Beet Western Yellows Virus Is Not an Important Component of Potato Leafroll Disease in Canada and the United States

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ABSTRACT

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We tested 801 samples of potato leaves with leafroll-like symptoms for potato leafroll virus (PLRV) and beet western yellows virus (BWYV) using virus-specific monoclonal antibodies in triple-antibody sandwich enzyme-linked immunosorbent assay (TAS ELISA). The samples represented 32 cultivars and originated in eight Canadian provinces and 12 states in the United States. None of the samples tested positive for BWYV, whereas 774 (96.6%) tested positive for PLRV. Neither virus could be recovered via aphid (*Myzus persicae*) transfers to indicator hosts from 18 samples that tested negative for both viruses. Tubers collected from 134 of the samples were sprouted and retested by both TAS ELISA and a polyclonal antiserum-based double-antibody sandwich (DAS) ELISA. These tests confirmed the initial results. Absorbance readings, when different, were consistently higher in TAS ELISA than in DAS ELISA. Aphid transmission attempts confirmed the ELISA results. In other aphid transmission trials, we were unable to transmit any of seven isolates of BWYV from infected groundcherry (*Physalis pubescens*) to potato, which indicated that potato is not a host of BWYV.

Potato leafroll disease is one of the most important diseases of potato (*Solanum tuberosum* L.) worldwide (17). Potato leafroll virus (PLRV) is usually considered to be the causal agent (2), but in North America (5,6) and Tasmania (7), beet western yellows virus (BWYV) has been reported to be a component virus in plants with typical leafroll symptoms.

The conclusion that BWYV occurs in potato was based on two assumptions: first, that shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.) is a host for BWYV but is not a host for PLRV, and second, that BWYV antiserum does not cross-react with PLRV. Both assumptions are challenged by more recent evidence (20,24). Several researchers have demonstrated that PLRV can indeed infect shepherd's purse (21,23), and others have reported that BWYV antiserum does cross-react with PLRV in some serological tests (15,16,18).

Immunological techniques are among the most important tools for detecting and identifying plant viruses (13). Recent advances in methodology, including enzyme-linked immunosorbent assay (ELISA) and monoclonal antibody production, have greatly improved the sensitivity, specificity, and ease of luteovirus diagnosis (4). The availability of a range of virus isolates, together with their specific polyclonal and monoclonal antisera, has enabled researchers to reexamine the causality of diseases attributed to luteoviruses. We attempted to determine whether BWYV is a component of a complex causing potato leafroll disease in Canada and the United States. A preliminary report has been published (8).

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MATERIALS AND METHODS

Collection of samples. We tested 801 samples, representing 32 cultivars and originating in eight Canadian provinces and 12 states in the United States, in 1986, 1987, and 1988. Most of the samples were collected in the seed potato certification winter test plots near Homestead, FL, and Oceanside, CA. Additional samples were provided by seed certification officials in Oregon and Canada. Foliar samples, and tuber samples when available, were collected from plants with secondary leafroll symptoms such as rolling of the lower leaves, chlorosis, and stunting. Samples were shipped in coolers to Vancouver, B.C., and indexed to determine whether they were infected with PLRV alone, BWYV alone, or both viruses.

Virus indexing by ELISA. Each of the 801 samples was tested initially by triple-antibody sandwich (TAS) ELISA as described previously (10). These tests were confirmed on leaf tissue samples obtained from the sprouted tubers of 134 of the samples. The tubers were stored at 4 C for 4 mo to break dormancy and were then planted in a greenhouse. In this second set of tests, TAS ELISA and double-antibody sandwich (DAS) ELISA (3) were compared. Positive and negative controls consisted of BWYV-infected groundcherry (*Physalis pubescens* L.), PLRV-infected potato, and virus-free groundcherry and potato. Infection with either BWYV or PLRV was indicated when absorbance readings (A_{405nm}) of samples were greater than twice the mean absorbance readings of five healthy controls.

Antisera and monoclonal antibodies.

The immunoglobulins of the PLRV polyclonal antiserum (PLRV-BC), prepared by Rowhani and Stace-Smith (19), were used at a concentration of 1 μg/ml for coating the ELISA plates. This polyclonal antiserum had been shown to react specifically against a range of PLRV isolates, did not cross-react with BWYV isolates, and gave low background reactions in the healthy control wells in ELISA tests (11,16).

We used a local isolate of BWYV (designated BWYV-BC) to prepare a polyclonal antiserum against BWYV. The virus was purified by the method of D'Arcy et al (4) and used to immunize a young white New Zealand rabbit. The first injection of 100 μg of virus emulsified with an equal volume of Freund's complete adjuvant was administered intramuscularly in a hind leg. The second injection of 200 μg of virus was given intravenously 10 days later. The next three injections of approximately 300, 200, and 100 μg were emulsified with Freund's incomplete adjuvant and injected intramuscularly in a hind leg at 2-wk intervals. Blood was collected at 2-wk intervals following the last injection. A booster injection of 100 μg of virus was given after the second bleeding. The antiserum titer was determined by agar gel double-diffusion tests as described by Rowhani and Stace-Smith (19).

The antisera were purified by Protein A affinity chromatography (1). The immunoglobulins were adjusted to approximately 1 mg/ml (A_{280nm} = 1.4) and stored in aliquots of 0.5 ml at −20 C. For use in ELISA, aliquots were thawed and diluted 1:1,000. The quality of the serum was improved in DAS ELISA by cross-absorption at the conjugate stage: 1 ml of healthy groundcherry sap was added to 9 ml of the conjugate diluted in PBS-Tween containing 0.2% nonfat milk powder (10) and incubated for 1 hr at 37 C before the conjugate was added to the wells.

Ellis and Wieczorek (10) described the production of specific monoclonal antibodies to PLRV and BWYV. For this study, we selected the PLRV-specific 26BE and the BWYV-specific 510H from the panel of available monoclonal antibodies because they exhibited no cross-reactivity and yet were capable of detecting a broad range of isolates of each virus (10).

Aphid transmission tests. We did not attempt aphid transmissions from the 801 leaf samples but did do so from the 134 tuber samples. Mature wingless green peach aphids (*Myzus persicae* (Sulzer)) were used in attempts to transmit virus from potato to the test species *P. pubescens* and *C. bursa-pastoris*. The *M. persicae* clone originated from a local potato source and was previously shown to be an efficient vector of several isolates of both BWYV and PLRV (9).

The potato leaves arising from the sprouted tubers were placed on moistened filter paper in petri dishes, and about 50 nonviruliferous aphids, reared on Chinese cabbage (*Brassica pekinensis* (Lour.) Rupr.), were placed on each leaf. After a 48-hr acquisition access, 10 aphids were transferred to each of the test species. Test plants with two to four true leaves were infested with aphids and caged in small plastic cylinders covered on top with fine wire mesh. After a 48-hr inoculation access, the plants were sprayed with pirimicarb at 0.25 g a.i./L (Chipman Inc., Stoney Creek, Ontario) to kill the aphids. The plants were moved to a greenhouse (15–20 C), and leaf samples were tested for PLRV and BWYV by TAS ELISA 6 wk after aphid inoculation.

Other aphid transmission trials were done in an attempt to determine whether BWYV could be transmitted from *P. pubescens* to potato. Five of the virus sources that were used were infected with BWYV alone, and two were infected with both BWYV and PLRV. Two sources infected with PLRV alone were included as controls. Acquisition access and inoculation test conditions were as outlined above. Aphids from each of the virus sources were transferred to two healthy groundcherry and two shepherd's purse seedlings in addition to 10 potato plants (cultivar Russet Burbank). Six weeks after the initial inoculation, transmissions were attempted from the inoculated potatoes to groundcherry by the same procedure.

The cultivar Russet Burbank was used as the test potato host in the initial attempts to aphid-transmit BWYV to potato. The trials were later extended to include the cultivars Chieftain, La Rouge, Norchief, Norchip, Norgold Russet, Norland, Red McClure, Russet Norkotah, Sebago, Superior, Viking, and White Rose.

RESULTS

Characteristics of the BWYV polyclonal antiserum. The titer of the BWYV antiserum as determined in agar gel diffusion tests was 1:256 for the first two test bleedings and 1:512, 1:1,024, and 1:512 for the next three bleedings, following the booster injection. Bleedings three, four, and five were combined and used to prepare immunoglobulins for the coating and conjugate steps in the DAS ELISA procedure. These immunoglobulins reacted with several different isolates of BWYV but showed no cross-reactivity in DAS ELISA with PLRV. The signal-to-noise ratio was initially high but was even higher when the immunoglobulin was cross-absorbed after conjugation. In control wells containing healthy tissue of groundcherry, potato, or shepherd's purse, absorbance readings after a 2-hr incubation rarely exceeded 0.015 when blanked on buffer, whereas antiserum that was not cross-absorbed gave readings in the 0.05–0.10 range. Infected groundcherry samples gave readings in the range of 1.00–2.00, and the absorbance readings did not change appreciably when the immunoglobulin was cross-absorbed. The polyclonal antiserum was therefore capable of attaining signal-to-noise ratios comparable to those achieved in the TAS ELISA using polyclonal antisera in the coating step and monoclonal antibodies for detection.

Table 1. Detection of potato leafroll virus (PLRV) and beet western yellows virus (BWYV) in samples of potatoes with symptoms of leafroll disease from Canada and the United States by triple-antibody sandwich enzyme-linked immunosorbent assay

Origin	No. of samples	No. of samples testing		
		PLRV-positive	BWYV-positive	PLRV/BWYV-negative
Province				
Alberta	6	6	0	0
British Columbia	28	25	0	3
Manitoba	2	2	0	0
New Brunswick	69	69	0	0
Nova Scotia	8	8	0	0
Ontario	11	11	0	0
Prince Edward Island	18	18	0	0
Quebec	9	9	0	0
State				
California	9	6	0	3
Colorado	45	45	0	0
Idaho	305	296	0	9
Maine	48	47	0	1
Michigan	25	24	0	1
Minnesota	7	7	0	0
Nebraska	20	19	0	1
North Dakota	22	22	0	0
Oregon	86	83	0	3
Utah	3	3	0	0
Wisconsin	76	70	0	6
Wyoming	4	4	0	0
Totals	801	774	0	27

Incidence of PLRV and BWYV in potato samples. Of the 801 potato leaf samples that were collected and indexed by TAS ELISA, 774 (96.6%) indexed positive for PLRV (Table 1). Some infected plants were detected in samples from each of the eight provinces and 12 states. None of the samples proved to be infected with BWYV (Table 1).

Infection with either virus was considered positive when absorbance readings in sample wells were greater than twice the mean absorbance of five healthy controls. Most samples that were scored as positive gave absorbance readings well above this threshold. When absorbance readings of samples obtained from different locations were plotted, the histogram patterns (Fig. 1) showed differences that could be attributed to the test sites. Samples collected from California (Fig. 1A) consistently gave higher absorbance readings than those from either Florida (Fig. 1B) or Oregon (Fig. 1C).

Comparison of TAS and DAS ELISA as detection techniques. TAS ELISA was also used to assay tissue samples arising from tubers. Viable tubers were collected from 134 of the 801 test samples. In an initial set of tests, 117 tubers were found to be infected with PLRV. In a second set of tests, both TAS and DAS ELISA were used with each sample. All 117 samples were positive for PLRV and negative for BWYV with both ELISA techniques, and the 17 samples that were negative in the first set of tests were also negative in the second set.

We found relatively few differences between the two ELISA techniques with respect to the intensity of reactions. In all cases the reaction was sufficiently strong to give an unequivocal result. When the readings differed, the TAS ELISA was consistently higher than the DAS ELISA. Histograms of absorbance readings from these samples were similar to those shown in Figure 1A.

Aphid transmission experiments. Foliage from the tuber samples was used as a virus source in attempts to transmit viruses from potato to *P. pubescens* seedlings using *M. persicae* as the vector. Results were in complete agreement with those obtained by ELISA; PLRV but not BWYV was transmitted to *P. pubescens* from each of the 117 infected potato plants, and neither virus was transmitted from the 17 plants that had indexed negative for both viruses.

Although our analysis of the 801 samples indicated that only one virus, PLRV, was involved in potato leafroll disease, it did not rule out the possibility that there is a strain of BWYV capable of infecting potato under some circumstances or that aphids can transmit BWYV to potato in mixed infections of BWYV and PLRV. We explored these two questions in aphid transmission tests with infected *P. pubescens* plants as virus sources and shepherd's purse, groundcherry, and Russet Burbank potato as test plants. BWYV was transmitted from all seven source plants to shepherd's purse and groundcherry but was not detected in or recovered from any of the exposed potato test plants (Table 2). In comparable trials with an additional 12 potato cultivars, BWYV was not recovered from any of the exposed potato test plants. Further, aphid transmission from sources containing PLRV showed a high level of transmission to groundcherry but a lower level of transmission to shepherd's purse (Table 2).

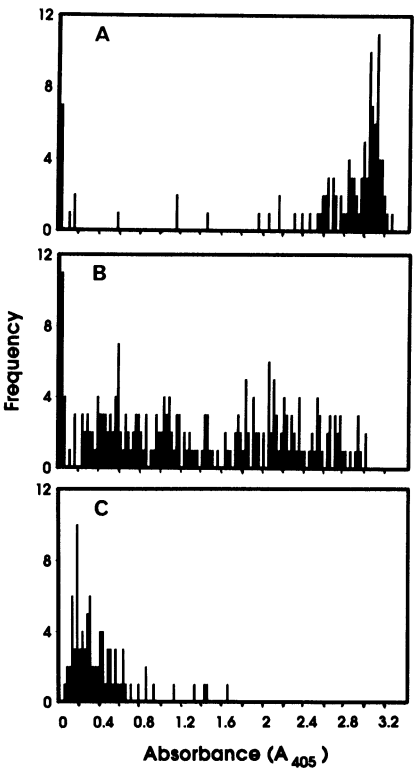

Fig. 1. Histogram of enzyme-linked immunosorbent assay results (mean of two tests) for potato leafroll virus from samples of potatoes with leafroll symptoms collected from seed potato winter test sites in California **(A)**, Florida **(B)**, and Oregon **(C)**.

DISCUSSION

The most significant result of our extensive indexing of potato plants with symptoms of leafroll disease representing a wide range of cultivars from different geographic areas in North America was that we found no evidence of BWYV. Our results are distinctly different from those first reported by Duffus (5,6) and later supported by the work of Gallenberg et al (11). These researchers reported that BWYV was common in leafroll samples from Canada and the United States.

There is a logical explanation for some of the discrepancies between our results and those reported by Duffus (5,6). Duffus used a density gradient precipitation technique, in which only antisera prepared against BWYV strains were used. This precluded the differentiation of authentic BWYV reactions from those caused by cross-reactivity between BWYV antiserum and PLRV.

We speculate that the differences between our results and those of Gallenberg et al (11) are due to differences in methodology. Gallenberg et al noted that the BWYV antiserum they used had a higher level of background interference and an overall lower level of reaction with infected samples than the PLRV antisera they used. They did not cross-absorb the γ-globulin to reduce nonspecific reactions and thereby increase the signal-to-noise ratio. Although many of their samples were rated as weak positives based on ELISA tests, these weak positives were not confirmed with aphid transmission tests. Under these test conditions, the possibility of false-positive readings is high, and without confirmatory biological tests, the false positives would remain undetected.

Table 2. Results of attempted aphid (*Myzus persicae*) transmissions from *Physalis pubescens* source plants infected with beet western yellows virus (BWYV), potato leafroll virus (PLRV), or both[a]

	Shepherd's purse		Groundcherry		Potato	
Virus source[b]	BWYV	PLRV	BWYV	PLRV	BWYV	PLRV
BWYV-BC1	+	−	+	−	−	−
BWYV-BC2	+	−	+	−	−	−
BWYV-BC3	+	−	+	−	−	−
BWYV-CA	+	−	+	−	−	−
BWYV-WA	+	−	+	−	−	−
(BWYV/PLRV)-WA1	+	+	+	+	−	+
(BWYV/PLRV)-WA2	+	−	+	+	−	+
PLRV-BC	−	+	−	+	−	+
PLRV-WA	−	+	−	+	−	+

[a] Plants were tested by triple-antibody sandwich enzyme-linked immunosorbent assay using virus-specific monoclonal antibodies (BWYV MAb 510H and PLRV MAb 26BE). A plus sign indicates a positive reaction, a minus sign a negative reaction.

[b] BWYV-BC1, -BC2, and -BC3 = BWYV from British Columbia isolated from sugar beet, *Senecio vulgaris*, and shepherd's purse, respectively. BWYV-CA = BWYV from California isolated from *Malva neglecta*. BWYV-WA = BWYV from Washington State isolated from shepherd's purse. (BWYV/PLRV)-WA1 and -WA2 = mixed infections of BWYV and PLRV from Washington State. PLRV-BC and -WA = PLRV from British Columbia and Washington State, respectively, isolated from potato.

Gunn and Pares (12) recently demonstrated the presence of a stress-induced antigen that copurified with PLRV. The antigen also appeared to be produced in uninfected but physiologically stressed potato plants, and it reacted with their PLRV antisera in ELISA tests, inducing false-positive results. It is possible that antibodies to a stress-induced protein may have been present in some of the BWYV polyclonal antisera previously used and may have caused some false-positive results.

We believe that the quality of the samples was the most critical factor in explaining the marked differences in the histogram patterns (Fig. 1) of the absorbance readings of samples from the three test sites. The time between collection and testing of the Florida samples was at least 1 wk and as long as 2 wk with many samples. Even though the samples were kept in a cooler during this period, the leaf tissue deteriorated considerably. We suspect that PLRV concentration diminished during tissue storage, resulting in the wide range of readings recorded (Fig. 1B). The Oregon samples were stored only a few days before indexing, yet these samples also varied considerably. We attribute this result to the fact that the Oregon samples were raised in a cool greenhouse where the mean temperature was well below the optimum for replication of PLRV. A significant finding in this work was that field samples could be held in cold storage for up to 2 wk before indexing and still provide reasonably reliable results.

The small number of samples (27/801) that were identified in the field as having leafroll symptoms but that tested negative for PLRV were probably affected by a physiological leafrolling. Impairment of carbohydrate translocation from the foliage may cause nonviral leafroll symptoms; when starch accumulates in the leaves, they become leathery and roll upward, and the symptoms are easily mistaken for potato leafroll disease (14). Rhizoctonia stem canker and other diseases, mechanical injury to the stems, and soil nutritional conditions such as nitrogen toxicity or boron deficiency may also cause symptoms that resemble those caused by PLRV (14). Despite the fact that about 3.4% of the samples tested negative for PLRV, the indexing results clearly demonstrated that visual inspection in the field is a reliable procedure for detecting PLRV in most cultivars.

The results presented here show that in Canada and the United States, potato leafroll disease is caused by one virus, PLRV. We found no evidence of BWYV as a component of potato leafroll disease. Moreover, since we were unable to transmit BWYV from *P. pubescens* to potato via the green peach aphid, we conclude that it is doubtful whether BWYV is a component of potato leafroll disease in North America or elsewhere. The results of our attempts to transmit BWYV to potato cultivars via aphids are in agreement with those reported from New Zealand by Webby and Close (25), who were unable to recover BWYV from six potato cultivars that they inoculated. Our results also support the work of Tamada et al (22) in Great Britain and Webby and Close (24) in New Zealand, who failed to recover BWYV from field samples of potato leafroll disease.

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