The Biology and Phylogenetics of *Potato virus S* Isolates from the Andean Region of South America

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Abstract

Biological characteristics of 11 *Potato virus S* (PVS) isolates from three cultivated potato species (*Solanum* spp.) growing in five Andean countries and 1 from Scotland differed in virulence depending on isolate and host species. Nine isolates infected *Chenopodium quinoa* systemically but two others and the Scottish isolate remained restricted to inoculated leaves; therefore, they belonged to biologically defined strains PVS^A and PVS^O, respectively. When nine wild potato species were inoculated, most developed symptomless systemic infection but *Solanum megistacrolobum* developed systemic hypersensitive resistance (SHR) with one PVS^O and two PVS^A isolates. Andean potato cultivars developed mostly asymptomatic primary infection but predominantly symptomatic secondary infection. In both wild and cultivated potato plants, PVS^A and PVS^O elicited similar foliage symptoms. Following graft inoculation, all except two PVS^O isolates were detected in partially PVS-resistant cultivar Saco, while clone Snec 66/139-19 developed SHR with two isolates each of PVS^A and PVS^O. *Myzus persicae* transmitted all nine PVS^A isolates but none of the three PVS^O isolates. All 12 isolates were transmitted by plant-to-plant contact. In infective sap, all isolates had thermal inactivation points of 55 to 60°C. Longevities in vitro were 25 to 40 days with six PVS^A isolates but less than 21 days for the three PVS^O isolates. Dilution end points were 10⁻³ for two PVS^O isolates but 10⁻⁴ to 10⁻⁶ with the other isolates. Complete new genome sequences were

obtained from seven Andean PVS isolates; seven isolates from Africa, Australia, or Europe; and single isolates from *S. muricatum* and *Arracacia xanthorrhiza*. These 17 new genomes and 23 from GenBank provided 40 unique sequences; however, 5 from Eurasia were recombinants. Phylogenetic analysis of the 35 nonrecombinants revealed three major lineages, two predominantly South American (SA) and evenly branched and one non-SA with a single long basal branch and many distal subdivisions. Using least squares dating and nucleotide sequences, the two nodes of the basal PVS trifurcation were dated at 1079 and 1055 Common Era (CE), the three midphylogeny nodes of the SA lineages at 1352, 1487, and 1537 CE, and the basal node to the non-SA lineage at 1837 CE. The *Potato rough dwarf virus/Potato virus P* (PVS/PRDV/PVP) cluster was sister to PVS and diverged 5,000 to 7,000 years ago. The non-SA PVS lineage contained 18 of 19 isolates from *S. tuberosum* subsp. *tuberosum* but the two SA lineages contained 6 from *S. tuberosum* subsp. *andigena*, 4 from *S. phureja*, 3 from *S. tuberosum* subsp. *tuberosum*, and 1 each from *S. muricatum*, *S. curtilobum*, and *A. xanthorrhiza*. This suggests that a potato-infecting proto-PVS/PRDV/PVP emerged in South America at least 5,000 years ago, became endemic, and diverged into a range of local *Solanum* spp. and other species, and one early lineage spread worldwide in potato. Preventing establishment of the SA lineages is advised for all countries still without them.

More than 50 viruses infect the potato crop, and *Potato virus S* (PVS; family *Betaflexiviridae*, genus *Carlavirus*) is one of the most widespread globally. It often causes mild symptoms or infects potato plants without producing symptoms; however, more severe symptoms develop following infection with its virulent strains, which are less common. When present alone, PVS diminishes tuber size and, although the yield losses it causes are normally minor, they can reach 20% (de Bokx and van der Want 1987; Jones 2014; Loebenstein et al. 2001; Stevenson et al. 2001; Valkonen 2007). Mixed infection with other viruses occurs commonly and may exacerbate the severity

of infection (Loebenstein et al. 2001); for example, coinfection with *Potato virus X* (family *Alphaflexiviridae*, genus *Potexvirus*) can increase the concentration of PVS virions in infected plants, resulting in enhanced foliar symptom expression (Nyalugwe et al. 2012). Because PVS is readily contact transmitted and its foliage symptoms are often subtle or absent, it is difficult to manage in seed potato production schemes, especially when these schemes depend entirely on visual observation for symptoms during crop inspections. Therefore, it is often the first virus to reinvade healthy seed potato stocks. Testing samples for PVS during the early phase of potato seed stock multiplication allows infected clones to be removed, and rigorous hygiene measures reduce contact transmission (de Bokx and van der Want 1987; Loebenstein et al. 2001; Stevenson et al. 2001). Breeding new potato cultivars for PVS resistance is possible by incorporating PVS hypersensitive resistance gene *Ns* (Baerecke 1967a; Makarov 1975; Pietrak 1985; Rothacker 1968) or the polygenic PVS resistance in cultivar Saco (Alfieri 1960; Alfieri and Stouffer 1957; Baerecke 1967b; Bagnall 1965; Bagnall and Young 1972; Larson and Oshima 1959). Several European potato breeding programs have used gene *Ns* for this purpose (Dziewonska and Pochitonow 1971; MacArthur 1986; Marczewski et al. 1998, 2002).

PVS was first reported infecting potato in Europe in 1952 (Wetter 1971), and soon afterward in potato from the Andean region of South America (McKee 1964). The first PVS isolates from the Andean region caused systemic infection in *Chenopodium quinoa* whereas those from elsewhere did not; therefore, the former were called PVS^A (Andean) and the latter PVS^O (ordinary). Although PVS^A

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was initially found only in the Andean region of South America (Hinostroza-Orihuela 1973; Jones 1981; Jones et al. 1982; Santillan 1979; Santillan et al. 1980), PVS isolates that invaded *Chenopodium* spp. systemically were found subsequently in Australia, Brazil, India, New Zealand, the United States, and several European countries (Dolby and Jones 1987, 1988; Duarte et al. 2012; Fletcher 1996; Garg and Vinayak 2000; Jones et al. 2009; Lambert et al. 2012; Rose 1983; Slack 1983; Weidemann and Koenig 1990). Despite this, the name PVS^A was kept for them. PVS^A was reported to be more efficiently aphid-borne than PVS^O and produce more severe foliage symptoms in potato plants (Dolby and Jones 1987; Rose 1983; Slack 1983; Wardrop et al. 1989). Also, the N-terminal ends of its nucleotide-binding protein (11K), 7K protein, and coat protein (CP) sequences contained blocks of amino acids which varied, to which differences in biological properties between PVS^A and PVS^O were attributed (Foster 1991; Foster and Mills 1992; Foster et al. 1990; Matoušek et al. 2000, 2005).

The same PVS^A and PVS^O nomenclature was adopted for the two major phylogroups found when CP gene sequences of PVS isolates were analyzed. However, when replicase gene sequences from European isolates defined biologically as belonging to PVS^O (20 isolates) and PVS^A (15 isolates) were examined by molecular probing, they all belonged to phylogroup PVS^O regardless of whether they invaded *C. quinoa* systemically (Matoušek et al. 2005). The term PVS^{CS} (CS = *Chenopodium* systemic) was therefore suggested to distinguish them. When the CP nucleotide sequences of 23 PVS isolates were subjected to phylogenetic analysis, they separated into major phylogroups, PVS^O (20 isolates) and PVS^A, the former subdividing into two minor phylogroups (Chikh Ali et al. 2008). However, there was disagreement between PVS^A defined biologically and PVS^A defined genetically. When Cox and Jones (2010) sequenced the CP of three biologically defined PVS^A isolates from Europe, they grouped with PVS^O isolates. They suggested use of the acronym PVS^{O-CS} for isolates that behave as PVS^O on phylogenetic analysis but spread

Table 1. Origins of *Potato virus S* isolates used in this study

Isolate sequences	Source species	Cultivar or breeding line name ^a	Germplasm accession number	Where collected or obtained	Isolation year	GenBank code	Isolate reference
New							
Co-1	<i>Solanum phureja</i>	Cuyo Blanco	CCC-4521	Ipiales, Narino, Colombia	1976	MF418035	Santillan (1979)
E-1	<i>S. tuberosum</i> subsp. <i>andigena</i>	Harbur	...	National Germplasm Bank (NGB), Santa Catalina, Ecuador	1976	MF418028	Santillan (1979)
E-2	<i>S. tuberosum</i> subsp. <i>andigena</i>	Maria	...	NGB, Santa Catalina, Ecuador	1976	MF418029	Santillan (1979)
E-3	<i>S. tuberosum</i> subsp. <i>andigena</i>	Santa Catalina	...	NGB, Santa Catalina, Ecuador	1976	No sequence	Santillan (1979)
E-4	<i>S. tuberosum</i> subsp. <i>andigena</i>	Puno	...	NGB, Santa Catalina, Ecuador	1976	No sequence	Santillan (1979)
P-1	<i>S. tuberosum</i> subsp. <i>andigena</i>	SA-289A	CIP-700021	National Potato Program, La Molina, Peru	1976	No sequence	Santillan (1979)
P-2	<i>S. curtilobum</i>	Yuracc Mallku	CIP-702116	Canas y Canchis, Cuzco, Peru	1976	MF418032	Santillan (1979)
B-1	<i>S. tuberosum</i> subsp. <i>andigena</i>	Quchulli	OCH-10581	Pacasi, Linares, Potosi, Bolivia	1976	MF418023	Santillan (1979)
B-2	<i>S. tuberosum</i> subsp. <i>andigena</i>	Sacampaya	OCH-10505	Azangaro, Frias, Potosi, Bolivia	1976	MF418024	Santillan (1979)
B-3	<i>S. tuberosum</i> subsp. <i>andigena</i>	Florentino	OCH-10584	Alcatuyo, Linares, Potosi, Bolivia	1976	MF418025	Santillan (1979)
CH-1	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	Cordillera	UA-0205	Isle of Chiloe, Chile	1976	MF418027	Santillan (1979)
S-1	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	G.429869	CIP-800877	Pentlandfield, Midlothian, Scotland	1976	MF418033	Santillan (1979)
ALV	<i>Arracacia xanthorrhiza</i>	Arracacha cultivar unknown	...	Huanuco, Peru	1975	MF496659	Phillips (1990)
PepLV	<i>S. muricatum</i>	Pepino cultivar unknown	...	Auckland, North Island, New Zealand (pepino cuttings from Chile)	1976	MF418034	Thomas et al. (1980); Dolby and Jones (1988)
Exodus	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	Exodus	...	NIAB, Cambridge, England (tuber from The Netherlands)	1984	MF418030	Dolby and Jones (1987)
78-22	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	78-22	...	England (tuber from The Netherlands)	1985	MF418022	Dolby and Jones (1987)
BA-365-4	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	BA-365-4	...	England (tuber from Germany)	1985	MF418026	Dolby and Jones (1987)
24	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	N/A	...	England (tuber from The Netherlands or Germany)	1985	MF418021	Dolby and Jones (1987)
Qld-1 (= BC22)	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	Kipfler	...	Perth, Australia (tuber from Queensland)	2008	MF375506	This study
Ken-1	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	N/A	...	Bomets region, Kenya	2013	MF418031	This study
GenBank WaDef	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	Defender	...	Othello, Washington State, United States	2008	FJ813512	Lin et al. (2009)

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^a N/A = not available.

^b Duplicates.

systemically in *Chenopodium* spp. The acronym PVS^{A-CL} (CL = *Chenopodium* localized) was suggested for any isolates found that fit within PVS^O biologically but within PVS^A on phylogenetic analysis (Cox and Jones 2010). When Lambert et al. (2012) compared the CP sequences of 17 biologically defined Australian PVS^O or PVS^A isolates, only PVS^O and PVS^{O-CS} were found.

Complete genomes of PVS isolates—16 of phylogenetically defined PVS^O and 7 of phylogenetically defined PVS^A—are available currently on GenBank. The PVS^O genomes come from China (YN and HB24), Australasia (Sw-14 and NZ-O), North America (Id4106 and WaDef), an unknown country (GAF318-16.1), and Europe (9 isolates); and the PVS^A genomes are from China (HB7), Europe (Vltava), New Zealand (NZ-A), and the Andean region (BB-AND, RVC, RL5, and Antioquia) (Blouin et al. 2016; Duarte et al. 2012; Gutiérrez et al. 2012, 2013; Lin et al. 2009, 2014; Matoušek et al. 2005; Pájtli 2015; Vallejo et al. 2016; Wang et al. 2016). The Vltava phylogenetic PVS^A isolate behaves as a PVS^O isolate biologically (Matoušek et al. 2000) but was found to be a recombinant between phylogroups PVS^O and PVS^A (Duarte et al. 2012). Therefore, it fits into the PVS^{A-CL} category.

In 1976 to 1978, biological properties of 11 PVS isolates from several potato species (*Solanum* spp.) from five Andean region countries

ranging from Colombia in the north to Chile in the south, including the potato's domestication center, and 1 from Scotland were studied. Nine Andean isolates belonged to biologically defined PVS^A and the other two Andean plus the Scottish isolate to biologically defined PVS^O. The results of biological studies with these 12 isolates were reported in an M.Sc. thesis in Spanish (Santillan 1979) and a brief abstract (Santillan et al. 1980) but never published in a refereed scientific journal. Subsequently, Dolby and Jones (1987) found biologically defined PVS^A in addition to PVS^O isolates in European potato samples and reported their biological properties. The aims of this article are to report the findings of (i) the original study on the biological properties of the 11 Andean isolates and the Scottish isolate (Santillan 1979) and (ii) our subsequent investigation of the phylogenetics of 17 new complete PVS genomes from nine of the isolates studied by Santillan (1979), four European potato isolates studied by Dolby and Jones (1988), and single PVS isolates, first from two Andean crops, *Solanum muricatum* (pepino) and the apiaceous root vegetable *Arracacia xanthorrhiza* (arracacha, Peruvian parsnip) (Dolby and Jones 1988; Hermann and Heller 1997; Phillips 1990; Thomas et al. 1980), and second from potato from Australia and Kenya. With aim (i), their host reactions following inoculation to indicator host species and both wild and

Table 1. (Continued from previous page)

Isolate sequences	Source species	Cultivar or breeding line name ^a	Germplasm accession number	Where collected or obtained	Isolation year	GenBank code	Isolate reference
Id4106	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	4106	A95053-61	Idaho, United States	2008	FJ813513	Lin et al. (2009)
Sw-14	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	Royal Blue	...	Myalup, Western Australia	2014	KP089978	...
NZ-O	<i>S. tuberosum</i> subsp. <i>andigena</i>	Tutai Kuri	(Maori cultivar)	Lincoln, New Zealand	2014	KU058656	Blouin et al. (2016)
NZ-A	<i>S. tuberosum</i> subsp. <i>andigena</i>	Tutai Kuri	(Maori cultivar)	Lincoln, New Zealand	2014	KU058657	Blouin et al. (2016)
YN	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	N/A	...	Yunnan, China	2010	KC430335	...
GAF318-16.1	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	393046.7	...	Unknown	<2001	KU586451	...
HB24	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	N/A	...	Hubei, China	2012	KU896945	Wang et al. (2016)
HB7	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	N/A	...	Hubei, China	2012	KU896946	Wang et al. (2016)
Leona ^b	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	Leona	...	Germany	<2004	AJ863509	Matoušek et al. (2005)
Leona ^b	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	Leona	...	Germany	<2004	NC007289	Matoušek et al. (2005)
Vltava	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	Vltava	...	Czech Republic	<2004	AJ863510	Matoušek et al. (2005)
89.249 (Hu1)	<i>Solanum</i> sp.	89.249	...	Kezthely genebank, Hungary	2009-2014	HF571059	Pájtli (2015)
Alex	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	Finka	...	Kiev, Ukraine	2014	LN851189	Pájtli (2015)
Bonita	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	Bonita ojo de perdiz	...	Budapest, Hungary	2014	LN851190	Pájtli (2015)
09.362	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	09.362	...	Kezthely genebank, Hungary	2009-2014	LN851191	Pájtli (2015)
Valery	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	Finka	...	Kiev, Ukraine	2014	LN851192	Pájtli (2015)
Irena	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	Finka	...	Kiev, Ukraine	2014	LN851193	Pájtli (2015)
Ewa	<i>S. tuberosum</i> subsp. <i>tuberosum</i>	Leona	...	Mlochow, Poland	2014	LN851194	Pájtli (2015)
BB-AND	<i>S. tuberosum</i>	N/A	...	Bueno Brandao, Minas Gerais, Brazil (tubers from Chile)	2008	JQ647830	Duarte et al. (2012)
RVC	<i>S. phureja</i>	Criolla Colombia	...	Santa Elena, Medellin, Colombia	<2012	JX419379	Gutiérrez et al. (2013)
RL5	<i>S. phureja</i>	Criolla Colombia	...	La Unión, Antioquia, Colombia	<2011	JX683388	Gutiérrez et al. (2012)
Antioquia	<i>S. phureja</i>	Criolla Colombia	...	Colombia	<2015	KR152654	Vallejo et al. (2016)

cultivated potato plants, their aphid and contact transmissibility, and their properties in sap were determined. With aim (ii), the phylogeny of the 35 nonrecombinants revealed three major lineages, two predominantly South American (SA) and one non-SA; and, using least squares dating (LSD) and nucleotide sequences, the results suggested that PVS emerged in South America, with one early lineage spreading worldwide.

Materials and Methods

Plants and mechanical inoculations. Indicator hosts and potato plants were grown in a sterile mix of soil, peat, and sand (1:1:1). Botanical seed of wild potato species was obtained from the Potato Introduction Station, Sturgeon Bay, WI. Their seedlings were propagated in peat blocks before transfer to pots. Healthy tubers of Andean, European, and North American potato cultivars were supplied by the seed potato programs of the Instituto Nacional de Investigaciones Agropecuarias de Ecuador (INIAP) and the International Potato Center (CIP), Peru. Tubers of PVS-resistant cultivars Saco and Snec 66/139-19 were supplied by the Scottish Plant Breeding Station, Pentlandsfield, UK. Leaf samples from all potato plants grown from tubers were tested before use by serology and indicator hosts to confirm that they were healthy. Plants were maintained in winter in insect-proof screen houses at approximately 20°C and in summer in insect-proof, air-conditioned glasshouse compartments at approximately 20°C.

Plants without soft leaves were kept in the dark for 24 to 48 h to increase their susceptibility to infection prior to inoculation. Mechanical inoculations to indicator hosts and potato plants were made by rubbing cotton swabs dipped in infective sap diluted in distilled water onto leaves previously dusted with 600-mesh-sized carborundum powder.

PVS isolates. Thirty PVS isolates were obtained in 1976 from plants growing in insect-proof screen houses from potato tubers belonging to recently made germplasm collections of native potato cultivars (land races) from five Andean countries. They were isolated by inoculation of sap extracted from infected leaf samples to the indicator hosts *C. quinoa*, *C. amaranticolor*, *Gomphrena globosa*, and *Nicotiana debneyi*. Based on symptoms induced in these indicator hosts and the absence of other viruses based on serological tests, 11 isolates that caused different types of symptoms were selected for study. These came from Bolivia ($n = 3$), Ecuador ($n = 4$), Colombia ($n = 1$), Peru ($n = 2$), and Chile ($n = 1$); nine came from *S. tuberosum* subsp. *andigena* and one each from *S. phureja*, *S. curtilobum*, and *S. tuberosum* subsp. *tuberosum* (Table 1; Fig. 1). An additional isolate selected as a control came from a Scottish *S. tuberosum* subsp. *tuberosum* breeding line. Based on whether they infected *C. quinoa* systemically, nine Andean isolates belonged to strain group PVS^A and the remaining two Andean isolates (E-4 and P-1) plus the Scottish isolate (S-1) to strain group PVS^O. They were cultured in plants of *C. quinoa* for PVS^A or *N. debneyi* for PVS^O isolates.

Serological tests. Serological tests to detect virus infection in samples from potato plants grown from tubers supplied as healthy stock were done using microprecipitin tests. Polyclonal antisera to common potato viruses were used. All other serological tests on leaf samples were done using latex tests. The procedures used for these two kinds of tests were described previously (Ball 1974; Bercks et al. 1972; Koenig and Bode 1978). They were used routinely for serological detection at CIP in the era before enzyme-linked immunosorbent assay (Fribourg et al. 1977a,b; Moreira et al. 1980). The polyclonal antiserum to PVS was made in Peru by C. E. Fribourg.

Evaluation of host reactions. In 1977, infective sap containing each PVS isolate was inoculated to three plants of each indicator host species and wild potato species evaluated, and three plants of each species were left uninoculated as healthy controls. The symptoms that developed were recorded. Samples from inoculated leaves of indicator hosts were back-tested for infection by inoculating to plants of *C. quinoa* for PVS^A or *N. debneyi* for PVS^O at 21 days postinoculation (dpi). Tip leaf samples from indicator hosts were back-tested in the same way by inoculating indicator host plants at 28 dpi. Samples of inoculated leaves of wild potato species were back-tested for

infection by the latex test and inoculation to the same two indicator host species at 21 dpi. At 30 and 60 dpi, tip leaf samples from each wild potato plant were back-tested for infection in the same way.

To observe the effects of primary infection with the different PVS isolates on potato plants, infective sap containing each isolate was inoculated to three plants of each potato cultivar, and three plants of each cultivar were left uninoculated as healthy controls. Six tubers were harvested for each isolate-cultivar combination and planted to observe secondary infection. The symptoms that developed in both infection cycles were recorded. Inoculated leaf samples from the primary infection cycle were back-tested for infection by serology (latex test) and inoculated to plants of *C. quinoa* for PVS^A or *N. debneyi* for PVS^O at 21 dpi. Tip leaf samples from each plant were back-tested for infection in the same way at 30 and 60 dpi (primary cycle) or 30 and 60 days postemergence (secondary cycle).

Evaluation of PVS-resistant potato lines. Scions taken from potato plants secondarily infected with each of the different PVS isolates were top-grafted as described by Noordham (1973) onto three plants each of PVS-resistant potato lines Saco and Snec 66/139-19. The symptoms that developed were recorded. Tip leaf samples from each potato plant were back-tested for infection by serology (latex test) and by inoculating indicator host plants (*C. quinoa* for PVS^A or *N. debneyi* for PVS^O) at 30 and 60 dpi.

Aphid transmission tests. For aphid inoculations, apterous aphids were taken from cultures of *Myzus persicae* maintained on plants of Chinese cabbage (*Brassica pekinensis*) kept in aphid cages. Aphids from these cultures were starved for 30 min before being transferred to symptomatic, systemically PVS-infected plants of *N. debneyi* or *C. quinoa* for 1-min acquisition access periods followed by transfer to healthy plants (5 aphids/plant) for inoculation access periods of 1 h. Insecticide was then applied to kill them. There were three experiments with each isolate and, in each experiment, 25 plants were aphid inoculated with each isolate and 10 plants were left uninoculated as controls. The symptoms that developed were recorded. To detect virus presence, tip leaf samples were taken from each plant at 28 dpi and inoculated to *C. quinoa* for PVS^A and *N. debneyi* for PVS^O. The maximum-likelihood (ML) estimator formula (Gibbs and Gower 1960) was used to calculate percent aphid transmission efficiencies.

Contact transmission tests. Contact transmission was studied by gently rubbing systemically PVS-infected leaves of *C. quinoa* or *N. debneyi* against the leaves of healthy plants of the same species. For each isolate, one infected *N. debneyi* leaf or one infected *C. quinoa* plant was rubbed against the leaves of three to four plants. There were three experiments with each isolate and, in each experiment, 10 plants were contact inoculated and 5 plants were left uninoculated as controls. The numbers of plants that developed symptoms were recorded. To confirm virus presence, tip leaf samples were taken from each plant at 28 dpi and inoculated to plants of *C. quinoa* for PVS^A and *N. debneyi* for PVS^O.

Properties in infective sap. Leaf sap from systemically infected leaves of *C. quinoa* for PVS^A or *N. debneyi* for PVS^O was filtered through muslin before use. To estimate dilution end points (DEP), using 10-fold steps, 0.5 ml of infective sap was diluted up to 10⁻⁷ with distilled water. Each serial 5.0-ml dilution was inoculated to four healthy test plants, starting at the greatest and ending at the least dilution. To establish thermal inactivation points (TIP), test tubes, each containing 1.8-ml aliquots of infective sap, were heated in a water bath for 10 min at 50, 55, 60, 65, 70, 75, and 80°C. Immediately after heating, the test tubes were cooled by placing them in a current of cold tap water, and the sap from each test tube was inoculated to four healthy test plants starting at the highest and ending at the lowest temperature treatment. To study longevity in vitro (LIV), 2-ml aliquots of infective sap were added to test tubes kept at room temperature (20 to 24°C) for 50 days. The sap from each test tube was inoculated to four test plants at regular intervals over this period. There were three experiments of each type with each PVS isolate. The numbers of plants that developed symptoms were recorded, and virus presence was established by back-testing tip leaf samples to test plants as described above.

Electron microscopy. With each isolate, crude sap preparations from infected leaves of *C. quinoa* for PVS^A and *N. debneyi* for PVS^O isolates were mixed with 2% sodium phosphotungstate (pH 6.5) and sprayed onto carbon-coated film on copper grids, as described by Roberts (1982). The grids were examined for virus particles using a JEM-100S electron microscope (JEOL USA Ltd.).

Sequence data. Twenty PVS isolates were selected for genome sequencing. These included the 11 from the Andean region and the Scottish isolate mentioned above (Table 1). They had been preserved over silica gel in Peru in 1978 before being sent to England, where they were stored for 37 years before being sequenced (Supplementary Fig. S1). In 1983, these 12 PVS isolates were still infectious when extracts from dried material were inoculated to *C. quinoa* and potato plants. No further inoculations were attempted, the test tubes containing the remaining dried samples being left unopened until the sequencing was undertaken. Four isolates (Exodus, 78-22, BA-365-4, and 24) from *S. tuberosum* subsp. *tuberosum* in Europe and one (PepLV) from *S. muricatum* cuttings originally from Chile were from previous work (Dolby and Jones

1987, 1988; Thomas et al. 1980), and another (ALV = “Arracacha latent virus”) was collected as a root sample from *A. xanthorrhiza* in Peru, sent to the United Kingdom in 1975, and isolated there (Phillips 1990). Recently, PVS was isolated again from *A. xanthorrhiza* in Peru (de Souza et al. 2018), but no complete genome of this isolate is available. Isolate Ken-1 was collected from *S. tuberosum* subsp. *tuberosum* in Kenya in 2013. They were freeze dried in England in 1984 (ALV), 1986 (Exodus, 78-22, BA-365-4, and 24), or 2013 (Ken-1) and kept frozen dried for 31 (ALV), 29 (Exodus, 78-22, BA-365-4, and 24), or 2 (Ken-1) years before being sequenced. Australian isolate Qld-1 was extracted and stored as RNA at -80°C in 2008. All isolates were sequenced in 2015 or 2016, apart from Qld-1, which was sequenced in 2013.

Total RNA was extracted from each sample using a Plant Total RNA kit (Qiagen, United Kingdom or Sigma-Aldrich, Australia), including the optional DNAase treatment. With all isolates except Qld-1, an indexed plant ribosome subtracted sequencing library was then produced from the total RNA using the Scriptseq complete plant leaf kit (Illumina) following the manufacturer’s instructions. The indexed

Fig. 1. Map of South America showing the Andean countries where the potato tuber collections originated that gave rise to the 11 Andean *Potato virus S* isolates named on the map.

library was then sequenced along with others on a MiSeq instrument (Illumina), using a 600 cycle V3 kit. The resulting paired reads were 3' trimmed to a quality score of 20 using Sickel (Joshi and Fass 2011). Contigs were assembled using Trinity (Grabherr et al. 2011) and compared with the GenBank nr and nt databases using BLAST+ (Camacho et al. 2009). Reads of viral origin were extracted using MEGAN (Huson et al. 2016). All isolates except E-3, E-4, and P-1 provided complete PVS genomic sequences. Their final nucleotide sequences were submitted to GenBank with accession numbers MF418021 to MF418035 and MF496659. No other viruses were found when the dried historic PVS samples from the Andes or Europe and the recent sample from Kenya were sequenced in England. With isolate Qld-1, total RNA extract was sent to the Australian Genome Research Facility for library preparation and sequenced with 23 other samples (i.e., with 24 samples/lane) before 100-bp single-end sequencing on an Illumina HiSeq2000. The reads obtained were first trimmed with a CLC Genomics Workbench 6.5 (CLCGW) (CLC Bio) using the quality score settings. Contigs were assembled using the de novo assembly function of CLCGW and sorted by length, as described by Kehoe et al. (2014). In addition, all reads were imported into Geneious 6.1.6 (Biomatters) and provided with a PVS reference sequence obtained from GenBank. Mapping was performed and a consensus between the contig of interest from CLCGW and the consensus from mapping was obtained, as described by Kehoe et al. (2014). The final nucleotide sequence was submitted to GenBank with accession number MF375506. The sample from which new PVS sequence Qld-1 was obtained also contained sequences of *Potato virus Y* (PVY) and *Potato virus A* (both genus *Potyvirus*, family *Potyviridae*).

The new PVS sequences and those retrieved from GenBank (Table 1) provided 40 unique full-length genomic sequences. They were separated into the constituent gene sequences found in all

Carlavirus genomes (i.e., RdRpase, 25K, 12K, CP, and 11K), including regions overlapping adjacent genes. The separated genes were then appended sequentially to give open reading frame (ORF) concatenates (concats); the 7K gene was omitted because it is totally overlapped by the CP gene. The genomic sequences were around 8,100 nucleotides (nt) long, and gave aligned concats of 8,391 nt (2,797 codons). The concats were tested for the presence of phylogenetic anomalies using the full suite of options in RDP4 (Boni et al. 2007; Gibbs et al. 2000; Holmes et al. 1999; Lemey et al. 2009; Martin and Rybicki 2000; Martin et al. 2005, 2015; Maynard-Smith 1992; McGuire and Wright 2000; Padidam et al. 1999; Posada and Crandall 2001). Anomalies found by five or fewer methods, and with a probability of occurring by chance greater than 10^{-5} , were ignored. Five significantly recombinant sequences were found and removed sequentially, starting with the recombinant which had the greatest statistical support. First, sequence AJ863510 was found recombinant by seven methods, with probabilities of anomalies occurring by chance of 10^{-118} to 10^{-34} . It was then removed and the remainder were retested, revealing a group of three nearly identical sequences (LN851189, LN851193, and LN851192), which were found by seven methods to have chance probabilities of having anomalies of 10^{-39} to 10^{-19} . They were removed, and the remainder checked again to find the fifth recombinant sequence, LN851194, detected by six methods, with chance probabilities of 10^{-20} to 10^{-7} .

Phylogenetic analysis. The 17 new PVS sequences and 23 nonduplicated sequences from GenBank (in June 2017) were sorted, checked, and grouped, and duplicates were removed using MAFFT (Katoh and Standley 2013), the neighbor-joining (NJ) facility in ClustalX (Jeanmougin et al. 1998), BioEdit (Hall 1999), and the TranslatorX server (Abascal et al. 2010) (<http://translatorx.co.uk>) with its MAFFT option. Using BioEdit, the 35 unique sequences were

Table 2. Reactions of 16 indicator host species belonging to four plant families to inoculation with 11 Andean and 1 European isolates of *Potato virus S*

Species ^b	Isolate ^a				
	Col-1	E-1	E-2	E-3	E-4
Amaranthaceae					
<i>Gomphrena globosa</i>	LCS, LNS, NS	LCR, LCS, LNS, SNS, SCS	LCR, LCS, LNS, NS	LCR, LCS, LNS, NS	LCR, LCS, LNS, NS
Chenopodiaceae					
<i>Chenopodium amaranticolor</i>	LCS, LNS, VC, SCS	LCS, LNS, VC, SCS	LCS, SCS	LCS, VC, SCS	LCS, LNS, NS
<i>C. capitatum</i>	SS	SS	SL, NS	SS	0
<i>C. ficifolium</i>	LCS, SCS	VC, SCS	SS	SS	LCS, LNS, SS
<i>C. foliosum</i>	LCS, SCS	LCS, SS	SL, NS	SL, NS	LCS, NS
<i>C. murale</i>	LCR, LCS, LNS, VC, LD	LCR, LCS, LNS, VC, LD	LCR, LCS, LNS, VC, LD	LCR, LCS, LNS, VC	LCS, LNS, NS
<i>C. quinoa</i>	LCS, LNS, VC, MM, LD, SCS, SNS, VN	LCS, LNS, VC, SM, LD, SCS, SNS, VN	LCS, LNS, VC, SM, SNS, VN	LCS, LNS, VC, MM, SCS, SNS, VN	LCS, LNS, NS
Fabaceae					
<i>Clitoria ternatea</i>	0	0	0	0	LNR, NS
<i>Cyamopsis tetragonoloba</i>	LNS, NS	LNS, NS	LNS, NS	SL, NS	LNS, NS
<i>Vigna unguiculata</i> 'Catjang'	LCS, NS	SL, NS	SL, NS	SL, NS	SL, NS
<i>V. unguiculata</i> 'Black'	SS	SS	SCS	SS	SCS
Solanaceae					
<i>Datura metel</i>	SS	SS	0	0	SS
<i>Nicotiana clevelandii</i>	SS	SS	0	SS	SS
<i>N. debneyi</i>	SL, VC, LD, SCS	SL, VC, LD, SCS	SL, VC, SCS	SL, VB, SCS	SL, VC, SCS
<i>Solanum esculentum</i> 'Rutgers'	0	0	0	0	0
<i>S. rostratum</i>	SS	SS	SS	SS	SS
<i>S. villosum</i>	LCR, LCS, NS	LCS, NS	SL, NS	SCS, NS	SL, NS

(Continued on next page)

^a Coded symptom descriptions: LCR = local chlorotic rings in inoculated leaves, LCS = local chlorotic spots in inoculated leaves, LNR = local necrotic rings in inoculated leaves, LNS = local necrotic spots in inoculated leaves, SL = symptomless local infection in inoculated leaves, VC = vein clearing, VB = vein banding, MM = mild mosaic, NS = no systemic infection, SM = severe mosaic, LD = leaf deformation, SCS = systemic chlorotic spots, SNS = systemic necrotic spots, VN = veinal necrosis, SS = symptomless systemic infection, St = stunting, and 0 = not infected.

^b Infective sap containing each isolate was inoculated to three plants of each species, and three plants of each species were left uninoculated as healthy controls. Inoculated leaf samples were back-tested for infection by inoculation to indicator host plants (*C. quinoa* or *N. debneyi*) at 21 days postinoculation (dpi). Tip leaf samples from each plant were similarly back-tested for infection by inoculation to indicator host plants at 28 dpi.

separated sequentially into their constituent ORF (i.e., RdRpase, 25K, 12K, CP, and 11K) and concat were made from them and tested for phylogenetic anomalies, as described above.

Nucleotide diversities (π) (Nei and Li 1979) were computed using DAMBE5 online (Xia 2013). Models for ML analysis of the aligned concat and their encoded protein sequences were compared using TOPALi (Milne et al. 2009) and the ProtTest 3 server (<http://darwin.uvigo.es>) (Darriba et al. 2011). Phylogenetic trees were inferred using the ML method PhyML 3.0 (Guindon and Gascuel 2003), and statistical support for their topologies was assessed using the SH option (Shimodaira and Hasegawa 1999). They were drawn using Figtree 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>) and a commercial computer illustration package, pairs of trees were compared using PATRISTIC (Fourment and Gibbs 2006) to test for mutational saturation, and the results were confirmed by the binary test of Xia (2013).

ML trees calculated from nucleotide sequences were rooted using the midpoint option in Figtree, and also using the nucleotide or encoded amino acid sequences of the carlaviruses *Potato rough dwarf virus* (PRDV) and *Potato virus P* (PVP) isolates NC-009759 and EU338239, respectively. The temporal signal in sets of aligned concat sequences, and the dates of the most common recent ancestor (TMRCA) and other nodes of inferred phylogenies, were estimated from ML trees of nucleotide sequences by TempEst (Rambaut et al. 2016) and the LSD method of To et al. (2015).

Results

Indicator host reactions. Depending on isolate and species inoculated, the 12 different PVS isolates produced diverse symptoms in indicator hosts, with infection sometimes limited to inoculated leaves (Table 2; Fig. 2). The symptom types found ranged from chlorotic or

necrotic spots or rings in inoculated leaves to systemic vein clearing, vein banding, mosaic, leaf deformation, chlorotic or necrotic spots or rings, and veinal necrosis. Symptom severity also varied, and symptomless localized or systemic infection developed with some isolate and species combinations. In *C. quinoa*, three isolates remained localized to inoculated leaves (E-4, P-1, and S-1) while the other nine caused systemic invasion (Fig. 2A). Therefore, the first three belonged to biological strain PVS^O but the other nine all to biological strain PVS^A. None caused symptomless infection. In *C. murale* and *C. amaranticolor*, the same three isolates (E-4, P-1, and S-1) plus one other (B-3) remained localized to inoculated leaves (Fig. 2B), whereas the other eight isolates developed systemic symptoms. Severe symptoms of systemic necrotic spots and veinal necrosis developed with all nine PVS^A isolates in *C. quinoa* but only one isolate (P-2) caused systemic necrotic symptoms in *C. murale* and none did this in *C. amaranticolor*. All nine PVS^A isolates infected *C. capitatum* but the three PVS^O isolates did not, even in inoculated leaves. All three PVS^O isolates invaded *C. ficifolium* systemically and one of them (P-1) also did so in *C. foliosum*. Only the PVS^O isolates infected *Clitoria ternatea*. Four isolates invaded *G. globosa* systemically whereas eight were restricted to inoculated leaves (Fig. 2C), and systemic movement was unrelated to whether they were PVS^O or PVS^A. Also, neither *Datura metel* nor *N. clevelandii* were infected by seven and one isolates, respectively; but, again, this was unrelated to whether they belonged to PVS^A or PVS^O. P-2 (PVS^A) was the only isolate to invade *S. villosum* systemically.

Using ability to infect 16 inoculated species systemically as a measure of virulence, the most virulent isolates were E-1, B-2, and CH-1 (all PVS^A), each infecting 11 to 12 of the 16 species systemically. The least virulent were S-1, E-4, P-1 (all PVS^O), and E-2 (PVS^A), which only invaded two (S-1) or five to six (E-2, E-4, and P-1)

Table 2. (Continued from previous page)

Isolate ^a						
P-1	P-2	B-1	B-2	B-3	CH-1	S-1
LCR, LCS, LNS, NS	LCR, LCS, NS	LCR, LCS, LNS, SNS, SCS	LCR, LCS, LNS, SNS, SCS	LCR, LCS, LNS, NS	LCR, LCS, LNS, SNS, SCS	SL, NS
LCS, LNS, NS	LCS, LNS, SS	LCS, LNS, SS	LCS, VC	LCS, LNS, NS	LCS, LNS, SS	SL, NS
0	SL, NS	SL, NS	SS	SS	SS	0
SS	SS	LCS, LNS, VC, SCS	SCS	SCS	VC, SCS	SS
LCS, SS	VC, SS	SL, NS	LCS, SCS	SCL, SCS	VC, SS	LCS, NS
LCS, LNS, LCR, NS	LCR, LCS, LNS, VC, SNS, LD	LCR, LCS, LNS, VC, LD	LCS, LNS, LCR, VC, SCS, LD	LCR LCS, LNS, NS	LCS, LNS, LCR, VC, SCS, LD	LCR, LNS, NS
LCS, LNS, NS	LCS, LNS, VC, MM, LD, SCS, SNS, VN	LCS, LNS, VC, SM, LD, SCS, SNS, VN	LCS, LNS, VC, SM, SCS, SNS, VN	LCS, LNS, VC, SM, SCS, SNS, VN	LCS, LNS, VC, SM, SCS, SNS, VN	LCS, LCR, LNS, NS
SL, NS	0	0	0	0	0	SL, NS
LNS, NS	SL, NS	LNS, NS	SL, NS	LNS, NS	SL, NS	SL, NS
LCS, NS	SL, NS	SL, NS	SL, NS	LCS, NS	SL, NS	SL, NS
SS	SS	SS	SS	SS	SCS	SS
0	0	SS	0	SS	0	0
SS	SL, NS	SS	SS	SS	SS	SL, NS
SL, VC, SCS	SL, VC, SCS	SL, VC, LD, SCS	SL, VC, SCS	SL, VC, LD, SCS	SL, SCS, VB	SL, VC, SCS
0	0	0	0	0	0	0
SS	SS	SS	SS	SS	SS	LCS, LNS, VC, LD, St
LCS, NS	LCR, VC	LCR, NS	LCS, NS	LCS, NS	LCR, NS	LCS, NS

species systemically. However, least-virulent isolate S-1 differed in being the only one that induced symptoms in *S. rostratum*. *Chenopodium ficifolium*, *N. debneyi*, *S. rostratum*, and *Vigna unguiculata* 'Black', which belong to three different families and were the only species that all isolates invaded systemically (Fig. 2D); tomato was the only nonhost species for all isolates. All isolates remained restricted to inoculated leaves of the legume species *V. unguiculata* 'Catjang' and *Cyamopsis tetragonoloba* (Fig. 2E).

Reactions of wild potato species. Symptomless systemic infection was the commonest response among the nine species inoculated. Severe systemic necrotic symptoms only developed in *S. megistacrobium*, in which they occurred with all isolates except P-1 (PVS^O), P-2, and CH-1 (both PVS^A) (Table 3). These necrotic symptoms consisted of veinal necrosis, systemic necrotic spotting (SNS), necrotic stem streaking, and apical necrosis. The only other example of systemic necrosis was the systemic necrotic spots or rings that formed with isolates S-1 (PVS^O), Col-1, B-1, and B-2 (PVS^A) in *S. chacoense* (Fig. 2F). *S. microdontum* developed mild or severe mosaic with all isolates except PVS^O isolate E-4. Other symptom types that developed with some combinations of PVS isolate and species included necrotic or chlorotic spots or rings in inoculated leaves and systemic vein clearing, systemic chlorotic spotting, rugosity, and stunting. Symptomless systemic infection occurred with all 12 PVS isolates in *S. acaule*, *S. demissum* Y, *S. mochosense*, and *S. stoloniferum*

and with 11, 10, and 8 isolates in *S. chancayense*, *S. demissum* A, and *S. chacoense*, respectively. PVS^O isolates S-1 and P-1 caused chlorotic rings in inoculated leaves of *S. demissum* A and S-1 caused mild mosaic in *S. chancayense*.

Using ability to infect the nine inoculated species systemically as a measure of virulence, PVS^O isolate S-1 was the most virulent, causing systemic symptoms in four species. The other two PVS^O isolates (E-4 and P-1) along with PVS^A isolate P-2 were the least virulent, each causing systemic symptoms in only one species. Thus, virulence was unrelated to whether isolates belonged to PVS^A or PVS^O. *S. berthaultii* was the only species which some isolates ($n = 3$) failed to infect, the other isolates only causing symptomless systemic ($n = 6$) or localized ($n = 3$) infection. Its responses to inoculation were unrelated to whether isolates belonged to PVS^A or PVS^O.

Reactions of PVS-susceptible and PVS-resistant potato plants. Sap inoculation with the 12 PVS isolates caused mostly symptomless primary infection in plants of potato cultivars (Table 4). The only exception was appearance of vein clearing and mild mosaic caused by PVS^O isolate S-1 in cultivar Revolution. By contrast, with secondary infection, most cultivars developed mild systemic symptoms. These consisted of green spots or blotches on senescent leaves (= premature lower leaf senescence), moderate or marked deepening of leaf veins, mild or obvious leaf rugosity (Fig. 2G), and mild mosaic. Premature lower leaf senescence was often associated with other symptoms but,

Fig. 2. Symptoms in leaves of indicator hosts and wild or cultivated potato plants caused by infection with different *Potato virus S* isolates. **A**, Systemic chlorotic mottle in a leaf of *Chenopodium quinoa* caused by infection with isolate E-2. **B**, Local necrotic spot lesions in an inoculated leaf of *Gomphrena globosa* caused by infection with isolate B-1. **C**, Local necrotic spot lesions surrounded by red rings in an inoculated leaf of *Nicotiana debneyi* caused by infection with isolate S-1. **D**, Systemic vein clearing in a leaf of *Nicotiana debneyi* caused by infection with isolate E-4. **E**, Local necrotic spot lesions in an inoculated leaf of *Cyamopsis tetragonoloba* caused by infection with isolate S-1. **F**, Coalescing systemic necrotic spots in *Solanum chacoense* foliage caused by infection with isolate S-1. **G**, Secondary symptoms of leaf rugosity and deepening of the veins in a leaf of cultivar Mi Peru secondarily infected with isolate B-2 (left) compared with a healthy leaf (right).

in several isolate–cultivar combinations, it developed in plants otherwise showing only symptomless infection, (e.g., with all five isolates infecting Revolucion, the most PVS-tolerant cultivar). Mild mosaic only developed with isolates B-3 in cultivar Renacimiento and S-1 in cultivar Ticahuasi. Symptomless systemic infection developed alone with four cultivar–isolate combinations. No obvious differences in virulence were evident between PVS^O and PVS^A isolates.

Following graft inoculation, Saco plants developed no symptoms and no virus was detected in leaf samples taken at 30 dpi (Table 4). However, at 60 dpi, the virus was detected with all isolates apart from PVS^O isolates S-1 and P-1. In axillary shoots of graft-inoculated Snec 66/139-19 plants, necrotic symptoms developed with seven isolates but not with the other five. Four isolates caused a severe systemic hypersensitive resistance (SHR) phenotype, consisting of SNS followed by shoot necrosis and plant death (E-1, E-3, E-4, and CH-1); three caused SNS without further necrosis (Co-1, B-2, and S-1); and the remaining five developed a fully susceptible phenotype (E-2, P-1, P-2, B-1, and B-3). There were PVS^O and PVS^A isolates within each of these three categories.

Aphid and contact transmission. All nine PVS^A isolates were transmitted at differing levels of efficiency by aphids but no aphid transmission was detected with the three PVS^O isolates (E-4, P-1, and S-1) (Table 5). Isolates E-1, B-1, and B-3 were most efficiently aphid and transmitted (9 to 11%); P-2, B-2, and CH-1 had intermediate transmission efficiencies (6 to 8%); and Co-1, E-2, and E-3 were least efficiently transmitted (1 to 3%). All 12 isolates were transmitted by contact with efficiencies of 10 to 40 and 7 to 50% for PVS^O and PVS^A isolates, respectively. Greatest contact-transmission efficiencies were found with isolates E-2, E-4, P-2, and B-3 (40 to 50%). The transmission efficiencies with E-1, B-2, and CH-1 were intermediate (17 to 27%), while Co-1, E-3, P-1, B-1, and S-1 were least efficiently transmitted (7 to 13%). Thus, although efficiency of contact transmission was unrelated to the strain group an isolate belonged to, aphid transmission only occurred with PVS^A.

Properties in infective sap. In LIV studies, all isolates remained infectious when sap was stored at approximately 22°C for long periods (Supplementary Table S1). However, survival durations varied between isolates and sometimes also between individual experiments with the same isolate. Isolates E-1 and CH-1 each remained infectious for up to 40 days, while infectivity of Co-1, P-2, B2, and B-3

survived for up to 25 to 28 days and E-2, E-3, E-4, and P-1 for up to 20 days. B-1 and S-1 only remained infectious for 10 to 17 and 7 to 16 days, respectively. In all three TIP experiments with each isolate, infectivity of isolates E-1, B-3, and CH-1 was destroyed at 65°C, while that of Co-1 and S-1 was destroyed at 60°C. With all other isolates, infectivity was destroyed at 60 or 65°C, depending on the experiment. In DEP studies with seven isolates, infectivity was lost at a dilution of 10⁻⁶ and with three others at 10⁻⁵ to 10⁻⁶. However, infectivity was only lost at 10⁻⁷ in one experiment with E-1. At the other extreme, no infectivity survived at 10⁻⁴ with PVS^O isolates E-4 and S-1. Thus, although the PVS^O and PVS^A isolates behaved similarly with regard to thermal inactivation, none of the three PVS^O isolates (E-4, P-1, and S-1) remained infectious for >20 days, and two of them (E-4 and S-1) had lower DEP values than any of the nine PVS^A isolates.

Electron microscopy. Filamentous virus particles approximately 650 nm in length and 12 nm wide were present in high concentration in electron micrographs prepared with sap from leaves of *Chenopodium quinoa* or *N. debneyi* infected with all 12 isolates.

Phylogenetic analysis. Five concat (AJ863510, LN851189, LN851192, LN851193, and LN851194) were found to be recombinants by at least six RDP4 methods, with probabilities of occurring by chance ranging from 10⁻¹¹⁸ to 10⁻⁷. The remaining 35 nonrecombinant sequences had a nucleotide diversity (π) (Nei and Li 1979) of 0.144 ± 0.068, and showed no evidence of translational saturation in graphs (Fourment and Gibbs 2006; Gibbs et al. 2015a,b) comparing patristic distances of nucleotide and encoded amino acid phylogenies (ML, LG + γ + I model) (Le and Gascuel 2008). An ML phylogenetic tree of the 35 nonrecombinant sequences calculated by PhyML using the GTR + γ + I model is shown in Figure 3 (Tavaré 1986). Most of the main nodes had >0.9 SH support (Shimodaira and Hasegawa 1999), and the midpoint root was calculated using the midpoint option in Figtree. The same root was found in ML analyses using LG + γ + I (Le and Gascuel 2008) and comparing the amino acid sequences of the RNA-dependent RNA polymerase (RdRp) proteins encoded by the 35 PVS sequences with that of PRDV NC-009759, which was closest in a GenBank Blastp search using NC-007289 as the PVS query sequence. Figure 3 also lists the host plant from which each isolate was obtained and the country where it occurred. There was a basal trifurcation of the phylogeny resulting in three major lineages, two evenly branched and predominantly SA

Table 3. Reactions of 10 wild potato species to inoculation with 11 Andean and 1 European isolates of *Potato virus S*

Species ^b	Isolate ^a											
	Col-1	E-1	E-2	E-3	E-4	P-1	P-2	B-1	B-2	B-3	CH-1	S-1
<i>Solanum acaule</i> PI 230554	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS
<i>S. berthaultii</i> PI 265857	SS	SS	0	0	SL	SL	0	SS	SS	SS	SL	SS
<i>S. chacoense</i> PI 275136	LNS, SNS, SNR	LNS, SS	LNS, SS	SS	LNS, SS	SS	SS	LNS, SNS, SNR	LNS, SNS, SNR	SS	LNS, SS	LNS, SNS, St
<i>S. chancayense</i> PI 338615	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	MM
<i>S. demissum</i> 'A'	SS	SS	SS	SS	SS	LDCR	SS	SS	SS	SS	SS	LDCR
<i>S. demissum</i> 'Y'	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS
<i>S. megistacrolobum</i> PI 265879	LNS, VC, VN, SNS, NSS, AN	LNS, VC, VN, SNS, NSS, AN	LNS, VN, NSS, AN	LNS, VN, NSS, AN	LNS, VN, NSS	LNR, SS	SS	VN, PN, NSS, AN	LNR, VN, NSS, AN	LNR, VN, NSS, AN	SS	LNS, VC, VN, SNS, NSS, AN
<i>S. microdontum</i> PI 218223	SM	SM, R, St	SM, R	SM R,	SS	MM	SM, R	SM, R	MM	SM, R	SM, R	LCS, SM R
<i>S. mochiense</i> PI 283114	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS
<i>S. stoloniferum</i> PI 230557	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS

^a Coded symptom descriptions: LDCR = local diffuse chlorotic rings in inoculated leaves, LCS = local chlorotic spots in inoculated leaves, LNR = local necrotic rings in inoculated leaves, LNS = local necrotic spots in inoculated leaves, LS = SL = symptomless infection in inoculated leaves, VC = vein clearing, MM = mild mosaic, SM = severe mosaic, R = rugosity, PN = petiole necrosis, St = stunting, SNR = systemic necrotic rings, SNS = systemic necrotic spots, VN = veinal necrosis, NSS = necrotic stem streaking, AN = apical necrosis, SS = symptomless systemic infection, and 0 = not infected.

^b Infective sap containing each isolate was inoculated to three plants of each species. Three plants of each cultivar were left uninoculated as healthy controls. Inoculated leaves were back-tested for infection by serology (latex test) and inoculation to indicator host plants (*Chenopodium quinoa* or *Nicotiana debneyi*) at 21 days postinoculation (dpi). Tip leaves from each plant were similarly back-tested for infection by serology and inoculation to indicator host plants at 30 and 60 dpi.

lineages and one non-SA with a single long basal branch with many short distal twigs containing isolates from other continents. The short distal twigs indicated a recent period of divergence, and the regular subdivisions indicated divergence over a much longer period. Taking the twigs of these three lineages that are most distant from each other and linked through the root in Figure 3, the nucleotide identities between the most distinct were 79.1% between MF418034 and JX683388, 78.3% between MF418034 and AJ863509, and 78.3% between JX683388 with AJ863509. The corresponding amino acid identity figures were 85.6% between MF418034 and JX683388, 85.1% between MF418034 with AJ863509, and 85.4% between JX683388 and AJ863509.

The recently diverged phylogroup contained PVS isolates, mostly from *S. tuberosum* subsp. *tuberosum* plants (18 of 19) collected worldwide, and 1 of *S. tuberosum* subsp. *andigena* (Maori potato) from New Zealand but none from South America. In contrast, the other two lineages contained mostly isolates from South America (14 of 16), with 1 each from New Zealand Maori potato and China, originally isolated as follows: 6 isolates from *S. tuberosum* subsp. *andigena*, 4 from *S. phureja*, 3 from *S. tuberosum* subsp. *tuberosum*, and 1 each from *S. muricatum* and *S. curtilobum*. The 16th isolate was from the nonsolanaceous host *A. xanthorrhiza*. However, there was no absolute difference in either host or provenance between the SA and non-SA lineages. Unlike the other isolates in the SA lineage, ALV from *A. xanthorrhiza* belongs to PVS^O (Phillips 1990); there were no sequences for the two PVS^O isolates studied from Andean potato (E-4 and P-1).

When all *Carlavirus* genomic sequences were retrieved from GenBank (in August 2017) and placement of PVS within the carlaviruses was determined using the RdRp protein sequences encoded by 134 *Carlavirus* and 3 *Foveavirus* outgroup genomes aligned by MAFFT (Fig. 4), PRDV and PVP were the closest to the PVS cluster. These are a pair of related viruses found in Brazil and Argentina (Nisbet et al. 2006). PVS, PRDV, and PVP are sister to a lineage of carlaviruses found first, or only, in the Americas (*Blueberry scorch virus*, *Hydrangea chlorotic mottle virus*, *Ligustrum necrotic ringspot virus*, and a carlavirus from *Opuntia ficus-indica* in Mexico), and a crown group of carlaviruses from other parts of the world (*Kalanchoe latent virus*, *Lily symptomless virus*, *Mirabilis jalapa mottle virus*, and *Pasiflora latent virus*). Other distinct carlaviruses have been isolated from potato, including *Potato latent virus*, *Potato virus M*, and *Potato virus H*. The RdRp protein taxonomy found that none of them were members of the PVS lineage.

Dating. TempEst analyses of NJ trees of concat nucleotide sequences gave TMRCA dates that ranged from 1125 to 1221 Common Era (CE). These analyses were used to assess the influence of PVS isolates with uncertain dates on the results. Removing all six isolates for which only the date of submission of the genomic sequence to GenBank was known (Table 1; isolates with “<2004”), increased the correlation between dates and phylogenetic relationships from 0.52 to 0.63. Furthermore, when the two isolates (HF571059 and LN851191) for which the likely date of collection was recorded as 2009 to 2014 (Table 1) were given either the earlier or later date, the correlation was marginally better with the earlier date, 0.6364 rather than 0.6354.

Table 4. Reactions of five Andean and one European potato cultivars to primary and secondary infection following sap inoculation, and of PVS-resistant clone Snec 66/139-19 and cultivar Saco following graft inoculation, with 11 Andean and 1 European isolates of *Potato virus S* (PVS)

Cultivar ^b	Isolate ^a											
	Col-1	E-1	E-2	E-3	E-4	P-1	P-2	B-1	B-2	B-3	CH-1	S-1
Primary infection												
Merpati	SS	–	–	–	–	SS	–	SS	–	–	–	–
Mi Peru	SS	SS	SS	SS	–	–	SS	SS	SS	SS	SS	–
Pentland Dell	–	SS	–	–	SS	SS	–	–	–	–	–	SS
Renacimiento	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS
Revolucion	–	SS	SS	SS	–	–	SS	–	–	–	–	VC, MM
Ticahuasi	SS	SS	SS	SS	SS	SS	SS	SS	SS	–	–	SS
Secondary infection												
Merpati	MLR, DLV, GSSL	–	–	–	–	MLR, DLV, GSSL	–	MLR, DLV, GSSL	–	–	–	–
Mi Peru	OLR, MDLV, GSSL	MLR, DLV, GSSL	MLR, DLV, GSSL	OLR, MDLV	–	–	MLR, DLV, GSSL	MLR, DLV, GSSL	OLR, MDLV, GSSL	MLR, DLV, GSSL	MLR, DLV, GSSL	MLR, DLV, GSSL
Pentland Dell	–	MLR, DLV, GSSL	–	–	MLR, DLV, GSSL	GSSL, SS	–	–	–	–	–	SS
Renacimiento	MLR, DLV	SS	MLR, DLV	SS	MLR, DLV, GSSL	GSSL, SS	MLR, DLV, GSSL	MLR, DLV, GSSL	MLR, DLV, GSSL	MM, GSSL	SS	GSSL
Revolucion	–	GSSL, SS	GSSL, SS	GSSL, SS	–	–	GSSL, SS	–	–	–	–	GSSL, SS
Ticahuasi	MLR, DLV, GSSL	SS	MLR, DLV, GSSL	MLR, DLV	GSSL, SS	MLR, DLV	GSSL, SS	MLR, DLV, GSSL	MLR, DLV	–	–	MLR, DLV, MM
Graft inoculation												
Snec 66/139-19 (Craigs Defiance × PI 25807)	SNS, LDR	SNS, LDR, SND	C	SNS, LDR, SND	SNS, LDR, SND	CGS	C, LDR	SS	SNS, LDR	C, LDR	CGS, SNS, LDR, SND	LC, SNS
Saco	DSS	DSS	DSS	DSS	DSS	0	DSS	DSS	DSS	DSS	DSS	0

^a Coded symptom descriptions: CGS = chlorosis with green spots/blotches, C = chlorosis, GSSL = green spots or blotches on senescent leaves, DLV = deepening of leaf veins, DSS = delayed systemic symptomless infection (no virus detected until 60 days postinoculation [dpi]), MDLV = marked deepening of leaf veins, LC = leaf curl, LDR = leaf drop, MM = mild mosaic, 0 = not infected, OLR = obvious leaf rugosity, MLR = mild leaf rugosity, SNS = systemic necrotic spots in some leaves, SND = systemic necrosis and plant death, SS = symptomless systemic infection, VC = vein clearing, and – indicates not tested.

^b To observe the effects of primary infection with PVS, infective sap containing each isolate was inoculated to three plants of each cultivar. Three plants of each cultivar were left uninoculated as healthy controls. Six tubers were harvested for each isolate–cultivar combination and planted to observe the effects of secondary infection. For graft inoculations, scions from plants infected with each isolate were top-grafted onto the three plants of each cultivar. Inoculated leaves from the primary infection cycle were back-tested for infection by serology (latex test) and inoculation to indicator host plants (*Chenopodium quinoa* or *Nicotiana debneyi*) at 21 dpi. Tip leaves from each plant were similarly back-tested for infection by serology and inoculation to indicator host plants at 30 and 60 dpi (primary infection) or at 30 and 60 days postemergence (secondary infection and graft inoculation).

Therefore, LSD was done using an ML tree of 27 PVS concats with unequivocal collection dates, plus two given a date of 2009, and the algorithm was used in the constrained mode with 1,000 replicate samplings to assess the confidence limits. The two nodes of the basal trifurcation were dated at 1079 and 1055 CE, giving a mean TMRCA of 1067 CE (confidence interval [CI]: 68 to 1369 CE), with an evolutionary rate of 4.8×10^{-4} substitutions/site/year. The node that is basal to the recent divergence of the non-SA lineage was dated as 1837 CE (CI: 1632 to 1905 CE); thus, that radiation is probably post-Columbian and occurred in Europe (i.e., after Christopher Columbus's 1492 voyage to the Americas). The three midphylogeny nodes in the SA lineages are dated 1352 CE (CI: 660 to 1562 CE), 1487 CE (CI: 945 to 1652 CE), and 1537 CE (CI: 1052 to 1686 CE) but their CI straddle the discovery of the Americas by Europeans, whereas none of the more recent nodes do. When the TMRCA was estimated by LSD using the ML phylogeny calculated from the amino acid sequences encoded by the 27 concats, it was 1170 CE (CI: 533 Before the Common Era [BCE] to 1493 CE). This estimate is between those from the concats although, as expected, it has a larger CI than that based on the nucleotide sequences.

The phylogeny of 134 *Carlavirus* RdRp protein sequences provided an opportunity to estimate the relative date of the PVS divergence from the PRDV-PVP pair (Fig. 4). The mean of the patristic distances linking the PRDV-PVS sequences to all sequences of the PVS cluster was 1.655 substitutions/site, whereas the mean of those between PVS sequences and passing through the basal node of the PVS cluster was 0.236 substitutions/site; a ratio of 7. If we assume that the TMRCA of the PVS cluster is 1,000 years before the present (YBP) and that patristic distances are linearly related to time, then PVS divergence from PRDV-PVP occurred around 7,000 YBP, although tests with subsets of the PVS sequences showed that this may be an overestimate resulting from the much larger number of PVS sequences than PRDV-PVP sequences in the ML comparison; therefore, 5,000 YBP is a more likely date for the divergence (i.e., 3,000 BCE).

Recombinant origins. The relationships of the five PVS recombinant concats and those of their "parents" are summarized in Figure 5. It also shows the crossover sites found in the recombinant concats (8,391 nt): at nucleotide 6,088 in AJ863510, at nucleotide 2,633 in LN851189/192/193, and at nucleotides 5,858 and 7,204 in LN851194. Four of the parents were from the non-SA lineage, one was itself a recombinant, and one was from the SA lineage. All the recombinants were from Eurasia, as were all but one of the parental isolates, the single exception (MF418023) being a member of a sublineage only found in Bolivia.

Discussion

This article constitutes the first detailed analysis of the biological and molecular properties of PVS isolates from countries spanning the entire Andean region of South America, including the potato's primary domestication center in Peru and Bolivia and its secondary domestication center in the Isla Grande de Chiloé in Chile (Hawkes 1978). For the first time, biologically defined PVS^O isolates were found in potato in the Andes, although most belonged to biologically defined PVS^A. In potato cultivars, primary and secondary infection mostly caused no symptoms or mild symptoms, respectively. Contrary to earlier suggestions that PVS^A isolates caused more severe symptoms in potato, symptom severity in wild and cultivated potato plants was unrelated to whether they were PVS^A or PVS^O. Plants of clone Snec 66/139-19, which carries hypersensitivity gene *Ns*, and of *S. megistacrolobum* PI265879, both developed strain-specific SHR; however, its occurrence was again unrelated to whether isolates were PVS^A or PVS^O. By contrast, other than two PVS^O isolates, all isolates were eventually detected following graft inoculation to partially PVS-resistant Saco. Moreover, although contact-transmission efficiency was unrelated to PVS strain, aphid transmission by *M. persicae* only occurred with PVS^A. Also, despite the stability of infectivity being similar for all isolates when infective sap was heated for 10 min at different temperatures, in general, infective sap remained infectious for longer with PVS^A than PVS^O when kept at room temperature or diluted in

10-fold dilution steps. The phylogeny of 17 new and 23 other complete nonrecombinant genomes revealed three major PVS lineages, two evenly branched and predominantly SA and one with a long basal branch and many distal subdivisions containing isolates from other continents. Furthermore, no relationship was found between biologically defined strains PVS^A and PVS^O and the SA and non-SA major lineages. The two SA lineages contained isolates from *S. tuberosum* subsp. *andigena* ($n = 6$) and *tuberosum* ($n = 3$), *S. phureja* ($n = 4$), *S. curtilobum* ($n = 1$), *S. muricatum* ($n = 1$), and the nonsolanaceous host *A. xanthorrhiza* ($n = 1$), whereas the non-SA lineage contained 18 isolates from *S. tuberosum* subsp. *tuberosum* and a single isolate from *S. tuberosum* subsp. *andigena*. The two nodes of the basal PVS trifurcation were dated at 1079 and 1055 CE; the three midphylogeny nodes of the SA lineages at 1352, 1487, and 1537 CE; and the basal node to the non-SA lineage at 1837 CE. We extrapolated these dates and found that the divergence of PVS from the PRDV-PVP lineage occurred 5,000 to 7,000 years ago. This suggests that a potato-infecting proto-PVS emerged in South America at least 5,000 years ago, became endemic, and diverged into a range of local *Solanum* spp. and other species, from which a single PVS lineage was spread around the world after potato were first transported to Europe.

When Rose (1983) graft inoculated a Dutch PVS^A isolate and a Scottish PVS^O isolate to potato plants, the former caused symptoms but the latter only asymptomatic infection. When Dolby and Jones (1987) sap inoculated three PVS^A isolates from Germany or The Netherlands to plants of 10 European potato cultivars, primary infection was mainly asymptomatic but, when infected tubers were planted, progeny plants belonging to most of the PVS^A isolate-cultivar combinations developed secondary symptoms. Results were similar when we sap inoculated PVS^A and PVS^O isolates to six Peruvian and one European potato cultivars because, although primary infection rarely caused symptoms, these often developed with secondary infection. However, in addition to the secondary symptoms of vein deepening, rugosity, chlorosis, premature senescence, and lower leaf loss observed in European cultivars, Peruvian

Table 5. Aphid and contact transmissibility of 12 Andean and 1 European *Potato virus S* (PVS) isolates^a

Isolate	Source host	Aphid transmission experiments ^b				Contact transmission experiments ^c			
		1	2	3	TE (%)	1	2	3	TE (%)
Co-1	<i>Chenopodium quinoa</i>	2	4	3	3	1	1	0	7
E-1	<i>C. quinoa</i>	10	11	9	10	2	1	4	23
E-2	<i>C. quinoa</i>	2	1	1	1	5	5	5	50
E-3	<i>C. quinoa</i>	3	3	4	3	0	2	2	13
E-4	<i>Nicotiana debneyi</i>	0	0	0	0	5	4	3	40
P-1	<i>N. debneyi</i>	0	0	0	0	1	1	1	10
P-2	<i>C. quinoa</i>	10	8	8	8	3	4	5	40
B-1	<i>C. quinoa</i>	11	10	8	9	1	0	1	7
B-2	<i>C. quinoa</i>	7	6	7	6	2	3	3	27
B-3	<i>C. quinoa</i>	12	10	11	11	6	4	4	47
CH-1	<i>C. quinoa</i>	7	7	9	7	1	1	3	17
S-1	<i>N. debneyi</i>	0	0	0	0	1	2	0	10

^a Data show number of plants out of 25 (aphid transmission) or 10 (contact transmission) that became infected. TE = transmission efficiency. With each PVS isolate, the infected source host species and the healthy test plant species were always the same. *Myzus persicae* was the species used in the aphid transmission experiments. In both types of experiment, 1 month after inoculation, tip leaf samples were taken from each plant and individually tested serologically for PVS presence. No PVS was ever detected in uninoculated control plants.

^b In each aphid transmission experiment, 25 test plants were used with each isolate and 5 aphids/test plant. Aphids were starved for 30 min beforehand and the durations of acquisition and inoculation access feeds were 1 min and 1 h, respectively. The maximum-likelihood formula of Gibbs and Gower (1960) was used to calculate percent aphid transmission efficiencies.

^c In each contact transmission experiment, 10 test plants were used with each isolate. Entire infected plants (*C. quinoa*) or detached infected leaves (*N. debneyi*) were brushed against test plants.

cultivars which, rather than being *S. tuberosum* subsp. *tuberosum* were *S. tuberosum* subsp. *andigena* or came from crosses between them, also developed mild mosaic and leaf curl. Secondary symptoms caused by PVS^A and PVS^O were of similar severity.

In graft-inoculated, partially PVS-resistant American Saco, no symptoms developed but all nine PVS^A and one of three PVS^O isolates were eventually detected. This resembles earlier findings in which, although not detected after sap inoculation, PVS was eventually detected following graft inoculation of Saco (Alfieri 1960; Alfieri and Stouffer 1957; Baerecke 1967a; Bagnall 1965; Bagnall and Young 1972; Larson and Oshima 1959). Graft-inoculated plants of clone Snec 66/139-19, which carries hypersensitivity gene *Ns* originally from *S. tuberosum* subsp. *andigena*, elicited full SHR with 4 of 12 and SNS with 3 of 12 isolates. PVS^O and PVS^A isolates occurred within each of the SHR, SNS, and susceptibility categories. Although the SHR phenotype found resembles that observed in

earlier studies with PVS^O isolates from Europe (Baerecke 1967a; Makarov 1975; Pietrak 1985), our study is apparently the first to find that *Ns* is a strain-specific hypersensitivity gene. This strain specificity reflects the presence of a resistance-breaking PVS strain unrelated to the PVS^A and PVS^O strains, which are defined by the ability to infect *C. quinoa* systemically. A PVS^O isolate from cultivar Desiree in the United Kingdom caused SHR when graft inoculated to a different clone (Snec 66/137/489) with gene *Ns* (R. A. C. Jones, unpublished data). When Bagnall (1972) evaluated wild potato species for responses to PVS infection, *S. megistacrolobum* was the only one that developed a hypersensitive phenotype. Similarly, in our study, *S. megistacrolobum* PI 265879 was the only one of nine wild potato species inoculated in which SHR occurred. Because it developed with nine isolates but not with two PVS^A and one PVS^O isolates, it was strain specific and, as with the SHR phenotype associated with gene *Ns*, avoidance of SHR development in *S. megistacrolobum* was independent of ability to invade *C. quinoa* systemically.

Fig. 3. Phylogenetic tree calculated by the maximum-likelihood method from the aligned concatenate genes of 35 nonrecombinant *Potato virus S* isolates. Nodes with green discs have statistical support of 1.0 for their topologies assessed by the SH option (Shimodaira and Hasegawa 1999) and those with yellow discs have >0.9 SH option support. Circles mark the parental isolates of recombinants. Accession codes, hosts, and provenance of the isolates are listed: S.t.t. = *Solanum tuberosum* subsp. *tuberosum*, S.t.and. = *S. tuberosum* subsp. *Andigena*, S.mur. = *S. muricatum*, S.curt. = *S. curtilobum*, S.phur. = *S. phureja*, and A.xan. = *Arracacia xanthorrhiza*.

When de Bokx (1970) inoculated six PVS isolates to 98 plant species belonging to 15 families, only species of the families Amaranthaceae, Chenopodiaceae, and Solanaceae became infected. Also, none invaded *C. quinoa* systemically; therefore, all were PVS^O. However, others had reported also infecting a species in the *Fabaceae* family (Bagnall et al. 1956; Yarwood and Gold 1955), and subsequent studies reported that PVS was experimentally transmissible to 56 solanaceous species and 33 species in 12 other families (Loebenstein et al. 2001). When Hinostroza-Orihuela (1973) inoculated a Peruvian PVS isolate to *C. quinoa*, it caused systemic symptoms; and, when Rose (1983) inoculated one PVS isolate each from The Netherlands and Scotland to *C. quinoa* and *C. amaranticolor* plants, the former caused systemic invasion but the latter only infected inoculated leaves. Similarly, when Slack (1983) inoculated two North American PVS isolates to *C. quinoa* and *C. amaranticolor* plants, one caused systemic invasion and the other did not; and, when the originally SA isolates PepLV from *S. muricatum* and ALV from *A. xanthorrhiza* were inoculated to *C. quinoa* plants, they caused systemic invasion (Thomas

et al. 1980) or remained in inoculated leaves (Phillips 1990), respectively. In this study, all 12 PVS isolates were inoculated to 16 indicator hosts within the four host families reported by Yarwood and Gold (1955), Bagnall et al. (1956), and de Bokx (1970). Nine caused systemic infection in *C. quinoa* but two others (E-4 and P-1) and the Scottish isolate (S-1) did not; therefore, they belonged to PVS^A and PVS^O, respectively. Three PVS^A isolates (E-1, B-2, and CH-1) were the most virulent because they invaded 11 to 12 species systemically, whereas the three PVS^O isolates and E-2 (PVS^A) were the least virulent because they only invaded 2 to 6 species systemically. There were also clear differences between PVS^O and PVS^A isolates in *C. capitatum* and *Clitoria ternatea* whereas, in *Chenopodium murale* and *C. amaranticolor*, only the three PVS^O isolates and one PVS^A isolate (B-3) remained restricted to inoculated leaves. By contrast, four species from three families (*C. ficifolium*, *N. debneyi*, *S. rostratum*, and *V. unguiculata* 'Black') were invaded systemically by all 12 isolates. When Slack (1983) inoculated the two North American isolates mentioned above to some of the same indicator hosts, (i) both caused systemic invasion

Fig. 4. Phylogenetic analysis showing a maximum-likelihood tree calculated from the sequences of 134 *Carlavirus* and three *Foveavirus* RNA-dependent RNA polymerase (RdRp) genes, the calculated date of radiation of the *Potato virus S* (PVS) population, and the inferred date of its divergence from *Potato rough dwarf virus* (PRDV) and *Potato virus P* (PVP). Details of the PVS, PRDV, and PVP lineage and its sister group are shown, and the four other clades of the tree are collapsed. Additional acronyms: BSV = *Blueberry scorch virus*, HCMV = *Hydrangea chlorotic mottle virus*, KLV = *Kalanchoe latent virus*, LNRV = *Ligustrum necrotic ringspot virus*, LSV = *Lily symptomless virus*, Of-IV = *Carlavirus HSO-2016a* (*Opuntia ficus-indica virus*), MjMtV = *Mirabilis jalapa mottle virus*, and PLV = *Passiflora latent virus*. Vertical dashed lines indicate the predominantly non-South American (non-SA), South American (SA), American, and global isolates.

in *C. murale*, which differed from our findings; (ii) his PVS^A but not his PVS^O isolate infected inoculated leaves of *G. globosa* whereas all 12 of our isolates did so; (iii) his PVS^A isolate infected tomato systemically whereas none of our isolates did this; and (iv) only his PVS^O isolate infected *C. tetragonoloba* whereas all of our isolates infected its inoculated leaves. These differences suggest that these four indicator hosts are unsuitable to use in differentiating PVS^A from PVS^O.

Studies on aphid transmission of PVS^O isolates from Europe and North America found that some are *M. persicae* transmitted and others are not (Bode and Weidemann 1971; MacKinnon 1974; Wardrop et al. 1989). During postentry quarantine testing in New Zealand, Thomas et al. (1980) isolated an *M. persicae*-transmitted carlavirus from pepino cuttings from Chile. They named it "Pepino latent virus" but Dolby and Jones (1988) subsequently found it to be PVS^A. Slack (1983) reported that his PVS^A but not his PVS^O isolate was *M. persicae* transmitted, and Fletcher (1996) obtained similar results with his PVS^A isolate from New Zealand. These findings agree with our study because all nine PVS^A isolates were *M. persicae* transmitted but no transmission occurred with the three PVS^O isolates. The aphid transmission efficiencies found varied from 9 to 11% (most efficient) to 1 to 3% (least efficient) depending on PVS^A isolate. By contrast, all 12 isolates were transmitted by contact at efficiencies of 10 to 40% (PVS^O) and 7 to 50% (PVS^A). Thus, contact transmission was unrelated to whether an isolate was PVS^O or PVS^A but aphid transmission only occurred with PVS^A, which implies that PVS^A may spread more readily in the field.

The reported stabilities of PVS^O isolate infectivity in infective sap are (i) a TIP of 55 to 60°C, DEP of 10⁻² to 10⁻³, and LIV for up to 4 days (Wetter 1971); (ii) a TIP of 75 to 80°C, DEP of 10⁻² to 10⁻⁴, and LIV of up to 70 days (Loebenstein et al. 2001); and (iii) a TIP of 60 to 65°C, DEP of 10⁻³, and LIV of up to 6 days (Thomas et al. 1980). In this study, survival of infectivity varied between isolates and sometimes between individual experiments with the same isolate. Our TIP values most resembled those of (i) and (iii) but were lower than those in (ii), varying from 60°C with three PVS^A isolates to 55°C with one PVS^A and two PVS^O isolates, with infectivity of the other isolates being lost at 55 to 60°C. Our DEP values were mostly greater than those in (i), (ii), and (iii), varying from 10⁻⁵ to 10⁻⁶ with

one PVS^A isolate and 10⁻⁵ with six others and a single PVS^O isolate, to 10⁻⁴ to 10⁻⁵ with the remaining two PVS^A isolates and 10⁻³ with two remaining PVS^O isolates. Our LIV values were of longer duration than those in (i) and (iii) but shorter than that in (ii), varying from 40 or 25 to 28 days with five PVS^A isolates to up to 20 days with one PVS^O and the three remaining PVS^A isolates, and up to 16 to 17 days with the two remaining PVS^O isolates. Thus, although the PVS^O and PVS^A isolates from this study behaved similarly with thermal inactivation, none of the three PVS^O isolates remained infectious for more than 20 days, and two of them had a lower DEP than any of the nine PVS^A isolates. Greater DEP values reflect higher virus titers in the plant, which would be expected to increase the efficiency of aphid and contact transmission, and higher LIV values reflect greater stability of infectivity in sap, which would increase the efficiency of contact transmission.

The International Committee on Taxonomy of Viruses discriminates members of different *Carlavirus* species, using pairwise sequence comparisons; the CP or polymerase genes of different *Carlavirus* spp. have less than 72% nucleotide identity and less than 80% amino acid identity (Adams et al. 2004; King et al. 2011). Using these criteria, all 35 nonrecombinant PVS sequences come from isolates of a single species. In addition, because (i) all five recombinants were isolated recently in Europe and all of their parental isolates except one belonged to the non-SA lineage, and (ii) the single parental isolate from the SA lineage belonged to a sublineage found only in Bolivia, this suggests that this parental isolate had also arrived in Europe where the recombination occurred but this parent has not yet been sequenced.

The most important deduction from our PVS phylogeny is that all three lineages from the root lead to existing populations infecting potato, only one of which dominated the recent global adoption of potato as a major food crop. The known PVS population is sister to two other carlaviruses infecting potato, PRDV and PVP, and their phylogeny indicates that there has probably been a group of potato-infecting carlaviruses in South America for 5,000 to 7,000 years. Such timing correlates with the first domestication of the potato in the Lake Titicaca region around 7,000 years ago rather than the end of the Tiahuanaco civilization in about 1000 CE (Browman 1978;

Fig. 5. Phylogenetic tree calculated by the maximum-likelihood method from the aligned concatenate genes of 40 *Potato virus S* isolates showing the relationships of the five recombinant sequences (X) and the five nearest parental sequences (O), with some irrelevant lineages collapsed. Accession codes are listed and the genome maps indicate the recombinant regions and their origins.

Brown and Henfling 2014; Hawkes 1978; Rumold and Aldenderfer 2016) and the start of the Inca civilization, which ended abruptly following the Spanish conquest in 1532 CE. The Inca empire eventually reached along the length of the Andes from modern-day Colombia in the north to modern-day Chile in the South. The Incas improved and expanded the irrigation systems and terraces already developed by previous cultures; excelled in road building, which connected the different provinces conquered; and stimulated or forced people to plant potato and other crops to feed the empire's growing population (Brown and Henfling 2014; Hawkes 1978). This expansion of potato production over large areas, which involved nine different cultivated potato species, would have provided ideal conditions for dissemination of a contact-transmitted virus such as PVS. Such dissemination among remote Andean valleys could have led to rapid diversification of the PVS population arising from the different environments, growing conditions, and cultivated potato species and landraces encountered. Only two potato introductions to Europe occurred in the late 16th century, and there were few other early potato introductions. *Phytophthora infestans*, the cause of potato blight disease, was introduced to Europe in the mid-19th century. It eliminated almost all potato cultivars due to their narrow potato gene pool. The new potato germplasm introduced to breed against this disease provided far greater genetic diversity (Glendinning 1983). Based on our study, we deduce that one isolate of PVS was inadvertently brought to Europe before the early 19th century, probably in tubers of *S. tuberosum* subsp. *andigena*. PVS was therefore apparently among the few early trans-Atlantic potato shipments, and part of the "Columbian exchange", which started around 1570 CE (Anonymous 1994). The increased genetic diversity of the potato in Europe following the introduction of potato blight could have led to the recent diversification of the PVS population there. Like several other potato virus pathogens, PVS then spread worldwide with the expanding potato crop.

These conclusions about the origin of the worldwide PVS population closely parallel and complement those reported recently for the world PVY population (Gibbs et al. 2017), which also invaded potato in the Andes and spread around the world in a similar way subsequently, and in a similar timeframe. The PVS and PVY phylogenies are closely similar in topology. Their datings are also closely similar, although that for PVS is more statistically robust, giving a better correlation with time, probably because more genomes of SA isolates of PVS than PVY are available, and these provide intermediate patristic distances in the phylogeny of the three mid-phylogeny nodes in the SA lineages, which are all in the CE range. PVY is a member of a distinct lineage of potyviruses, most of which were first found in the Americas or have only been found there. There is a similar, geographical correlation among the carlaviruses most closely related to PVS. Its closest relatives are PRDV and PVP, only found in South America, and half their sister lineage is of "American viruses". Nonetheless, our conclusions about the similar recent evolutionary histories of the PVS and PVY populations are clearly well supported. It is interesting that two viruses with such similar ecological histories, driven by a shared host, have no known phylogenetic links and seem primordially distinct; PVS is an α -like virus and PVY a picorna-like virus (Goldbach 1987; Koonin 1991; Koonin et al. 2008).

Our studies suggest that isolates of the two SA lineages may (i) generally reach higher concentrations in the plant and be more stable in infective sap, both of which would favor their aphid and contact transmission in the field, and (ii) be more readily aphid transmitted. Although biologically defined PVS^o is widely distributed outside South America, the SA lineages are not. Therefore, plant quarantine and biosecurity authorities would be well advised to take precautions to prevent establishment the SA lineages in countries from which they are still absent.

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Western Australia (1 Australian isolate). Botanical seed of wild potato species was from the U.S. Potato Introduction Station, Sturgeon Bay, WI, healthy tubers of potato cultivars were from INIAP and CIP, and PVS-resistant cultivar Saco and Snec 66/139-19 were from the Scottish Plant Breeding Station. F. W. Santillan was a seconded employee of the Ministerio de Agricultura y Ganadería del Ecuador in 1977 to 1979. R. A. C. Jones was an employee of CIP in 1973 to 1978. He and C. E. Fribourg were supervisors of F. W. Santillan's M.Sc. project. Technical assistance in Lima was provided by J. Nakashima in the laboratory and J. Rojas in the glasshouse; help was also provided by C. Chuquillanqui, H. Espinosa, and N. Melendez. In Canberra, Australia, technical assistance with LSD software was provided by H. To. O. Gascuel. J. Kreuzer, S. Wylie, J. Fletcher, A. Blouin, and L. Palkovics provided background information about previously sequenced genomes for Table 1.

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