Properties of Strains of Potato Virus Y^N in North America

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ABSTRACT

McDonald, J. G., and Kristjansson, G. T. 1993. Properties of strains of potato virus Y^N in North America. Plant Dis. 77:87-89.

A tobacco veinal necrosis strain of potato virus Y (PVY^N) was detected in tobacco production in Ontario in 1989. Subsequent surveys of potato production in Ontario, Prince Edward Island, New Brunswick, Nova Scotia, and Québec and of table potatoes imported into Canada from California yielded similar isolates of PVY^N. The host range and serological properties of several of these isolates were compared to a PVY^N strain from Scotland and other PVY strains from the United States (PVY-NN, PVY-MN, and PVY-36) that had been reported to cause necrosis in tobacco. These other PVY strains did not belong to the PVY^N strain group, because they did not systemically infect potato cultivars and differed in host range and serological reactions.

Additional keywords: monoclonal antibodies, PVY^O

There is considerable confusion in the literature regarding the classification of strains of potato virus Y (PVY) that can cause veinal necrosis symptoms in tobacco. The traditional classification scheme, widely used by workers investigating isolates from potato (*Solanum tuberosum* L.), has been to place such strains in the tobacco veinal necrosis (PVY^N) group (1). An additional characteristic of such strains is that they induce systemic mottle in *Physalis floridana* Rybd. and very mild mottling in practically all potato cultivars.

Gooding and Tolin (5) classified strains of PVY that caused necrosis on tobacco on the basis of the reaction of flue-cured tobacco cultivars that were either susceptible or resistant to root-knot nematodes. A number of strains that caused necrosis were defined with this scheme (4,5), including the MN and NN strains, which occur in the United States. These workers (5) stated that although they had not made direct comparisons (and had not investigated the susceptibility of *S. tuberosum* cultivars), the symptomatology of the NN strain was very similar to that of the tobacco veinal necrosis strain (PVY^N) reported from other countries. The MN strain had previously been reported to be distinct from the PVY^N group (8).

Heath et al (7), in Australia, identified two necrotic strains of PVY from tobacco that could be placed, respectively, in the MN and NN groups. However, they concluded that these two strains could not be placed in the PVY^N group, because a number of potato cultivars reacted to these strains with localized lesions and without systemic invasion.

Makkouk and Gumpf (9) reported a strain of PVY from pepper (PVY-36) in California that had characteristics similar to those described earlier as the tobacco veinal necrosis strain (PVY^N group), and they stated that this was the first report of such a strain occurring naturally in the United States. However, these workers did not investigate the susceptibility of potato cultivars to this isolate.

Correct classification of necrotic strains of PVY is particularly important from a plant quarantine standpoint, because a number of countries (including Canada and the United States) have no tolerance for PVY^N in potatoes and, if it is found, would attempt to eradicate infected stocks.

In 1989, a tobacco veinal necrosis disease was observed in a number of fields in southern Ontario (R. Reeleder, *personal communication*). Preliminary evidence indicated that a strain of PVY was the cause (L. Stobbs, *personal communication*). An outbreak of a PVY^N-like virus had occurred in this area in 1969 (R. Singh, *personal communication*) but was believed to have been eradicated as a result of measures taken by Agriculture Canada. Isolates similar to the 1989 isolate have been found in subsequent surveys (by Agriculture Canada) of potato production in Prince Edward Island, New Brunswick, Nova Scotia, Québec, and Ontario, as well as of table potatoes imported into Canada from California. This study was initiated to identify the strain of PVY apparently associated with the 1989 outbreak and to compare it to other tobacco necrotic strains of PVY reported from North America.

Accepted for publication 30 August 1992.

MATERIALS AND METHODS

Virus isolates and cultures. A virus culture, TVN-Ont, was isolated in 1989 from tobacco leaf samples showing veinal necrosis. Other cultures were obtained from a potato leaf survey in Québec (TVN-Q) and from table potatoes imported into Canada from California (TVN-Cal). PVY^O strains from potato, PVY^O-1 and PVY^O-12, were supplied, respectively, by G. Hawkins, New Brunswick Department of Agriculture, and R. P. Singh, Agriculture Canada Research Station, Fredericton, New Brunswick. Cultures of PVY-NN and PVY-MN were supplied by G. V. Gooding of North Carolina State University; of PVY^N from Scotland (PVY^N-C3) (12), by G. Rose (Agricultural Scientific Services, East Craigs, Edinburgh); and of PVY-36 (9), by D. Gumpf (University of California, Riverside).

Virus cultures were maintained in *Nicotiana tabacum* L. cv. Samsun by mechanical inoculation, using 0.01 M potassium phosphate buffer (pH 8) containing 0.01 M Na_2SO_3. The absence of potato virus X in these isolates and cultures was confirmed by the absence of local lesions on inoculation to *Gomphrena globosa* L. and negative reaction in enzyme-linked immunosorbent assay (ELISA).

Indicator plants. The indicator plants tested were the *N. tabacum* cultivars Samsun, NC95, NC2326, and Burley 21; the *Lycopersicon esculentum* Mill. cultivar Sheyenne; the *Capsicum frutescens* L. cultivar Calwonder; the *S. tuberosum* cultivars Atlantic, Jemseg, Kennebec, Red Pontiac, Russet Burbank, and Sebago; *S. demissum* Lindl., *P. floridana, P. angulata* L., *Chenopodium amaranticolor* Coste & Reyn., and *C. quinoa* Willd. Plants were grown in an insect-proof glasshouse at 20–25 C. All trials were conducted at least twice.

ELISA. Direct double-antibody ELISA was used as previously described (10) with a polyclonal antiserum to PVY^O (10), a monoclonal antibody to PVY^N (Bioreba Company, Basel, Switzerland), and a monoclonal antibody to PVY^O, Mab-2 (produced by A. Cepica, Univer-

sity of Prince Edward Island, and J. McDonald). Concentrations of the antibodies were adjusted to give a similar range of optical density readings. Test antigens were raised in *N. tabacum* cv. Samsun. Leaf sap of each test sample was diluted 1:10, 1:100, 1:1,000, 1:10,000 and 1:100,000 (v/v) in ELISA sample buffer. Reactions were read with a colorimeter at 405 nm after incubation of substrate for about 1 hr.

Electron microscopy leaf cell extracts were negatively stained with 2% ammonium molybdate, pH 7, using the leaf-chopping method (11) and were examined with a Hitachi electron microscope.

RESULTS

Symptoms on indicator plants. The symptoms induced by TVN-Ont, TVN-Q, TVN-Cal, PVY^N-C3, PVY^O-1, PVY^O-12, PVY-NN, PVY-MN, and PVY-36 on a range of indicator hosts are summarized in Table 1. TVN-Ont, TVN-Q, TVN-Cal, and PVY^N-C3 caused very similar symptoms, although some differences in severity were noted on certain hosts. On the tobacco cultivars, TVN-Q induced relatively mild veinal necrosis. On Jemseg potato, PVY^N-C3 caused severe systemic necrosis, and TVN-Ont and TVN-Q caused mild leaf cupping; TVN-Cal was intermediate by showing more severe stunting.

Both PVY^O strains caused similar symptoms. They were distinct from the TVN isolates by inducing local lesions on the *Chenopodium* spp., infecting Calwonder pepper, causing more severe mosaic on the potato cultivars, and being nonnecrotic on the tobacco cultivars.

The PVY-NN and PVY-MN strains were quite distinct from the TVN isolates by not causing veinal necrosis on flue-cured tobacco cultivars that lacked the gene for RKN resistance (NC95). They also caused local lesions on the six potato cultivars evaluated, and efforts to recover virus from systemic leaves failed. They induced local lesions on the two *Chenopodium* spp. Symptom reaction of these two strains on the other solanaceous indicators were always more severe than that of the TVN and PVY^O strains.

PVY-36 caused symptoms on the tobacco cultivars very similar to those induced by the TVN isolates; the major difference was the appearance of distinct local lesions on the inoculated leaves. However, this strain failed to induce any visible symptoms on *S. demissum* and the potato cultivars; attempts to recover infectivity from systemic leaves failed. Symptom reaction on the other solanaceous indicators was very severe, and this strain induced local lesions on the two *Chenopodium* spp.

ELISA. TVN-Ont, TVN-Q, TVN-Cal, and PVY^N-C3 reacted in a similar manner to the three antibodies, and high absorbance readings with the monoclonal antibody to PVY^N, moderate readings with the polyclonal antibody to PVY^O, and no reaction with the monoclonal antibody to PVY^O were obtained. In contrast, PVY-NN and PVY-MN did not react with the monoclonal antibody to PVY^N, but did react with either the monoclonal or polyclonal antibody to PVY^O. PVY-36 did not react with any of the antibodies. High readings with the two antibodies to PVY^O were obtained, but the two PVY^O isolates reacted differently with the monoclonal antibody to PVY^N. The PVY^O-1 reacted strongly, and PVY^O-12 reacted minimally, with the PVY^N antibody. Typical reactions of TVN-Ont, TVN-Cal, PVY^N-C3, PVY^O-1, PVY^O-12, PVY-NN, and PVY-MN with the antibodies are shown in Figure 1.

Electron microscopy. Flexuous rod-shaped particles about 700 nm long and 12 nm wide (at least 20 particles counted) as well as cylindrical inclusion scrolls (2) were observed were observed in leaf dip extracts of all the virus isolates and cultures. There was no evidence of laminated aggregate inclusion bodies.

DISCUSSION

The results of the indicator plant study, ELISA, and electron microscopy indicate that TVN-Ont, TVN-Q, and TVN-Cal share the reported properties of the PVY^N group of PVY strains (1). In direct comparison with a member of this group, PVY^N-C3, very similar reactions were recorded. However, some differences in severity of symptoms were noted on the potato cultivar Jemseg, on which PVY^N-C3 induced systemic necrosis and death, TVN-Ont and TVN-Q induced much milder symptoms of leaf-cupping and slight stunting, and TVN-Cal caused an intermediate reaction. The cultivar Jemseg has some hypersensitive resistance to PVY^O (14). TVN-Q could be differentiated from the other PVY^N

Table 1. Reaction of potato virus Y (PVY) strains on selected indicator plant species

	Viruses[a]								
Indicator plant	**TVN-Ont**	**TVN-Q**	**TVN-Cal**	**PVY^N-C3**	**PVY^O-1**	**PVY^O-12**	**PVY-NN**	**PVY-MN**	**PVY-36**
Nicotiana tabacum									
Samsun	VCN	VCN	VCN	VCN	VCM	VCM	VC1N	VCM	LL/VCN
NC95	VCN	VCN	VCN	VCN	VCM	VCM	VC1N	SN	LL/VCN
NC2326	VCN	VCN	VCN	VCN	VCM	VCM	VC1N	VCM	LL/VCN
Burley 21	VCN	VCN	VCN	VCN	VCM	VCM	VCM	VCM	LL/VCN
Lycopersicon esculentum									
Sheyenne	M	M	M	M	M	M	SM	SM	SM
Capsicum frutescens									
Calwonder	O[b]	O[b]	O[b]	O[b]	M	M	SM	SM	SM
Solanum tuberosum									
Atlantic	M	M	M	M	SM/LD	SM/LD	LL[b]	LL[b]	O[b]
Jemseg	LC/LD	LC/LD	LC/LD	LL/LD/SN	LL/LD/SN	LL/LD/SN	LL[b]	LL[b]	O[b]
Kennebec	M	M	M	M	SM/LD	SM/LD	LL[b]	LL[b]	O[b]
Red Pontiac	M	M	M	M	SM	SM	LL[b]	LL[b]	O[b]
Russet Burbank	M	M	M	M	SM	SM	LL[b]	LL[b]	O[b]
Sebago	LC	LC	LC	LC	LL/LD	LL/LD	LL[b]	LL[b]	O[b]
S. demissum	LL	LL	LL	LL	LL	LL	LL	LL	O[b]
Physalis floridana	M	M	M	M	M	M/LD	SM/LD	SM/LD	SM/LD
P. angulata	M	M	M	M	M	M	SM/LD	SM/LD	SM/LD
Chenopodium amaranticolor	O	O	O	O	LL	LL	LL	LL	LL
C. quinoa	O	O	O	O	LL	LL	LL	LL	LL

[a] VCN = veinclearing followed by veinal necrosis, VCM = veinclearing followed by mottle, VC1N = veinclearing followed by mottle and interveinal necrosis, M = mild mosaic, SM = strong mosaic, O = no symptom, LL = local lesions on inoculated leaves, LD = leaf drop, SN = systemic necrosis leading to death, and LC = cupping of uninoculated leaves.

[b] No systemic infection detected by infectivity to a susceptible indicator host.

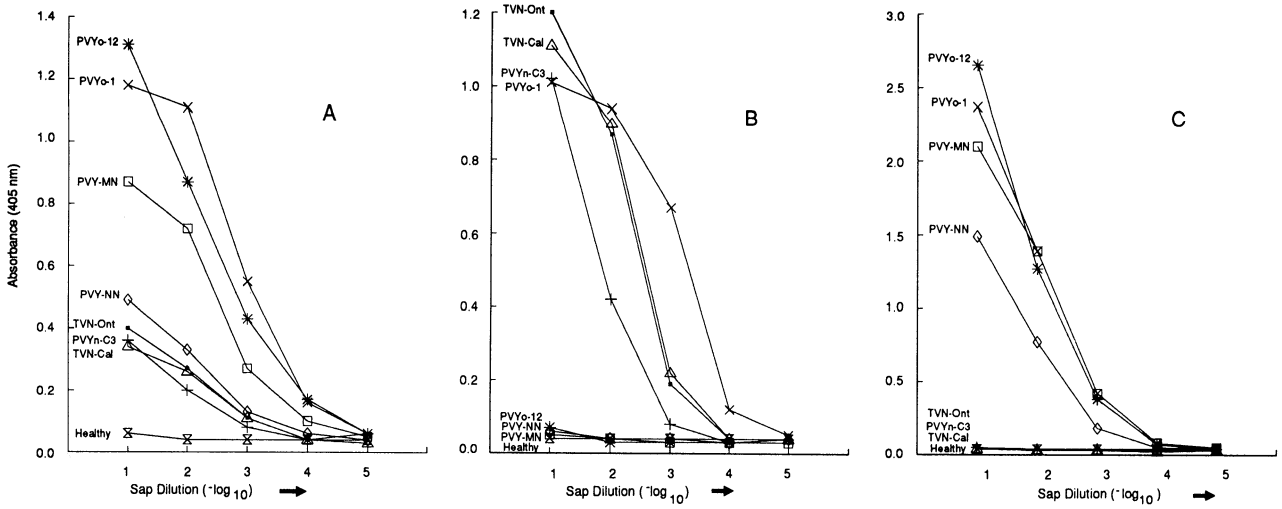

Fig. 1. Comparative absorbance of seven potato virus Y (PVY) isolates and cultures in double-antibody sandwich enzyme-linked immunosorbent assay with **(A)** the polyclonal antibody to PVY^O, **(B)** the monoclonal antibody to PVY^N (Bioreba), and **(C)** the monoclonal antibody to PVY^O (Mab-2). Dilutions (10-fold) were made from infected sap of *Nicotiana tabacum* cv. Samsun.

strains by its induction of milder veinal necrosis symptoms on the tobacco cultivars.

In comparison with the PVY^N group strains, PVY-NN, PVY-MN, and PVY-36 were distinctly different and could not be classified in this group. Most importantly, these three strains failed to systemically infect six common potato cultivars, but they induced local lesions on the two *Chenopodium* spp. None of these strains reacted with a monoclonal antibody to PVY^N that has broad reactivity against PVY^N group strains (6). Surprisingly, PVY-36 did not react with the two antibodies to PVY^O. However, Makkouk and Gumpf (9) had reported only weak reaction of this strain with a polyclonal antibody to PVY^O in sodium dodecyl sulfate agar gel double-diffusion test.

Gooding and Tolin (5) did not report the susceptibility of potato cultivars to PVY-NN and PVY-MN. However, Heath et al (7) reported that two tobacco isolates of PVY from Australia (JF and 18) that appeared to share the properties of PVY-NN and PVY-MN gave necrotic local lesions, without systemic invasion, on a number of potato cultivars, including Red Pontiac and Kennebec. These workers speculated that JF and 18 had lost the ability to systemically infect potato as a result of continued propagation in tobacco. In this study, the fact that the PVY-NN and PVY-NM also failed to systemically infect the selected potato cultivars suggests that this is a general characteristic of such strains and one that may be used to distinguish them from PVY^N group strains.

PVY-NN and PVY-MN share some of the characteristics of the PVY^C group of strains (1), but their definitive classification will require further work. However, PVY-36 is quite distinct from these and the other PVY strains included in this study. That the strain did not react in ELISA with the three antibodies and gave no evidence of infection of the *Solanum* spp. and cultivars suggests that it may be so distantly related to other PVY strains as to require classification as a distinct virus.

Serology, particularly using monoclonal antibodies, can be a powerful tool in classifying virus strain groups. The reaction of the different strains with the PVY^N and PVY^O monoclonals demonstrates the potential of this approach for differentiating strains. With a much wider panel of monoclonal antibodies, it may be possible to much better define the strains of PVY.

A number of workers (3,12,13) have used ELISA with a monoclonal antibody to PVY^N (sometimes the same as in this study) as the sole basis for identifying PVY^N. In this study, results of ELISA for the two different PVY^O strains indicate that some strains (e.g., PVY^O-1) will cross-react to a significant degree with this monoclonal antibody to PVY^N, suggesting that false positive reactions might result if there is exclusive reliance on this antibody for diagnosis.

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