Etiology

# Characterization of a New Potyvirus from Potato

C. E. Fribourg and J. Nakashima

Universidad Nacional Agraria, Apartado 456, Lima, Peru and International Potato Center, Apartado 5969, Lima, Peru, respectively.
We thank R. A. C. Jones and R. Koenig for critically reading the manuscript, and Ernesto Velit for preparing the electron micrograph.
Accepted for publication 1 June 1984.

## ABSTRACT

Fribourg, C. E., and Nakashima, J. 1984. Characterization of a new potyvirus from potato. Phytopathology 74:1363-1369.

A virus with flexuous filamentous particles that failed to react serologically with antisera to potato virus Y (PVY) and potato virus A (PVA) was isolated in central Peru from a potato clone showing symptoms of mosaic, veinal necrosis, and leaf drop. The host range of the virus was restricted to 20 species in the family Solanaceae. Symptoms induced in potato cultivars infected by grafting were mainly mild mosaics, systemic chlorotic spots, partial vein necrosis of the leaf undersides, and premature senescence of lower leaves. The virus (code-named UF) and a similar isolate previously reported from the Netherlands ($PVY^C$-Gl) did not induce a hypersensitive response in potato cultivars carrying gene *Nc*. UF was transmitted with difficulty by *Myzus persicae*. Sap or purified preparations contained flexuous filaments with a normal length of 760 nm. In serological comparisons made by using partially purified antigens and six different antisera, UF was found to be distantly related to PVA and to isolates belonging to the three groups of PVY strains presently recognized, but it was closely related to $PVY^C$-Gl and another potyvirus isolated from potato in Northern Ireland ($PVY^C$-AB). The results show that all three should be considered as isolates of a distinct potyvirus different from PVY and PVA and we propose that the virus be named PVV (potato virus V).

A virus code-named UF was isolated from a clone of *Solanum tuberosum* subsp. *andigena* Juz. et Buk. growing in a screenhouse at the International Potato Center facilities in the Andean highland locality of Huancayo, Peru. UF had a longevity in vitro of about 3 wk and flexuous filamentous particles (~750 nm) like those of potato virus Y (PVY) and potato virus A (PVA); however, it did not induce local lesions in clone A6, a diagnostic indicator plant for PVY and PVA (21). Also, it failed to react serologically with antiserum to both PVY (15) and PVA. Three groups of PVY strains are recognized ($PVY^C$, $PVY^O$, and $PVY^N$) all of which have the same antigenic properties (7). Rozendaal et al (25) and Bokx et al (7) reported that a deviating strain of $PVY^C$ ($PVY^C$-Gl) isolated from the cultivar Gladblaadje in the Netherlands had a negligible relationship with other strains of PVY, and Calvert et al (9) showed that $PVY^C$-Gl was very similar to strain $PVY^C$-AB isolated from cultivar Arran Banner in Northern Ireland. When UF was tested against antiserum to $PVY^C$-Gl, it reacted strongly. Therefore, a detailed study was started to determine the relationship between UF and PVY strains.

This paper describes the results of an investigation of the properties of UF, its relationship to $PVY^C$-Gl and $PVY^C$-AB, and its reaction and symptomatology in certain potato cultivars.

## MATERIALS AND METHODS

**Isolation and culture.** UF was first isolated in 1979 from a plant of the Peruvian potato clone Hua-760 (*S. tuberosum* subsp. *andigena*) showing symptoms of strong mosaic, veinal necrosis, and leaf drop. *Nicotiana glutinosa* L., *N. tabacum* L. 'White Burley,' and *N. occidentalis* Wheeler were used to maintain the virus and as sources of inocula for the experiments. These two indicator plants and *N. clevelandii* Gray were used to culture the following potyviruses (names of donors are given in parentheses): potato virus Y strains $PVY^N$-R, $PVY^C$-Gl (J. A. de Bokx); $PVY^C$-R, $PVY^C$-AB (R. Copeland); PVA (R. A. C. Jones); Peru tomato virus strain PTV M-4 (E. N. Fernandez-Northcote); and wild potato mosaic (WPMV) and $PVY^O$ which were from previous work (17).

**Plants.** Indicator hosts were grown in pots containing a mixture of sterilized soil, sand, and peat. Wild tuber-bearing *Solanum* spp. came initially from true seed supplied by the Potato Introduction Station, Sturgeon Bay, WI, in the United States. Later, some were

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

propagated by cuttings rooted in peat blocks. Potato cultivars were grown from cuttings or tubers supplied by the International Potato Center's seed program. Some British cultivars were supplied by R. A. C. Jones, Ministry of Agriculture, Fisheries and Food, Plant Pathology Laboratory, Virology Section, Hatching Green, Harpenden, Herts., England. Plants were grown under greenhouse and/or insect-proof screenhouse conditions at 18–22 C. Mechanical inoculations were made by rubbing leaves dusted with 22-$\mu$m (600-mesh) Carborundum with sap inoculum. All grafts to potato cultivars were done by top grafting with infected scions of *S. chancayense* Ochoa. Plants were tested for infection by back-inoculation to *N. occidentalis*. To study the properties of UF in infective sap, inoculations were made to groups of three to six plants of *N. occidentalis* or *N. clevelandii*.

**Aphid transmission tests.** *Myzus persicae* Sulz. reared on Chinese cabbage (*Brassica pekinensis* (Lour.) Rupr.) were used for all transmission tests. Source plants were infected *N. occidentalis*, and test plants were young seedlings of this species. Aphids were starved for 1 hr in a petri dish, and allowed to feed individually for 15–30 sec or 1.5–2.0 min. Then, five aphids were transferred to each healthy seedling, left for 15 min and killed by spraying with 0.1% Tamaron (amido-*O*-methyl-*S*-methyl phosphate).

**Purification and serology.** UF was purified from systemically infected leaves of *N. occidentalis* harvested 3 wk after inoculation. After storage overnight at 4 C, leaves were homogenized in a Waring blender with 0.2 M phosphate buffer, pH 8, containing 0.15% 2-mercaptoethanol and 0.01 M EDTA (1 g of leaf tissue: 2 ml of buffer). After low speed centrifugation (LSC) at 8,000 rpm (usually for 15–20 min) in a J-17 rotor of a Beckman J2-21 centrifuge or 10,000 rpm in No. 30 or 40 rotors of a Beckman L2 65B ultracentrifuge, the supernatant was stirred for 3 hr at 4 C with the detergent Triton X-100 (1%, v/v). Virus was then precipitated by adding 40 g of polyethylene glycol 6,000 per liter and NaCl to make 0.2 M, stirring and incubating the mixture for 1.5 hr. The precipitate was pelleted by LSC and then resuspended by shaking for 2 hr in an amount of 0.2 M phosphate buffer, pH 8, containing 1% Triton X-100, equal to one-tenth of the original volume of supernatant. This resuspension was continued by stirring overnight at 4 C. After a further LSC to remove insoluble material, the preparation was then given two cycles of differential centrifugation consisting of a high speed centrifugation (40,000 rpm for 1 hr in a No. 65 rotor of a Beckman L2 65B ultracentrifuge) (HSC) and an LSC. After the first HSC, pellets were resuspended in 0.05 M phosphate buffer, pH 8, and after the second HSC, in 0.01 M phosphate buffer, pH 7.3.

An antiserum was prepared by injecting a rabbit intramuscularly three times at weekly intervals with 2–4 mg of virus in 0.5 ml of buffer emulsified with 0.5 ml of Freund's incomplete adjuvant. Comparative serological tests were done with UF, PVA, $PVY^C$-AB, $PVY^C$-Gl, $PVY^C$-R, $PVY^N$-R, $PVY^O$, and WPMV and their homologous antisera except $PVY^C$-AB. Antigens were prepared for this by using the above purification procedure but omitting the second HSC; purified virus was diluted in 0.05 M $PO_4$ buffer, pH 7.3. For titration of antibodies, a modification of the microprecipitin test described by Ball (2) was used. Drops of the antigens were placed in plastic petri dishes in serial two-fold dilutions made in 0.05 M $PO_4$ buffer, pH 8 (range: 1/2–1/4,096), and drops of the different antisera similarly diluted in saline (range: 1/2–1/64,000) were added. The dishes were then put on a mechanical shaker for 15 min, incubated in a humid chamber for 2 hr and reactions were observed by using a stereoscopic microscope with lateral illumination.

**Electron microscopy.** Samples prepared from diluted infective sap of *N. occidentalis* or from purified preparations were stained with 2% phosphotungstic acid, pH 7.0. Measurements of particles were determined in sap preparations deposited on carbon grids coated with UF antiserum diluted 1/1,000 as described by Roberts and Harrison (24). The magnification of the electron microscope JEOL JEM-100S was checked with a carbon replica of a diffraction grating with 2,160 lines per millimeter. For thin sectioning, systemically infected leaves of *N. occidentalis* were fixed by vacuum infiltration with 5% glutaraldehyde/PIPES and postfixed with 0.2% osmium tetroxide in 0.1 M sodium cacodylate buffer. The samples were stained overnight with 5% uranyl acetate, ethanol-dehydrated, and embedded in an Araldite resin mixture.

TABLE 1. Symptomatology of UF virus in indicator hosts and in wild and cultivated potatoes after mechanical inoculation

| Species, subspecies, PIs, and cultivars | Symptoms[a] |
|---|---|
| *Datura metel* L. | LCS,SCS,MM |
| *Lycopersicon esculentum* Mill. | |
| 'Marglobe' and 'Rutgers' | SS |
| *Nicandra physaloides* Gaertn. | SS |
| *Nicotiana* spp. | |
| *bigelovii* Wats. | MM |
| *clevelandii* Gray | MM,VB |
| *debneyi* Domin. | LCS,SCS |
| *glutinosa* L. | MM,VB |
| *occidentalis* Wheeler | VC,SM,VB |
| *tabacum* L. | |
| 'Samsum' and 'White Burley' | MM |
| *Physalis floridana* Rybd. | SCS,MM |
| *Solanum* spp. | |
| *brachycarpum* Corr. | |
| (PI 275180) | MM |
| *berthaultii* Hawkes | |
| (PI 265857) | LNS,SNS,SAN |
| *chancayense* Ochoa | |
| (PI 338615) | SM,LD,SNS |
| *chacoense* Bitt. | |
| (PI 275136) | LNS,SNS,SAN |
| *curtilobum* Juz. & Buk. | |
| (PI 186181) | MM |
| *demissum* Lindl. | |
| (PI 230579) | LNS,VN,SAN |
| *demissum* × *tuberosum* L. | |
| 'A6' | VN |
| *mochicense* Ochoa | |
| (PI 283114) | SM,LD |
| *raphanifolium* Card. & Hawkes | |
| (PI 210048) | SS |
| *tuberosum* ssp. *tuberosum* L. | |
| 'Atzimba' | MM |
| 'Kennebec' | LGR,SS |
| 'Radosa' | LGR,SS |

[a] Coded symptom descriptions: LCS = local chlorotic spots; LNS = local necrotic spots; LGR = local green spots and rings; SCS = systemic chlorotic spots; SNS = systemic necrotic spots; MM = mild mosaic; SM = strong mosaic; VB = veinbanding; VC = veinclearing; LD = leaf deformation; VN = vein necrosis on leaf undersides; SAN = systemic apical necrosis; SS = symptomless systemic infection.

TABLE 2. Symptomatology of UF virus in potato cultivars inoculated by grafting

| *Solanum* species, subspecies and cultivars | Symptoms[a] | |
|---|---|---|
| | Primary | Secondary |
| *tuberosum* ssp. *tuberosum* | | |
| 'Atzimba' | MM | MM |
| 'Atlantic' | MM,C | MM,VN,C |
| 'Clavela' | SCS,LD | SCS,LD |
| 'Kennebec' | MM | S,SGS,LDr,VN |
| 'María Tropical' | SCS,LD | SCS,LD |
| 'Norland' | SS | SS |
| 'Radosa' | MM | S,SGS,LDr,VN |
| 'Wauseon' | SCS,MM,C | S,SGS,LDr,SCS,VN |
| ssp. *tuberosum* × ssp. *andigena* | | |
| 'Tomasa Condemayta' | SCS,SNS | SCS,SNS |

[a] Coded symptom descriptions: MM = mild mosaic, LD = leaf deformation, C = crinkling, S = premature senescence of lower leaves, SGS = systemic green spots and rings in lower leaves, LDr = leaf drop, VN = vein necrosis on leaf undersides, SCS = systemic chlorotic spots, SNS = systemic necrotic spots, and SS = symptomless systemic infection.

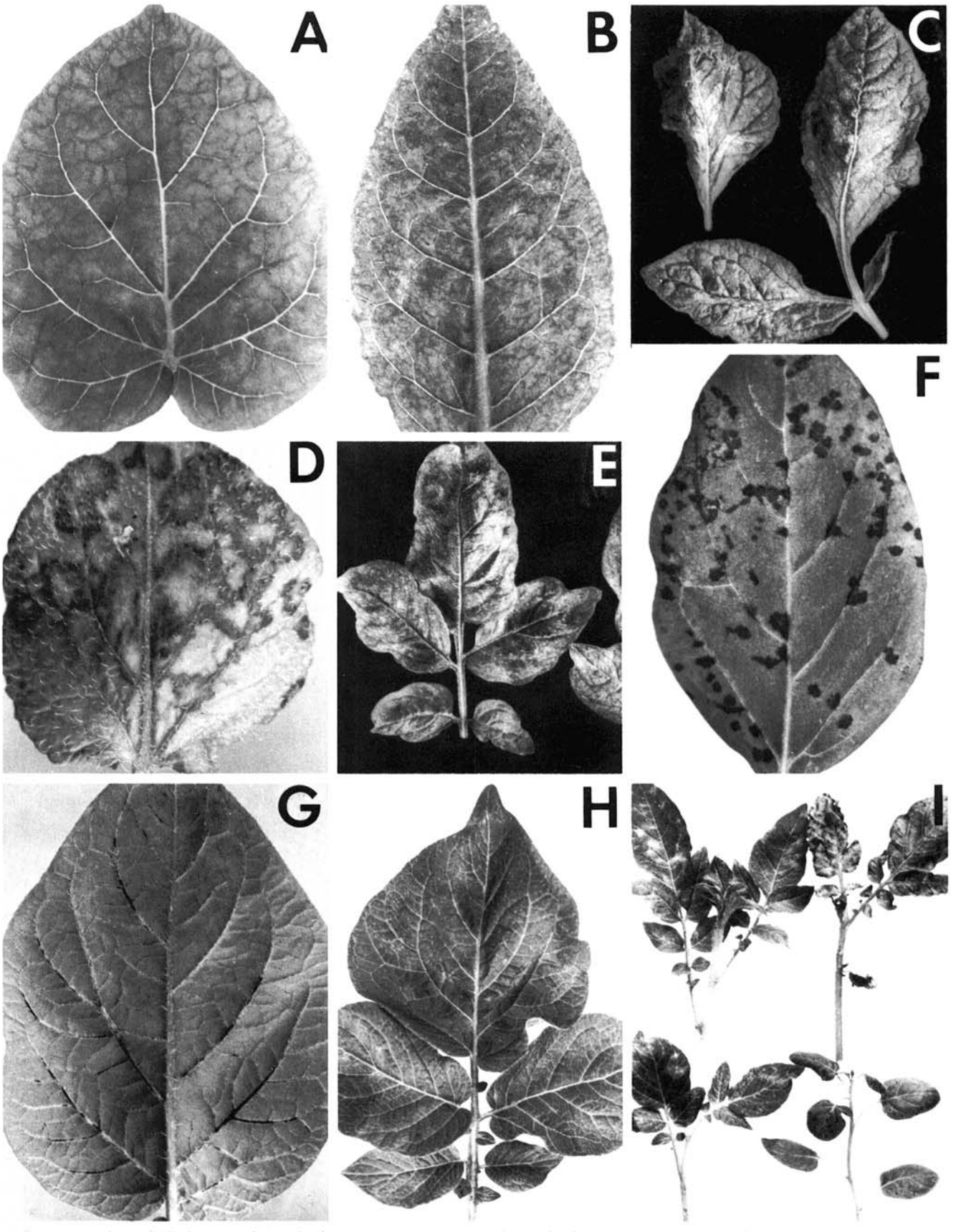


**Fig. 1.** Symptomatology of UF virus: **A,** veinbanding in *Nicotiana glutinosa*; **B,** veinbanding in *N. occidentalis*; **C,** mosaic and leaf deformation in *Solanum chancayense*; **D,** green spots and rings in potato cultivar Kennebec; **E,** chlorotic spots and leaf deformation in potato cultivar María Tropical; **F,** local necrotic spots in *S. demissum* (PI 230579); **G,** partial vein necrosis of the leaf underside in potato cultivar Atlantic; **H,** mild mosaic and crinkling in potato cultivar Wauseon; **I,** left column—systemic chlorotic spots induced by UF in potato cultivars King Edward (top) and Maris Piper (bottom); right column—systemic apical necrosis induced by $PVY^{C}$-R in the same cultivars.

Thin sections were stained with lead citrate before examination in the electron microscope.

## RESULTS

**Host range and symptomatology.** UF infected 20 solanaceous species (Table 1). The following 27 species in nine different families developed no symptoms when inoculated with UF and no virus was detected in them by backtesting to indicator hosts: Amaranthaceae—*Amaranthus edulis* L. and *Gomphrena globosa* L.; Balsaminaceae—*Impatiens balsamina* L.; Chenopodiaceae—*Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., and *C. murale* L.; Cruciferae—*Mathiola incana* (L.) R. Br.; Cucurbitaceae—*Cucumis sativus* L. and *C. melo* L.; Leguminosae—*Cajanus cajan* (L.) Millsp., *Clitorea ternatea* L., *Cyamopsis tetragonoloba* Taub., *Dolichos biflorus* L., *D. lablab* L., *Phaseolus aborigineus* Burkart, *P. acutifolius* Gray, *P. calcaratus* Roxb., *P. vulgaris* L. (cultivars 'Monroe,' 'Pinto,' and 'Top-crop'); *Vigna unguiculata* (L.) Walp., ssp. *unguiculata* 'Black,' and *V. unguiculata* ssp. *cylindrica* 'Catjang'; Papaveraceae—*Papaver* sp.; Pedaliaceae—*Sesamum indicum* L.; Solanaceae—*Capsicum annuum* L. (cultivars 'Agronomico' and 'Avelar'), *Datura stramonium* L., *Lycopersicon chilense* Dun., *L. pimpinellifolium* (Jusl.) Mill., *Solanum cardiophyllum* Lindl. (PI 275215), *S. stoloniferum* Schlechtd. (PI 230557), and *S. demissum* Lindl. (PI 175404) which reacts specifically with PVA (32).

Symptoms in indicator plants were usually mild mosaics (Table 1). The most useful hosts were *N. glutinosa* and *N. clevelandii* which developed a distinct systemic veinbanding (Fig. 1A). *N. occidentalis* reacted initially with veinclearing and later with mosaic and veinbanding (Fig. 1B). Eight of 10 wild tuber-bearing *Solanum* species inoculated with UF virus became infected systemically (Table 1). *S. chancayense* Ochoa (PI 338615) reacted with symptoms of strong mosaic, leaf deformation, and necrosis of some leaves (Fig. 1C), *S. mochicense* Ochoa (PI 283114) reacted similarly but without necrosis. *S. chacoense* Bitt. (PI 275136) and *S. demissum* Lindl. (PI 230579), both of which react hypersensitively to PVY (5,33), developed local necrotic spots followed by systemic necrosis (Fig. 1F). *Solanum berthaultii* Hawkes (PI 265857) behaved similarly. Repeated inoculations of whole plants or detached leaves of clone A6 which is a diagnostic host for PVY and PVA did not result in production of local lesions but in partial systemic necrosis of leaf veins (visible from the undersides of the leaves) and stem streaking.

Mechanical inoculations in three cultivars of *S. tuberosum* induced very mild mosaics or no visible systemic infection (Table 1). However, inoculated leaves of two cultivars became chlorotic, showed green spots or rings with a chlorotic center, and were shed from the plant. Symptoms after grafting nine cultivars with infected scions of *S. chancayense* were mainly mild mosaics (Fig. 1H) or systemic chlorotic spots (Table 2). The strongest symptoms developed in cultivars Clavela and María Tropical. These started as irregular chlorotic spots or blotches distributed along the leaflet margins which were followed by leaf deformation (Fig. 1E). When tubers harvested individually from each of the inoculated plants of clone A6 and of all cultivars that showed symptoms were planted and the plants that grew were tested for secondary infection, UF was transmitted to all tubers of cultivars Wauseon, Kennebec, Radosa, and Atlantic and clone A6, but only to 17–70% of tubers of cultivars María Tropical, Clavela, and Tomasa Condemayta. In addition to the symptoms of primary infection, secondary symptoms (Table 2) also included a premature senescence of lower leaves with the formation of green spots and rings similar to those obtained by mechanical inoculation (Fig. 1D), plus partial vein necrosis on the undersides of the leaves of cultivars Kennebec, Radosa, Wauseon, and Atlantic (Fig. 1G)

**Comparative tests of PVY$^C$ isolates in potato cultivars.** Isolates UF, PVY$^C$-Gl, and PVY$^C$-R were graft-inoculated to seven potato cultivars (Table 3). PVY$^C$-R induced a typical systemic hypersensitive reaction consisting of necrotic spots and systemic apical necrosis in cultivar King Edward which carries hypersensitivity genes *Nc* and *Na* (11,34). A similar reaction was also induced in cultivars Maris Piper and Maris Peer, but not in the other four cultivars tested which developed mosaic and/or chlorotic spots. PVY$^C$-Gl and UF both induced systemic chlorotic spots but no necrosis in cultivar King Edward (Fig. 1I), but cultivars Maris Peer and Pentland Dell reacted with apical necrosis to both isolates. Cultivar Pentland Crown reacted with necrotic spots and stem streaking and Spunta with necrotic dots and mosaic. Thus, the reactions of UF and PVY$^C$-Gl were similar in each of five cultivars, but in cultivars Maris Piper and Pentland Ivory, PVY$^C$-Gl developed necrotic spots and stem streaking, symptoms which were not observed with UF. In contrast, PVY$^C$-R induced symptoms that differed from those of UF and PVY$^C$-Gl in all cultivars tested except Maris Peer.

**Symptomatological comparisons of PVY$^C$ isolates in *Nicotiana* spp.** Plants of *N. debneyi* and *N. glutinosa* were inoculated with

TABLE 3. Reactions of some potato cultivars to graft-inoculation with UF and two isolates of PVY$^C$

| Cultivar | Host reactions[a] to: UF | PVY$^C$-Gl | PVY$^C$-R |
|---|---|---|---|
| King Edward | CS | CS | NS,SN |
| Maris Peer | NS,SN | NS,SN | NS,SN |
| Maris Piper | CS | CS,NS,S | NS,SN |
| Pentland Crown | NS,S | NS,S | M |
| Pentland Dell | NS,SN | NS,SN | CS,M |
| Pentland Ivory | CS | CS,NS,S | CS,M |
| Spunta | ND,M | ND,M | CS,M |

[a] Coded symptom descriptions: CS = chlorotic spots; M = mosaic; ND = necrotic dots; NS = necrotic spots; SN = systemic necrosis and death of growing points; and S = streaking of stems.

TABLE 4. Reactions of six indicator hosts to mechanical inoculation with UF, some PVY strains, Peru tomato virus, and wild potato mosaic virus[a]

| Host | Reactions[b] to: PVY$^O$ | PVY$^C$-R | PVY$^C$-AB | PVY$^C$-Gl | UF | PTV-M4 | WPMV |
|---|---|---|---|---|---|---|---|
| *Solanum chancayense* Ochoa (PI 338615) | M,D | M,D | M,D | M,D | M,D | M,D | M,D |
| *S. chacoense* Bitt. (PI 275136) | NS | NS | NS | NS | NS | 0 | – |
| *S. demissum* Lindl. (PI 230579) | NS | NS | NS | NS | NS | 0 | 0 |
| *S. demissum* × *S. tuberosum 'A6'* | NS | M | VN | VN | VN | 0 | 0 |
| *Lycopersicon esculentum* 'Marglobe' | SS | SS | SS | SS | SS | M,D | SS |
| *Capsicum annuum* 'Avelar' | 0 | 0 | 0 | 0 | 0 | E,NS | 0 |

[a] Back inoculations to *Nicotiana occidentalis* were made to confirm the presence of the different viruses.
[b] Coded symptom descriptions: M = mosaic, D = leaf deformation, E = epinasty, NS = local and/or systemic necrotic spots, VN = vein necrosis from the undersides, SI = symptomless infection in inoculated leaves only, SS = symptomless systemic infection, 0 = not infected, and – = not tested.

isolates PVY$^O$, PVY$^C$-R, PVY$^C$-AB, and PVY$^C$-Gl to see if the symptoms produced were similar to those caused by UF. Like UF, PVY$^C$-Gl and PVY$^C$-AB produced local and systemic chlorotic spots or rings in *N. debneyi* similar to those obtained by Calvert et al (9), but the symptoms were stronger than with UF. In contrast, PVY$^O$ and PVY$^C$-R both induced veinclearing and mosaic. In *N. glutinosa*, PVY$^C$-Gl and PVY$^C$-AB produced a uniform veinbanding, which was somewhat milder than that caused by UF. In contrast, PVY$^O$ and PVY$^C$-R induced mainly mild mosaic and leaf rugosity or crinkling. The symptoms of PVY$^C$-Gl and PVY$^C$-AB, therefore, closely resemble those of UF in these two *Nicotiana* species.

**Symptomatological comparisons with other potyviruses.** PVY$^C$-R, PVY$^C$-AB, PVY$^C$-Gl, PTV-M4, WPMV, and an isolate of PVY$^O$ were mechanically inoculated to three *Solanum* spp., clone A6, tomato, and peppers to see if they induced symptoms similar to those of UF (Table 4). Like UF, PVY$^O$, PVY$^C$-R, PVY$^C$-AB, and PVY$^C$-Gl all caused local necrotic spots in *S. chacoense* and *S. demissum* PI 230579, hosts that have been reported to react to PVY with local lesions (5,33). In clone A6, only PVY$^O$ induced the typical necrotic lesions reported for this virus (21); PVY$^C$-R, PVY$^C$-Gl, and PVY$^C$-AB all infected clone A6 without causing symptoms in inoculated leaves but the latter two isolates produced the same systemic vein necrosis of leaf undersides as did UF. There are only two previous reports of PVY strains that do not induce local lesions in clone A6, a PVY$^O$ strain (7) and PVY$^C$-AB (9). PTV and WPMV did not infect *S. demissum* and clone A6; this reaction differentiated them from the other viruses tested. In addition, PTV and WPMV could be differentiated from one another because only the former induced strong symptoms in tomato cultivar Marglobe and pepper cultivar Avelar as previously reported (12,13). In contrast, WPMV infected only tomato causing symptomless infection. UF behaved similarly to PVY$^C$-Gl and PVY$^C$-AB in all hosts tested, but differed from the other viruses in one or more of the hosts.

**Aphid transmission.** UF was transmitted by *M. persicae* from infected to healthy *N. occidentalis* by using acquisition feeding periods of 15–30 sec, but only when a large number of aphids were used (~20 per test plant). When groups of five viruliferous aphids were transferred from source leaves infected with UF, PVY$^C$-R or PVY$^C$-Gl to individual test plants, no transmission was detected with UF and PVY$^C$-R, but 7 of 10 plants became infected with PVY$^C$-Gl and 9 of 10 with PVY$^O$. In further experiments with acquisition feeding periods of 1.5–2.0 min and groups of five aphids per test plant, no transmission was obtained with UF and PVY$^C$-R. Unlike PVY$^C$-Gl and PVY$^C$-AB which are readily aphid transmitted (9), UF resembles some strains of group PVY$^C$ in being difficult to transmit by aphids when no helper virus is present (4). However, the inefficiency of transmission with UF perhaps could also be attributed to the maintenance of the culture in indicator hosts for a long period of time by manual inoculation resulting in decreased efficiency of aphid transmission as has been reported previously with other aphid transmitted viruses (1,19,26).

**Cross protection.** Groups of four *N. tabacum* cultivar White Burley plants infected with UF were mechanically inoculated with PVY$^O$ and PVY$^N$. Similar groups of healthy plants were also inoculated as checks. After 2 wk, plants inoculated with PVY$^O$ showed the same veinbanding as the plants infected with PVY$^O$ alone. Similarly, plants inoculated with PVY$^N$ showed the same vein necrosis as those infected only with PVY$^N$. UF therefore does not protect tobacco plants from infection with PVY$^O$ and PVY$^N$ strains, and resembles PVY$^C$-AB in this respect (9).

**Electron microscopy.** Virus particles in sap preparations were measured on enlarged electron micrographs of negatively stained grids, classified as to lengths at intervals of 10 nm and a histogram

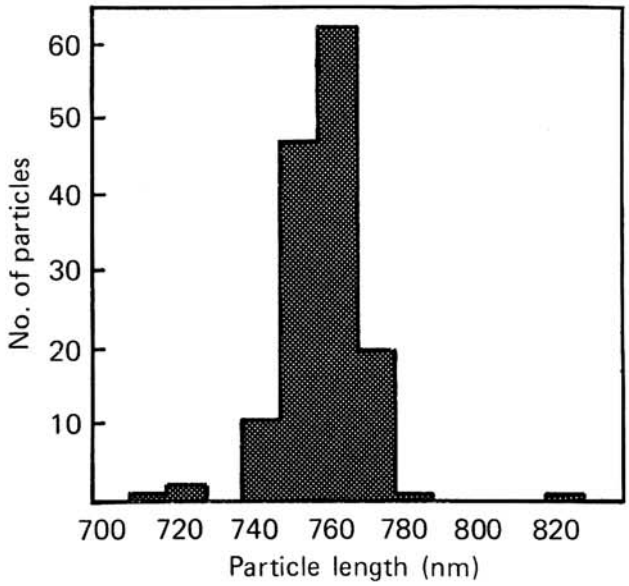


**Fig. 2.** Histogram particle length distribution of UF virus in crude preparations from infected *Nicotiana occidentalis*.

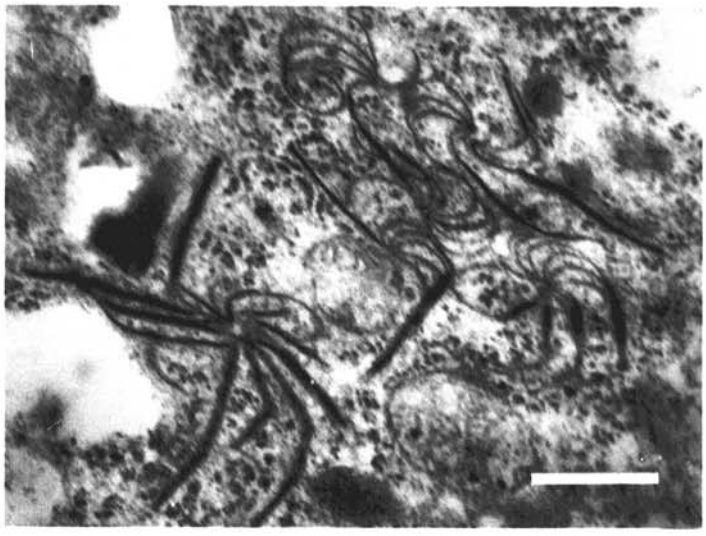

**Fig. 3.** Electron micrograph of ultrathin sections of UF virus-infected tissue of *Nicotiana occidentalis* showing pinwheel inclusions. Bar = 500 nm.

TABLE 5. Homologous and heterologous serological reactions between UF virus, some PVY strains, PVA, and wild potato mosaic virus

| | Antigen | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Antiserum | PVY$^O$ | PVY$^C$-R | PVY$^N$-R | PVY$^C$-AB | PVY$^C$-Gl | UF | WPMV | PVA |
| PVY$^O$ | 32,000[a] | 32,000 | 8,000 | 128 | 64 | 32 | 64 | ··· |
| PVY$^N$-R | 4,096 | 8,000 | 8,000 | 8 | 32 | 8 | 256 | ··· |
| PVY$^C$-Gl | 8 | 4 | 0 | 2,048 | 4,096 | 1,024 | 256 | ··· |
| PVA | ··· | ··· | ··· | ··· | ··· | 0 | ··· | 128 |
| UF | 16 | 32 | 0 | 2,048 | 2,048 | 2,048 | 16 | 8 |
| WPMV | 64 | 32 | 64 | 512 | 256 | 128 | 4,096 | ··· |

[a] Reciprocal values of titers in microprecipitin grid tests. ··· = not tested.

of the particle length distribution was prepared. The normal length based on 148 particles that corresponded to the main maximum (8) was 760 nm (Fig. 2). Thin sections of tissue revealed the presence of pinwheels typical of the potyviruses group (10) (Fig. 3).

**Purification and serology.** Final purified preparations were apparently free from impurities when observed with the electron microscope. Their $A_{260}/A_{280}$ ratios were about 1.31–1.35. The value obtained for a preparation after rate zonal centrifugation in sucrose gradients was 1.25. These ratios are within the range of 1.2–1.37 previously reported for other potyviruses (22,23,27). Virus yields based on an extinction coefficient $E^{0.1\%}_{1\,cm}$ 260 nm of 2.8 (28) were 13–30 mg/kg of fresh leaves. Partially purified preparations did not react against a PVA antiserum with a titer of 1/128 (14).

UF antiserum obtained from bleedings taken 3 wk after the first injection had a titer of 1/2,048 in microprecipitation grid titrations using purified antigen. A close serological relationship was demonstrated among $PVY^O$, $PVY^C$-R, and $PVY^N$-R (Table 5). This close relationship among the three recognized PVY strain groups was expected (7). Similarly $PVY^C$-AB, $PVY^C$-Gl, and UF showed a close mutual relationship. However, the first group showed a distant relationship with the second group in reciprocal reactions. These results have been confirmed by direct ELISA using monoclonal and polyclonal antibodies against a strain of $PVY^N$ (16). The relationships are more clearly seen when the viruses are listed according to their serological differentiation indices (SDIs) which are considered reliable for distinguishing serological cross-reactivity between viruses (29–31) (Table 6). Values estimated were average SDIs obtained from reciprocal tests (RT-SDIs) (20). RT-SDIs for UF–$PVY^C$-Gl and for $PVY^O$–$PVY^N$-R were low (1.0–1.5) indicating a very close relationship. By contrast, values for UF–$PVY^O$, $PVY^C$-Gl–$PVY^O$, $PVY^C$-Gl–$PVY^N$-R, and UF–$PVY^N$-R were between 8.5 and 10.5, all far higher than 4.0 which is the threshold commonly used in deciding whether serological relationships between two viruses are distant (30). It can also be seen that the SDIs among $PVY^C$-Gl, $PVY^N$-R, UF, $PVY^O$, and WPMV place WPMV as intermediate between the two virus groups.

## DISCUSSION

UF resembles other members of the potyvirus group in particle size and shape, in its properties in infective sap, in being acquired in brief probes by aphids and in inducing pinwheel inclusions in infected cells. It seems to be a third potyvirus from potato because it is very distantly related serologically to PVY and differs from this virus in having a host range apparently restricted to the Solanaceae, and in its symptomatology in certain indicator hosts. Besides, the symptoms of mild mosaics, systemic chlorotic spots, partial vein necrosis of the leaf undersides, and premature senescence of lower leaves induced by UF in some cultivars do not resemble the more necrotic type of symptoms of $PVY^O$ and are more severe than those induced by $PVY^N$. Also, they are different from the stipple-streak or systemic apical necrosis induced by $PVY^C$ in many cultivars (4,6,18). Similarly, UF differs from PVA in its lack of serological reaction with PVA/As, in the type of symptoms induced in potato cultivars (18) and because it remains infectious in sap for a much longer time (3). However, UF closely resembles $PVY^C$-AB (9) and $PVY^C$-Gl (7,24) in symptomatology and in being serologically closely related to them. WPMV, a virus found in the wild potato species *S. chancayense* and that does not infect cultivated potato (17) was intermediate serologically between PVY and the UF, $PVY^C$-Gl, and $PVY^C$-AB group. We propose the name potato virus V (PVV) for this new group of strains.

Previous workers found that $PVY^C$-Gl (7,25) and $PVY^C$-AB (9) were only very distantly related to PVY; however, they still classed it as a member of the $PVY^C$ strain group. This was presumably because both isolates caused hypersensitive reactions when inoculated to potato cultivars known to carry the Nc gene to which all members of the strain group respond hypersensitively (11,34). The results of our grafting experiments clearly show that UF and $PVY^C$-Gl do not respond to Nc. For example, in cultivar King Edward in which isolate $PVY^C$-R gave a strong hypersensitive response, UF and $PVY^C$-Gl induced only systemic chlorotic spots. Also, in Pentland Dell $PVY^C$-R gave only a mosaic but UF and $PVY^C$-Gl both gave a severe hypersensitive response. Indeed, UF and $PVY^C$-Gl seemed to be responding to a different hypersensitivity gene (or genes) which was present with Nc only in one of the cultivars tested, Maris Peer.

Although UF, $PVY^C$-Gl, and $PVY^C$-AB may resemble PVY and PVA by the symptoms they induce in some indicator plants and potato cultivars, they can be clearly distinguished as isolates of a different virus primarily because their distant serological relationship with both PVY and PVA is reciprocal, not one-sided (3) and because they induce a hypersensitive response in potato cultivars susceptible to PVY and PVA (9) and symptoms of mosaic in cultivars that carry the hypersensitivity genes *Nc* or *Na* (eg, cultivar King Edward).

Calvert et al showed that in Northern Ireland $PVY^C$-AB was only common in a restricted number of mostly old cultivars. If this is true for most European countries, it is presumably another reason why the virus has always been confused with PVY in the past. Little is known of its dissemination in the Andean region or in the other parts of the world outside western Europe. However, it is likely to be present everywhere and care should be taken not to overlook it in routine tests in certification schemes that rely on using clone A6 or on antisera to PVY or PVA, none of which will detect it.

TABLE 6. Serological relationships between UF virus, some PVY strains, PVA, and wild potato mosaic virus as determined by the serological differentiation index

| Antigen 1 | Antigen 2 | RT-SDI[a] |
|---|---|---|
| UF | $PVY^C$-Gl | 1.0 |
| $PVY^O$ | $PVY^N$-R | 1.5 |
| $PVY^C$-Gl | WPMV | 4.0 |
| $PVY^N$-R | WPMV | 5.5 |
| UF | WPMV | 6.0 |
| $PVY^O$ | WPMV | 7.5 |
| UF | PVA | 7.5 |
| UF | $PVY^O$ | 8.5 |
| $PVY^C$-Gl | $PVY^O$ | 9.0 |
| $PVY^C$-Gl | $PVY^N$-R | 10.0 |
| UF | $PVY^N$-R | 10.5 |

[a]Obtained by averaging SDI values from reciprocal tests (RT).

## LITERATURE CITED


1. Badami, R. S. 1958. Changes in the transmissibility by aphids of a strain of cucumber mosaic virus. Ann. Appl. Biol. 46:554-562.
2. Ball, E. M. 1974. Serological tests for the identification of plant viruses. The American Phytopathological Society, St. Paul, MN. 31 pp.
3. Bartels, R. 1971. Potato Virus A. Descriptions of Plant Viruses No. 54. Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England.
4. Beemster, A. B. R., and Rozendaal, A. 1972. Potato viruses: properties and symptoms. Pages 115-143 in: Viruses of Potatoes and Seed-Potato Production. J. A. de Bokx, ed. PUDOC, Wageningen, The Netherlands. 233 pp.
5. Bokx, J. A. de 1974. Some remarks on the use of $TE_1$ (*Solanum chacoense*) as a test plant for potato virus Y. Potato Res. 17:323-326.
6. Bokx, J. A. de 1980. Potato Virus Y. Pages 70-71 in: Compendium of Potato Diseases. W. J. Hooker, ed. American Phytopathological Society, St. Paul, MN. 125 pp.
7. Bokx, J. A. de, Kratchanova, B., and Maat, D. Z. 1975. Some properties of a deviating strain of potato virus Y. Potato Res. 18:38-51.
8. Brandes, J. 1966. Identification of plant viruses by electron microscopy. Pages 218-229 in: Viruses of Plants. A. B. R. Beemster and J. Dijkstra, eds. North Holland Publ. Co., Amsterdam. 342 pp.
9. Calvert, E. L., Cooper, P., and McClure, J. 1980. An aphid transmitted strain of $PVY^C$ recorded in potatoes in Northern Ireland. Rec. Agric. Res. 28:63-74.
10. Christie, R. G., and Edwardson, J. R. 1977. Light and electron microscopy of plant viruses. Fl. Agric. Exp. Stn., Monogr. Ser. 9. 155 pp.
11. Cockerham, G. 1943. The reaction of potato varieties to viruses X, A, B and C. Ann. Appl. Biol. 30:338-344.
12. Fernandez-Northcote, E. N., and Fulton, R. W. 1980. Detection and

characterization of Peru tomato virus strains infecting pepper and tomato in Peru. Phytopathology 70:315-320.
13. Fribourg, C. E. 1979. Host plant reactions, some properties, and serology of Peru tomato virus. Phytopathology 69:441-445.
14. Fribourg, C. E., and de Zoeten, G. A. 1970. Antiserum preparation and partial purification of potato virus A. Phytopathology 60:1415-1421.
15. Fribourg, C. E., and Nakashima, J. 1981. Propiedades de un aislamiento de PVY que no induce lesions locales en el clon de papa A6. (Abstr.) Fitopatología 17:10.
16. Gugerly, P., and Fries, P. 1983. Characterization of monoclonal antibodies to potato virus Y and their use for virus detection. J. Gen. Virol. 64:2471-2477.
17. Jones, R. A. C., and Fribourg, C. E. 1979. Host plant reactions, some properties, and serology of wild potato mosaic virus. Phytopathology 69:446-449.
18. Jones, R. A. C., Fribourg, C. E., and Slack, S. A. 1982. Potato Virus and Virus-Like Diseases. Set No. 2, in: Plant Virus Slide Series. O. W. Barnett and S. A. Tolin, eds. Clemson University, Clemson, SC. 59 pp. (140 slides).
19. Kamm, J. A. 1969. Change in transmissibility of bean yellow mosaic virus by aphids. Ann. Entomol. Soc. Am. 62:47-50.
20. Koenig, R. 1976. A loop-structure in the serological classification system of tymoviruses. Virology 72:1-5.
21. Köhler, E. 1953. Der *Solanum demissum* bastard "A6" als testpflanze verschiedener Mosaikviren. Züchter 23:173-176.
22. Makkouk, K. M., and Gumpf, D. J. 1976. Characterization of potato virus Y strains isolated from pepper. Phytopathology 66:576-581.
23. Moghal, S. M., and Francki, R. I. B. 1976. Towards a system for the identification and classification of potyviruses. Virology 73:350-362.
24. Roberts, I. M., and Harrison, B. D. 1979. Detection of potato leaf roll and potato mop-top viruses by immunosorbent electron microscopy. Ann. Appl. Biol. 93:289-297.
25. Rozendaal, A., Binsbergen, J. van, Anema, B., Slogteren, D. H. M. van, and Bunt, M. H. 1971. Serology of a deviating potato virus $Y^C$ strain in the potato variety Gladblaadje. (Abstr.) Potato Res. 14:241.
26. Sako, N. 1980. Loss of aphid-transmissibility of turnip mosaic virus. Phytopathology 70:647-649.
27. Shepherd, R. J., and Purcifull, D. E. 1971. Tobacco etch virus. Descriptions of Plant Viruses No. 55. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England.
28. Stace-Smith, R., and Tremaine, J. H. 1970. Purification and composition of potato virus Y. Phytopathology 60:1785-1789.
29. Van Regenmortel, M. H. V. 1975. Antigenic relationships between strains of tobacco mosaic virus. Virology 64:415-420.
30. Van Regenmortel, M. H. V. 1982. Serology and immunochemistry of plant viruses. Academic Press, New York. 302 pp.
31. Van Regenmortel, M. H. V., and von Wechmar, M. B. 1970. A re-examination of the serological relationship between tobacco mosaic virus and cucumber virus 4. Virology 41:330-338.
32. Webb, R. E., and Buck, R. W., Jr. 1955. A diagnostic host for potato virus A. Am. Potato J. 32:248-252.
33. Webb, R. E., and Wilson, D. R. 1978. *Solanum demissum* PI 230579 a true seed diagnostic host for potato virus Y. Am. Potato J. 55:15-23.
34. Wiersema, H. T. 1972. Breeding for resistance. Pages 174-187 in: Viruses of Potatoes and Seed Potato Production. J. A. de Bokx, ed. PUDOC, Wageningen, The Netherlands. 233 pp.