

## Characterization of Potato Virus Y Strains Isolated from Pepper

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### ABSTRACT

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Five strains of potato virus Y (PVY) could be differentiated by their reaction on different indicator plants. Three strains (PVY-4, PVY-10, and PVY-82) produced symptoms on *Nicotiana tabacum* that resembled (with slight variation) those produced by the common type (PVY-NC), and one strain (PVY-36) produced symptoms of the necrotic type. Potato virus Y strains NC, 10, and 82 were serologically identical and also closely related to strains 4 and 36 which likewise were serologically identical. Cross protection tests showed that PVY strains NC, 10, and 82 protected almost completely against PVY-4 but only partially against PVY-36. Strain PVY-4 was transmitted more readily by aphids

(*Myzus persicae*) than were strains NC, 10, and 82. Strain PVY-36 showed the lowest level of aphid transmission. Ultraviolet light absorption spectra of purified preparations of the five PVY strains were identical, with a minimum at 245 nm, a maximum at 260 nm, and a slight shoulder at 290 nm. The  $A_{260}:A_{280}$  ratio varied between 1.22 and 1.28, and the  $A_{260}:A_{245}$  ratio varied between 1.14 and 1.18 depending on the virus strain. All five PVY strains migrated anodically in agarose-acrylamide gel electrophoresis at pH 8.2. Strain PVY-36 migrated most rapidly followed by strains NC and 4, and strains 10 and 82 migrated more slowly and at the same rate.

Several strains of PVY have been reported (2, 7, 21). Common strains of the virus produce vein clearing followed by mottling in tobacco (*Nicotiana tabacum* L.), whereas several strains cause necrotic symptoms (1, 2, 13, 19, 22). The first report of a necrotic strain of PVY was made by Smith and Dennis (22) in 1940. Bawden and Kassanis (2) reported a serological relationship between the tobacco veinal necrosis strain (PVY-TVN) and other PVY strains, but did not demonstrate cross protection. Ramirez et al. (17) reported that PVY protects against PVY-TVN in tobacco. Kahn and Monroe (7) in 1963 reported PVY-TVN in two introductions of *Solanum cardenasii* and one of *S. andigenum* from Bolivia growing in quarantine at the U.S. Plant Introduction Station, Glen Dale, Maryland. This strain is not known to occur naturally in the United States (7).

Therefore, this work was initiated to present a comparative study, based on selected properties of the virus, of naturally occurring PVY strains.

### MATERIALS AND METHODS

**Virus cultures and virus purification.**—Five PVY strains were used in this study. One strain (NC-57) was provided by G. V. Gooding of North Carolina State University and designated in this work as PVY-NC. Strains, PVY-4, PVY-10, PVY-36, and PVY-82, were obtained from pepper fields in Ventura, Santa Barbara, Orange, and Ventura counties, respectively. These strains were selected from 50 PVY isolates obtained from pepper fields in California, because of symptom type and host reaction (11). All strains were submitted to at least two

single-lesion serial passages in *Physalis floridana* or tobacco cultivar Havana 425 and increased in tobacco. Systemically infected tobacco leaves, dried and stored in the freezer, served as stock cultures throughout this work. Virus was purified using a method described previously (10).

**Insect transmission.**—The method used to study differential transmission of PVY strains by aphids is essentially that described by Norman and Pirone (14). A 10-mm diameter disk was cut from a systemically infected tobacco leaf with a cork borer. Aphids (*Myzus persicae*) were fasted for 1-2 hours before being placed on the disk for a 1 minute acquisition feeding time. Probing was observed through a magnifying lens or a dissecting microscope. Following the probing period, three aphids were transferred to each of a series of healthy tobacco plants. Local lesion assay of the disks used for the acquisition feeding were made on fully expanded leaves of *Chenopodium amaranticolor*. Extracts that were used for inoculation were prepared by grinding each leaf disk in 20 ml of water.

**Serology.**—Potato virus Y antiserum was produced in rabbits by first injecting 3 mg of purified virus intravenously, followed by three additional intramuscular injections of 1 ml antigen (3-4 mg virus/ml) mixed with one ml of Freund's incomplete adjuvant administered at weekly intervals. Serum was collected 7 days after the last injection.

Serological reactions were examined using the agar double-diffusion test. Gel plates contained 0.8% agarose, and 0.1% sodium azide. Virus preparations to be tested were made 1% with respect to SDS and incubated for 1

TABLE 1. Reaction of five strains of potato virus Y (PVY) on different indicator plants

Indicator plant	PVY strain				
	NC	4	10	36	82
<i>Nicotiana tabacum</i>					
'Havana 425'	S <sup>a</sup>	Cl.Sp. <sup>b</sup> ,S	S	Cl.Sp.,S	S
<i>Datura stramonium</i>	I <sup>c</sup>	I	I	I	I
<i>Capsicum frutescens</i>					
'Tabasco'	S	LL <sup>d</sup>	S	LL	S
<i>Physalis floridana</i>	LL,S	S	LL,S	LL,S	LL,S
<i>Solanum demissum</i>	LL	LL	LL	O <sup>e</sup>	LL
<i>Chenopodium amaranticolor</i>	LL	LL	LL	LL	LL
<i>C. quinoa</i>	LL	LL	LL	LL	LL

<sup>a</sup>S = systemic infection.

<sup>b</sup>Cl.Sp. = chlorotic spot produced first, and then turned necrotic.

<sup>c</sup>I = immune.

<sup>d</sup>LL = necrotic local lesions produced.

<sup>e</sup>O = no symptoms.

hour at room temperature before they were placed in the wells in the agar plates.

**Cross protection.**—Since PVY strains 4 and 36 gave local lesions on *N. tabacum* "Havana 425", and strains PVY-NC, PVY-10, and PVY-82 were systemic, it was possible to quantitate protection offered by systemic strains against those that induced the local reaction. Six half-leaves of "Havana 425" tobacco were inoculated with PVY-NC, PVY-10, and PVY-82. Five days later the entire leaf was inoculated with either PVY-4 or PVY-36. Eight days after the second inoculation local lesions were counted.

**Polyacrylamide-gel electrophoresis of PVY.**—A 75- to 100- $\mu$ l sample of the purified virus preparation which contained 1 mg virus per ml was layered on top of gels composed of 1% polyacrylamide together with 0.5% agarose. The temperature of the agarose and acrylamide solutions was adjusted to 50 C prior to mixing and then poured rapidly into the tubes. Ammonium persulfate was added to the agarose solution just before mixing. The buffer system used was composed of 0.04 M Tris, 0.03 M sodium phosphate (monobasic), and 1 mM EDTA pH 8.2. The buffer was used for both the gels and reservoirs. Electrophoresis was carried out at 5 mA/gel for 6 hours.

**Linear-log density-gradient centrifugation.**—Samples (0.5 ml) of each of the five PVY strains containing 1 mg purified virus/ml were layered on linear-log sucrose density gradients designed for the Spinco SW 41 rotor (4). Buffer used as the sucrose solvent was 0.02 M orthoborate containing 1 mM EDTA pH 8.2. The gradients were placed at 4 C for 16-18 hours before use, and were centrifuged at 25,000 rpm for 3 hours at 6 C. Gradients were scanned at 254 nm using the ISCO density-gradient fractionator. Brome grass mosaic virus (BMV), and tobacco mosaic virus (TMV) were used as standards for the estimation of sedimentation coefficients.

## RESULTS

**Symptomology.**—Some of the PVY strains isolated from peppers induced readily distinguishable reactions in the indicator plants (Table 1). For instance, strains 4 and 36 induced local lesions on tobacco cultivar Havana 425 and pepper cultivar Tabasco. Strain 36 could be

differentiated from strain 4 because it induced systemic vein necrosis of tobacco leaves (Fig. 1-A) as well as severe necrosis of the stems (Fig. 1-B). Strain 4 did not induce systemic vein necrosis on the leaves of tobacco, and only limited stem necrosis (Fig. 1-B). In addition, PVY-4 was more easily transmitted mechanically to tobacco (100%) than was PVY-36 (20-30%). Symptoms induced by strains PVY-10 and PVY-82 on different indicator plants were similar (Table 1) except that vein clearing symptoms with PVY-82 in tobacco were more distinct, because of the wide clear areas around the veins. The common strain of PVY (PVY-NC) produced no symptoms on the bell pepper variety Yolo Y, whereas all other strains produced variable symptoms. Strain PVY-36 was the most severe. Anaheim Chili pepper was affected by all five PVY strains including PVY-NC, and the reduction in growth and fruit size was greater than that in Yolo Y. Strain PVY-36 again produced the most severe symptoms.

**Serology.**—Antisera, with titers of 128 and 16 when reacted with the homologous antigens, were produced against PVY-NC and PVY-4, respectively. These titers were determined by agar double diffusion tests against purified SDS-degraded virus preparations. When the same antisera were reacted with the heterologous antigens, titers of 16 and 4 were determined. Adsorption of PVY-NC or PVY-4 antisera with the heterologous antigen produced a strain-specific antiserum which reacted only with the homologous antigen. Purified SDS-degraded preparations of the five PVY strains NC, 4, 10, 36, and 82 all reacted positively with the PVY-NC antiserum, giving a single precipitin band by agar double diffusion (Fig. 2). In a few instances a faint second band was also formed by PVY-4 and PVY-36. The five PVY isolates also reacted positively with PVY-4 antiserum. Potato virus Y strains NC, 10, and 82 were serologically identical. Strains 4 and 36 were likewise serologically identical. However the presence of a spur in reactions against strains NC, 10, and 82 in the same tests indicates that 4 and 36, are only closely related to NC, 10, and 82. Antisera produced against PVY strains NC and 4 did not react with SDS-degraded tobacco etch virus (TEV) in agar double diffusion tests.

**Cross protection.**—The results of cross protection tests (Table 2) show that Havana 425 tobacco infected with PVY strains NC, 10, and 82 were almost completely

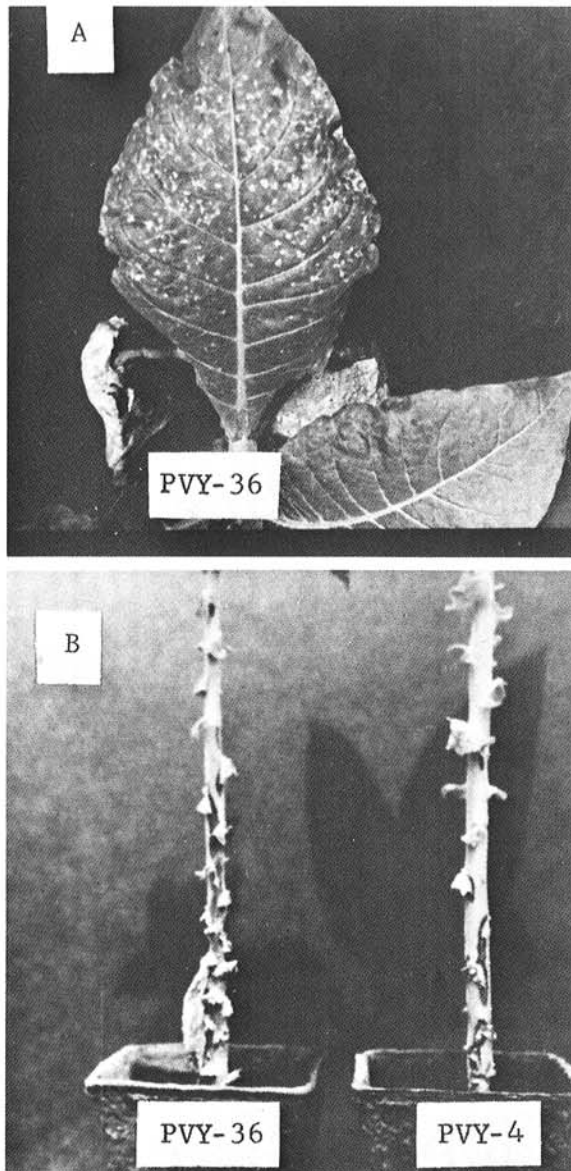


Fig. 1-(A,B). A) Tobacco leaves infected with potato virus Y strain 36 showing veinal necrosis. B) Stem necrosis induced in tobacco by PVY-4 and PVY-36.

protected against the challenge of PVY-4 since only occasional lesions formed on the protected half-leaf. Protection against PVY-36 was only partial, as indicated by a reduction in the number of lesions produced when they were challenged by PVY-36. Strain PVY-82 demonstrated more protection against PVY-36 than the other two, with an eight-fold reduction in lesions.

**Aphid transmission.**—Donor plants used for virus acquisition were tobacco plants singly infected with each of the five PVY strains. These plants were mechanically inoculated 2-4 weeks earlier and showed typical systemic symptoms. Comparative transmission tests were repeated

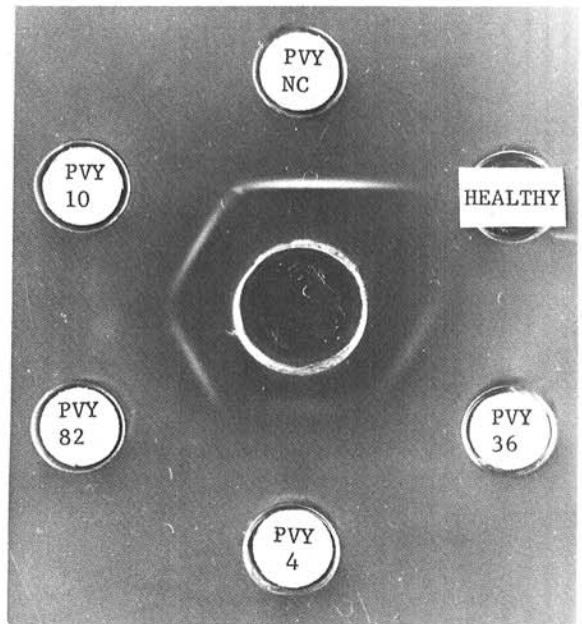


Fig. 2. Agar-gel double diffusion test using purified SDS-degraded preparations of five PVY strains and healthy extract in the peripheral wells. The center well was filled with PVY-NC antiserum.

TABLE 2. Cross protection between potato virus Y (PVY) strains systemic in *Nicotiana tabacum* 'Havana 425' (PVY-NC, PVY-10, and PVY-82) and PVY strains that induce local lesions (PVY-4 and PVY-36)

Challenge strain <sup>b</sup>	Number of lesions produced <sup>a</sup> by local lesion challenge strains after right half-leaf was first inoculated with protecting strain:					
	PVY-NC		PVY-10		PVY-82	
	R <sup>c</sup>	L <sup>c</sup>	R	L	R	L
PVY-4	3	95	2	92	1	84
PVY-36	34	97	43	118	18	114

<sup>a</sup>Average number of lesions produced on six half-leaves.

<sup>b</sup>Whole leaf was inoculated with the challenge strain five days after the right half-leaf was inoculated with the protecting strain.

<sup>c</sup>R = right half-leaf; L = left half-leaf.

three times with each strain under similar conditions. Although there was variation among experiments, strain PVY-4 was transmitted more efficiently than the other four strains, even though the virus concentration in the acquisition disks, as measured by local lesion assay was no higher than that of PVY-NC, PVY-10, or PVY-82 (Table 3). These experiments suggested that the low rate of transmission of PVY strain 36 was not due to differences in virus concentration.

**Purification.**—The method used for purifying PVY has been published earlier (10), except that the preparation was digested with deoxyribonuclease (DNase) 50  $\mu$ g/ml for 12-16 hours before subjecting it to density-gradient centrifugation. Following sucrose density-gradient centrifugation, one strong light scattering band and a

faint band just below it was observed. Material collected from both zones was infectious on Havana 425 tobacco, and produced typical PVY symptoms. Several of these zones were collected separately, concentrated by high speed centrifugation, and resuspended in the orthoborate buffer for serological testing. Both fractions reacted positively with PVY antiserum in agar double diffusion tests, suggesting the faster sedimenting material was an aggregate of the virus. Purified preparations frozen and thawed prior to density-gradient centrifugation showed an increase in the proportion of the leading band. Precipitation of the virus from the clarified extract with polyethylene glycol also appeared to increase the proportion of faster sedimenting material. In spite of this tendency to increase aggregation, polyethylene glycol precipitation was adopted because of convenience and because the volume of the clarified extract could be reduced (90%) before subjecting it to high speed centrifugation.

Tubes holding the density gradients always contained an infectious pellet of aggregated virus following centrifugation. These pellets were resuspended and incubated at 4 C for 20-25 hours in 0.02 M orthoborate buffer, pH 8.2, containing 0.5 M urea, and again subjected to density-gradient centrifugation. An infectious light scattering zone appeared in the gradient where PVY monomers usually sediment. This suggests that 0.5 M urea is effective in releasing single particles from the aggregated virus. The yield of purified virus by this procedure varied from 9 (PVY-NC) to 23 (PVY-4) mg/kg of leaves depending on virus strain.

**Ultraviolet light absorption.**—The UV absorption spectrum of the purified PVY solution was typical of long flexuous rods. The spectra of the five PVY strains in 0.02 M orthoborate, pH 8.2, were identical, with a minimum at 245 nm, a maximum at 260 nm, and a slight shoulder at 290 nm. The 260:280 ratio varied between 1.22 and 1.28, and the 260:245 ratio varied between 1.4 and 1.18, depending on virus strain. Each value was the average of five separate determinations.

**Linear-log density gradient centrifugation.**—When virus preparations were centrifuged through linear-log density-gradient columns in a Beckman SW41 rotor at 25,000 rpm for 3 hours, all strains produced one strong light scattering band and a faint faster sedimenting band (Fig. 3). The ratio of material in the slower sedimenting

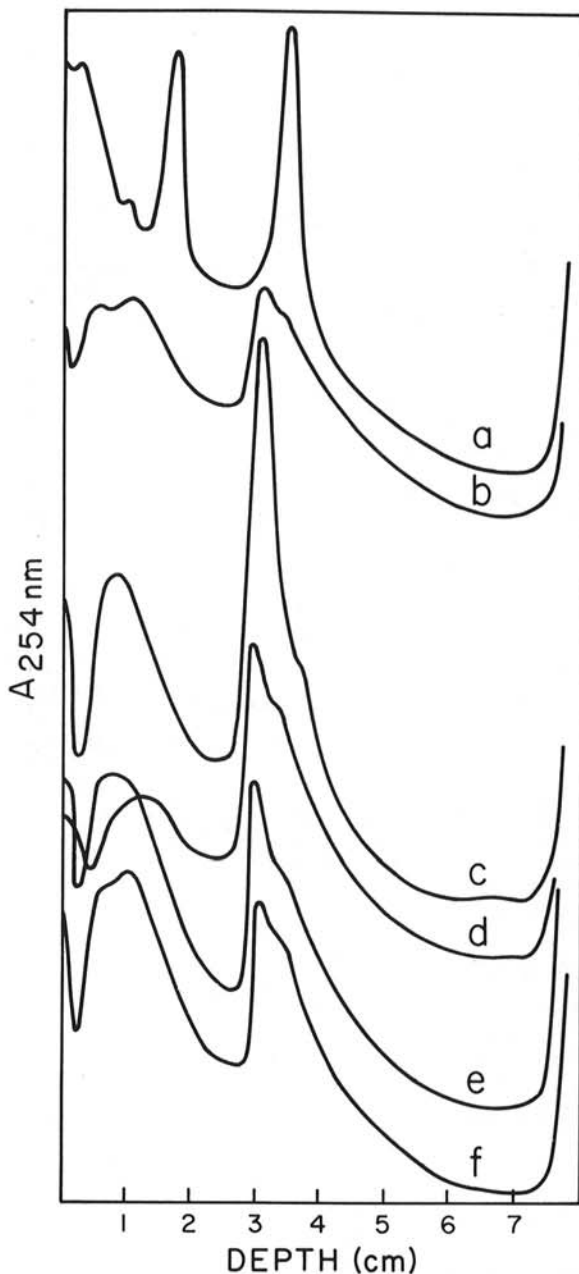


Fig. 3. Ultraviolet light absorbancy scanning profiles of potato virus Y strains centrifuged in linear-log sucrose density gradient at 6 C at 25,000 rpm in Beckman SW 41 rotor for 3 hours (a) Brome grass mosaic virus and tobacco mosaic virus, (b) PVY-NC, (c) PVY-4, (d) PVY-10, (e) PVY-36, and (f) PVY-82.

TABLE 3. Transmission of five strains of potato virus Y acquired by *Myzus persicae* from infected *Nicotiana tabacum* 'Havana 425'

Virus strain	Transmission <sup>a</sup> (%)	Local lesions <sup>b</sup> (no. per leaf)
PVY-NC	58	67
PVY-4	73	46
PVY-10	51	181
PVY-36	9	27
PVY-82	56	185

<sup>a</sup>Percentage of plants infected using three aphids per plant, three replicates of 15 plants per treatment.

<sup>b</sup>Average number of lesions produced on six leaves of *Chenopodium amaranticolor*.

band to that in the faster sedimenting band was different for each PVY strain, highest with PVY-4 and lowest with PVY-NC, suggesting that aggregation was less with PVY-4. Estimates of the sedimentation coefficient of the five PVY strains, using TMV and BMV as standards, gave an average value of 151S. This value is in agreement with the 150S value obtained by Stace-Smith and Tremaine (23) and the 154S obtained by Delgado-Sanchez and Grogan

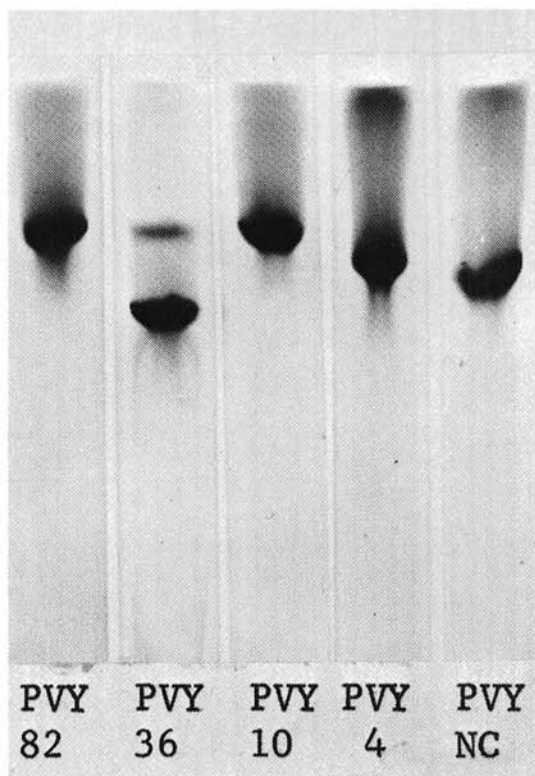


Fig. 4. Electrophoresis of five strains of potato virus Y in 1% polyacrylamide and 0.5% agarose gels, using a tris-phosphate buffer, pH 8.2. Electrophoresis was carried out at 5mA/gel for 6 hours. Migration was toward the anode.

(5). No difference in sedimentation coefficient value among PVY strains was detected.

**Electrophoretic mobility of PVY.**—The electrophoresis of PVY strains in 1% polyacrylamide together with 0.5% agarose at pH 8.2 (Fig. 4) showed that all five strains migrated toward the anode. Four of the strains migrated as a single infectious band. Strain PVY-36, however, gave one fast major band and a second slower migrating minor band, both of which were infectious. The faster migrating material of PVY-36 was the most mobile followed by PVY-NC and PVY-4. Strains PVY-10, PVY-82, and the slow moving material of PVY-36, migrated slowest. All five strains migrated slower than TMV or cowpea mosaic virus (CPMV).

#### DISCUSSION

No previously reported PVY strains in California were of the necrotic type. Our strain PVY-36, however, has characteristics that are similar to those described earlier as tobacco vein necrosis strain (2). Apparently the isolation of strain PVY-36 is the first report of a necrotic strain occurring naturally in the United States. It should be mentioned, however, that Kahn and Monroe (7) earlier reported such a strain in two potato introductions from Bolivia growing in quarantine at the U.S. Plant Introduction Station, Glen Dale, Maryland. The spread of this strain into tobacco or potato plants in high

production areas could cause very serious damage. The isolation of strain PVY-4 from pepper has been reported earlier (11).

Serologically, the five PVY strains can be divided into two groups; strains NC, 10, and 82 in one and strains 4 and 36 in the other. The formation of spurs in gel double diffusion plates between members of the two groups suggest they were closely related, but not identical. Cross protection studies indicated that PVY strains NC, 10, and 82 offered more protection to PVY strain 4 than to strain 36, also suggesting the relatedness of these strains to strain 4 is closer than they are to the necrotic strain PVY-36. Also, the protection of PVY-82 to strain PVY-36 was greater than the protection offered by PVY strains NC and 10 to PVY 36. On the basis of symptomatology, serology, cross protection, and chemical studies, the diminishing order of relatedness of the five PVY strains is: PVY-NC, PVY-10, PVY-82, PVY-4, and PVY-36.

The transmissibility by *M. persicae* of PVY strain 4 was higher than strains NC, 10, 82, and 36. This increased transmission of PVY-4 cannot be due to a high concentration of the virus in the donor tissue since the number of lesions, as measured by local lesion assays, produced by strain 4 was less than the number produced by PVY-10 or PVY-82. Relating variation in aphid transmission to variation in virus concentration in donor tissue, as measured by mechanical assays, might be misleading since local lesion assays detect both whole virus and naked RNA, while aphids probably transmit only whole virus (15). There was a positive correlation, however, between the transmission of PVY-36 by aphids, which was very low, and the number of lesions produced which was also the lowest. Watson (24) suggested that transmissibility depends on some chemical or structural property of the virus particle which makes them bind specifically with sites on the aphid's mouth parts. The amino acid analyses as well as the reaction with cyanogen bromide of coat proteins from the five PVY strains (12) indicated that differences between PVY-4 and PVY-36 are less than differences between the other PVY strains. Since the aphid transmission work was not done with purified virus preparation, the idea of specific binding of the virus with the insect really cannot be accepted or rejected. Norman and Pirone (14) did not find any difference in the transmission of purified preparations of four strains of cucumber mosaic virus CMV by aphids. When aphids acquired the virus from infected leaves, however, there were differences among strains, suggesting the possibility of a host-virus interaction. Such an interaction might explain the differences in aphid transmission of the five PVY strains. Govier and Kassanis (6) recently found that a host component induced by PVY is needed for aphid transmission of PVY, lending further support to the host-virus interaction hypothesis.

Few procedures have yielded pure nonaggregated preparations of PVY (5, 23). Using a modification of the procedure described by Purcifull and Gooding (16), higher yields of purified, relatively nonaggregated virus were obtained. The yield varied with the virus strain being purified, presumably because the tendency to aggregate varied among different strains. Aggregation is a serious problem encountered during the purification of long flexuous viruses, especially those of the PVY group (18, 20, 23). Even in highly purified material some aggregation

can be found as indicated by the presence of an infectious band below the main band in density-gradient columns. Pellets observed at the bottom of density-gradient tubes were also infectious, which indicated the presence of aggregated virus. When these pellets were dissolved in buffer containing 0.5 M urea and again subjected to density-gradient centrifugation a band appeared in the gradient where the monomer of PVY usually sediments. When this band was collected and inoculated to tobacco plants it produced typical PVY symptoms. These results suggest that 0.5 M urea has little or no harmful effect on the virus, and is effective in solubilizing single virus particles from the aggregated material. Introducing 0.5 M urea into the resuspension buffer in the early steps of purification might increase the yield of purified unaggregated virus. The effectiveness of urea in dispersing this virus suggests that aggregation of PVY particles is most likely due to hydrophobic-type interactions. The presence of one band in 8.0 M urea SDS gels (12) suggests that the adopted purification procedure resulted in satisfactorily purified preparations.

Wolf and Caspar (25) recently reported electrophoresis of an elongated virus (PVX) in agarose (0.5%)–acrylamide (2%) gels. Substituting 1% acrylamide for 2%, resulted in gels with larger pore sizes, allowing fine differences in the electrophoretic mobilities of the five PVY strains to be observed. This is apparently the first report of electrophoresing a long flexuous virus in polyacrylamide gels. The five PVY strains could be classified in three groups according to their electrophoretic mobility. Strain PVY-36 migrated the most rapidly and PVY-10 and PVY-82 (which migrated at the same rate) were the slowest. Strains PVY-NC and PVY-4 were intermediate. Strain PVY-36 separated into two infectious bands in the gels. Electron micrographs showed that the faster moving bands contained primarily single particles, and that the slower moving bands contained aggregated virus. Density-gradient centrifugation of the different PVY strains, however, showed that the tendency to aggregate was almost the same for strains PVY-NC, PVY-10, PVY-36 and PVY-82. The reason PVY-36 separates into two bands during agarose–acrylamide gel electrophoresis is not clear.

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