

# Potential Role of Winter Rapeseed Culture on the Epidemiology of Potato Leaf Roll Disease

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## ABSTRACT

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Rapeseed was an efficient overwintering host of beet western yellows virus (BWYV), one of two viruses that has been associated with potato leaf roll disease. However, rapeseed was not an overwintering host of potato leafroll virus (PLRV), which also causes the disease. Although all unprotected potato plants in the test area were infected with PLRV, no rapeseed plants were infected with PLRV among 3,040 plants assayed in 72 cultivars over a 2-yr period. In contrast, BWYV infected many plants of all winter rapeseed cultivars in the fall, survived winter in these plants, and spread to nearly all remaining plants of every cultivar in the spring. Although small differences in incidence of infection and in BWYV antigen content of infected plants were detectable among the rapeseed cultivars, all cultivars were highly susceptible to the virus. Two aphid vectors of BWYV and PLRV, the green peach aphid (*Myzus persicae*) and, rarely, the potato aphid (*Macrosiphum euphorbiae*), were found on rapeseed plants in the Columbia Basin of Washington in both fall and spring, but neither aphid species survived winter on rapeseed. Both species returned to rapeseed in an early spring migration, colonized the crop, and began to migrate away from rapeseed as the crop ripened in early summer.

Rapeseed, *Brassica napus* L. (Argentine type) and *B. campestris* L. (turnip or Polish type), is grown in many countries for both edible and industrial oils, and for protein supplements in animal feeds (8). Winter rape cultivars that qualify as canola (rapeseed for human consumption) have been developed during the last 10 yr (22). There is a growing interest in rapeseed production in the United States, particularly in the Northwest where canola production increased from near zero in 1983 to more than 12,000 ha in 1987 (7).

The potential impact of this expanding winter rapeseed hectareage on the epidemiology of potato leaf roll disease became an important concern in the Northwest when both beet western yellows virus (BWYV) and potato leafroll virus (PLRV) were implicated in

the etiology of the leaf roll disease of potato (2,3). Susceptible winter rapeseeds could provide excellent overwintering sources of both viruses, since the crops are seeded in the fall and ripen late the following spring, and since they are excellent hosts of the major aphid vector of both viruses, the green peach aphid (GPA) (*Myzus persicae* Sulzer). To be an overwintering host, however, a plant must be infected with the virus in nature. While the susceptibility of rapeseed species to BWYV is known (6, 16), their susceptibility to PLRV is still in question. Both viruses were isolated from winter annual Brassica weeds in Washington (20). Both were transmitted to shepherd's purse (*Capsella bursa-pastoris* L.) (9) and Jim Hill mustard (*Sisymbrium altissimum* L.) (4), but only BWYV could be transmitted to *B. chinensis* L., *B. nigra* (L.) W. Koch, and three varieties of *B. oleracea* L. (9). Although Salaman and Wortley (15) transmitted PLRV to matthiola, turnip, and brussels sprouts, Helson and Norris (10) could not transmit PLRV to these or to other crucifers.

BWYV and PLRV are distinct but closely related luteoviruses and are persistently transmitted by the GPA and a few other aphid species (23).

Development of potato leaf roll disease in the Columbia Basin of Washington has been associated with a massive aphid flight (18) that occurs there routinely (13,14) at about the time the winter rapeseed crop is ripening. Potato

leaf roll, which reduces the yield and quality of potato tubers, is a serious disease of potato throughout the world (5,21). BWYV can cause serious economic losses in rapeseed (16).

Because we recently discovered high levels of resistance to BWYV among cultivars of both *B. napus* and *B. campestris* that are commonly used for forage (19), it seemed probable that resistance would also occur among cultivars of the same species used for rapeseed production.

The objectives of this study were to determine the potential impact of winter rapeseed culture on epidemiology of potato leaf roll disease, to determine the extent to which rapeseed cultivars are infected with BWYV and PLRV, and to identify resistant cultivars that could eliminate the overwintering of the viruses in rapeseed.

## MATERIALS AND METHODS

**Field-plot organization.** The National Rapeseed Variety Trials conducted at the Washington State University Irrigated Agriculture Research and Extension Center at Prosser, Washington, were used in our studies. These trials are given no pesticide or biological treatments for aphid control.

The spring rapeseed cultivar trials were seeded on 5 June 1989, and the winter rapeseed trials were seeded on 26 August 1988 and 1 September 1989, in a randomized complete block design with four replicates. Plots within blocks were 0.9 × 9 m in size with rows spaced 23 cm apart. The rapeseed plantings followed wheat in the rotation. The soil, Warden very fine sandy loam, tested above S and K response levels. Ammonium nitrate and superphosphate were broadcast and incorporated at the rate of 145 kg/ha of N and 12 kg/ha of P. Trifluralin (Treflan 5E) was preplant incorporated to 10 cm at 0.9 kg a.i./ha to control weeds. The plots were furrow irrigated at 7–14-day intervals after planting in the fall, and beginning 20 April in the spring.

The incidence of infection and the relative virus-antigen concentration in the infected plants were determined in late May 1989 and 1990, just before the plants began to ripen. The study was expanded in the second year to determine the extent to which infection occurred

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in the fall, or in the following spring for winter rapeseed, and to determine the aphid species associated with the crop.

**Serological assays.** Plants were assayed for virus content with a two-step adaptation (11) of the standard double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) (1). All antisera were prepared in our laboratory by using rabbits. The BWYV antiserum was prepared against an isolate (supplied by J. E. Duffus, USDA, ARS, Salinas, CA) originally isolated from radish (*Raphanus sativus* L.) and designated RY1R. The PLRV antiserum was prepared against an isolate designated as LR-7. This antiserum has produced a positive assay in every instance among more than 3,000 independent cases of leaf roll-diseased potato analyzed from throughout the western United States over 6 yr. In reciprocal tests, the PLRV antiserum did not detect BWYV, and BWYV antiserum did not detect PLRV. The LR-7 isolate produces severe net necrosis leaf roll symptoms in potato. It was isolated in the Yakima Valley of Washington and supplied by Lee Fox (USDA, ARS, Yakima, WA).

The conditions and ingredients for all ELISAs within each year were identical. Tissue samples (four 3-mm-diameter leaf disks from each of three young leaves of each plant—about 30 mg) were triturated in 0.1 ml of ELISA buffer in a conical-shaped, 1.5-ml polyethylene centrifuge tube with a spinning Teflon pestle. An additional 0.5 ml of ELISA buffer was added to each tube and mixed, and 0.1 ml of the final tissue macerate was added to 0.1 ml of conjugate in an ELISA plate well precoated with gamma globulin. The ELISA plates were incubated in a moist chamber at 4 C for 18 hr, then washed. *p*-Nitrophenyl phosphate substrate solution was added and  $A_{505}$  was read spectrophotometrically after 1 hr at 23 C.

A sample dilution of 1:40 was used in ELISAs and was selected from the log portion of a sample dilution series. The dilution series was prepared from a composite of tissue taken from three infected rapeseed plants (cultivar K8-5) growing in the experimental plots.

Healthy and control standards were included in each ELISA plate. New standards were prepared each year from greenhouse-grown turnip (healthy) or *Physalis floridana* L. (infected) plants. To provide for constancy in the standards between assays, bulk samples of standard tissues were divided into aliquots and held at -70 C until needed.

Forty plants of each cultivar (10 plants from each of four replicates) were assayed for PLRV and BWYV antigen in each 96-well ELISA plate. The healthy, infected, and buffer standards were replicated four times in each ELISA plate.

**Aphid-transmission assays.** Aphid-

transmission assays were conducted on selected plants to supplement and confirm ELISA results. Nonviruliferous GPA were held for about 30 hr on the leaf tissue of each plant to be assayed in a petri plate on moist filter paper. The aphids were then caged on the index host (*P. floridana*) seedlings for 48 hr under a plastic cylinder with one end covered with nylon screen. Aphids were killed by fumigation with nicotine sulfate. The index host plants were observed for virus symptom development and were assayed for virus antigen by ELISA after 4 wk.

**Aphid counts.** Plants of each rapeseed cultivar were examined visually for vector aphids in the fall and spring of both test years. In addition, the numbers of aphids of each species on winter rapeseed were determined by the Berleze-Tulgren funnel method (17) in the fall of the second year. On 9 October 1990, the number of aphids of various species was determined by examining one lower leaf harvested from each of 10 randomly selected plants from each of four replicate plots of each cultivar.

The arrival of flying aphids in the vicinity of the plots in the spring was determined with yellow pan water traps (12). One 29-cm-diameter pan was placed on each side of the plots. The traps were filled with fresh water each Monday morning, and aphids collected each day for the next four days were bulked.

## RESULTS

**Virus symptoms.** Neither obvious yellows-type disease symptoms nor reductions in the size or vigor of plants were observed in any winter rapeseed cultivars in either 1989 or 1990.

**PLRV infection in rapeseed.** Among the 3,040 rapeseed plants ELISA tested for PLRV in 1989 and 1990 (cultivars are listed in Tables 1 and 2), seven plants produced  $A_{505}$  readings above 0.100, 53 produced readings between 0.020 and 0.100, and the remainder produced readings below 0.020. The 60 plants with  $A_{505}$  readings of 0.020 and above were all assayed by insect transmission. No transmission of PLRV occurred. Potato plants grown in nearby plots at the same

**Table 1.** Field susceptibility to beet western yellows virus (BWYV) among cultivars in the National Winter Rapeseed Variety Trials, 1989

Cultivar	Incidence of infection <sup>y</sup>	Relative virus antigen concentration <sup>z</sup>		
		Healthy control	Infected control	Test plants
Dwarf Essex	40/40	0.006	0.494	0.855 a
Librabon	40/40	0.010	0.470	0.706 ab
WW 988	39/40	0.006	0.452	0.703 ab
Glacier	40/40	0.004	0.465	0.703 ab
K8-3	36/40	0.004	0.373	0.695 b
SVO 261	40/40	0.005	0.324	0.683 b
K8-2	40/40	0.005	0.324	0.680 b
Liporta	40/40	0.003	0.452	0.655 bc
WW 1045	39/40	0.007	0.472	0.653 bcd
Bienvenu	38/40	0.010	0.412	0.641 b-e
AWR 0107	40/40	0.006	0.366	0.602 b-f
WW 1031	39/40	0.008	0.458	0.602 b-f
SVO 253	39/40	0.005	0.439	0.588 b-g
SVO 223	40/40	0.008	0.348	0.586 b-g
WW 1058	40/40	0.006	0.494	0.585 b-g
Crystal	40/40	0.006	0.366	0.585 b-g
85 WRB	50/50	0.005	0.373	0.575 b-h
RBR 82	38/40	0.008	0.358	0.562 b-i
K8-5	39/50	0.008	0.458	0.560 b-i
RBR 83	37/40	0.007	0.472	0.521 c-i
WW 1052	40/40	0.003	0.490	0.517 c-j
Cascade	39/40	0.005	0.594	0.499 d-j
AWR 0110	40/40	0.006	0.452	0.496 e-j
Jet Neuf	40/40	0.001	0.391	0.496 e-j
Lindora	39/40	0.005	0.594	0.485 f-j
K8-4	40/40	0.010	0.470	0.465 f-j
Bridger	40/40	0.012	0.412	0.463 f-j
K8-1	39/40	0.004	0.373	0.447 g-j
WW 1011	37/40	0.003	0.490	0.427 hij
RBR 72	40/40	0.003	0.452	0.414 ij
SVO 220	40/40	0.005	0.439	0.410 ij
SVO 238	40/40	0.004	0.464	0.385 j

<sup>y</sup>Ratio: Number of plants infected/number assayed. Number infected estimated by ELISA and aphid-transmission assays (see Table 3).

<sup>z</sup>Mean ELISA reading ( $A_{505}$ ) for BWYV-infected winter rapeseed plants among 40 plants assayed, 16 assays of greenhouse-grown, infected control plants (*Physalis floridana*), and 16 assays of greenhouse-grown healthy control plants (turnip). Sample dilutions were selected to produce ELISA readings in the linear area of the dilution curve. Analysis of variance by SAS, general linear models procedure. Means not followed by the same letter are statistically different ( $P = 0.05$ ).

time were all infected by PLRV.

**BWYV infection of rapeseed.** In the 1989 trials, nearly all plants of all winter rapeseed cultivars were infected with BWYV by late May (Table 1). All plants in 19 of 32 cultivars were infected. Only 22 plants among the 1,280 plants assayed did not contain detectable virus. No significant differences in incidence of infection were detected among the cultivars. At most, only four of 40 plants escaped infection in any one cultivar (K8-3).

Infection pressure was lower in the 1990 than in the 1989 rapeseed trials. Among six cultivars included both years (Cascade, SVO 261, Bienvenue, Lindora, Bridger, and Glacier), infection incidence was 98% in 1989 compared with 83% in 1990. In contrast to the results of 1989, the 1990 cultivars differed significantly

in incidence of infection (Table 2). However, all cultivars were highly susceptible, because 32% was the lowest incidence of infection.

Infection of the winter rapeseed crop with BWYV was well underway in the fall of 1989 (Table 2) before the aphids were killed by winter frost. Nearly half (44%) of the infected plants had already been infected in the fall. The remainder were infected the following spring. Differences in incidence of fall infection among the cultivars were small, and there was little correlation between incidence of infection in fall and in spring.

There was a strong correspondence between  $A_{505}$  values of ELISA and the actual incidence of infection as determined by aphid transmission. Virus was transmitted from only two of 23 plants

with  $A_{505}$  readings lower than 0.020, from five of 11 with  $A_{505}$  readings from 0.020 to 0.059, and from 23 of 24 with  $A_{505}$  readings from 0.050 to 0.099. Virus was transmitted from all of the 64 plants assayed with  $A_{505}$  readings above 0.100. Based on these results, all assays with  $A_{505}$  readings above 0.050 in the 1990 trials were counted as positive.

Based on the strength of ELISA reactions, the BWYV antigen content of winter rapeseed plants varied significantly among cultivars in both test years. However, all cultivars accumulated high concentrations of virus antigen. Data is given for the 32 cultivars in 1989 (Table 1). The cultivars lowest in virus content contained about the same amount of virus as the infected, greenhouse-grown *P. floridana* plants used as positive controls, and those highest in content contained considerably more antigen than the infected *P. floridana*.

**Aphid vectors.** Among aphids known to be vectors of BWYV or PLRV, only *Myzus persicae* and *Macrosiphum euphorbiae* Thomas were found on plants or in water traps. The vector aphids found on plants in the fall (Table 2) were predominantly *Myzus persicae*. Although numbers of aphids varied markedly among cultivars, there was no correlation between aphid number and incidence of infection.

No aphids were found on plants on 1 March following a mild (1991-92) or a severe (1990-91) winter. However, a few *Macrosiphum euphorbiae* were captured in the first trapping of the spring on 13 April 1990, and the first *Myzus persicae* were taken on 20 April (Table 3). Peak numbers of *Myzus persicae* were captured on 11 May and 15 June as the crop began to ripen.

With the exception of *Myzus persicae* and *Macrosiphum euphorbiae*, the only other species captured in sufficient numbers to account for significant virus dissemination was *Brachycaudus helichrysi* Kaltenbach (Table 3), a species that has not been reported as a vector of the viruses under study. The first of this species was captured on 20 April, and large numbers were captured from 4 to 18 May.

The numbers of *Brevicoryne brassicae* L., a nonvector aphid found on plants in the fall, varied markedly among cultivars (Table 3).

## DISCUSSION

This work demonstrated that the culture of rapeseed in the same region with potatoes may represent a threat to the potato crop, because rapeseed served as an overwintering host of BWYV, one of the two viruses reported to be involved in the etiology of potato leaf roll disease. Under heavy exposure to infection, rapeseed did not overwinter PLRV, which also causes potato leaf roll disease. The rapeseed crop itself was almost com-

**Table 2.** Incidence of infection with beet western yellows virus (BWYV) and degree of aphid infestation among cultivars in the National Winter Rapeseed Variety Trials, 1990

Cultivar	Incidence of infection <sup>y</sup>		Aphid species counts		
	Fall	Final	<i>Myzus persicae</i> al/ap/ny/tot <sup>z</sup>	<i>Macrosiphum euphorbiae</i>	<i>Brevicoryne brassicae</i>
SVO 255	12/40	40/40 a	0/0/3/3	0	~30
Norsman	21/40	40/40 a	0/0/4/4	0	~100
Cascade	19/40	40/40 a	0/1/20/21	0	~800
Ceres	16/40	39/40 ab	0/1/2/3	1	~30
KWC 4	17/40	39/40 ab	0/2/24/26	1	~500
SVO 261	21/40	39/40 ab	0/0/0/0	0	~20
2/9001	21/40	37/40 abc	0/0/3/3	0	~30
Humus	13/40	37/40 abc	0/0/3/3	0	~1000
KWC 655	21/40	37/40 abc	0/3/29/32	0	~300
Bienvenu	17/40	37/40 abc	0/3/2/5	0	~500
LD 9443	18/40	36/40 abc	0/0/1/1	0	10
KWC 361	16/40	36/40 a-d	0/1/18/19	0	~20
Lira Donna	24/40	36/40 a-d	0/0/3/3	0	~40
Capricorn	22/40	36/40 a-d	2/1/22/25	0	~1000
2/9009	19/40	36/40 a-d	0/1/18/19	0	~20
OWYX	17/40	35/40 a-e	0/0/1/1	0	9
Lindora	21/40	35/40 a-e	1/0/5/6	0	194
AWR 173	14/40	35/40 a-e	0/1/0/1	0	~100
CoBRA	15/30	35/30 a-e	1/0/3/3	0	100
DNK 5/87	15/30	35/30 a-e	0/0/1/1	0	~200
KWC 158	19/40	35/40 a-e	3/4/76/83	0	366
Arabella	14/40	35/40 a-f	0/2/7/9	0	~200
Tapidora	12/40	34/40 a-f	0/0/0/0	0	~400
KWC 121	14/40	33/40 a-f	0/1/3/4	0	~100
LEI III	15/40	32/40 a-f	0/0/2/2	0	~700
Aspen	13/40	33/40 a-g	0/1/4/5	2	~20
Crystal	13/40	32/40 a-h	0/0/21/21	0	~150
Samourai	10/40	31/40 a-i	0/2/9/11	0	~50
SVO 506	21/40	30/40 b-i	0/1/1/2	0	~500
Cantana	10/40	29/40 c-i	0/0/1/1	0	~400
SVO 508	10/40	27/40 d-j	0/1/1/2	0	~50
KWG 616	8/40	26/40 e-j	0/0/6/6	0	~200
Bridger	11/40	26/40 e-j	0/0/2/2	0	~300
LD 9431	14/40	25/40 f-j	0/2/2/4	0	~20
AWR 243	12/40	25/40 f-j	0/0/3/3	0	~100
ES 8918	9/40	24/40 g-k	0/0/4/4	0	~70
Olein	8/40	23/40 h-k	0/0/4/4	0	~50
ES 8916	10/40	22/40 i-l	0/0/4/4	0	~20
AWR 238	10/40	22/40 i-l	0/0/0/0	0	~500
Glacier	10/40	22/40 i-l	0/2/17/19	0	~50
ES 20917	10/40	17/40 jkl	0/2/33/35	0	~150
KWC 386	7/40	15/40 kl	0/2/49/51	0	~50
DNK 17/88	3/40	13/40 l	0/0/0/0	1	~800
Diadem	4/40	13/40 l	0/0/1/1	0	7

<sup>y</sup>Ratio: Number of plants infected/number assayed. Number infected estimated by ELISA and aphid-transmission assays (see Table 3). Analysis of variance by SAS, general linear models procedure. Means not followed by the same letter are statistically different ( $P = 0.05$ ).

<sup>z</sup>al = alatae, ap = apterous, ny = nymph, tot = total.

**Table 3.** Yellow pan trap collections from National Rapeseed Trial, 1990

Aphid species	Number of aphids captured <sup>2</sup>							
	4/13	4/20	5/4	5/11	5/18	5/25	6/8	6/15
<i>Myzus persicae</i>	0	4	17	24	13	3	8	60
<i>Acyrtosiphon lactucae</i>	0	1	0	0	0	0	0	0
<i>Macrosiphum euphorbiae</i>	2	3	6	9	2	0	4	7
<i>Cavariella aegopodii</i>	0	0	0	1	0	0	0	0
<i>Brachycaudus helichrysi</i>	0	2	67	60	24	9	10	5
<i>Acyrtosiphon pisum</i>	1	0	0	0	0	0	0	1
<i>Capitophorus eleagni</i>	0	0	0	0	1	1	0	0
<i>Dysaphis</i> sp.	0	0	0	1	0	1	0	0
<i>Capitophorus hippophaes</i>	0	1	1	0	0	0	0	0
<i>Sitobion avenae</i>	0	0	0	0	0	0	0	1
<i>Pemphigus?</i> sp.	0	0	0	0	0	0	1	0
<i>Dactynotus taraxaci</i>	0	1	0	0	0	0	1	1
<i>Lipaphis erysimi</i>	1	0	0	0	0	0	0	2
<i>Hyperomyzus lactucae</i>	0	2	0	0	0	0	0	0
<i>Hyperomyzus</i> sp.	0	1	0	0	0	0	0	0
<i>Ovatus crataegarius</i>	0	0	1	1	0	0	0	1
<i>Brevicoryne brassicae</i>	0	0	1	1	1	3	67	263
<i>Aphis armoraciae</i>	0	0	0	1	0	0	0	0
<i>Diuraphis noxia</i>	0	0	0	0	0	0	4	0
<i>Acyrtosiphon malvae?</i>	0	0	0	0	0	0	0	1
<i>Acyrtosiphon</i> sp.	0	0	0	0	0	0	0	1
<i>Dactynotus</i> sp.	0	0	0	0	0	0	0	1
<i>Aphis fabae</i>	0	0	0	0	0	0	0	1

<sup>2</sup>Each number represents the number of aphids captured in four traps over a period of four days previous to the date indicated.

pletely infected by BWYV and probably suffered serious losses from the disease, even though virus infection did not produce obvious foliage symptoms or loss of vigor.

The major vector of BWYV in seed rapes of the Northwest is probably *Myzus persicae*. It is abundant there in the fall (13,14,18) and was the most numerous of the two vector species found on and near the rapeseed crop in these studies both in the fall and in the spring.

The virus was introduced to winter rapeseed during a short period in the fall between seeding and frost. Neither of the aphid species that transmit BWYV overwintered on rapeseed; but both returned to rapeseed in an early spring migration, colonized the crop, spread the virus to uninfected plants within the crop, and began a massive migration away from rapeseed as the crop began to ripen in early summer (15 June). The timing of this migration preceded by about 15 days the massive GPA flight of early summer that has been associated with the initiation of leaf roll disease epidemics in the potato crop in the Northwest (18). This

introduces the possibility that rapeseed culture could move the initiation of leaf roll epidemics forward about 15 days and cause more severe crop losses.

Although small differences were demonstrated among cultivars in the BWYV antigen content of infected plants and in the incidence of infection, all cultivars were highly susceptible. However, resistance sufficient to prevent overwintering of BWYV is available in both rapeseed species (19), and this presents an opportunity to breed BWYV-resistant rapeseed.

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