Recurrence of Natively Occurring Potato Yellow Dwarf Virus in Minnesota

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ABSTRACT

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Potato yellow dwarf virus (PYDV), which has not been reported from the midwestern United States for the past 40 years, occurred naturally during 1986–1988 in Minnesota in ornamental tobacco (*Nicotiana alata*), marigold (*Tagetes erecta*), zinnia (*Zinnia elegans*), and four-o'clock (*Mirabilis jalapa*), causing severe stunting, chlorosis, vein yellowing, and systemic vein and leaf necrosis. PYDV also occurred naturally in white clover (*Trifolium repens*) in mixed infections with clover yellow vein virus. The Minnesota PYDV was identified serologically as a PYDV-S (*sanguinolenta*) isolate, and its biological properties were similar to those of previously described PYDV-S isolates. Perennial *Trifolium* spp. may provide reservoirs of both PYDV and its cicadellid vector, *Aceratagallia sanguinolenta,* which is common in Minnesota.

Potato yellow dwarf virus (PYDV) occurred naturally in the northeastern United States and Canada in the 1930s and 1940s and was thought to be occasionally spread elsewhere in infected potato tubers (1). PYDV was reported to occur in potatoes in the north-central United States 40 years ago. These reports were based entirely on visible foliar and tuber symptoms (9,11), without experimental virus transmission either mechanically or by vectors. Reports of transmission of what was presumed to be PYDV by aphids (7) and by the potato leafhopper, *Empoasca fabae* (Harris) (8), raise questions about the fidelity of the diagnosis in some instances. Apart from a description of PYDV infection in ornamental *Catharanthus roseus* (L.) G. Don (ex *Vinca rosea* L.) in California (3), there have been no recent reports of PYDV occurrence in the United States or elsewhere.

PYDV is a member of the proposed subgroup II (10) of the plant rhabdovirus group. PYDV isolates have been subdivided into two groups on the basis of vector specificity and serological differences (1,4). PYDV-S is transmitted by *Aceratagallia sanguinolenta* (Provancher) and other *Aceratagallia* spp. but not by *Agallia constricta* (Van Duzee), the vector of PYDV-C (1). PYDV-S and PYDV-C are serologically related but not identical (1,4).

This report concerns the association of PYDV-S with naturally occurring diseases of ornamental tobacco (*Nicotiana alata* Link & Otto), marigold (*Tagetes erecta* L.), zinnia (*Zinnia elegans* Jacq.), and four-o'clock (*Mirabilis jalapa* L.) during three successive years in Minnesota.

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MATERIALS AND METHODS

Disease occurrence, symptoms, virus transmission, and virus culture. Viruslike diseases of ornamental tobacco, marigold, zinnia, and four-o'clock were observed in several locations in St. Paul, MN, in the summers of 1986–1988. Infected plants were severely stunted and showed varying degrees of systemic vein and leaf necrosis (Figs. 1–5).

Crude leaf extracts of diseased plants contained rhabdovirus particles (Fig. 6), which were transmissible by mechanical inoculation to *Nicotiana* spp. One sample of naturally infected *N. alata,* which also had mosaic symptoms, contained in addition to rhabdovirus particles, filamentous particles later identified as those of potato virus Y (PVY). The rhabdovirus was transmitted by mechanical inoculation from naturally infected *N. alata* to healthy *N. benthamiana* Domin, in which the virus culture was maintained. The rhabdovirus culture was verified by electron microscopic, serological, and indicator plant tests to be free of PVY, tobacco mosaic virus, cucumber mosaic virus, alfalfa mosaic virus, broad bean wilt virus, tobacco streak virus, tobacco ringspot virus, and tomato ringspot virus. These viruses occur in weed hosts and various ornamental plants in Minnesota.

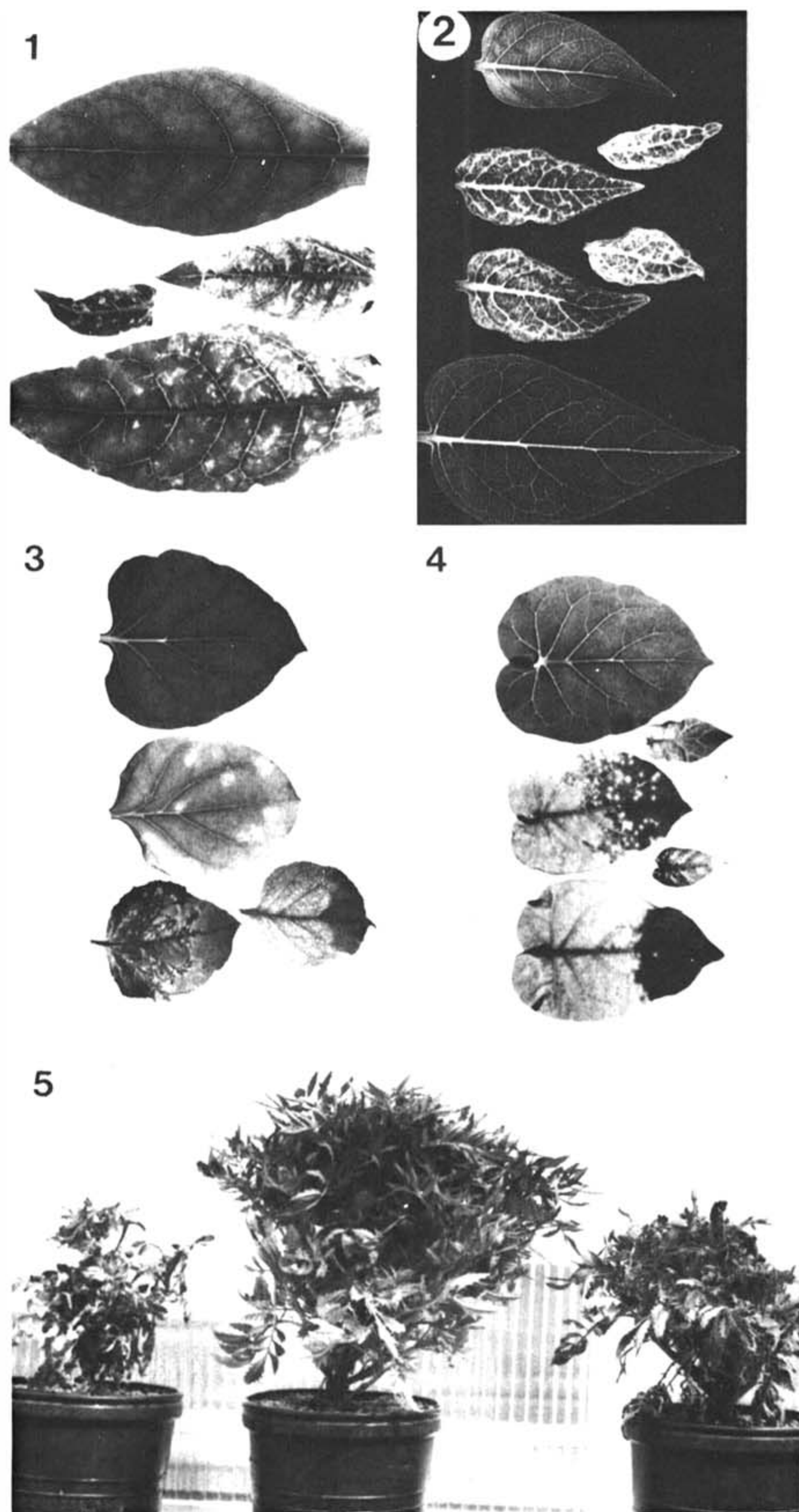
Indicator plants were mechanically inoculated using crude extracts prepared by grinding a mixture of infected *N. benthamiana* leaf tissue and 600-mesh Carborundum in 1% K_2HPO_4 containing 0.2% Na_2SO_3. All plants were kept in a greenhouse at 28–30 C. No inoculations were made from virus-infected marigold, four-o'clock, or zinnia.

Electron microscopy and serological tests. Crude leaf extracts were examined following negative staining, without prior fixation, using 2% ammonium molybdate, pH 6.8 (AM), or following fixation in 2.5% phosphate-buffered glutaraldehyde and negative staining with 2% sodium phosphotungstate, pH 7.0 (PTA). Immunodiffusion tests were done in 0.8% agarose, 0.2% NaN_3 prepared in distilled water. Undiluted, untreated leaf extracts of healthy and infected tobacco, marigold, four-o'clock, and zinnia were used as test antigen in all cases. Enzyme immunoassay (EIA) was performed by the standard double-antibody sandwich method (2) using alkaline phosphatase-immunoglobulin G (IgG) conjugate. Coating IgG was applied at 1 μg/ml and IgG-enzyme conjugate at a dilution of 1/1000. Results were measured spectrophotometrically at 405 nm using an EIA microplate reader. Virus sources and preparation of antisera to PYDV-S, PYDV-C, and tomato vein-yellowing virus (TVYV) were as described previously (3). Six sample wells were used per treatment.

RESULTS AND DISCUSSION

Mechanical transmission and symptoms on indicator plants. The rhabdovirus found in naturally infected *N. alata* was transmitted readily to healthy *N. alata, N. benthamiana, N. glutinosa* L., *N. debneyi* Domin, and *N. rustica* L. The symptoms produced on *N. alata* were identical to those observed on the original *N. alata* source plants (Fig. 1). Local and systemic chlorotic spotting, vein yellowing, and chlorosis were produced on *N. benthamiana* (Fig. 3), *N. debneyi,* and *N. glutinosa* (Fig. 4). Typical rhabdovirus particles (Fig. 6) were present in extracts of all these test plants. The symptoms produced were similar to those reported previously for PYDV infection on these indicator plants (1,3,5). No symptoms were produced on *Cucurbita pepo* L. 'Fordhook Zucchini,' *Phaseolus vulgaris* L. 'Pinto,' *Pisum sativum* L. 'Dwarf Gray Sugar,' or *Vigna unguiculata* (L.) Walp. 'California Blackeye.'

Electron microscopy and serology. Bullet-shaped particles were observed in extracts of infected *N. alata* (Fig. 6), marigold, and zinnia when the extracts were stained with AM without prior fixation. These particles measured approximately 175–180 × 85–90 nm. In unfixed extracts, the particles were completely disrupted by PTA, as reported previously for PYDV and TVYV (3). After fixation in 2.5%



Figs. 1–5. Symptoms of potato yellow dwarf virus (PYDV-S) infection in naturally and experimentally infected plants. **(1)** Leaves of *Nicotiana alata* with symptoms of natural PYDV-S infection; healthy leaf at top. **(2)** Vein chlorosis and deformation in leaves of four-o'clock (*Mirabilis jalapa*) infected naturally with PYDV-S; healthy leaves at extreme top and bottom. **(3)** Local chlorotic lesions (middle leaf) and systemic chlorosis, veinal chlorosis, and veinal necrosis (bottom leaves) in leaves of *N. benthamiana* mechanically inoculated with PYDV-S; healthy leaf at top. **(4)** Systemic chlorosis, chlorotic spotting, and vein yellowing in leaves of *N. glutinosa* mechanically inoculated with PYDV-S; two top leaves are healthy. **(5)** Severely stunted marigold (*Tagetes erecta*) plants naturally infected with PYDV-S (left and right); middle plant is healthy; all three plants are the same cultivar and age and were grown at the same location.

glutaraldehyde in 0.2 M phosphate buffer, pH 7.0, particles were stable in PTA, and undamaged bacilliform particles (Fig. 7) were observed. These particles measured 240–275 × 75–80 nm.

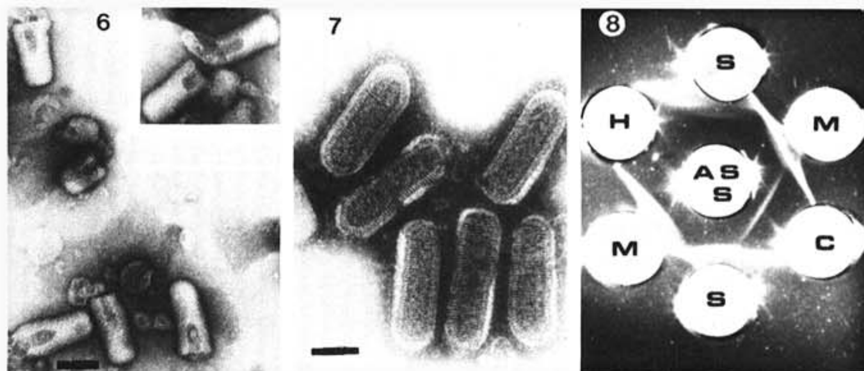
In immunodiffusion tests, extracts of rhabdovirus-infected *N. alata,* marigold, and zinnia reacted with antiserum to both PYDV-S and PYDV-C. The precipitin lines produced were confluent with that of PYDV-S and spurred with that of PYDV-C (Fig. 8). It was concluded that the rhabdovirus occurring in *N. alata,* marigold, zinnia, and four-o'clock was a PYDV-S isolate.

In EIA tests, extracts of infected *N. alata,* marigold, zinnia, and four-o'clock reacted positively with anti-PYDV-S but not with anti-PYDV-C IgG. Positive A_{405} readings were 0.995–2.036, and negative readings were 0.002–0.103.

Occurrence of PYDV-S in white clover. PYDV has been reported to occur naturally in several hosts, including clover and the oxeye daisy, *Chrysanthemum leucanthemum* L. var. *pinnatifidum* Lecoq. & Lamotte, and was transmitted experimentally to plants in several other dicotyledonous families (6,12). Several leguminous and composite species growing in the vicinity of naturally PYDV-infected ornamentals in Minnesota were tested by electron microscopy and serology for infection by PYDV-S. The virus was detected only in several samples of white clover, *Trifolium repens* L. Electron microscopy revealed rhabdovirus particles in very low concentration in crude leaf extracts. These particles were identified as PYDV-S by EIA tests, although the virus concentration was apparently too low to react in immunodiffusion assays. No attempt was made to isolate PYDV-S from white clover. All white clover plants infected by PYDV-S were also infected by clover yellow vein virus, which occurs commonly in *Trifolium* spp. in Minnesota, and no distinctive symptoms could be attributed to PYDV-S infection.

These results confirm the natural recurrence of PYDV-S in Minnesota during three successive years. Although white clover was the only weed in which PYDV-S was detected, the virus may occur in weed hosts in other families susceptible to infection (12). In terms of providing a source of primary inoculum for infection of annual crop plants, perennial virus hosts such as *Trifolium* spp. would be more important than annual weed hosts of the virus. The clover leafhopper, *Aceratagallia sanguinolenta,* which is the principal vector of PYDV-S (1), is ubiquitous in Minnesota and other areas of the United States, and PYDV-S infection in potatoes and other cultivated or wild host plants may be more frequent than is diagnosed and reported. One reason for the decline in incidence of PYDV-S symptoms in potato crops may be the use of cultivars such as Russet Burbank, which have been

reported to appear tolerant to PYDV-S infection (8). Despite the lack of reports on PYDV occurrence during the past 40 years, the virus has obviously not disappeared from perennial weed hosts like *T. repens* and *T. pratense* L. (6), and it is apparent from this report that PYDV can occasionally cause disease in cultivated plants. The occurrence of PYDV-S on cultivated ornamentals in Minnesota during three successive years suggests that movement of the virus from weed hosts to cultivated plants may occur regularly. Symptoms of PYDV-S infection in marigold, for example, included severe chlorotic stunting and development of red pigmentation in leaves and resembled symptoms produced by, and normally attributed to, infection by aster yellows mycoplasmalike organisms. Although PYDV-S infection could be distinguished from aster yellows by the occurrence of distinctive vein yellowing in terminal leaflets, these two diseases may be easily confused. In areas in which PYDV occurrence has been documented, it may be useful to consider the possibility of PYDV-S infection in the etiology of diseases characterized by yellowing and stunting symptoms.



Figs. 6–8. Particle morphology and serological reaction of the Minnesota *Nicotiana alata* isolate of potato yellow dwarf virus (PYDV). **(6)** Bullet-shaped particles of the virus in unfixed leaf dip preparations from *N. benthamiana*, negatively stained with 2% ammonium molybdate, pH 6.8. Scale bar represents 100 nm. **(7)** Bacilliform particles of the virus in a leaf dip preparation from *N. benthamiana*, after fixation in 2.5% phosphate-buffered glutaraldehyde and negative staining in 2% sodium phosphotungstate, pH 7.0. Scale bar represents 100 nm. **(8)** Immunodiffusion reaction between antiserum to PYDV-S (AS-S) and homologous antigen (S), the Minnesota *N. alata* PYDV isolate (M), and PYDV-C (C); all antigens in undiluted infected leaf sap. H = sap from healthy *N. benthamiana*.

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