The Occurrence of PVY^O, PVY^N, and PVY^{N:O} Strains of *Potato virus Y* in Certified Potato Seed Lot Trials in Washington and Oregon

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ABSTRACT

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Totals of 960 and 286 certified potato seed lots from locations across North America were planted in trials in Washington and Oregon, respectively, in 2001 to 2003 and tested for strains of *Potato virus Y* (PVY). The incidence of PVY^O-infected lots averaged 16.4 and 25.9% in the Washington and Oregon trials, respectively. There was a general trend of increasing incidence of the PVY^O, PVY^{N:O}, and PVY^N strains during this period, as evidenced by more infected cultivars, sites of seed origin, and number of seed growers providing infected seed lots. In particular, there was a dramatic increase in seed lots with the PVY^{N:O} strain from 2002 to 2003. PVY^{N:O}, in contrast to PVY^O, which only causes yield reduction, also causes internal and external damage to tubers, making them unmarketable. In 2003, PVY^{N:O} occurred in seed lots originating in eight states and three Canadian provinces. The increased incidence of PVY^{N:O} was likely due to the difficulty in differentiating this strain from PVY^O. The prevalence of PVY in potato seed lots documented herein poses a threat to potato production in the United States and suggests that current measures to reduce the incidence of this virus are inadequate.

Additional keywords: ELISA, monoclonal antibodies, potyvirus, RT-PCR

Potato virus Y (PVY) is the type member of the family *Potyviridae* and occurs worldwide wherever potatoes are grown (8). The virus exists as a number of strains or biological types that differ in host range, symptomology, serology, and molecular characteristics (2,4,18). The common or ordinary strain of PVY (PVY^O) causes a systemic mottle in potatoes and tobacco. Although the symptoms of PVY^O are variable based on the potato cultivar, the reduction in yield can be significant even in cultivars that exhibit mild mosaic symptoms (14,19,25). Isolates of PVY that cause systemic vein necrosis in tobacco belong to the PVY^N strain group, which produces milder symptoms in potato than

does PVY^O. Viruses in the PVY^N group are widespread in Europe (1) and are economically important due to their yield effects on the crop and quarantine/certification issues (4). PVY^N was reported in eastern Canada in the early 1990s (16) and in the potato-producing regions of the western United States in 2002 (6). Isolates of PVY^N that produce internal and external tuber symptoms (potato tuber necrotic ringspot disease [PTNRD]) are classified as the PVY^{NTN} strain (4). Enzyme-linked immunosorbent assays (ELISA) using strain-specific PVY^O and PVY^N monoclonal antibodies (11,23) are used to differentiate PVY^O from PVY^{NTN}.

Recent reports from the United States and Canada (6,7,26) indicate the presence of PVY isolates in several states and provinces that have characteristics of both PVY^O and PVY^N. Some isolates were designated PVY^{N:O} because they were serologically classed as PVY^O yet produce necrosis on tobacco (7,17). Additionally, some PVY^{N:O} isolates produced PVY^{NTN}-like symptoms in potato tubers of some cultivars (20). ELISA would identify

PVY^{N:O} isolates as PVY^O and allow these infections to “escape” detection (26).

Reverse transcription-polymerase chain reaction (RT-PCR) has been widely utilized for detection and differentiation of strains of PVY (2,3,12,17,18,24,27,28). Using RT-PCR, PVY^O has been differentiated from PVY^{N:O/NTN} (7,17). Also, results of RT-PCR and sequence analysis of amplified products showed that genetic recombination between strains of PVY occurs in nature (13,22).

Heretofore, seed potatoes from Canada testing positive for PVY^N strains were quarantined, and movement of infected seed into the United States was restricted (4). However, discovery of PVY^N in the Pacific Northwest (6) in a seed lot originating in the Midwest clearly demonstrated that PVY^N occurred within the United States. Since tuber-borne virus is the single most important source of PVY in a potato field (15), information on the incidence of PVY in seed lots within the United States was needed. The survey described herein was conducted to determine the incidence of seed-borne infections of PVY^O and PVY^{N:O} in certified potato seed lots being produced in North America.

MATERIALS AND METHODS

Field plot design and locations. The Washington trials were planted at the Washington State University research farm near Othello. The Oregon trials were planted at the Hermiston Agricultural Research and Extension Center, Oregon State University, Hermiston. The trials were conducted from 2001 to 2003 using commercially available certified seed lots originating in several states and Canadian provinces.

Numbers of certified seed lots, cultivars, origins, and seed growers are listed in Table 1. Two hundred or 300 single drop seed pieces per seed lot were planted in single rows in Oregon and Washington, respectively. Plant spacing was 25 cm and rows were 85 cm apart. Fertilization, irrigation frequency, and insect, weed, and disease control practices were consistent

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with commercial potato production practices in the Columbia Basin. Planting dates varied each year, depending on receipt of seed lots, but ranged from late April to late May. Seed potatoes originated in the states of California, Colorado, Idaho, Minnesota, Montana, Nebraska, North Dakota, Oregon, Washington, Wisconsin, and Wyoming, and the Canadian provinces Alberta, British Columbia, Manitoba, Prince Edward Island, and Saskatchewan.

Plant sampling and ELISA. After shoot emergence, generally at rosette, half of the plants per row were individually staked and numbered and then sampled in groups of 10. A single leaf per plant was removed and placed into a Ziploc bag with other leaves from that group of 10. Bags were placed into coolers and then into walk-in coolers at approximately 4°C until processed, usually within 48 h. A single leaflet was removed from each of the 10 leaves per bag, stacked together, and 4-mm disks were removed with a cork borer. Each stack of 10 disks was placed into separate ELISA wells precoated with PVY polyclonal antiserum from Agdia (Elkhart, IN) or Phyto Diagnostics (North Saanish, BC, Canada) in standard coating buffer (5). Each well also contained 200 µl of PEP buffer (phosphate buffered saline [PBS] containing 0.02% Tween 20, 2% polyvinyl pyrrolidone, and 0.2% egg albumin [5]). A total of four sample stacks were removed from each group of 10 leaflets, and two stacks each were tested individually for PVY^O and PVY^N serotypes. Consistent readings from the two replicate wells/serotype was required or samples were retested. Samples were tested as described below.

Tissue disks were incubated overnight at 4°C, wells were then emptied, and the plates were washed three times with PBS-Tween. PVY^N serotype-specific alkaline phosphatase-conjugated monoclonal antibody 1F5 (Agdia) was diluted in PEP buffer and used according to the manufacturer's recommendations. A triple antibody sandwich ELISA (5) was conducted for PVY^O using specific monoclonal antibody MAb2 (Phyto Diagnostics) as suggested by the manufacturer. The specificity of monoclonal antibody 1F5 (PVY^N) and MAb2 (PVY^O) have been reported (10,11). Absorbance values at 405 nm were measured with a spectrophotometer 1 to 2 h after adding *p*-nitrophenyl phosphate substrate. Samples producing an absorbance value greater than twice that of healthy controls were considered positive. ELISA positives with monoclonal antibody 1F5 were considered to be PVY^{N/NTN}. Samples positive with MAb2 were considered PVY^O serotype, yet could be either PVY^O or PVY^{N:O}.

When a composite sample tested positive for PVY^O or PVY^N, plants within that 10 composite sample were individually retested with the appropriate antiserum to

determine the number of infected plant(s). Usually only two composites from each row with PVY^O positive plants were tested in this manner, but up to four were tested in some situations where PVY^N and PVY^O plants occurred in separate composite samples. Plants confirmed as PVY^O positive were further tested by RT-PCR (only in 2002 and 2003 because PVY^{N:O} isolates were unknown in 2001) to differentiate between PVY^O and PVY^{N:O}.

Nucleic acid extraction and RT-PCR.

Total nucleic acid was extracted from approximately 200 mg of leaf tissue using the procedures of Presting et al. (21). Nucleic acid pellets were resuspended in 400 µl of sterile distilled water. A coupled, one-tube, duplex RT-PCR (cRT-PCR) was conducted as previously described (7). Primers

Anti/S3 amplified a product of 745 bp from PVY^N, PVY^{N:O}, and PVY^{NTN} isolates, while primers Anti/S7 produced a product of 281 bp for PVY^O isolates (7,17). Virus isolates that produced the 745-bp cRT-PCR product and were detected with MAb2, indicating they were O serotype, were classified as PVY^{N:O}.

RESULTS

Totals of 960 and 286 certified potato seed lots were evaluated in Washington and Oregon, respectively (Table 1), representing 40 cultivars from 212 seed growers in 11 states and 5 Canadian provinces. Cultivars Russet Burbank, Ranger Russet, Russet Norkotah, Umatilla, and Shepody made up more than 80% of the seed lots tested (Tables 2 and 3). Incidence of PVY

Table 1. Numbers of certified potato seed lots, cultivars, states/provinces of origin, and seed growers of seed lots evaluated in Washington and Oregon from 2001 to 2003

Year	Seed lots ^a	Cultivars	States/provinces of origin	Seed growers
Washington				
2001	313	24	8/3	122
2002	312	21	7/3	116
2003	335	22	8/3	117
Total ^b	960	40	9/4	196
Oregon				
2001	81	13	6/3	45
2002	99	9	7/3	57
2003	106	16	8/4	59
Total ^b	286	23	9/4	97
Total of both states ^c	1,246	40	16 ^d	212

^a Lots may occasionally overlap between Washington and Oregon since a seed grower may have supplied identical seed lots to both states.

^b Totals are not additive except for total number of lots. Numbers of cultivars, states/provinces, and seed growers represent numbers that are different.

^c Overall totals from both locations. Not additive except for total number of lots. Numbers of cultivars, states/provinces, and seed growers represent numbers that are different.

^d States: California, Colorado, Idaho, Minnesota, Montana, Oregon, Nebraska, North Dakota, Washington, Wisconsin, and Wyoming. Provinces: Alberta, British Columbia, Manitoba, Prince Edward Island, and Saskatchewan.

Table 2. Incidence of *Potato virus Y* strain PVY^O in certified potato seed lot trials planted in Washington from 2001 to 2003

Cultivar	Seed lots ^a	Percent lots planted ^b	PVY ^O infected lots ^c	Percent infected ^d
Alturas	46	4.8	5	10.8
Atlantic	1	<1	0	0
Cal Red	6	<1	2	33.3
Gem Russet	10	1.0	6	60.0
Nooksack	6	<1	2	33.3
Russet Norkotah	83	8.6	23	27.7
Russet Norkotah 3	32	3.3	23	71.9
Russet Norkotah 8	23	2.4	10	43.5
Other Russet Norkotah ^e	4	<1	0	0
Russet Burbank	279	29.1	24	8.6
Ranger Russet	223	23.2	17	7.6
Shepody	62	6.5	29	46.8
Umatilla Russet	130	13.5	11	8.5
Yukon Gold	11	1.1	1	9.1
Others	42	4.4	4	9.5
Unknown	2	<1	0	0
Total	960	...	157	16.4

^a Number of lots planted for each cultivar.

^b Percentage of each cultivar planted compared with total number of all lots planted.

^c Number of lots positive for PVY^O for each cultivar.

^d Percentage of lots positive for each cultivar.

^e Additional Russet Norkotah lines represented.

Table 3. Incidence of *Potato virus Y* strain PVY^O in certified potato seed lot trials planted in Oregon from 2001 to 2003

Cultivar	Seed lots ^a	Percent lots planted ^b	PVY ^O infected lots ^c	Percent infected ^d
Alturas	10	3.5	0	0
Atlantic	9	3.1	3	33.3
Cal Red	0	0	0	0
Gem Russet	3	1	1	33.3
Nooksack	3	1	3	100.0
Russet Norkotah	69	24.1	21	42.0
Russet Norkotah 3	4	1.4	3	75.0
Russet Norkotah 8	5	1.7	3	60.0
Other Russet Norkotah ^e	0	0	0	0
Russet Burbank	60	21.0	14	23.3
Ranger Russet	60	21	2	3.3
Shepody	31	10.8	18	58.1
Umatilla Russet	6	2.1	1	1.7
Yukon Gold	7	2.5	0	0
Others	11	3.8	4	36.4
Unknown	8	2.8	1	1.3
Total	286	...	83	25.9

^a Number of lots planted for each cultivar.

^b Percentage of each cultivar planted compared with total number of all lots planted.

^c Number of lots positive for PVY^O for each cultivar.

^d Percentage of lots positive for each cultivar.

^e Additional Russet Norkotah lines represented.

Table 4. Incidence of *Potato virus Y* strain PVY^O in certified potato seed lots planted in Washington and Oregon from 2001 to 2003

Year	Seed lots PVY ^O positive/no. tested ^a	Cultivars ^b	States/provinces of origin	Seed growers
Washington				
2001	53/313	12	7/2	34
2002	34/312	10	7/1	25
2003	70/335	9	8/1	36
Total ^c	157/960	16	9/3	70
Oregon				
2001	25/81	10	5/2	17
2002	24/99	5	5/1	22
2003	34/106	7	6/4	25
Total ^c	83/286	15	9/4	52
Total of both states ^d	240/1,246	20	9/4	88

^a Lots may occasionally overlap between Oregon and Washington since a seed grower may have supplied identical seed lots to both states.

^b The various Russet Norkotah lines are counted separately.

^c Totals are not additive. Numbers represent total number different over the 3 years.

^d Overall totals from both locations. Not additive. Numbers represent total number different over the 3 years.

in Russet Norkotah and Shepody appeared higher than in Ranger Russet, Russet Burbank, and Umatilla at both locations (Tables 2 and 3). Russet Norkotah and Shepody represented 8.6 and 6.5%, respectively, of the lots submitted, and the incidence of lots with PVY was 27.7% for Russet Norkotah and 46.8% for Shepody at the Washington location. In Oregon, these two cultivars represented 24.1 and 10.8% of the lots submitted, respectively, and 42.0% of the Russet Norkotah and 58.1% of Shepody lots were infected with PVY. Over the 3 years of the study, PVY was detected in 16.4 and 25.9% of seed lots tested in Washington and Oregon, respectively (Tables 2 and 3).

PVY^O occurred in 16 cultivars, originating from 12 states/provinces and 70 seed growers in the Washington trials and was detected in 15 cultivars, originating from

13 states/provinces and 52 seed growers in the Oregon trials (Table 4). In total, 240 seed lots were positive for PVY^O, representing 20 cultivars, originating in 13 states/provinces, from 88 seed growers. The incidence of PVY^O fluctuated over the 3 years of the study, but was generally higher in 2003 than in 2001 (Table 4), whereas the incidence of PVY^N remained relatively constant (Table 5). In contrast, the incidence of PVY^{N:O} approximately doubled in the number of states/provinces and seed growers where this strain was found between 2002 and 2003.

DISCUSSION

The presence of PVY^O in leaf tissue from plants grown from certified seed of 20 cultivars produced in diverse locations in North America was not surprising. This virus is widely distributed wherever pota-

toes are grown (8). PVY^O is known to reduce yields, particularly when seed-borne (14,19,25). Therefore, seed-producing states and provinces have agencies that certify or refuse certification of seed lots based on incidence of PVY. Certification standards may or may not require a winter grow out test accompanied by some degree of ELISA testing. Most potato-producing states require the planting of certified seed lots, even though certification standards differ among individual states and provinces. While the objective during this study was not to quantify the incidence of PVY^O infection in each seed lot, many were infected at levels that would preclude certification, even in the most liberal certification states (unpublished data). Clearly, the methods used by some certification agencies do not always adequately identify lots with high levels of virus infection.

The PVY^N strain is widespread in Europe (1,13) and was found in potatoes in the western United States relatively recently (6,16). In 2000, the PVY^N strain was considered a quarantine pathogen between Canada and the United States. As late as 2001, seed certification agencies in the United States were not commonly testing seed lots for this strain of PVY. Even so, the number of seed lots infected was low, since PVY^N was detected in only 8 cultivars, 4 states/provinces, and 12 seed growers in 2001 (Table 5). After the detection of PVY^N, certification agencies moved quickly to restrict further dissemination of the necrotic strain. This was easily accomplished by large-scale testing with a monoclonal PVY^N serotype-specific antiserum. These efforts allowed the level of PVY^N to remain relatively constant through the 3 years of testing reported here. A new agreement (PVY^N Management Plan) between the United States and Canada, after recognizing the apparently widespread occurrence, now allows shipment of potatoes even if PVY^N is present.

The recent identification of PVY^{N:O} poses a potentially serious threat to the potato industry. Besides yield loss, this virus was associated with tuber symptoms in some cultivars (20). Also, the work described herein allows conjecture that the PVY^{N:O} strain is becoming the predominant form of the virus and suggests it may have been misdiagnosed as PVY^O in serological tests (26). Certification agencies have typically only used serological techniques to differentiate PVY^O from PVY^N, whereas PVY^{N:O} is only easily recognizable by using both serological and PCR techniques (7,20). While PVY^O is regulated, no certification agency has a zero tolerance for this virus, so some level of PVY^O is permitted, allowing PVY^{N:O} to be maintained. A simple test that would allow easy differentiation of PVY^{N:O} from PVY^O is needed. Much more research is needed to confirm yield and quality reductions by

Table 5. Occurrence of *Potato virus Y* strains PVY^N and PVY^{N:O} in certified potato seed lots evaluated in Washington and Oregon from 2001 to 2003

Year	PVY ^N			PVY ^{N:O}		
	Cultivars ^a	Origin ^b	Seed growers	Cultivars ^a	Origin ^c	Seed growers
2001	8	4	12	1 ^d	1	1
2002	7	5	12	5	4	8
2003	10	4	11	6	7	17

^a The various Russet Norkotah lines are counted separately.

^b Number of states and provinces providing seed each year. PVY^N was confirmed in seed originating from the states of California, Colorado, Idaho, Oregon, Montana, North Dakota, and Washington, and the province of Alberta.

^c Number of states and provinces providing seed each year. PVY^{N:O} was confirmed in seed originating from the states of California, Colorado, Idaho, Oregon, Montana, Nebraska, North Dakota, and Washington, and the provinces of Alberta, British Columbia, and Prince Edward Island.

^d Plants testing positive for PVY^O were not tested further by reverse transcription-polymerase chain reaction because PVY^{N:O} was unknown in 2001. However, this isolate produced tuber symptoms and was later transmitted to tobacco and confirmed as PVY^{N:O}.

PVY^{N:O} strains, particularly in relation to different cultivars and environments.

The identification of recombinant isolates such as PVY^{N:O} has increased the complexity of the strain concept within PVY. Classically, the PVY^{NTN} designation has been assigned to virus isolates producing systemic vein necrosis in tobacco and rings or arcs in or on tubers (1,4). PVY^{N:O} isolates also produce symptoms in or on the surface of tubers of some cultivars (20; J. M. Crosslin and P. B. Hamm, *personal observations*) and cause systemic vein necrosis in tobacco (7,20). This raises the question as to whether or not symptom-producing PVY^{N:O} isolates should be considered PVY^{NTN}. The PVY^{N:O} designation, however, is more descriptive of the genotype of the isolate (7), and the PVY^{NTN} designation remains a biological descriptor.

Seed certification inspectors are faced with the difficulty of visually recognizing PVY infection in seed potato fields. Shepody and Russet Norkotah are considered latent carriers because PVY^O symptoms in these cultivars are extremely mild (9), as evidenced by the high virus levels found during this survey, and only the most experienced personnel can reliably recognize PVY^O infections. Furthermore, some cultivars, like Ranger Russet and Liberty, exhibit a shock reaction, or plant die-back, in response to PVY^O, yet these cultivars produce a much less severe reaction to PVY^N. In addition, some newly released cultivars such as Gem Russet and Gem Star resemble Shepody and Russet Norkotah in their response to PVY. Unless newly released cultivars clearly show foliar symptoms of PVY infection, seed growers and certification personnel will continue to have difficulty in recognizing and roguing infected plants. These same plants may be unrecognized sources of PVY infection to nearby potato plantings.

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