Biological Properties of *Potato virus X* in Potato: Effects of Mixed Infection with *Potato virus S* and Resistance Phenotypes in Cultivars from Three Continents

Eviness P. Nyalugwe, School of Plant Biology, Faculty of Natural and Agricultural Sciences, University of Western Australia, Crawley, WA 6009, Australia; **Calum R. Wilson**, Tasmanian Institute of Agricultural Research, University of Tasmania, New Town Research Laboratories, New Town, TAS 7008, Australia; and **Brenda A. Coutts** and **Roger A. C. Jones**, Department of Agriculture and Food, Baron-Hay Court, South Perth, WA 6151, Australia; and School of Plant Biology, Faculty of Natural and Agricultural Sciences, University of Western Australia, Crawley, WA 6009, Australia

Abstract

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Interactions between *Potato virus X* (PVX) and *Potato virus S* (PVS) were studied in potato plants, and isolates of PVX were inoculated to potato cultivars from four continents to identify occurrence of PVX resistance genes. Mixed infection with PVX and PVS increased the titer of PVS and enhanced expression of foliar symptoms in primarily and secondarily infected plants of 'Royal Blue'. PVX isolates belonging to strain groups 1 and 3 (WA1+3) or 3 (XK3 and TAS3) were sap and graft inoculated (1 to 3 isolates each) to 38 cultivars and one breeding line. Presence of extreme PVX resistance gene *Rx* was identified in four Australian ('Auski', 'Billabong', 'Flame', and 'Ruby Lou') and two European ('Mondial' and 'Rodeo') cultivars, and in a clone of North American 'Atlantic'. PVX hypersensitivity gene *Nx* was identified for the first time in two Australian ('Bliss' and 'MacRusset'), four European ('Almera', 'Harmony', 'Maxine', and 'Nadine'), and one

North American ('Ranger Russet') cultivars, and in Australian breeding line 98-10713. PVX hypersensitivity gene *Nb* was identified for the first time in one Australian ('White Star'), five European ('Innovator', 'Kestrel', 'Kipfler', 'Laurine', and 'Royal Blue'), and one North American ('Shepody') cultivars. Probable ancestral sources of the resistance genes found in different cultivars were identified. Thus, although PVX resistance genes often occur in parents used in crosses, knowledge of their occurrence in parents and cultivars is often lacking. On sap inoculation, systemic hypersensitive phenotypes that caused shoot death often developed in cultivars with *Nx* but not necessarily in all shoots. This phenotype caused severe necrotic symptoms in infected tubers. In some instances, passage through cultivars with *Nb* separated strain group 3 from mixed isolate WA1+3.

Potato virus X (PVX; family *Alphaflexiviridae*, genus *Potexvirus*) is one of over 30 viruses that infect potato crops (31,36,57). Infection normally causes mild mosaic or is latent but severe mosaic sometimes occurs, with tuber yield losses of 5 to 20%. Mixed infection of potato plants with PVX and *Potato virus Y* or *Potato virus A* (PVY or PVA, respectively; family *Potyviridae*, genus *Potyvirus*) causes a synergistic reaction resulting in the classical foliage diseases 'rugose mosaic' (PVX + PVY) and 'crinkle' (PVX + PVA) and considerably greater yield losses (20,36,48,57). Mixed infection of potato plants with PVX and *Potato virus S* (PVS; family *Betaflexiviridae*, genus *Carlavirus*) occurs commonly. It can cause tuber yield losses when none occur with either virus alone (67) but there are no reports of synergism between them involving enhanced virus titer or symptom expression.

Although there has never been active breeding for PVS resistance in potato, in the past, in Europe and North America, there was active breeding aimed at incorporating PVX hypersensitivity genes *Nx* and *Nb* or extreme resistance gene *Rx* into cultivars (13,16,25,52,53). Information on whether some older cultivars carry hypersensitivity genes *Nx* or *Nb* is available for the United Kingdom (14), Germany (50), and North America (5). The achievements of breeding for resistance to PVX and other potato

viruses since the 1930s were reviewed extensively (9,16,19,49, 55,56,59,60). However, little emphasis is now being placed on breeding for PVX resistance worldwide. Also, although data on whether new cultivars have some resistance is sometimes provided, information about resistance genes is lacking (4,51).

PVX is divided into four strain groups defined by phenotypes induced by inoculation of isolates to potato cultivar differentials with *Nb* or *Nx*. Group 1 strains overcome neither gene, group 2 strains *Nx* only, group 3 strains *Nb* only, and group 4 strains both genes (15). The PVX 25-kDa movement protein gene elicits hypersensitivity in plants with *Nb* but the coat protein (CP) gene elicits hypersensitivity in plants with *Nx* and extreme resistance in plants with *Rx* (22,39,42). When the CP genes of 85 different PVX isolates were analyzed phylogenetically, clade I isolates were from six continents but those in subclades II-1 and II-2 were only from Europe or the Americas, respectively. Clade I contained isolates in strain groups 1, 3, and 4; and subclades II-1 and II-2 isolates in strain groups 2 and 4 (18).

In Australia, potato cultivars originally bred in Australia, Europe, or North America are widely grown. PVX and PVS infection occurs in seed potato stocks and high infection incidences of both are sometimes found in ware crops (23,24,29,40,63). Studies on PVX resistance in potato were published in 1940 to 1952 (27,28). Subsequently, Wilson and Jones (66) found PVX strain groups 1 and 3, and that Australian-bred 'Coliban' and 'Geographie' carry gene *Nb*. This study (i) examined the effects of mixed infection with PVX and PVS on symptom expression and virus titer in potato plants and (ii) used sap and graft inoculation with different PVX isolates to 38 cultivars from Australia, Europe, North America, or southeast Asia to determine the occurrence of resistance genes *Rx*, *Nb*, and *Nx*. Putative parental sources were identified for the resistance genes found. New information also was obtained on development of tuber necrosis associated with *Nx* presence, expression of the systemic hypersensitive phenotype

Corresponding author: R. A. C. Jones,
E-mail: roger.jones@agric.wa.gov.au

Current address of E. P. Nyalugwe: Department of Crop Development, Ministry of Agriculture and Food Security, P.O. Box 30145, Capital Hill, Lilongwe, Malawi.

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associated with *Nx*, and separation of components from PVX strain group mixtures.

Materials and Methods

Virus isolates, inoculations, and antiserum. PVX isolates XK3 and TAS3 belong to strain group 3. XK3 is a subisolate of Western Australian isolate XK from potato ‘Kennebec’ (64), which is a mixture of strain groups 1 and 3 (66). Tasmanian isolate TAS3 (18) was originally obtained by Dr. Gradon Johnstone from an unknown potato cultivar. West Australian isolate WA1+3 is also from an unknown cultivar and is a mixture of strain groups 1 and 3. No strain group 1 isolates were available, and strain group 2 has never been identified definitively in Australia (18). PVS ordinary strain isolate SK was from Kennebec (17,65). Isolates XK3, TAS3, and WA1+3 were maintained in infected potato plants by serial planting of infected tubers of ‘Royal Blue’, Coliban, and ‘Desiree’, respectively. Isolates XK3 and WA1+3 also were maintained in plants of *Nicotiana glutinosa* by serial subculture using sap inoculation. Isolate SK was maintained in plants of Royal Blue. Sap from leaves from XK3-infected, SK-infected, or healthy potato plants were used as positive or negative controls in enzyme-linked immunosorbent assay (ELISA). Sap inoculations were done by rubbing infective leaf sap diluted in 0.05 M phosphate buffer (pH 7.2) containing 0.05 M sodium sulfite (Perth), or 0.2 M phosphate buffer (pH 7.5) containing 1% cysteine (Hobart), and mixed with “celite” abrasive onto leaves of healthy potato plants. Mock

inoculations differed in that the sap extracts came from healthy potato plants. Graft inoculations were top grafts in which PVX-infected potato scions were grafted onto healthy potato plants. In Perth, the scions used came from infected plants of Royal Blue, ‘Kestrel’, or ‘FL-1867’ (isolate XK3) or Desiree, ‘White Rose’, or FL-1867 (isolate WA1+3). In Hobart, they were from infected plants of Coliban (isolate TAS3) or Desiree (isolate WA1+3). Polyclonal PVX and PVS antibodies used in ELISA were from Loewe Biochemica (Sauerlach, Germany) or Agdia Inc. (Elkhart, IN).

Potato cultivars and growing conditions. Healthy tubers of the 38 different potato cultivars and one potato breeding line used were supplied by Peter Dawson, Ian McPharlin, Jeff Mortimore, or David Tooke of the Department of Agriculture and Food for Western Australia (DAFWA), or by Iain Kirkwood or Leonie White of the University of Tasmania (UTAS). The only exception was healthy tubers of ‘Kipfler’, which were selected from shop-purchased tubers. Details of cultivar names, original country of origin, parentage, and known presence of PVX resistance genes *Nb*, *Nx*, or *Rx* are provided in Table 1. Potato tubers were planted in steam-sterilized potting mix (mix of soil, sand, and peat) in 25-cm-diameter pots. The plants were kept throughout in air-conditioned, insect-proof glasshouses maintained at 15 to 20°C, or a controlled environment cabinet (Contherm Scientific Ltd, Petone, New Zealand) maintained at 20°C with a 16-h photoperiod. Pots were widely spaced to ensure that interplant contact did not occur.

Table 1. Origins and parentage of the 38 potato cultivars and one breeding line studied, and published occurrence of *Potato virus X* (PVX) resistance genes

Cultivar	Country (year of release) ^w	Parentage	PVX resistance genes ^x
Almera	The Netherlands (1999)	2102/77/2698 × AR 80-31-20	Unknown
Atlantic	United States (1976)	Wauseon × Lenape	<i>Rx</i>
Auski	Australia (NR)	Denali × 79-5-2	Unknown
Billabong	Australia (NR)	Mondial × 85-30-12	Unknown
Bismark ^y	Germany (1889)	Sachsische zwiebel × Erste von Fromsdorf	Unknown
Bliss	Australia (2000)	Atlantic × Lindsey	Unknown
Carlingford	United Kingdom (1982)	S 62-47-1 × Dr. McIntosh	Unknown
Challenger	The Netherlands (2008)	Aziza × Victoria	Unknown
Cherry Red	United States (1999)	ND 4750-2R × LA 1858	Unknown
Coliban	Australia (1974)	Kennebec × V 28-12	<i>Nb</i>
Desiree	The Netherlands (1962)	Urgenta × Depesche	None
Fergifry	Australia (2003)	Mondial × 85-30-12	Unknown
FL-1867 ^z	United States (2003)	FL162 × Atlantic	Unknown
Eben	CIP, Peru/Philippines (2000)	Y84.025 × 378015.16	Unknown
Flame	Australia (NR)	Foxton × 69-32T-4	Unknown
Forester	Australia (NR)	Crispa × Kaimai	Unknown
Harmony	United Kingdom (1998)	Nadine × Stamina	Unknown
Kestrel	United Kingdom (1992)	Seedling × Cara	Unknown
King Edward	United Kingdom (1902)	Magnum Bonham × Beauty of Hebron	<i>Nx</i>
Kipfler	Austria (1956)	Unknown	Unknown
Igorota (=PO3)	CIP, Peru/Philippines (1987)	True potato seed selection	Unknown
Innovator	The Netherlands (1999)	Shepody × RZ-84-2580	Unknown
Laurine	The Netherlands (NR)	Not available	Unknown
MacRusset	Australia (1996)	Lemhi Russet × Maris Piper	Unknown
Maris Piper	United Kingdom (1963)	(Y 22/6 × Ulster Night) × (Arran Cairn × Herald)	<i>Nb</i> , <i>Nx</i>
Maxine	United Kingdom (1994)	Antar × 93/2	Unknown
Monaro (97-102-1)	Australia (NR)	91-158-6 × Yankee Supreme	Unknown
Mondial	The Netherlands (1987)	Spunta × VE 66-295	Unknown
Nadine	United Kingdom (1987)	<i>Solanum vernei</i> polycross	Unknown
Ranger Russet	United States (1991)	Butte × A-6595-3	Unknown
Rodeo	The Netherlands (1999)	Mondial × Bimonda	Unknown
Royal Blue	The Netherlands (1995)	Asterisk × VE 76-46	Unknown
Ruby Lou	Australia (1998)	Foxton × 69-32 T-4	Unknown
Russet Burbank	United States (1914)	Mutant from Burbank	None
Shepody	Canada (1980)	Bake King × F050	Unknown
Tasman	Australia (1974)	(Duke of York × BC 0/4) × (Crana × 11-79)	Unknown
White Rose (= Delaware) ^y	United States (1893)	Seed ball from ‘Jackson’	None
White Star	Australia (2006)	Gladiator × 91-158-6	Unknown
98-10713 ^z	Australia (NR)	89-12-1 × MacRusset	Unknown

^w NR = not yet released.

^x References to occurrence of resistance genes (5,26,32,33,62,66).

^y Bismark is believed to be a selection from the German-bred (1889) Bismarck of parentage Sachsische zwiebel × Erste von Fromsdorf, and White Rose is called Delaware in Australia.

^z FL-1867 is a commercially grown cultivar that never received a different name, and 98-10713 is a breeding line.

Virus detection. Leaf samples were extracted (1 g per 20 ml) in phosphate-buffered saline (10 mM potassium phosphate, 150 mM sodium chloride, pH 7.4) with Tween 20 at 5 ml/liter and polyvinyl pyrrolidone at 20 g/liter using a mixer mill (Retsch, Germany). Sample extracts were tested for the presence of PVX or PVS antigens by double-antibody sandwich ELISA (12). All samples were tested in duplicate wells in microtiter plates, and sap extracts from PVX- or PVS-infected and healthy potato leaf samples were included in paired wells as controls. The substrate used was *p*-nitrophenyl phosphate at 0.6 mg/ml in diethanolamine, pH 9.8, at 100 ml/liter. Absorbance values at 405 nm (A_{405}) were measured in a Bio-Rad model 680 microplate reader (Bio-Sys, Australia). Positive absorbance values were always more than three times those from healthy sap controls.

Interactions between PVX and PVS. Primary infection. At 3 to 4 weeks after planting healthy tubers of potato Royal Blue, plants were trimmed so that only one stem remained and their leaves were mock inoculated with healthy potato sap or inoculated with infective sap containing PVX-XK3, PVS-SK, or a mixture of both isolates (four plants per treatment). To prepare the mixed inoculum, equal fresh weights of Royal Blue leaves infected with PVX-XK3 or PVS-SK were ground together and the extract mixed before inoculation. The pots were then arranged in a randomized block design, each block containing a single replicate (pot) of each treatment. Foliage symptoms were recorded weekly for 12 weeks after planting. Samples were taken from tip leaves of each plant 32 and 62 days after planting (dap) and tested individually for PVX and PVS by ELISA. At maturity, the tubers from each pot were harvested separately and kept in individually labeled paper bags, numbers of tubers counted, and yields weighed.

Secondary infection. Tubers harvested from the primarily infected Royal Blue plants were stored until they sprouted. Then, nine tubers from each of the four treatments were planted and the pots arranged in a randomized block design. Foliage symptoms were recorded weekly for 12 weeks and samples taken from tip leaves of each plant 32, 66, and 87 dap and tested individually for PVX and PVS by ELISA. After harvest, tuber number and yields were determined as above.

Data analysis. ELISA optical density (A_{405}) values were transformed exponentially (e^x) to obtain a linear relationship between them and virus titer as described by Wilson and Jones (65). Data for virus titer, mean numbers of tubers per plant, and gross tuber yield were subjected to analysis of variance using Genstat for Windows, release 10.

Reactions of cultivars of known PVX resistance status. Plants of PVX cultivar differentials Desiree (without *Nx* or *Nb*), Coliban (with *Nb* only), 'King Edward' (with *Nx* only), and 'Maris Piper' (with *Nx* and *Nb*) (32,33,66) were sap inoculated with extracts containing isolates XK3, TAS3 and WA1+3 (two to six plants per isolate). In addition, plants of 'Atlantic' for which presence of *Rx* is disputed (58) (10 plants/isolate) and 'Russet Burbank' (without *Nx* or *Nb*; 66) (2 plants/isolate) were sap inoculated with extracts containing the same three isolates; and plants of White Rose (without *Nx* or *Nb*; 66) (4 to 6 plants/isolate) with extracts containing isolates XK3 and WA1+3. Plants of each cultivar were also mock inoculated with healthy sap extract. All plants were observed for virus symptoms for 10 weeks after inoculation. Samples taken from inoculated and tip leaves 4 weeks after inoculation, and again from tip leaves 8 to 10 weeks after inoculation, were tested for PVX by ELISA. Where systemic necrosis developed, these tip leaf samples were always from the necrotic shoots.

Resistance gene identification. Sap extracts infected with isolates WA1+3, XK3, or TAS3 were inoculated to two to seven plants of 31 cultivars and one breeding line (one to three isolates each). These inoculations were first done at the two- to three-leaf stage and then repeated 1 week later. Two mock-inoculated control plants of each cultivar were also present in each test. All plants were observed for up to 10 weeks after first inoculation for development of virus symptoms. Samples were taken from inoculated leaves of each plant 4 weeks after inoculation and from their tip

leaves after 4 to 10 weeks. Where systemic necrosis developed in some or all shoots on a plant, unless otherwise stated, tip leaf samples were always from necrotic shoots. All samples were tested for PVX by ELISA. Harvested tubers were examined for symptoms with some combinations of isolate and cultivar. Hypersensitive and extreme resistance phenotypes were deduced as described previously (32,33,66).

Confirmatory graft inoculations. Scions infected with isolates WA1+3, XK3, or TAS3 were graft inoculated onto plants of 20 potato cultivars (one to three isolates each, up to six scions per plant), which included all seven cultivars that remained uninfected after sap inoculation with PVX (2 to 7 plants/isolate for all cultivars, except for 40 plants/isolate with Atlantic). Axillary shoots that grew out beneath graft unions were observed for symptoms weekly for 9 to 12 weeks after graft inoculation. Tip leaf samples from axillary shoots on each plant were taken 4, 9, and 12 weeks after graft inoculation and tested for PVX by ELISA. Harvested tubers were examined for symptoms. Hypersensitive and extreme resistance phenotypes were deduced as above.

Results

Interactions between PVX and PVS. Primary infection. Mock-inoculated potato plants developed no symptoms. PVS-infected plants developed faint bronzing and undulating margins on upper leaves, mild leaf mosaic formed on PVX-infected plants, and plants infected with both viruses developed severe mosaic symptoms which were much more obvious than those caused by either virus alone (Fig. 1Ai-iv). When virus titer values were compared, at 32 dap there were significant differences between those for PVX and PVS but not between those for single and mixed infection with the same virus (Table 2). By contrast, at 62 dap, although there was still no significant difference between the two values for PVX, the PVS value for mixed infection was significantly higher than that for PVS alone. There were no significant differences between any treatments in tuber numbers per plant or yield (Table 3).

Secondary infection. Unlike the situation with primary infection, the PVS-infected plants resembled the mock-inoculated control plants in being asymptomatic, and the mild mosaic caused by PVX was transient. In contrast, the plants with mixed infection developed an obvious severe mosaic and chlorosis which commenced at 28 dap and intensified as they approached maturity (Fig 1Bi-iv and Ci-iv). Also, although there were, again, no significant differences between titer values for PVX alone and in mixed infection at 32, 66, and 87 dap, the PVS value for mixed infection was always significantly higher than that for PVS alone (Table 2). There were no significant differences in tuber numbers per plant between any of the four treatments but tuber yield was significantly greater for mock-inoculated control plants than for any of the other treatments (Table 3). There were no significant yield differences between any of the three virus treatments but yield was decreased most by mixed infection (23%).

Reactions to sap inoculation. Cultivars of known resistance status. When inoculated with infective sap containing isolates WA1+3, XK3, or TAS3, plants of Desiree and 'Russet Burbank' developed susceptible phenotypes (Table 4). Coliban plants behaved similarly with XK3 and TAS3 but WA1+3 induced a localized hypersensitive phenotype followed by systemic infection without necrosis. All three isolates induced systemic hypersensitive phenotypes in plants of King Edward and Maris Piper and extreme resistance phenotypes in Atlantic, while XK3 and WA1+3 induced susceptible phenotypes in White Rose. The findings with standard differentials confirm that isolate WA1+3 is a mixture of strain groups 1 and 3 while isolates XK3 and TAS3 contain only strain group 3.

Cultivars of unknown resistance status. When four cultivars were sap inoculated with isolates WA1+3 and TAS3, plants of 'Bismark', 'Fergify', and 'Tasman' always developed susceptible phenotypes (Table 4). In contrast, 'Shepody' behaved like Coliban (Fig. 2Ai). When plants of 17 cultivars were inoculated with isolates WA1+3 and XK3, 'Mondial', 'Ruby Lou', and 'Rodeo' showed extreme resistance phenotypes but 'Challenger' and

Fig. 1. Leaves of potato 'Royal Blue' (Nb) with systemic **A**, primary or **B and C**, secondary infection with *Potato virus S* (PVS) isolate SK, *Potato virus X* (PVX) isolate XK3, or both viruses. **A**, Leaves at 44 days after planting (dap) infected by i, neither virus (mock-inoculated control) without symptoms; ii, PVS alone showing undulating leaflet margins and faint bronzing; iii, PVX alone showing a mild mosaic; or iv, a mixture of the two viruses showing a severe mosaic. **B**, Leaves at 47 dap infected by i, neither virus (mock-inoculated control) showing no symptoms; ii, PVS alone causing symptomless infection; iii, PVX alone causing mild mosaic; or iv, a mixture of the two viruses causing severe mosaic. **C**, Leaves at 81 dap infected by i, neither virus (mock-inoculated control) showing no symptoms; ii, PVS alone causing symptomless infection; iii, PVX alone causing symptomless infection; or iv, a mixture of the two viruses causing severe mosaic and chlorosis.

FL-1867 developed susceptible phenotypes. In 'Almera', 'Harmony', and 'Maxine', both isolates induced localized hypersensitive phenotypes without systemic movement. 'Nadine' and 'Ranger Russet' behaved like this with one isolate but not with the other, which induced systemic hypersensitive phenotypes (Fig. 2Aii and iii), while 'Bliss' developed systemic hypersensitive phenotypes with both (Fig. 2Aiv). XK3 induced a susceptible phenotype in plants of 'Innovator', Kestrel, Kipfler, 'Laurine', Royal Blue, and 'White Star'. WA1+3 caused localized hypersensitive phenotypes in Innovator, Kipfler, and Laurine but Kestrel, Royal Blue, and White Star developed localized hypersensitive phenotypes (Fig. 2Av) followed by systemic infection. The systemic symptoms induced were initial necrotic spotting (Fig. 2Avi) followed by mosaic and leaf deformation in Kestrel and Royal Blue (Fig. 2Avii) but necrosis and shoot death in White Star. When plants of 10 cultivars and one breeding line were inoculated with XK3 alone, extreme resistance phenotypes developed in 'Auski', 'Billabong', and 'Flame' but systemic hypersensitive phenotypes in '98-10713' and 'MacRusset'. The remaining six cultivars developed susceptible phenotypes consisting of symptomless systemic infection ('Cherry Red', 'Forrester', 'Igorota', and 'Monaro') or mosaic ('Carlingford' and 'Eben').

Occurrence of extreme resistance phenotypes suggested the presence of *Rx* in six additional cultivars (Table 4). Similarly, appearance of hypersensitive phenotypes with XK3 or TAS3 suggested *Nx* presence in another eight cultivars and one breeding line. Presence of *Nb* could be deduced for the eight additional cultivars that developed hypersensitive phenotypes with WA1+3 but not XK3 or TAS3. However, when hypersensitive phenotypes developed with WA1+3 and XK3 in Almera, Bliss, Harmony, Maxine, Nadine, and Ranger Russet, it was impossible to deduce whether they also contained *Nb*. Similarly, it was impossible to say whether cultivars inoculated with XK3 alone had gene *Nb*.

When Ranger Russet tubers harvested from plants that developed systemic hypersensitive phenotypes with isolate WA1+3 were

examined, deep, sunken necrotic areas surrounding tuber eyes were present in 13 of 16 tubers (Fig. 3i). In some, the sunken areas had coalesced, causing enlarged, sunken necrotic regions that encompassed much of the affected tuber. No symptoms were present in tubers of Kestrel or Royal Blue from plants that developed initial systemic necrotic spotting followed by mosaic with WA1+3, or where susceptible phenotypes formed: XK3 in Kestrel and Laurine or either isolate in Challenger and FL-1867.

Occurrence of systemic hypersensitive phenotype. When systemic hypersensitive phenotypes developed in plants infected by sap inoculation, normally all shoots were affected. However, sometimes these phenotypes developed in some shoots only (e.g., in MacRusset and 98-10713 with isolate XK3 and Bliss with WA1+3; Table 4). Also, systemic hypersensitive phenotypes developed rapidly in some cultivars but slowly in others (e.g., with XK3 they appeared within 3 to 4 weeks of inoculation in MacRusset but took 7 weeks to commence in Bliss). In Nadine, XK3 normally caused a systemic hypersensitive phenotype within 3 to 4 weeks but, on one occasion, inoculation induced only a localized hypersensitive phenotype. Also, with WA1+3, Nadine only developed a localized hypersensitive phenotype. In contrast, in Ranger Russet, WA1+3 induced a systemic hypersensitive phenotype within 3 to 4 weeks of inoculation but XK3 only a localized hypersensitive phenotype. In Almera, Harmony, and Maxine, both isolates induced only localized hypersensitive phenotypes. Thus, in some cultivars with *Nx*, expression of systemic hypersensitive phenotypes was slow or sporadic or did not occur. In cultivars with *Nb* but without *Nx*, all developed susceptible phenotypes with XK3 but only White Star developed a systemic hypersensitive phenotype with WA1+3. The others developed localized hypersensitive phenotypes or local necrotic spot lesions associated with initial systemic necrotic spotting followed by mosaic.

Separation of strain group 3 from isolate WA1+3. Development of local necrotic spot lesions followed by systemic mosaic with isolate WA1+3 in Shepody indicates separation of strain group 3

Table 2. Effect of mixed and single infection with *Potato virus X* (PVX) and *Potato virus S* (PVS) on virus titer in leaves of primarily and secondarily infected plants of potato 'Royal Blue'

Virus	Second virus	Virus titer (days after planting) ^y				
		Primary infection		Secondary infection		
		32	62	32	66	87
PVX	None	3.06 (1.10) c	1.89 (0.63) b	1.71 (0.54) b	1.47 (0.38) b	1.40 (0.34) b
PVX	+ PVS	3.50 (1.25) c	1.90 (0.64) b	1.70 (0.53) b	1.35 (0.29) ab	1.48 (0.39) b
PVS	None	1.90 (0.60) b	2.88 (1.05) d	1.76 (0.55) b	2.09 (0.68) c	3.63 (1.26) c
PVS	+ PVX	2.23 (0.79) b	4.32 (1.45) a	1.99 (0.68) c	2.78 (1.01) d	3.93 (1.37) d
None	None	1.00 (0.00) a	1.02 (0.02) a	1.00 (0.00) a	1.01 (0.01) a	1.00 (0.00) a
<i>P</i>	...	<0.001	<0.001	<0.001	<0.001	<0.001
LSD ^z	...	0.68	0.71	0.21	0.36	0.19
df	...	12	8	32	32	32

^y Virus concentrations in leaf extracts were measured using exponentially transformed enzyme-linked immunosorbent assay optical density (absorbance at 405 nm) values. Figures in parentheses are original optical density values. Means followed by the same letter are not significantly different at *P* < 0.05.

^z Least significant difference.

Table 3. Effect of mixed and single infection with *Potato virus X* (PVX) and *Potato virus S* (PVS) on tuber yield of primarily and secondarily infected plants of potato 'Royal Blue'^y

Virus	Primary infection		Secondary infection	
	Number of tubers/plant	Tuber yield (g/plant)	Number of tubers	Tuber yield (g/plant)
PVX	8.7	171.5	5.8	350.4 a (12)
PVS	5.0	313.5	5.0	318.9 a (20)
PVX + PVS	4.3	202.2	5.8	305.7 a (23)
None	5.0	205.9	7.2	397.6 b
<i>P</i>	ns	ns	ns	0.01
LSD ^z	...	...	...	45.36
df	...	...	...	24

^y Data are mean number of tubers/plant and mean tuber weight/plant. Means followed by the same letter are not significantly different at *P* < 0.05; ns = not significant. Numbers in parentheses are percent yield losses.

^z Least significant difference.

from strain group 1, the latter being restricted to inoculated leaves (32,33,66). Although systemic necrotic spotting and shoot death developed with WA1+3 in 8 of 12 shoots of White Star, absence of any mosaic-affected tissue and lack of detection of any PVX in symptomless tissue (by ELISA or inoculation to plants of Royal Blue) indicated lack of separation of strain groups 1 and 3. As mentioned above, in Kestrel and Royal Blue, WA1+3 induced systemic necrotic spotting initially but mosaic and leaf deformation subsequently (Fig. 2Av–vii). When extracts from later-formed leaves were inoculated to further plants of Royal Blue (three plants each), the Royal Blue extract induced chlorotic blotch local lesions followed by systemic mosaic and leaf deformation, indicating separation of strain group 1 from strain group 3 (32,33,66). In contrast, the Kestrel extract still induced some local necrotic spot lesions in addition to faint chlorotic blotch local lesions (>5:1 ratio of chlorotic/necrotic lesions) followed by systemic mosaic and leaf deformation without necrosis. This indicates that group 1 was still present at a low level in the extract from Kestrel leaves without necrosis. When an extract from mosaic-affected leaves from one of the plants inoculated previously with Royal Blue extract was inoculated to Nadine (which carries *Nx*), local necrotic spots (Fig. 2Aviii) formed in inoculated leaves followed by the systemic necrotic phenotype in two of three shoots. Deep, sunken necrotic eyes were present in two of seven tubers from these Nadine plants infected with isolate WA3 (i.e., with the group 3 component of WA1+3; Fig. 3ii).

Confirmation by graft inoculation. No symptoms developed and no PVX was detected in plants of Atlantic graft inoculated with all three PVX isolates despite repeated testing amounting to inoculation of 40 plants/isolate (Table 5). Similarly, no symptoms developed and no PVX was detected in plants of Mondial, Rodeo, or Ruby Lou graft inoculated with WA1+3 and XK3 (Fig. 2Bi), or Auski, Billabong, or Flame graft inoculated with XK3. These extreme resistance phenotypes confirm the presence of *Rx*. Systemic hypersensitive phenotypes developed in plants of Bliss, King Edward, Maris Piper, and Nadine graft inoculated with isolate WA1+3 (Fig. 2Bii) and XK3 or TAS3, and in Maxine graft inoculated with XK3 (Maxine had developed a localized necrotic phenotype previously after sap inoculation with WA1+3). This confirms *Nx* presence in these cultivars. Coliban and Shepody developed systemic necrotic phenotypes with isolate WA1+3 (Fig. 2Biii) but not TAS3, and Kipfler with WA1+3 (Kipfler had developed a susceptible phenotype previously after sap inoculation with XK3), confirming *Nb* presence. Susceptible phenotypes developed in five other cultivars graft inoculated with WA1+3 or TAS3.

When tubers harvested from plants of five combinations of cultivar and isolate that developed hypersensitive phenotypes on graft inoculation were examined, deep, sunken necrotic areas that surrounded tuber eyes, especially those at their rose ends, were observed with the following: Maxine with XK3, Nadine with WA1+3, and Bliss with both isolates (Fig. 3iii–iv). In a few affected tubers, deep, sunken necrotic areas were also centered on

Table 4. Responses of 32 potato cultivars and one breeding line to sap inoculation with one to three isolates of *Potato virus X* (PVX)^w

Cultivar, breeding line	Response ^x			<i>R</i> gene ^y
	Isolate WA1+3	Isolate XK3	Isolate TAS3	
Almera	LNS, NS	LNS, NS	...	<i>Nx</i>
Atlantic	NI	NI	NI	<i>Rx</i>
Auski	...	NI	...	<i>Rx</i>
Billabong	...	NI	...	<i>Rx</i>
Bismark	MM	...	SM	...
Bliss	LNS, SNS, NSST, SD ^z	LNS, SNS, NSST, SD	...	<i>Nx</i>
Challenger	MM	MM	...	...
Coliban	LNS, MM	FLNS, FSNS, SS	SM	<i>Nb</i>
Desiree	SM	SS	SM	...
Fergifry	MM	...	SM	...
FL1867	MM	MM	...	...
Flame	...	NI	...	<i>Rx</i>
Harmony	LNS, NS	LNS, NS	...	<i>Nx</i>
Innovator	LNS, NS	MM	...	<i>Nb</i>
Kestrel	LNS, SNS, SM, LD	MM	...	<i>Nb</i>
King Edward	LNS, SNS, NSST, SD	LNS, SNS, NSST, SD	LNS, SNS, NSST, SD	<i>Nx</i>
Kipfler	LNS, NS [MLN]	MM, FSNS, [MLN]	...	<i>Nb</i>
Laurine	LNS, NS	MM	...	<i>Nb</i>
Maris Piper	LNS, SNS, NSST, SD	LNS, SNS, NSST, SD	LNS, SNS, NSST, SD	<i>Nx</i>
MacRusset	...	LNS, SNS, NSST, SD ^z	...	<i>Nx</i>
Maxine	LNS, NS	LNS, NS	...	<i>Nx</i>
Mondial	NI	NI	...	<i>Rx</i>
Nadine	LNS, NS	LNS, SNS, NSST, SD	...	<i>Nx</i>
Ranger Russet	LNS, SNS, NSST, SD ^z	LNS, NS	...	<i>Nx</i>
Rodeo	NI	NI	...	<i>Rx</i>
Royal Blue	LNS, SNS, SM, LD	MM	...	<i>Nb</i>
Ruby Lou	NI	NI	...	<i>Rx</i>
Russet Burbank	MM	SS	SM	...
Shepody	LNS, MM	...	SM	<i>Nb</i>
Tasman	MM	...	SM	...
White Rose	MM	SM	...	...
White Star	LNS, SNS, SD ^z	SS	...	<i>Nb</i>
98-10713	...	LNS, SNS, NSST, SD ^z	...	<i>Nx</i>

^w In all, 2 to 7 plants were inoculated for each isolate-cultivar combination, except Atlantic, with which it was 10 plants/isolate. Plants were kept for 5 to 10 weeks after inoculation. Samples from inoculated and noninoculated tip leaves were tested for PVX presence by enzyme-linked immunosorbent assay (ELISA) after 4 and 4 to 10 weeks, respectively.

^x NLS = local necrotic spots in inoculated leaves; FNLS = few faint local necrotic spots in inoculated leaves; MM = mild mosaic; SM = severe mosaic; LD = leaf deformation; SNS = systemic necrotic spotting; FSNS = few systemic necrotic spots; NSST = necrotic stem streaking; SD = systemic necrosis and shoot death; SS = symptomless systemic infection; NS = not infected systemically (confirmed by ELISA testing); NI = not infected; [MLN] = non-viral marginal leaf necrosis symptoms.

^y Resistance gene.

^z Systemic necrosis developed in some but not all shoots on inoculated plants.

the point of attachment to the stolon and, in several others, the necrotic areas around eyes had coalesced, causing enlarged, sunken necrotic regions that encompassed much of the affected tuber. In Bliss, the sunken areas were also cracked (Fig. 3v). In contrast, necrotic blisters and rings not associated with eyes or stolons were present in 1 of 13 Kipfler tubers from plants graft inoculated with WA1+3. As expected, no symptoms were seen in tubers from plants of Atlantic, Mondial, Ruby Lou, or Rodeo graft inoculated with either isolate.

Discussion

This study shows that mixed infection between PVS and PVX can increase both PVS titer and severity of foliar disease symptoms in potato plants. It also shows that, although PVX resistance genes *Rx*, *Nx*, and *Nb* are often present in the parents used in crosses made in potato breeding programs in different continents, knowledge of their occurrence in parental lines and released cultivars is often lacking. Of the 38 potato cultivars and single breeding line evaluated, PVX resistance genes were found in 25 of them which came from three continents (Australia, Europe, and North America). Only *Nx* in Maris Piper and King Edward (26,32,33), *Nb* in Coliban and Maris Piper (32,33,66), and *Rx* in Atlantic (62) were documented previously, but five of the others (Innovator, Maxine, Mondial, Nadine, and Ranger Russet) were reported to have "PVX resistance" in cultivar descriptions (4,46,51). Information was also obtained on tuber necrosis associated with the systemic hypersensitive phenotype; its often sporadic expression in shoots of cultivars sap inoculated with gene *Nx*; separation of strain group 3 from a mixed strain group 1+3 isolate; and the parental origins of *Nx*, *Nb*, and *Rx* in cultivars from different continents.

In a field experiment with Russet Burbank potato, despite latent infections with PVX or PVS alone having no effect on tuber yield, combined infection with both of them significantly decreased yield without causing symptoms (67). In our pot experiment with both primarily and secondarily infected plants of Royal Blue, mixed infection with PVX and PVS induced much more severe foliar symptoms than single infection with either virus. Also, PVX presence significantly increased PVS titer. In contrast, PVX titer was not increased by PVS presence. Thus, the possibility that a synergistic interaction that increased PVS concentration might have been responsible for the enhanced disease symptom expression

was indicated. To prove beyond doubt that synergism was involved, additional research would be required (e.g., experiments involving inoculations with controlled amounts of both viruses and quantitation of virus titer in infected plants using relative absorbance in ELISA or quantitative RT-PCR methodologies to determine precise concentrations of both viruses in infected plants). Mixed viral infections have synergistic effects because one virus suppresses host gene silencing mechanisms which would normally keep the second virus in check. For example, the *Potyvirus* P1/HC-Pro is a suppressor of post-transcriptional gene silencing, allowing PVX to replicate to higher levels and cause more dramatic symptoms in plants with mixed infections (3,48). Thus, in mixed infection with PVX and PVS, repression of host gene silencing by PVX might allow PVS to replicate to higher levels. In our study, although mixed infection decreased gross tuber yield by 23%, its effect in increasing PVS concentration and symptom severity was not reflected in tuber yield reductions significantly greater than those caused by single infection with either virus ($P < 0.05$). This was probably a consequence of the small pot size, which was insufficient to allow tuber development to occur unimpeded. Additional experiments are needed to reveal whether mixed infection with these two viruses causes similar effects in other cultivars showing susceptible phenotypes, establish the influences of different strains of either virus in mixtures, and evaluate effects on tuber yield in situations where tuber development is unrestricted.

Sap and graft inoculations with different PVX isolates revealed the presence of extreme resistance gene *Rx* in four Australian (Auski, Billabong, Flame, and Ruby Lou) and two European (Mondial and Rodeo) potato cultivars. Tavantzis et al. (58) working in Maine in the United States, reported widespread infection with PVX in North American Atlantic which contrasts with the initial report that it carries *Rx* (62). However, Wilson and Jones (66) found *Rx* in the clone of Atlantic grown in Australia. In this study, sap and graft inoculations with three different PVX isolates (WA1, TAS3, and XK3) again confirmed the presence of *Rx* in this clone of Atlantic. Possibly, Tavantzis et al. (58) found PVX in a different clone from the one originally released. Another possibility is that the PVX strain detected in Maine resembles resistance-breaking PVX strain HB in overcoming gene *Rx* (44). However, thus far, HB has been reported only from the center of origin of the potato in the

Table 5. Responses of 20 potato cultivars to graft inoculation with one to three isolates of *Potato virus X* (PVX)^x

Cultivar	Isolate ^y			<i>R</i> gene ^z
	XK3	TAS3	WA1+3	
Atlantic	NI	NI	NI	<i>Rx</i>
Auski	NI	...	...	<i>Rx</i>
Billabong	NI	...	...	<i>Rx</i>
Bismark	...	SM	MM	...
Bliss	SNS, NSST, SD	...	SNS, NSST, SD	<i>Nx</i>
Coliban	...	SM	SNS, NSST, SD	<i>Nb</i>
Desiree	...	SM	MM	...
Flame	NI	...	...	<i>Rx</i>
Fergifry	...	SM	MM	...
King Edward	...	SNS, NSST, SD	SNS, NSST, SD	<i>Nx</i>
Kipfler	...	...	SNS, NSST, SD	<i>Nb</i>
Maris Piper	...	SNS, NSST, SD	SNS, NSST, SD	<i>Nx</i>
Maxine	SNS, NSST, SD	...	...	<i>Nx</i>
Mondial	NI	...	NI	<i>Rx</i>
Nadine	SNS, NSST, SD	...	SNS, NSST, SD	<i>Nx</i>
Rodeo	NI	...	NI	<i>Rx</i>
Ruby Lou	NI	...	NI	<i>Rx</i>
Russet Burbank	...	SM	MM	...
Shepody	...	SM	SNS, NSST, SD	<i>Nb</i>
Tasman	...	SM	MM	...

^x Numbers of plants inoculated were normally 2 to 7 for each isolate-cultivar combination but were 40 for each isolate with Atlantic. Samples from tip leaves of axillary shoots were tested for PVX presence by enzyme-linked immunosorbent assay at 4, 6, and 9 weeks post inoculation.

^y Axillary shoots: NI = no symptoms developed and no PVX detected; MM = mild mosaic; SM = severe mosaic; SNS = systemic necrotic spotting; NSST = necrotic stem streaking; SD = systemic necrosis and shoot death.

^z Resistance gene found.

Fig. 2. Leaves or axillary shoots of potato plants infected with *Potato virus X* (PVX) by **A**, sap or **B**, graft inoculation. **Ai**, Plant of 'Shepody' (Nb) inoculated with isolate TAS3 showing a severe leaf mosaic; **Aii**, leaf of 'Nadine' (Nx) inoculated with isolate XK3 showing necrotic spot local lesions; **Aiii**, plant of 'Ranger Russet' (Nx) inoculated with isolate WA1+3 showing systemic necrotic spotting, necrotic stem streaking, and shoot necrosis; **Aiv**, leaf of 'Bliss' (Nx) inoculated with isolate XK3 showing necrotic spot local lesions; **Av**, leaf of 'Kestrel' (Nb) inoculated with isolate WA1+3 showing necrotic spot local lesions; **Avi**, lower leaf of 'Royal Blue' (Nb) infected with isolate WA1+3 showing systemic necrotic spotting; **Avii**, upper leaf of 'Royal Blue' (Nb) infected with isolate WA1+3 showing a severe mosaic and leaf deformation; and **Aviii**, inoculated leaf of 'Nadine' (Nx) with necrotic spot local lesions caused by PVX group 3 isolate (WA3) derived from isolate WA1+3 by passage through a plant of 'Royal Blue'. **Bi**, Plant of 'Mondial' (Rx) showing a scion infected with isolate WA1+3 and a healthy axillary shoot; **Bii**, leaf of 'Nadine' (Nx) growing from an axillary shoot infected with isolate WA1+3 showing systemic necrotic spotting; and **Biii**, plant of 'Coliban' (Nb) showing a scion infected with isolate WA1+3 and axillary shoots with necrotic stem streaking and apical necrosis.

Andean region of South America (18,44,47). Based on CP nucleotide sequence comparisons, with the exception of U.S. strain group 2 isolate WA2, HB belongs to a different phylogenetic grouping (clade II-2) from all other PVX isolates sequenced to date from outside the Andes (18). However, at the time of writing this paper, the CP nucleotide sequences of only two North American PVX isolates (WS2 and Canadian) are available in GenBank for comparison; therefore, there is little information on the phylogenetic position of North American isolates (18). Sequencing of additional PVX isolates might provide an explanation for the findings in Maine.

PVX hypersensitivity gene *Nx* was found in two Australian (Bliss and MacRusset), five European (Almera, Harmony, Maris Piper, Maxine, and Nadine), and one North American (Ranger Russet) cultivars, and in Australian breeding line 98-10713. Unlike PVX strain groups 1 and 2, which hypersensitivity gene *Nb* restricts to inoculated leaves, when strain groups 1 and 3 are sap inoculated to cultivars with *Nx*, development of necrotic local lesions in inoculated leaves is followed by systemic necrosis and shoot death (systemic hypersensitive phenotype; 2,32,33,66). In this study, following sap inoculation of cultivars with *Nx*, the systemic hypersensitive phenotype developed in most isolate-cultivar combinations but not in Almera, Harmony, and Maxine (with isolates WA1+3 or XK3); Nadine (with WA1+3 and, on one occasion, with XK3); and Ranger Russet (with XK3 only). There were also four combinations of isolate and cultivar where systemic invasion followed by necrosis occurred in only some shoots on the

same plant. In addition, although systemic necrosis often started to develop within 3 to 4 weeks of inoculation, it sometimes took much longer. Possible reasons for slow, incomplete or absence of systemic invasion include "mature plant resistance", such that systemic movement through stems of older plants is prevented (10); the presence of other genes (genetic background) that enhance the ability of *Nx* to localize infection within inoculated leaves; early shedding of inoculated leaves before systemic infection takes place; development of few infection foci, such that spread out of inoculated leaves never occurred (only a few necrotic local lesions formed with either isolate in Almera, Harmony, and Maxine); or different combinations of these scenarios. Graft inoculations induced systemic necrotic phenotypes readily in Bliss and Maxine with XK3 and in Nadine with WA1+3; therefore, no slow, incomplete, or lack of systemic invasion was evident with this more potent inoculation procedure. Adams et al. (2) found that keeping plants at 10 and 25°C but not 15 and 20°C after sap inoculation with a group 3 isolate prevented or greatly delayed development of systemic necrosis. However, in our studies, temperature was always held within a range of 15 to 20°C.

Examples of other useful resistance genes that behave like *Nx* in causing systemic hypersensitive resistance phenotypes include *Nc*, *Nv*, and *Nytr* in potato; *Nbm-1* in narrow-leaved lupin (*Lupinus angustifolius*); *Nam-1* in button medic (*Medicago orbicularis*); and *Rsv1-s* in soybean (*Glycine max*) (11,30,34,38,41,45,61). Such genes are effective in the field because they remove within-crop sources of virus infection for acquisition and further spread by

Fig. 3. Potato tubers harvested from plants showing systemic necrotic phenotypes caused by infection of cultivars carrying gene *Nx* with different *Potato virus X* isolates. i, Tubers from plants of 'Ranger Russet' infected with isolate WA1+3 by sap inoculation showing deep, sunken necrotic eyes (central tubers) and a large necrotic region arising from coalescence of sunken necrotic eyes (left) or without symptoms (right). ii, Tuber with a sunken necrotic eye from a plant of 'Nadine' infected with isolate WA3 by sap inoculation. iii, Tubers with sunken necrotic eyes from plants of Nadine infected with isolate WA1+3 by graft inoculation. iv, Tuber with sunken necrotic eyes from a plant of 'Maxine' graft inoculated with isolate XK3. v, Tubers from a plant of 'Bliss' graft inoculated with isolate XK3 showing cracked sunken eyes (left) or no symptoms (right).

vectors or by contact (35,37,43). Potato breeders first used them more than 60 years ago (13,25,50,53).

PVX hypersensitivity gene *Nb* was found in two Australian (Coliban and White Star), five European (Innovator, Kestrel, Kipfler, Laurine, and Royal Blue), and one North American (Shepody) cultivars. As with Maris Piper (26,32,33), some cultivars with *Nx* might also carry *Nb* but this could not be established because a PVX strain group 2 isolate was unavailable in Australia to use in comparisons. When cultivars with *Nb* were sap inoculated with mixed isolate WA1+3, the two strains separated in some instances but not others. In Coliban and Shepody, strain group 3 became systemic but strain group 1 remained in inoculated leaves. In Royal Blue, both moved systemically but only strain group 3 invaded later-formed leaves. In Kestrel and White Star, both moved systemically but failed to separate. In Innovator, Kipfler, and Laurine there was no systemic movement. Because few necrotic local lesions formed, only strain group 1 might have been present in inoculated leaves or strain group 3 was unable to move systemically for one or more of the reasons given above with *Nx*-containing cultivars. When isolate WA1+3 was graft inoculated to plants of Kipfler, severe systemic necrosis developed with this stronger inoculation procedure.

Early studies mentioned necrotic tuber symptoms associated with systemic hypersensitive phenotypes caused by PVX in potato plants (7). Jones (32) described necrotic symptoms in tubers from plants of Maris Piper and 'Pentland Dell' (with both *Nx* and *Nb*) infected with a group 3 isolate. These consisted of sunken, necrotic areas usually associated with eyes or the point of stolon insertion. These necrotic areas expanded during storage, completely killing some affected tubers. In this study, we found sunken necrotic areas mostly associated with eyes in tubers from plants of cultivars with *Nx* infected with WA1+3 (Ranger Russet), XK3 (Maxine and Nadine), either isolate (Bliss), or WA3 selected from WA1+3 (Nadine).

Wilson and Jones (66) attributed the frequent occurrence of PVX strain group 1 in Australia to widespread cultivation of cultivars lacking *Nx* and *Nb*. This contrasted with the situation in the United Kingdom, where group 3 was regarded as the "common" strain of PVX (1,16,19) and *Nb* was common in old cultivars (14) which selected against group 1 strains (1). The supposition that the

common strain of PVX was group 3 led to the erroneous typing of the common strain in Peru as group 3 rather than group 2 (21), which was later resolved using U.K. cultivar differentials with *Nx* and *Nb* (44). Whether strain group 2 occurs currently in Australia is unknown. It occurs among clade II-1 isolates from Europe and II-2 isolates from North and South America but was not detected among the 11 Australian isolates sequenced so far (18). Bald and Pugsley (6) reported that the historical potato virus B, which was included within PVX soon afterward (8), occurs in Australia but the isolate was lost. Such isolates normally fit into clade II-1 (18) and are found infecting cultivars with *Nx* but without *Nb*, such as U.K. 'Epicure' and King Edward (1,15,16,19,32).

The original parental sources of *Rx* used in potato breeding were 'USDA 41956' and 'CPC 1673' (52,54), and the original source of *Rx* for USDA 41956 was Chilean 'Villaroela' (47). USDA 41956 was a distant ancestor of Atlantic and Australian Auski, while CPC 1673 was an ancestor of Netherlands Mondial (Table 6). Australian Billabong and Netherlands Rodeo have Mondial as a parent. 'Foxton' is a parent of Australian Flame and Ruby Lou, and is listed as possessing "high" resistance to PVX (4), but our study of its ancestry revealed no known *Rx* carrier. The original source of *Nx* in U.K. cultivars was 'Paterson's Victoria' (19), which is a distant ancestor of Maris Piper, King Edward, and Pentland Dell. Maxine has Maris Piper as a grandparent, and Nadine has Maris Piper and Pentland Dell as polycross parents and is itself a parent of Harmony. Within the Australian cultivars and breeding line with *Nx*, Maris Piper is a parent or grandparent of Bliss and MacRusset, and MacRusset is a parent of 98-10713. In the United States, parent 'Butte' of Ranger Russet is described as having PVX resistance (51) and seems the probable *Nx* source. The *Nx* source is unclear for Almera. Parents 'V 28-12' and Shepody are the probable sources of *Nb* for Coliban and Innovator, respectively. 'Excelsior' was a parent or great grandparent of White Star and Shepody and, therefore, may have contributed *Nb*. Which parent *Nb* came from in Kestrel, Kipfler, Laurine, and Royal Blue is unknown.

Identification of PVX resistance genes using inoculation with PVX isolates belonging to strain groups 2 and 3 is a relatively simple procedure (15,32,33). Therefore, although PVX resistance may

Table 6. Putative sources of resistance genes in cultivars within known parentage

Cultivar	Typed resistance gene	Ancestor with typed resistance gene	Ancestral generation with gene	Reference
Almera	<i>Nx</i>	None known	?	...
Atlantic	<i>Rx</i>	USDA 41956	4	62
Auski	<i>Rx</i>	USDA 41956	6	This study
Billabong	<i>Rx</i>	Mondial	1	This study
Bliss	<i>Nx</i>	Maris Piper	2	This study
Coliban	<i>Nb</i>	V 28-12 ^w	1	66
Flame	<i>Rx</i>	Foxton? ^x	1	This study
Harmony	<i>Nx</i>	Nadine	1	This study
Innovator	<i>Nb</i>	Shepody	1	This study
Kestrel	<i>Nb</i>	None known	?	...
King Edward	<i>Nx</i>	Paterson's Victoria	?	19, 26, 32
Kipfler	<i>Nb</i>	Unknown parentage	?	...
MacRusset	<i>Nx</i>	Maris Piper	1	This study
Maris Piper	<i>Nx</i>	Paterson's Victoria	?	19, 26, 32
Maxine	<i>Nx</i>	Maris Piper	2	This study
Mondial	<i>Rx</i>	CPC 1673-20	3	This study
Nadine	<i>Nx</i>	Maris Piper and Pentland Dell (both <i>Nx</i>)	2	This study
Laurine	<i>Nb</i>	Unknown parentage	?	...
Ranger Russet	<i>Nx</i>	Butte? ^y	1	This study
Rodeo	<i>Rx</i>	Mondial	1	This study
Royal Blue	<i>Nb</i>	None known	?	...
Ruby Lou	<i>Rx</i>	Foxton? ^x	1	This study
Shepody	<i>Nb</i>	Excelsior? ^z	3	This study
White Star	<i>Nb</i>	Excelsior? ^z	1	This study
98-10713	<i>Nx</i>	MacRusset	1	This study

^w Kennebec (second parent of Coliban) lacks *Nx* and *Nb* (66); therefore, V 28-12 is the probable *Nb* source.

^x Foxton is a common parent to Flame and Ruby Lou. Foxton is listed as possessing high resistance to *Potato virus X* (PVX) (4).

^y Butte (parent of Ranger Russet) is listed as possessing resistance to PVX (51).

^z Excelsior is a parent of White Star and a great grandparent of Shepody. However, no information is available on PVX resistance in Excelsior.

not always be a major criterion in selection of new cultivars, potato breeding programs should test parental lines and potential new cultivars before release to establish whether they are present.

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