Effect of infection by the Tradescantia potyvirus in *Rhoeo discolor*. Healthy plant at left, infected plant at right.

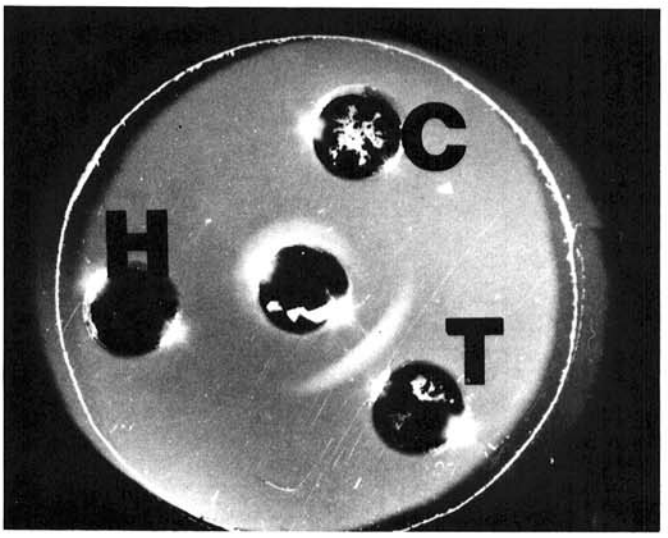

Fig. 4. Reaction of the Tradescantia potyvirus antiserum (center well) with crude sap from healthy *Tradescantia albiflora* (H), and from *T. albiflora* infected with the Tradescantia potyvirus (T) and with Commelina mosaic virus (C).

munication). A Florida isolate of CoMV, provided by F. W. Zettler, was transmitted both mechanically and by *M. persicae* to *T. albiflora*, *Z. pendula*, and *R. discolor*. The symptoms of CoMV infection on these plants consisted of faint mottle and line-mosaic patterns, and were clearly distinguishable from those produced by the Minnesota Tradescantia virus. The presence of CoMV in infected test plants was verified by electron microscopic examination of leaf-dip preparations. No symptoms were produced by the Tradescantia virus in, and no viruslike particles were observed in leaf-dip preparations from, inoculated or new leaves of any of the following test plants: *Apium graveolens* L., *Datura stramonium* L., *Capsicum annuum* L., *Cucumis sativus* L., *Cucurbita pepo* L., *C. maxima* Dene., *Chenopodium amaranticolor* Coste and Reyn., *C. quinoa* Willd., *Gomphrena globosa* L., *Phaseolus vulgaris* L. 'Bountiful' and 'Red Kidney,' *Lactuca sativa* L., *Nicotiana clevelandii* Gray, *N. glutinosa* L., *N. tabacum* L., *N. debneyi* Domin, *Pisum sativum* L., *Vicia faba* L., *Vigna unguiculata* (L.) Walp., *Physalis floridana* Rydb., *Petunia hybrida* Vilm., *Brassica chinensis* L., *B. rapa* L., *Beta vulgaris* L., *Helianthus annuus* L., *Zinnia elegans* Jacq., *Avena sativa* L., *Hordeum vulgare* L., *Secale cereale* L., *Triticum aestivum* L., *Zea mays* L., and *Tulipa gesneriana* L.

Aphid transmission. In aphid transmission tests the virus was transmitted by both *M. persicae* and *R. padi* to each of six replicate test plants in separate experiments.

Electron microscopy and virus purification. Numerous flexuous rods were present in PTA-stained crude preparations from infected, but not from healthy, leaves of *Tradescantia* spp., *Z. pendula*, and *R. discolor*. The average length of 100 such particles was 754 nm. Pinwheel and tubular inclusions were observed in ultrathin sections of infected, but not of healthy, tissue. The form of these inclusions was similar to those associated with potyviruses in subdivision II as classified by Edwardson (1).

The purification method described above yielded clean virus preparations with little aggregation. In sucrose gradients the virus sedimented as a single major peak with a small shoulder consisting of aggregated particles. Samples taken from this peak contained only flexuous rods and produced typical symptoms when inoculated mechanically to *T. albiflora* and *Z. pendula*.

Serology. The antiserum produced against the Tradescantia virus had a homologous titer of 1/16 in gel diffusion tests. A single band of precipitate was produced with purified virus and with crude sap from artificially infected *Tradescantia* spp., *Z. pendula*, and *R. discolor* as well as with naturally infected *Tradescantia* spp. and *Z. pendula* from two commercial greenhouses. There was no reaction either with sap from healthy plants or with sap from *C. diffusa*, *T. albiflora*, or *R. discolor* infected with CoMV (Fig. 4). Similarly, no serological reaction was obtained with unidentified potyviruses occurring in a *Tradescantia* sp. from Cuba, in *Tradescantia virginiana* L. from Minnesota, and in three unidentified Commelinaceae from Cameroun.

In immunodiffusion tests performed by F. W. Zettler, University of Florida, Gainesville, using crude extracts from infected *T. albiflora* and *R. discolor*, no reaction was obtained with antisera to the following potyviruses: bidens mottle, dasheen mosaic, bean yellow mosaic, bean common mosaic, blackeye cowpea mosaic, potato virus Y, pepper mottle, tobacco etch, watermelon mosaic 1, and watermelon mosaic 2. The homologous reaction was positive in all tests. In similar tests at Minnesota, the Tradescantia virus also failed to react with antisera to maize dwarf mosaic virus A (MDMV-A) and MDMV-B.

DISCUSSION

Based on particle morphology, aphid transmissibility, and induction of pinwheel cytoplasmic inclusions, the Tradescantia virus is assigned to the potyvirus group. An examination of the host ranges of viruses in this group (2) and lack of serological reaction of the Tradescantia virus with any of the potyvirus antisera tested, suggest that the virus is possibly a new member of this group. Further reciprocal serological testing with a wider range of potyviruses, especially those infecting other monocots, will have to be done before the identity of this virus can be established. This virus differs from CoMV, which occurs in uncultivated *Commelina diffusa* in Florida, in the type of cytoplasmic inclusion produced (5) as well as in failing to infect *C. diffusa*. However, because CoMV itself does not appear to infect all selections of *C. diffusa* (F. W. Zettler, *personal communication*), it is possible that the same may hold for the Tradescantia potyvirus. In our inoculation tests, CoMV was transmissible to *Trandescantia* spp., *Z. pendula*, and *R. discolor*, all three of which represent new hosts of this virus. The difference between the Tradescantia virus and CoMV in symptom expression on these plants and the failure of CoMV to react with antiserum to the Tradescantia virus, suggest that these two viruses are distinct from each other.

C. diffusa in Florida was found frequently to be infected with both CoMV and cucumber mosaic virus (CMV). No CMV was isolated from any samples of potyvirus-infected *Tradescantia* spp. and *Z. pendula* from commercial greenhouses in Minnesota. A single sample of *Z. pendula* from one greenhouse was infected with CMV only.

The Tradescantia virus is widespread in greenhouses in Minnesota and causes economically important damage by affecting the growth and appearance of ornamental *Tradescantia* and *Zebrina* spp. Effective control of this virus may be achieved by recognition of the symptoms and avoiding propagation from diseased plants, as well as by taking steps to reduce the possibility of aphid transmission from diseased to healthy stock.

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