Reduced Field Spread of Potato Leafroll Virus in Potatoes Transformed with the Potato Leafroll Virus Coat Protein Gene

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ABSTRACT

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Russet Burbank potato was transformed with plant expression vectors containing the potato leafroll luteovirus (PLRV) coat protein (CP) gene. Transgenic potato lines contained a gene expression cassette with two copies of a PLRV CP gene in which the nucleotide sequence was modified to improve expression of the gene. In addition, the two copies of the PLRV CP gene were each driven by a different promoter. Field test screening for PLRV resistance identified 15 lines which showed moderate resistance to PLRV infection and virus titer build-up and a longer incubation period for systemic infection. By conducting field resistance assays during a period when the vector of PLRV was not present, it was possible to test whether the observed resistance was sufficient to restrict aphid transmission of PLRV in a field test. Two years of field testing demonstrated that PLRV-spread from an infected plant to adjacent healthy plants of the same line was severely restricted in nearly all the transgenic lines in the field. These lines have useful resistance to PLRV and could aid in managing PLRV disease in Russet Burbank potato.

Additional keywords: Columbia Basin, environmental quality, food safety, genetic engineering, *Myzus persicae*, net necrosis

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Potato leafroll virus (PLRV) causes a costly disease of potatoes in the Columbia Basin of the northwestern United States (35) and in most, if not all, countries where potatoes are grown (2,27). Limited control of the disease is achieved in the Columbia Basin by planting seed stocks that contain little or no PLRV, and applying aphicides to control the vector of the virus (17,33). However, incidence of PLRV infection routinely approaches 100% in potato crops in the Columbia Basin and in other regions when aphicides are not used (33,34), even when seed stocks are essentially free of the virus. Control of PLRV in the Columbia Basin is costly (35) and not totally effective. Furthermore, regulations to ensure food safety and environmental quality could lead to withdrawal of the aphicides

on which PLRV control is currently dependent. When PLRV is not controlled, the disease markedly reduces yield (25,37) and causes an internal tuber defect called net necrosis (13) in some cultivars, including Russet Burbank, the leading cultivar grown in the Columbia Basin. Net necrosis renders tubers unfit for fresh market and all except the least profitable of processed products. The industry could not sustain the losses in yield and tuber quality that would be associated with the loss of aphicides. Thus, resistance to PLRV in potatoes that meet market and cultural requirements is urgently needed. Immune commercial cultivars do not exist, and resistant cultivars have not been generally accepted.

An alternative strategy to traditional breeding is the introduction of resistance genes into proven cultivars by genetic engineering (17). A distinct advantage of this approach over traditional breeding is that a single characteristic may be added to a proven cultivar without changing other characteristics of the cultivar. The cultivated potato is a highly heterozygous tetraploid. Potato cultivars are seedling clones maintained by vegetative propagation. Thus, adding a characteristic by traditional breeding methods entails developing a new cultivar. This may be achieved only if genes that control the characteristic are available. Transgenic lines of potatoes are the original seedling clones that have been transformed. The transformants must

also be maintained vegetatively as clones. Since genetic crossing is not involved, there is no segregation of genes in development of transgenic potato cultivars.

In efforts to achieve virus disease resistance, virus coat protein (CP) genes have been introduced into a number of different crop species (14), and some measure of resistance to the homologous virus has been achieved in all reported applications of this approach. Virus CP gene expression in potatoes has produced high levels of resistance to infection by potato virus Y (PVY) and potato virus X (PVX; 20). Low to moderate levels of resistance to PLRV were achieved in potato by van der Wilk et al. (36), Kawchuk et al. (23), and Barker et al. (4,5) by expression of the viral CP gene, but none were resistant enough to replace existing cultivars. Modified, single gene constructs of the PLRV CP gene made by Presting et al. (32) did not produce resistant lines among CP gene transgenics, but some vector controls were somewhat resistant. The basis for this resistance has not been determined.

We (19) previously found that reduced PLRV (tomato yellow top strain) titer in the acquisition host can result in a longer transmission latent period and reduced probability of transmission of PLRV by the green peach aphid (GPA; *Myzus persicae* Sulz.). Barker and Harrison (3) came to the same conclusion, and suggested that reduced PLRV titer in a potato cultivar should reduce the spread of virus in the field. They pointed out that typical resistance assessment methods, including direct inoculation and the use of susceptible spreader plants, provide an estimate of susceptibility to infection but do not take into account other factors that may affect the potency of a genotype as a source of inoculum for spread of virus in the field. However, assessment of resistance to secondary spread in the field may be difficult, since plots are easily contaminated with outside sources of virus by migratory vectors.

Secondary spread of PLRV from primary sources accounts for most infection by PLRV in the Columbia Basin. Primary sources of PLRV inoculum originate in the potato crop, either from virus-infected tuber seed or introduction by migratory alatae of aphid vector species (30,32). In the Columbia Basin, the virus is vectored almost exclusively by the GPA (32). Alatae

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of the first seasonal flight of this aphid from its overwintering peach tree host in early May colonize potatoes, and their apterous progeny spread virus from tuber seed sources already present (32). However, new, primary sources of PLRV are rarely, if ever, introduced to potatoes by aphids until the mid-summer flight that begins about July 1 (30,33). Under current cultural conditions, the number of primary sources of infection that occur in a field is often in the order of 1 to 4 per hectare (32,33), but there are exceptions when a higher incidence of infection occurs in the tuber seed. Secondary spread of virus from these few primary sources by the apterous offspring of migratory alatae appears to account for spread of the virus to the remainder of the crop (32,33). The virus is spread in expanding concentric areas around primary inoculum sources as the aphids move from plant to plant. The areas of infection may be oblong, following the rows, if virus spread begins before canopy closure, since apterous aphids have difficulty crossing an open space without a foliage bridge. Infected plants at the periphery of the infection site exhibit the earliest stages of symptom development, while those at the center have advanced symptoms. These expanding infection sites will coalesce and may engulf entire fields within a few weeks if the aphid vectors are not controlled (27,33).

Many types of field experiments involving controlled inoculation with PLRV are rendered difficult or impossible by contamination with PLRV introduced from outside sources by migratory alatae (22). The fact that PLRV is rarely, if ever, introduced to potatoes from outside sources until the mid-summer flight begins in the Columbia Basin (33) provides a window during which controlled inoculation trials

might be performed without undue interference.

Since plant-to-plant spread accounts for most of the PLRV disease in potato crops around the world (2,12,17,33), resistance to plant-to-plant spread of PLRV could be highly effective even in genotypes that are susceptible to initial infection. In this paper we report the transformation of Russet Burbank potatoes with two copies of a modified gene construct. Some of the lines from these gene constructions had reduced symptoms, reduced virus titer, and a longer incubation period requirement for systemic infection compared with single CP gene constructs or nontransformed controls. Although resistance to initial infection in these lines was only moderate, it seemed possible that the combination of resistance characteristics they possessed could reduce secondary spread of PLRV in the field. A major objective of this study was to test whether secondary spread of PLRV was restricted in selected double gene construct lines. We also wanted to develop a method to test for resistance to secondary spread of persistent, aphid-transmitted viruses.

MATERIALS AND METHODS

PLRV purification. A Washington State field isolate of PLRV, LR-7, was selected for isolation of the CP gene. Virus was purified from infected leaves of *Datura stramonium* by homogenization in 0.1M citrate buffer, pH 6.0, containing 0.01M EDTA, 0.3% (wt/vol) DIECA (diethylthiocarbamic acid, sodium salt), 5% (vol/vol) 2-mercaptoethanol, and 1.5% (wt/tissue wt) Rohment (Rohm Enzymes, Huls America Inc., New Jersey) using a tissue:buffer ratio of 1:3 (wt/vol). Homogenate was stirred at room temperature for 2.5 h and then for an

additional 0.5 h after addition of 1.0% Triton X-100 (vol/vol). The homogenate was then emulsified with 20% (vol/vol) of a 1:1 n-butanol:chloroform mixture. After low-speed centrifugation, the aqueous phase was subjected to PEG precipitation (8% [wt/vol] of PEG 8000 and 1% [wt/vol] NaCl) at room temperature. Low-speed centrifugation pellets were dissolved overnight in about 25% (vol/original tissue wt) of 0.1M citrate buffer, pH 6.4, containing 0.01M EDTA. After low-speed centrifugation, the supernatant was centrifuged in a 45Ti rotor (Beckman, Palo Alto, CA) for 2 h at 30,000 rpm. The pellets were resuspended in about 1% (vol/original tissue wt) of 50 mM citrate, pH 6.4, containing 5 mM EDTA. Low- and high-speed centrifugation steps were repeated, scaling down the volume to 12 ml. Final purification was by sucrose density gradient centrifugation (10 to 40%) in a SW28 rotor (Beckman) for 2 h at 25,000 rpm. The virus band was collected using syringe and ISCO model 640 gradient fractionator.

Cloning of PLRV coat protein gene and site directed mutagenesis. The PLRV CP gene was cloned from RNA purified from PLRV virions using expression cloning of the cDNA in Lambda gt10 library, according to the protocols outlined in the Amersham cDNA synthesis system, RPN.1256, and Amersham cDNA cloning system, Lambda gt10. CP-containing clones were identified by antibody reaction on colony lifts. The cDNA was sequenced using United States Biological Sequenase with synthetic primers, according to the manufacturer's instructions. The cDNA coding sequence for the native PLRV LR-7 CP gene comprises 624 nucleotide bases of open reading frame that encodes a protein of 208 amino acids.

Fig. 1. Plant expression vectors: (A) 8517 pFMV/PLRV modCP::p35S/PLRV mod CP; (B) 8515 pGS/PLRV mod CP::p35S/PLRV mod CP.

The CP gene was subcloned from the lambda clone as an AluI/EcoRI fragment into PUC 118. The PLRV CP gene was modified by site-directed mutagenesis (26) to alter 2 AUG codons which may initiate the translation of a 17 kDa ORF, and by changing the TAG amber termination codon to TAA. Nucleotide +27 was changed from a thymidine to a cytosine, and nucleotide +33 was also changed from a thymidine to a cytosine using mutagenesis primer 1. The termination codon was modified beginning at nucleotide +625 using the mutagenesis primer 2. This mutagenesis also eliminated all 3 PLRV sequence to the CP coding sequence. The coat protein coding sequence was not altered with these changes. Two copies of the modified CP genes were cloned into plant expression vectors behind different promoters. These vectors are pMON8515 and pMON8517. pMON8515 contains a copy of modified PLRV CP gene with transcription driven by the enhanced 35S promoter (P-e35S) from CaMV (24), and a second copy of the modified PLRV CP gene with transcription driven by the glutamine synthetase promoter (P-GS) from

tobacco, a phloem specific promoter (11). pMON8517 contains a copy of the modified PLRV CP gene with transcription driven by the P-e35S promoter and a second copy of the modified CP gene driven by the figwort mosaic virus promoter (P-FMV; 15). All of the PLRV-modified CP gene expression cassettes are transcriptionally terminated by the E93 terminator region from pea rubisco (6). The selection of transformed potato by regeneration of kanamycin-resistant plants in tissue culture is conferred by expression of the neomycin phosphotransferase II enzyme (NPTII). This expression cassette is contained in both pMON8515 and pMON8517. The plasmid maps of these two vectors are shown in Figure 1. Mutagenesis primer 1: GGTCGTGGTTAAAGGAAACGTCAAC GGTGGTGTACAACAACC; Mutagenesis primer 2: GCAAAACCCCAAATAAGA ATTCTCCGGATCAGAG.

Transgenic potato and plant propagation. The CP gene vectors (pMON8515 and pMON8517) were mated into *Agrobacterium tumefaciens* by the triparental mating procedure (10). Russet Burbank potatoes were transformed with *Agrobacte-*

rium containing the double gene constructs (28). Kanamycin-resistant plants were produced for each construct. Regenerated plants identified to be transformed were propagated as clones in vitro. Plants of these clones (called lines) were analyzed for gene expression and for virus resistance in growth chamber and field screening experiments.

All 15 lines selected as resistant (see below) were transgenic. NPT-II protein was detected by enzyme-linked immunosorbent assay (ELISA) for these lines. Transgenic DNA presence was confirmed by Southern analysis, and RNA was detected by Northern analysis. PLRV coat protein was detected only in a few lines by Western blot, but not during all growing stages. When detected, CP levels were very low. CP could not be detected in any of the 15 lines by ELISA. Therefore, it did not interfere with virus assays.

Selection of lines to be assessed for resistance to secondary spread. Sixty transgenic Russet Burbank potato lines containing pMON8517 and 42 lines containing pMON8515 were challenged with PLRV by inoculation with viruliferous

Table 1. Summary of performance of virus coat protein gene transgenic lines of Russet Burbank potato when assessed for resistance to initial infection by potato leafroll virus (PLRV)^a

Line identification		Foliage symptom severity ^b				Infection (%) ^c				Titer ^e	Tuber symptom ^d (net necrosis)		
		1991		1992		1991		1992			Incidence	Severity	
Construct	Line	4-wk	12-wk	7-wk	12-wk	4-wk	12-wk	7-wk	12-wk	(ng/mg)	(%)	Severity	(plot)
8515	2-2	1.2* ^f	3.0	3.0	3.0	95.0	100	77.0	97.5	0.74*	51.7	1.68	92
8515	2-3	1.2*	3.0	3.0	3.0	80.0	100	27.5*	40.0*	0.64*	na	na	na
8515	2-4	1.2*	3.0	3.0	3.0	85.0	100	62.5*	80.0	0.65*	51.7	1.63	83
8515	2-5	1.2*	2.0	2.0	3.0	57.5*	100	32.5*	77.5*	0.42*	12.5*	1.65	27*
8515	2-6	1.3*	3.0	2.5	2.5	57.5*	100	55.0*	75.0*	0.57*	50.0	1.56	75*
8515	2-7	1.0*	3.0	2.0	2.0	90.0	100	87.5	95.0	0.65*	na	na	na
8517	3-1	1.0*	2.0	2.0	2.5	17.5*	100	52.5*	100.0	0.37*	60.8	1.79	110
8517	3-2	1.0*	3.0	2.0	2.0	42.5*	100	50.0*	70.0*	0.55*	na	na	na
8517	3-3	1.4*	3.0	2.0	2.5	62.5*	100	52.5*	87.5	0.39*	54.2	2.06	112
8517	3-4	1.3*	2.0	1.5	2.0	52.5*	100	20.0*	60.0*	0.43*	50.0	1.76	88
8517	3-5	1.2*	na	na	na	70.0*	100	na	na	0.49*	52.5	2.45	129
8517	3-6	1.2*	3.0	2.5	3.0	67.5*	100	40.0*	85.5	0.58*	44.2	2.12	85
8517	3-7	1.0*	3.0	3.0	3.0	30.0*	100	27.5*	72.5*	0.61*	37.5	1.82	68*
8517	3-8	1.0*	3.0	2.0	2.0	40.0*	100	35.0*	72.5	0.42*	43.3	1.88	82
8517	3-9	1.0*	2.0	1.0	1.0	32.5*	100	17.5*	80.5	0.33*	18.3*	1.60	30*
RB cont 1		3.0	3.0	3.0	3.0	95.0	100	90.5	97.5	1.16	56.7	2.27	128
RB cont 2		3.0	3.0	3.0	3.0	87.5	100	87.5	92.5	1.35	56	2.00	112
Mean transgenic		1.1	2.7	2.3	2.46	58.9	100	45.5	77.9	0.52	43.9	1.84	0.82
Mean controls		3.0	3.0	3.0	3.00	91.3	100	90.0	95.0	1.25	56.3	2.14	1.20
Difference <i>P</i> value ^g		<0.0001	na	na	na	<0.0001	na	<0.0001	0.0060	<0.0001	0.0444	0.1187	0.0087
Error deg freedom		17	na	na	na	17	na	16	16	153	13	13	13

^a Significance was evaluated using one-way analysis of variance model *t*-statistics corresponding to linear contrasts between the control means and the individual (or grouped) transgenic means. All test were one-tailed since the effect of any effect was known. na = not available. Statistical significance could not be determined for severity in 1992 and at the 12-week stage in 1991.

^b Symptom severity on plants that expressed symptoms. Severity was rated on a 1 to 3 scale: 1 = very mild, 2 = intermediate, and 3 = equal to severity on Russet Burbank control. The 4-week reading for 1991 is the mean of individual plant readings in two 20-plant replications. All other symptom data represent the mean of two 20-plant replications where overall severity for each replication was rated.

^c Percent infection is given as the mean of two 20-plant replications. The data is based on symptom readings where accuracy of the readings was verified with enzyme-linked immunosorbent assay (ELISA).

^d Incidence and severity of net necrosis was determined by cutting 3 tubers from each of 20 plants in two replications in the field at harvest time in 1991. Severity was rated on a 1 to 5 scale: 1 = netting about 1 cm deep at the stem end only and 5 = at least 50% of tuber netted. Severity (plot) = indication (%) × severity.

^e Titer is the mean antigen content of 10 plants regrown during the winter in a greenhouse from tubers produced in the field in 1991. Young leaves of each plant were subsampled three times, and titer was determined by quantitative ELISA. The titer curve was established using purified PLRV of known concentration. The ELISA baseline was determined using juice extracts from healthy plants of each line.

^f * Indicates the line is significantly different from the mean of the Russet Burbank controls at the 5% one-tailed level of significance.

^g The observed one-tailed significance level from a comparison of the transgenic mean to control mean; e.g., *P* values less than 0.05 indicate statistical significance at the 5% level.

aphids in the field. Forty plants per line were tested in two replicated sets of 20 plants each in 1991, and again in 1992. Two replicated sets of Russet Burbank nontransgenic lines were included as infection controls. Each plant was inoculated with apterous GPA from colonies propagated on *Physalis floridana* (Rydb) plants infected with LR-7. A leaflet or portion of a leaflet bearing 10 to 15 aphids was attached near the growing point of each plant using a straight pin. The plots were treated with the organophosphate aphicide, methamidophos, 5 days post-inoculation. Symptoms were recorded at 4 and 12 weeks post-inoculation in 1991, and at 7 and 12 weeks post-inoculation in 1992. Leaf samples of each of the 40 plants of each transgenic and control line were tested by ELISA (22) at 4 weeks post-inoculation in 1991, and at 7 weeks post-inoculation in 1992. Selected plants were tested by ELISA at 12 weeks post-inoculation in both 1991 and 1992 to verify the accuracy of symptom readings. Fifteen lines, 6 of pMON8515 and 9 of pMON8517, exhibited moderate levels of resistance to PLRV and were selected to be assessed for resistance to secondary spread of PLRV in the field.

Assessment of resistance to field spread. To avoid contamination of plots with PLRV from outside sources, the trials were conducted between 1 May and 1 July, when contamination with PLRV from out-

side sources is minimal (33). Spread of virus in the plots was terminated by treatment with methamidophos on 1 July. Additional applications were made at intervals thereafter to control aphid infestation.

The experiment was designed to simulate introduction of PLRV to the field from an outside source of virus followed by secondary spread from the initially infected plant. The spread of PLRV was measured from a centrally located, inoculated source plant of each transgenic line to other plants of the same line in single row plots. Each plot consisted of 20 plants of a line spaced 30 cm apart in a row. The plots were planted on 1 May of 1993 and 1994, using vigorously growing *in vitro*-produced plantlets that had been transferred to soil flats and were approximately 10 cm tall. The center plant of each plot served as the virus source plant. To insure that it was infected, the source plant was massively inoculated 1 week after planting with approximately 50 viruliferous GPA from a colony propagated on *P. floridana* infected with the LR-7 isolate of PLRV. Aphids were confined on the central plant for 10 days after inoculation using a floating screen, then killed by treatment with the contact aphicide, pirimicarb. The floating screen was removed, and 15 noninfective, apterous aphids were placed on each central plant 2 days later. The single-row plots were arranged side by side, 1.8 m apart, in each of four blocks spaced 3.5 m apart.

Plots and blocks were arranged in a randomized complete block design with one replication for each test line in each of four blocks. The plots were observed at weekly intervals to determine time of symptom development in the central inoculated plants. To determine the distance of virus spread from the central infected plant, symptoms were read at 5 weeks after spread of virus was terminated by methamidophos treatment, 53 days after the center plant was inoculated. Leaf samples taken at this time were assayed for PLRV by ELISA (21) to confirm the accuracy of symptom readings.

The mean number of plants to which virus had spread by 1 July among test and control lines was compared using the 1-tailed *t* test. The square-root transformation was used in all statistical analyses to equalize within line variances. Differences due to blocks were not significant and were ignored in the analysis.

RESULTS

Selection of lines. The field screening of 102 lines of PMON8515 and PMON8517 resulted in the selection of 15 lines with somewhat greater resistance to infection than nontransformed Russet Burbank potatoes (Table 1). However, levels of resistance were low. Symptoms tended to be less severe and to develop more slowly in transgenic than nontransformed control plants. Significantly fewer trans-

Fig. 2. Spread of potato leafroll virus in 4 transgenic Russet Burbank lines expressing the potato leafroll virus coat protein gene compared with that in the non-transformed Russet Burbank parental line. The position of infected plants (solid circles) and healthy plants (open circles) in relationship to the central spreader plant (ellipsoids) is given for 4 replicate plots of each line in 1993 and 1994.

genic than control plants had developed symptoms or antigen content detectable by ELISA at 4 weeks post-inoculation in 1991, and the symptoms on infected transgenic plants were significantly less severe at this stage of development. By 12 weeks post-inoculation, however, all plants were infected and symptoms were not significantly reduced. In 1992, symptoms on transgenic plants were fully developed by 7 weeks post-inoculation. Again, incidence of infection, although high in transgenic lines, was significantly lower in some transgenic lines than in control lines, even after 12 weeks. Virus titer in greenhouse plants produced from tubers infected in the field in 1991 was significantly reduced in all transgenic lines. Incidence of net necrosis was significantly reduced in lines 2 through 5 and 3 through 9 (Table 1). Severity of net necrosis, though somewhat lower, was never significantly lower in transgenic than in control lines.

Among both transgenic and control lines, ELISA assays were positive only for plants that had symptoms, and plants with symptoms were always ELISA-positive.

Resistance to PLRV spread in the field.

The spread of PLRV in transgenic lines compared with that in control lines is illustrated in Figure 2. The position of infected plants in relationship to the central inoculated plant is shown for the 4 most resistant transgenic lines and the nontransformed control line in 1993 and 1994. The statistical comparison between PLRV spread in all transgenic lines and control lines is given in Figure 3. Spread of PLRV was significantly reduced ($P > 0.05$) in 14 of the 15 transgenic lines tested in 1993 (Fig. 3A), and in all lines assayed in 1994 (Fig. 3B).

Leafroll symptoms appeared on the central inoculated plant of the Russet Burbank control line in the third week after inoculation. Symptoms appeared 1 week later in the central, inoculated, transgenic plant among the test lines.

Aphids were observed on all plants of all plots when spread of virus was terminated by insecticide treatment on 1 July, 53 days after the central plant had been inoculated. Many plants in the nontransformed control plots had advanced leafroll symptoms at this date. In contrast, transgenic plants adjacent to the central inoculated plant in the transgenic plots were just beginning to show symptoms. At 5 weeks post-insecticide treatment, symptoms were well-developed on both transgenic and control plants. Those with symptoms assayed positive and symptomless plants assayed negative for PLRV by ELISA. Just before harvest in September, some additional plants had begun to express symptoms. These symptoms occurred randomly in the plots and clearly were not associated with spread from the central infected plants, but were likely the result of late season infestation by alate aphids arriving from outside of the test area.

Fig. 3. Statistical variability in resistance of transgenic Russet Burbank lines expressing the potato leafroll virus coat protein gene compared with that of the non-transformed Russet Burbank parental line. Two coat protein constructs for transgenic lines are designated 8515 and 8517. The square root transformation was used to equalize within line variances. Block (replication) differences were non-significant ($df = 3$, $P = 0.7870$ in 1993, 0.9604 in 1994) and were ignored in the analyses. (A) 1993, overall $df = 16$, $P = 0.0016$ (including block effects) or 0.0010 (excluding block effects). (B) 1994, overall $df = 16$, $P < 0.0001$ (including block effects) or < 0.0001 (excluding block effects).

DISCUSSION

Secondary spread of PLRV was markedly restricted in double gene construct CP-transgenic lines that were only moderately resistant to initial infection, titer build-up, and development of systemic infection. These results illustrate the point suggested by Barker and Harrison (2) that methods used to assess genotypes for resistance to PLRV may fail to detect effective resistance to secondary spread of the virus. Methods of assessing resistance typically rely on direct inoculation of the test plants or the use of highly susceptible, infected spreader plants (25). Such methods tend to select for resistance to infection and do not take into account plant characteristics and epidemiological factors that may limit virus spread under natural conditions.

Methods to assess resistance to secondary spread have not been examined in detail (2,25). However, the factors that may contribute to resistance to secondary spread should be evaluated in light of the complex of processes that must be involved in secondary spread of PLRV. It is clear that aphids carry virus from a central source to adjacent healthy plants, and these plants must become systemically infected before they can serve as sources of virus for further spread. If distance traversed by aphids from the central virus source plant is distributed normally, many aphids reach plants nearest to the central source but few reach maximum distances. Thus, the nearest plants are exposed to larger numbers of aphids from the virus source than more distant plants. They are also the first exposed after aphids acquire virus when transmission efficiency of aphids is highest (7), they are exposed at a younger, more susceptible age (8), they are exposed longer, and they are exposed to younger aphids that transmit PLRV most efficiently (29).

Our experiments were designed to simulate natural introduction of virus to plantings of transgenic and nontransgenic lines, followed by secondary spread from the initially infected plant to other plants of the same line. We do not know the plant characteristics that restricted spread of virus in the transgenic lines, but can speculate about how some known characteristics of the transgenic lines might have contributed toward restricted spread.

It is logical that the longer incubation period required for systemic infection of the transgenic lines (28 days), compared with the incubation period required for nontransgenic controls (24 days), would delay spread of virus at each stage in the process of plant-to-plant spread. This delay in availability of virus for dissemination to neighboring plants could induce further delays, since it results in plants being older and progressively more resistant to infection (8) at the time of inoculation. Clearly, the longer incubation period required for

systemic infection in the transgenic plants could account for much of the reduced spread observed in the transgenic lines.

Efficiency of virus transmission is a function both of virus titer of the source plant (3,19) and resistance to infection of the recipient plant (9). These factors could be complementary in effecting a reduced level of secondary spread of PLRV among the transgenic lines. Thus, the moderate levels of resistance to infection characteristic of the transgenic lines may become more effective in reducing transmission as virus content of the aphid vector decreases. Such an interaction could contribute to the reduced spread of PLRV observed. Since resistance to infection may be overcome by high inoculum pressure (8), transmission efficiency may not be an important factor governing infection of plants immediately adjacent to the source plant that are exposed to large numbers of aphids. But transmission efficiency should become increasingly important at greater distances, where plants are exposed to fewer aphids with reduced capacity to transmit.

Other factors that could account for resistance to secondary spread include reduced capacity of transgenic plants to serve as a host of the aphid, or an interference in aphid acquisition or transmission of PLRV. The latter seems plausible if virus coat protein or other viral products produced in the transgenic plants blocked receptor sites for the virus in the aphid vectors.

PLRV infection may be somewhat more difficult to detect by ELISA in the transgenic lines described here than in nontransgenic lines, since virus titer is reduced in the transgenic lines. This could pose some increased difficulty for seed certification agencies in clinical detection of infection. However, our results suggest that infection would be as reliably detected by symptoms in transgenic as in nontransgenic lines. Although symptom severity was somewhat reduced, the symptoms were no less distinct in transgenic lines than in the nontransgenic parent.

This work demonstrated that trials to measure spread of PLRV in potatoes can be conducted in the Columbia Basin before the summer GPA flight with little or no contamination from outside sources. Termination of virus spread by insecticide treatment on 1 July was necessary to prevent PLRV contamination from outside sources (30) and to prevent complete infection of all control plots. Differences in rate of spread could no longer be compared reliably beyond the stage where virus had spread to all control plants.

The efficacy of resistance to secondary spread for control of PLRV is illustrated by the effectiveness of the systemic aphicides, aldicarb and methamidophos, currently used in the potato industry to control the disease. Because they do not kill aphids quickly enough to prevent transmission of PLRV, neither of these aphicides limits

introduction of PLRV into potato fields by alatae. Like the transgenic resistance we have described here, these insecticides control PLRV, not by preventing introduction of PLRV by aphids, but by limiting secondary spread (1,38).

The efficacy of the resistance described here was demonstrated under natural exposure in experiments conducted by G. L. Reed at Hermiston, Oregon (34). Plots in these trials were initiated with virus-free seed but were not treated with insecticides to control aphids. At harvest, incidence of PLRV infection was nearly four times higher in nontransformed Russet Burbank than in Line 3-4, and incidence of net necrosis was six times higher in Russet Burbank than in 3-4. Clearly, 3-4 provided practical resistance to PLRV under natural exposure. This resistance could be useful in many situations to reduce dependence on insecticides.

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