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Short and long distance spread of potato leafroll luteovirus: effects of host genes and transgenes conferring resistance to virus accumulation in potato

Peter M. Derrick† and Hugh Barker

Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

Potato leafroll luteovirus (PLRV) movement through phloem of PLRV-resistant potato clones was examined in experiments in which stem pieces were grafted either between infected rootstocks and virus-free susceptible scions or between infected scions and virus-free susceptible rootstocks. These test plants permitted either upwards or downwards virus movement into the susceptible tissue. Resistant potato clones had either host gene-mediated resistance (H-MR) or transgene-mediated resistance (T-NR, conferred by transformation with the PLRV coat protein gene) to PLRV accumulation. The rate of PLRV movement was similar whether stem tissue was taken from H-MR, T-MR or susceptible potato clones. Virus movement through two graft unions began around 7 days after grafting and was generally complete by about 14 to 16 days. Virus movement occurred soon after acquiring functional phloem continuity across grafts as demonstrated by tracing with 6(5)-carboxyfluorescein, a phloem-mobile dye. Most of the delay in virus detection after grafting probably resulted from the time necessary to develop new phloem strands across graft unions; subsequent movement of PLRV was rapid suggesting a passive process. PLRV infection was largely excluded from external phloem bundles in stem tissue of clones with either H-MR or T-MR. This trait was less pronounced as tissue aged. The mechanism limiting PLRV invasion of external phloem bundles of the T-MR clones appears to be similar to that operating in the H-MR clones. Results are discussed in the context of a proposed model of PLRV movement.

Introduction

There are two recognized phases of virus spread within a host plant, the first being characterized by a relatively slow short distance or cell-to-cell movement via plasmodesmata and the second by rapid long distance movement along vascular traces from metabolic source to sink tissues. There have been many studies of the movement of viruses that invade most parts of their host plant and are known to encode at least one protein (movement protein) that mediates short distance movement through plasmodesmata (reviewed by Mushegian & Koonin, 1993; Lucas & Gilbertson, 1994). Remarkably little is known about the movement of members of the genus *Luteovirus* in plant tissues. Potato leafroll luteovirus (PLRV) is introduced into the vascular tissue of plants by vector aphids and is believed to remain largely confined to sieve element–companion cell complexes throughout the life of the plant (Kojima *et al.*, 1969). However, Barker (1987*a*) observed that 0·2% of mesophyll protoplasts isolated from PLRV-infected *Nicotiana clevelandii* leaves contained PLRV antigen. Van den Heuvel *et al.* (1995) found that PLRV was not exclusively limited to the phloem tissue in infected potato plants. They found that mesophyll cells neighbouring minor phloem vessels were PLRV-infected by immunogold labelling of EM sections. Furthermore, in *N. clevelandii* plants co-infected with PLRV and one of several sap-transmissible 'helper' viruses (e.g. potato virus Y) the proportion of PLRV-infected mesophyll cells increased about 10-fold (Barker, 1987*a*, 1989). A similar enhanced invasiveness of the phloem-limited bean golden mosaic geminivirus has been reported in mixed infection with cowpea mosaic tobamovirus (Carr & Kim, 1983*a*, *b*). Thus, PLRV appears to lack an effective movement function and the very limited movement of PLRV in mesophyll tissue can be incompletely complemented by some other viruses in mixed infections. Indeed, no movement protein has been identified

Author for correspondence: Hugh Barker.
Fax +44 1382 562 426. e-mail HBARKER@scri.sari.ac.uk

† **Present address:** The Samuel Roberts Noble Foundation Inc., PO Box 2180, Sam Noble Parkway, Ardmore, OK 73401, USA.

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for luteoviruses. However, protein with a molecular mass of 17 kDa ('17K protein'), translated by internal initiation of an open reading frame nested within the coat protein gene of PLRV and expressed to high levels *in vivo*, has been identified as a single-strand nucleic acid binding-protein (Tacke *et al.*, 1991, 1993). These workers suggested that the 17K protein may have a function as a phloem-specific movement protein because it has properties in common with other viral movement proteins.

Not only does PLRV differ from the majority of sap-transmissible viruses in its inability to move unaided from cell-to-cell within mesophyll tissue, PLRV also infects only a small proportion of available phloem companion cells in a plant (H. Barker, unpublished results). It may be that this phenomenon is due to a host reaction that limits PLRV infection in order to protect the phloem from catastrophic damage.

Host gene-mediated resistance (H-MR) and transgene-mediated resistance (T-MR) in plants transformed with the PLRV coat protein gene have been shown to be effective in decreasing PLRV accumulation in infected potato plants (Barker & Harrison, 1985; Kawchuk *et al.*, 1990; Van der Wilk *et al.*, 1991; Barker *et al.*, 1992; Wilson & Jones, 1992). Furthermore, in plants with either of these types of resistance, PLRV-infected cells were found to be restricted largely to internal (adaxial) phloem bundles (Barker & Harrison, 1986; Derrick & Barker, 1992). A third form of PLRV-specific resistance is recognized as decreased virus movement from foliage to tubers. For example, many virus-free progeny tubers developed from plants with a primary (current season) infection of PLRV in potato cv. Bismark (Hutton & Brock, 1953) and clone G7445(1) (Barker, 1987*b*). Wilson & Jones (1992) found that the rate of systemic movement of PLRV through grafted stem sections of cv. Bismark was considerably slower than through stem tissues of susceptible potato cultivars.

In this paper we report an investigation into the rate of PLRV movement along phloem strands in potato clones possessing H-MR and T-MR to PLRV and an examination of the distribution of virus in grafted stem tissue in an attempt to reveal more about the mechanisms controlling virus movement in the tissues of potato clones that are resistant to PLRV accumulation.

Methods

■ **Plant material and virus.** Plants were grown from tubers of cvs Pentland Crown, Pentland Squire and Désirée [Scottish Crop Research Institute breeding clones G7445(1), G8107(1), G7032(5), G7714(1), G6867(2) and G8176(1) and transgenic lines C4 and C10 derived from cv. Pentland Squire] and transformed with the PLRV coat protein gene (Barker *et al.*, 1992). Plants were grown in a soil-less potting compost in an aphid-proof glasshouse at about 20 °C. A stock isolate of PLRV was used (Barker & Harrison, 1985) for primary (plants with a current season infection, obtained by graft inoculation as described below, and secondary (plants grown from infected tubers, produced by graft-inoculated plants the previous season) infection.

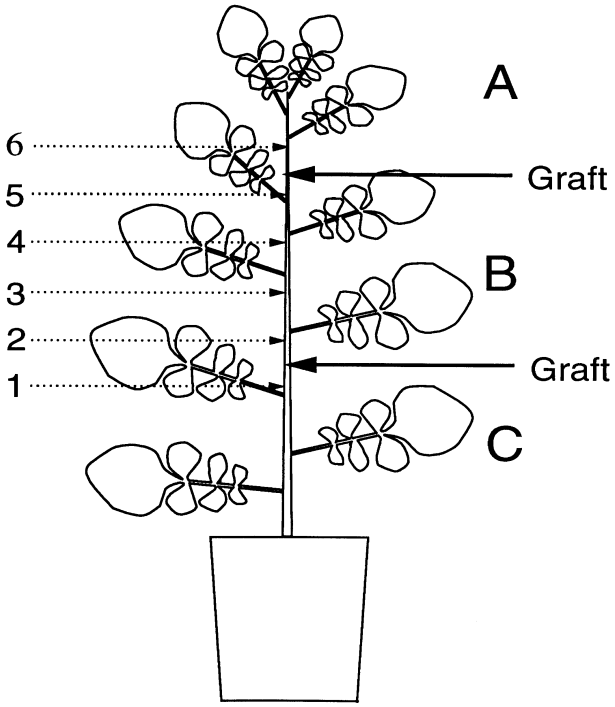

Fig. 1. PLRV graft inoculation procedures. Sections A, B and C represent different tissue pieces used for grafting as detailed above. Positions 1 to 6 represent areas of stem tissue used for tissue printing. Positions of graft unions are indicated.

(*a*) **Rootstock inoculation.**
A, Initially virus-free Maris Piper receptor scion.
B, Test stem section.
C, PLRV-infected Maris Piper rootstock.

(*b*) **Scion inoculation.**
A, PLRV-infected Maris Piper scion.
B, Test stem section.
C, Initially virus-free Maris Piper receptor rootstock.

(*c*) **Leaf inoculation.**
A, Initially virus-free Maris Piper receptor scion.
B, Test stem section.
C, Virus-free Maris Piper rootstock onto which three PLRV-infected leaves were grafted 14 days after the initial stem grafts.

■ **Graft inoculation.** Three different types of graft (illustrated in Fig. 1) were used in which test stem pieces were grafted between PLRV-susceptible (cv. Maris Piper) rootstocks and scions as follows. (1) 'Rootstock-inoculated grafts' in which virus-free test stem pieces were cleft-grafted onto PLRV-infected rootstocks, and virus-free scions taken from the tips of young stems were then cleft-grafted onto the top of the test stem pieces. (2) 'Scion-inoculated grafts' which were similar to the above except that rootstocks were virus-free and inoculum was supplied by PLRV-infected scions. (3) 'Leaf-inoculated grafts' in which the rootstock, test stem piece and scion were all grafted together from virus-free material, then, 14 days after grafting, three leaves on the rootstock were removed and replaced by grafting in PLRV-infected leaves. Grafted parts of plants were enclosed within polythene bags for 6 days following grafting to minimize transpiration. Virus-free test stem pieces used for the grafts were taken from the upper sections of young (390–460 mm tall) plants. Test stem sections were either two internodes long and bearing one leaf or four internodes long and bearing three leaves.

■ **Testing for phloem continuity across grafts.** The 6(5)-carboxyfluorescein phloem tracing technique of Grignon *et al.* (1989) was

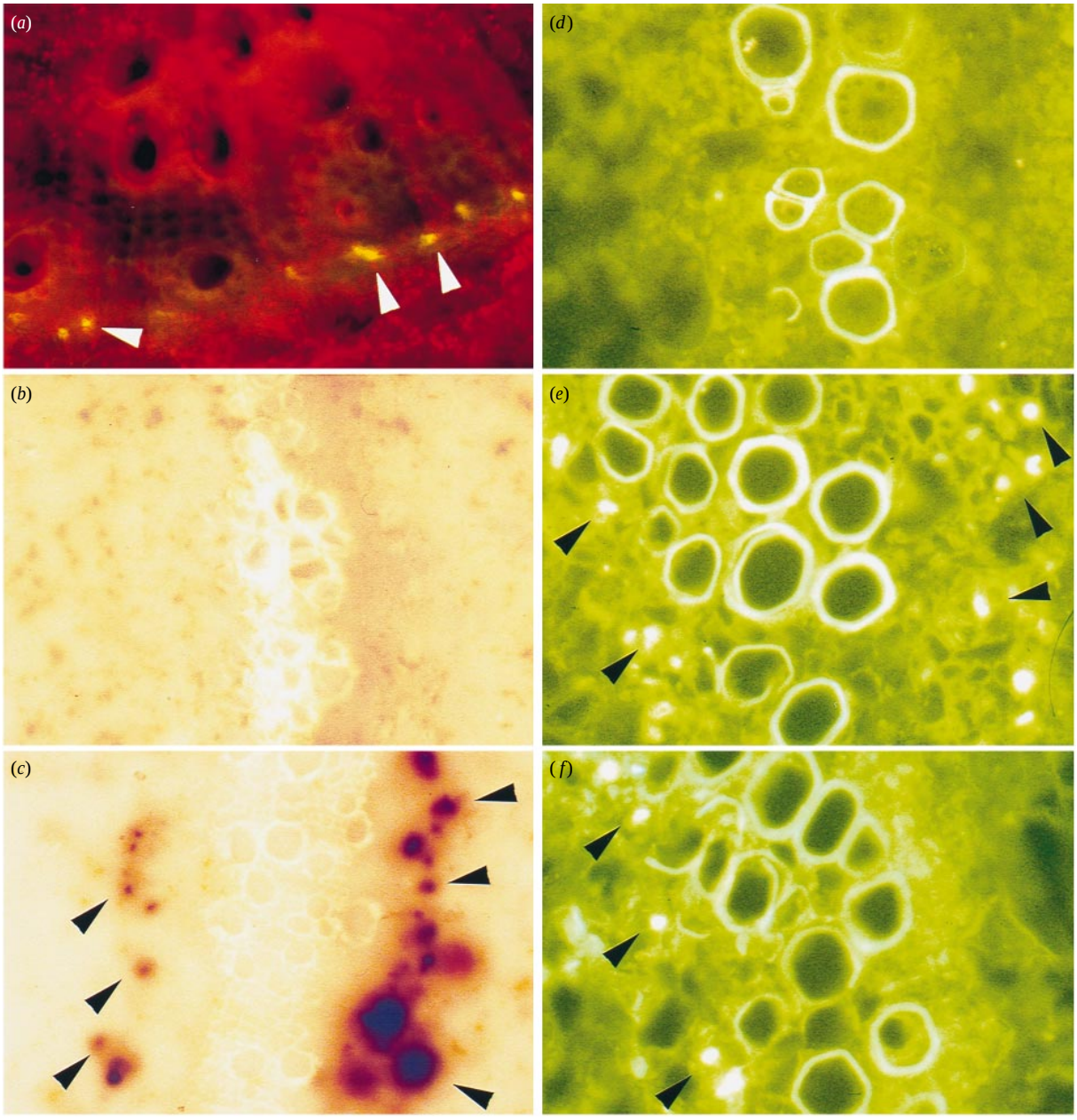

Fig. 2. Detection of a phloem-mobile tracer dye and PLRV antigen in phloem cells of potato tissue. (*a*) Movement of 6(5)-carboxyfluorescein tracer in phloem sieve tubes of external (abaxial) phloem bundles from a labelled leaf of a rootstock-inoculated plant to stem tissue immediately below the test stem–rootstock graft. The presence of fluorescent cells (marked with arrowheads) in external phloem bundles was detected in freehand sections examined with an epifluorescence microscope; large xylem vessels can be seen above these cells. (*b*, *c*) Tissue prints of transverse sections of stem tissue from virus-free (*b*) and PLRV-infected (*c*) Maris Piper plants. Stained foci (marked with arrowheads) representing infected cells are readily seen with a light microscope [the PLRV-infected print in (*c*) has been over-developed in order to facilitate photography]. The internal (adaxial) phloem bundle are to the left and the external phloem bundles are to the right of the imprint left by the xylem vessels, which can be seen in the centre of the prints. (*d*, *e*, *f*) Cryostat sections of potato petiole tissue from virus-free Maris Piper (*d*), PLRV-infected Maris Piper (*e*) and PLRV-infected Pentland Crown plants (*f*), after treatment with fluorescein-conjugated γ-globulin to the PLRV coat protein. Sections were examined with an epifluorescence microscope and stained companion cells in phloem bundles are visible as brightly stained spots (marked with arrowheads). The internal phloem bundles are to the left and the external phloem bundles are to the right of the xylem vessels which are visible by their autofluorescence and can be seen in the centre of the prints.

used to detect the presence of functional phloem connections. A 1 mM solution of 6(5)-carboxyfluorescein (Sigma), adjusted to pH 6·3, was applied to leaves above grafts as 50 µl droplets onto 10 × 5 mm areas of leaf which had been abraded with carborundum. A glass coverslip was pressed onto a ring of petroleum jelly applied to the leaf to prevent evaporation of the tracer. Two droplets were applied to each of the two leaves attached to the test stem pieces of rootstock-inoculated plants. Plants were left for 5 h before sampling. Freehand sections were cut from stem tissue immediately above and immediately below the test stem–rootstock graft, mounted in 90% glycerol and examined using a Nikon Optiphot epifluorescence microscope to detect fluorescent tracer in phloem tissue (see Fig. 2).

■ **Tissue printing.** Unless stated otherwise, PLRV antigen was detected in grafted plants by tissue printing using 2 mm thick stem sections, essentially as described by Wisniewski *et al*. (1990) except for the following: tissue prints were blocked for 1 h at 37 °C with an extract of virus-free potato stem tissue (1 g macerated into 20 ml of 3% non-fat milk powder in 10 mM Tris, 0·9% NaCl, pH adjusted to 7·4) and then incubated for 1 h at 37 °C with rabbit anti-PLRV polyclonal IgG conjugated to alkaline phosphatase. Two sections, from each of two plants, were printed from each sample point at the six stem positions indicated in Fig. 1. Developed prints were examined with a microscope and stained foci counted (see Fig. 2).

■ **Fluorescent antibody staining.** PLRV antigen was detected in sections cut from frozen tissue using a microtome mounted in a cryostat and stained by indirect fluorescein-labelled antibody staining as described by Derrick & Barker (1992). Sections were examined in an epifluorescence microscope and the numbers of stained cells counted (see Fig. 2).

■ **ELISA.** PLRV antigen was detected in leaf extracts by the double antibody sandwich form of ELISA as described by Barker & Solomon (1990).

Results

PLRV detection by fluorescent antibody staining and tissue printing

PLRV antigen was revealed on cryostat sections as brightly fluorescent foci within phloem tissue (Fig. 2). On tissue prints the presence of PLRV was apparent as discrete foci of purple indoxyl precipitate (Fig. 2). Because the outlines of most cells could be readily observed on stem tissue prints (well illustrated by Varner & Ye, 1994) the precise location of individual infected cells could be discerned by microscopy. Additionally, probably because PLRV antigen is not diluted in the tissue printing method, the sensitivity of this technique was found to be greater than that of ELISA for detection of PLRV (P. M. Derrick, unpublished results; also see Whitworth *et al*., 1993). Tissue printing thus provided a sensitive and semi-quantitative technique for detecting PLRV.

The distribution of PLRV-infected cells in stem tissue was assessed by fluorescein-labelled antibody staining of tissue sections taken from secondarily infected plants of several potato clones. In the susceptible clones G7714(1) and G6867(2), virus was found in companion cells of many internal and external phloem bundles (Fig. 3). By contrast, PLRV-infected cells were almost entirely restricted to internal phloem bundles in five resistant clones (Fig. 3). In a previous study we found that PLRV-infected cells were similarly distributed in petioles of these genotypes (Derrick & Barker, 1992). The distribution of PLRV in stem tissue was also assessed by tissue printing at different positions in the stems of plants of the H-MR clone G7445(1) and the susceptible cv. Maris Piper. In young stems, and in young parts of mature stems of G7445(1), very few virus-infected cells were observed in the external phloem (Fig. 4*a*, *b*). However, in older stems of G7445(1) there

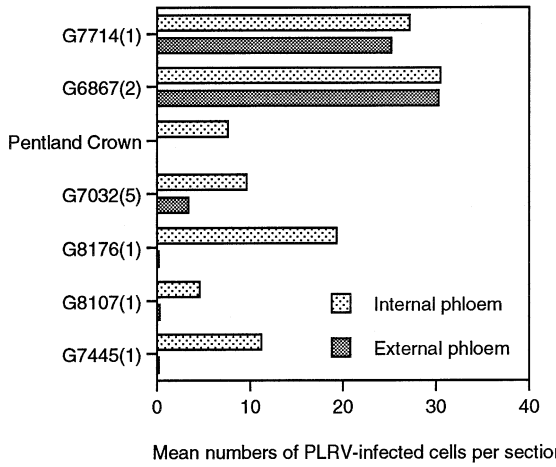

Fig. 3. Distribution of PLRV-infected cells in internal and external phloem bundles in secondarily infected stem tissue of potato clones differing in resistance to virus accumulation; G7714(1) and G6867(2) are susceptible, other clones are resistant. Data given are mean numbers of PLRV-infected cells per section, which were determined by staining with fluorescein-labelled antibody three sections sampled from each of four plants per potato clone.

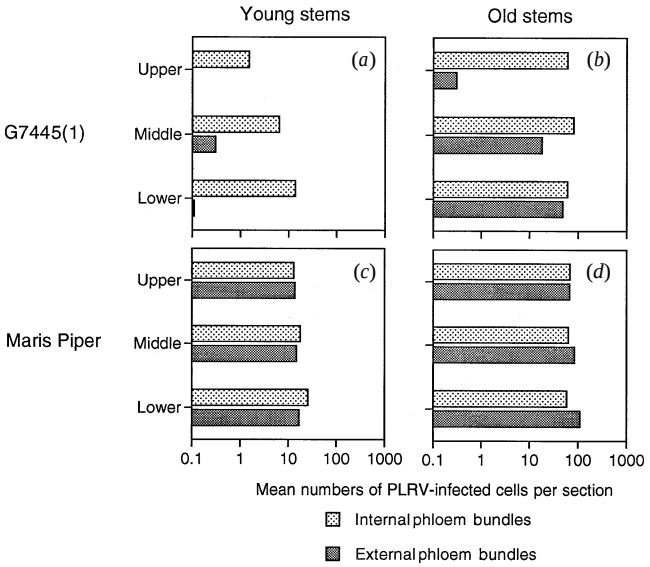

Fig. 4. Distribution of PLRV-infected cells in internal and external phloem bundles in secondarily infected stem tissue of G7445(1) (*a*, *b*) and Maris Piper (*c*, *d*) (*a*, *c*, young stems; *b*, *d*, old stems). Maris Piper is susceptible to PLRV accumulation, G7445(1) is resistant. Data given are mean numbers of PLRV-infected cells per section which were determined by tissue printing two sections sampled at each stem position (upper, middle and lower) from each of three plants.

Table 1. Timescale for the development of functional phloem continuity and upward movement of PLRV across newly formed grafts

Test clone	Resistance/ susceptibility to PLRV accumulation*	Movement of 6(5)-carboxyfluorescein† Days after grafting: 5	7	14	Movement of PLRV‡ Days after grafting: 5	7	14
Pentland Squire	S	0/5	2/5	5/5	0/5	2/5	5/5
Pentland Squire C4	T-MR	0/5	2/5	5/5	0/5	0/5	5/5
Pentland Squire C10	T-MR	0/5	5/5	5/5	0/5	1/5	5/5
G7032(5)	H-MR	0/4	2/4	5/5	0/4	0/4	5/5

* S, susceptible; T-MR, transgene-mediated resistance; H-MR, host gene-mediated resistance.
† Data are given as no. of plants in which dye movement was detected/no. tested. Dye movement is defined as presence of dye in position 1 after loading at positions 2 to 5 shown in Fig. 1.
‡ PLRV was detected by tissue printing. Data are given as no. of plants in which PLRV was detected within test stem pieces/no. tested.

Table 2. Upward movement of PLRV through grafted stem sections

Rootstock-inoculated plants (see Fig. 1). PLRV was detected by tissue printing. Data are given as no. of plants in which PLRV was detected within test stem pieces/no. tested.

Test clone	Resistance/ susceptibility to PLRV accumulation*	Short stem segments† Days after grafting: 9	13	16	22	Long stem segments‡ Days after grafting: 9	13	16	22
Désirée	S	0/4	3/4	4/4	4/4	1/4	2/4	4/4	4/4
Pentland Squire	S	0/7	7/7	7/7	7/7	0/7	7/7	5/7	7/7
Pentland Squire C4	T-MR	0/7	7/7	7/7	7/7	0/7	3/6	5/7	7/7
Pentland Squire C10	T-MR	0/7	7/7	6/7	7/7	0/7	5/6	7/7	7/7
Pentland Crown	H-MR	0/4	0/4	3/4	4/4	0/4	1/4	4/4	4/4
G7461(1)	H-MR	0/4	0/4	3/4	4/4	0/4	2/4	4/4	4/4
G7445(1)	H-MR	1/4	1/4	4/4	4/4	0/4	1/4	4/4	4/4

* S, susceptible; T-MR, transgene-mediated resistance; H-MR, host gene-mediated resistance.
† Short stem segments were two internodes long.
‡ Long stem segments were four internodes long.

was an equal proportion of infected cells in the internal and external phloem in the lower part of the stem (Fig. 4*b*). In young and mature stems of Maris Piper, infected cells were evenly distributed between internal and external phloem tissue (Fig. 4*c*, *d*). Not surprisingly, infected cells were more numerous in both types of phloem tissue in older stems of Maris Piper and G7445(1) than in younger tissues.

Timing of the movement of 6(5)-carboxyfluorescein and PLRV across grafts

To determine the effect of graft annealing on the lag time observed before PLRV was detected in sink tissues, the ability of grafts to transmit the phloem tracer 6(5)-carboxyfluorescein was compared temporally with ability to transmit PLRV. Following leaf application of 6(5)-carboxyfluorescein, the dye became trapped within phloem tissue (Grignon *et al.*, 1989) and was rapidly transported out of labelled leaves (at several cm/h), predominantly towards the root (Fig. 2). In this study, 6(5)-carboxyfluorescein almost always moved downwards in external phloem bundles of ungrafted potato stems. It was, however, occasionally seen moving downwards in internal phloem cells. Once carboxyfluorescein had traversed a graft, the tracer was commonly found in cells of both internal and external phloem bundles. In petioles and stems, 6(5)-carboxyfluorescein fluorescence was observed within sieve elements and most intensively within companion cells, highlighting the multiple vacuoles typical of these cells.

Table 3. Downward movement of PLRV through stem sections

Scion-inoculated plants (see Fig. 1). Data are given as no. of plants infected/no. tested. Infection was determined by tissue printing of stem samples at position 1.

Test clone	Resistance/ susceptibility to PLRV accumulation*	Days after grafting: 7	14
Maris Piper	S	3/4	4/4
Pentland Squire	S	1/4	4/4
Pentland Crown	H-MR	0/4	4/4
G7445(1)	H-MR	0/4	4/4

* S, susceptible; H-MR, host gene-mediated resistance.

Five days after grafting, 6(5)-carboxyfluorescein applied to leaves attached to a stem segment above grafts was observed immediately above but not below the lower graft interface (Table 1) indicating that at this time wound phloem had not yet developed to bridge the graft. Seven days after grafting, 6(5)-carboxyfluorescein was able to cross 11 out of 19 grafts. Tracer dye was able to cross all grafts by 14 days after grafting. PLRV was detected above the lower graft union by tissue printing of some plants 7 days after grafting and through all stem sections by 14 days after grafting (Table 1). The movement of virus, therefore, appeared to occur very soon after the establishment of functional phloem connections within grafts.

Rate of upward PLRV movement through stem sections of different lengths

To determine whether the rate of PLRV movement through stem sections was influenced by the length of the test stem section, short (two internodes long) and long (four internodes long) stem pieces were grafted using the rootstock inoculation method (Fig. 1). Virus movement occurred through 62% of stem segments after 13 days, 92% after 16 days and 100% after 22 days. The rate of movement was not influenced by the length of stem segment and was similar using tissue from susceptible, H-MR and T-MR potato clones (Table 2).

Downwards movement in scion-inoculated grafts

PLRV detection in rootstocks, after downward movement through test stem sections from infected scions, showed that virus movement had occurred in all plants by 14 days after grafting. Even though virus had moved through some susceptible test stem sections at 7 days after grafting, there was no marked difference between the rate of virus movement through resistant or susceptible stems (Table 3).

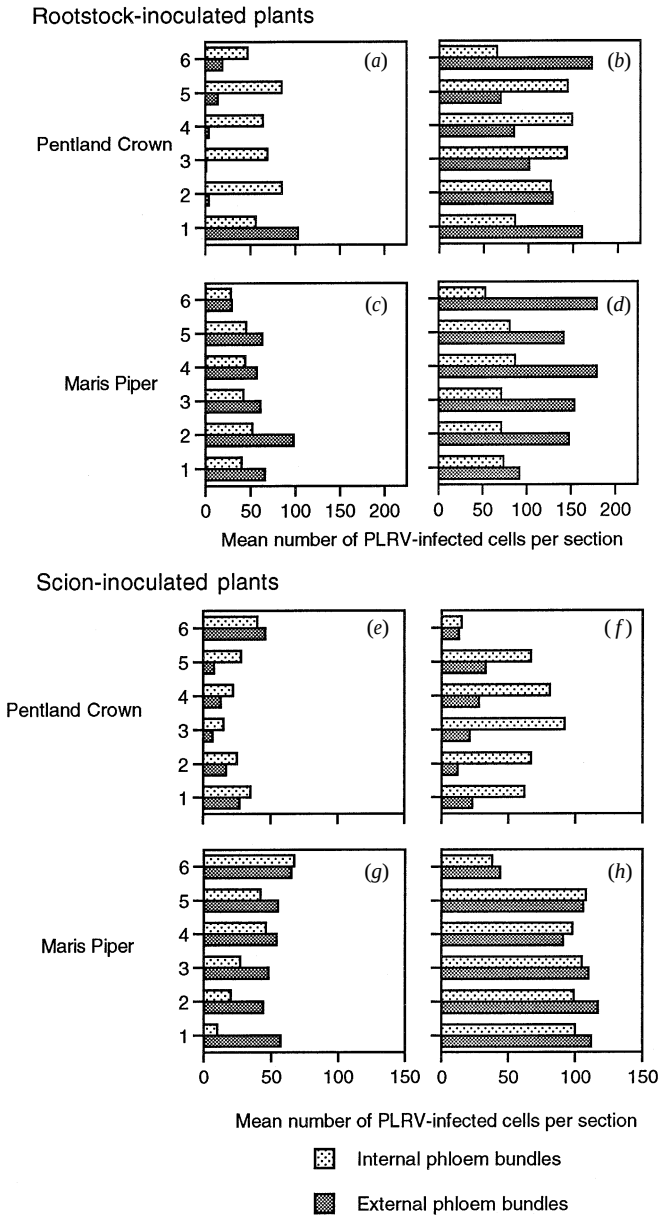

Fig. 5. Distribution of PLRV-infected cells in internal and external phloem bundles after upward movement from rootstock-inoculated plants (*a*, *b*, *c*, *d*) or downwards movement from scion-inoculated plants (*e*, *f*, *g*, *h*). Numbers 1 to 6 on the vertical axis represent different tissue sampling positions (details given in Fig. 1). Test stem tissue was from Maris Piper (susceptible to PLRV accumulation) and Pentland Crown (resistant). Data given are mean numbers of PLRV-infected cells per section which were determined by tissue printing two sections from each of two plants sampled at each position. Samples were taken from plants 21 days (*a*, *c*), 28 days (*b*, *d*), 7 days (*e*, *g*) and 14 days (*f*, *h*) after grafting.

Influence of the magnitude of PLRV inoculum in determining rate of virus movement

The possibility was considered that the rapid movement of PLRV through grafts during establishment of the union, and the lack of impedance in stem tissue from resistant clones, was

due to the large load of virus provided by infected rootstock or scion tissues. Tests were made using the 'leaf inoculation' method (Fig. 1) to determine whether resistance to the rate of virus movement might be observed if relatively small amounts of virus inoculum were applied to plants after the development of graft unions. PLRV was detected in some plants inoculated in this way at 10 days after introducing the source of inoculum, and in all plants by 17 days. There was no difference observed in the rate of virus movement through stem tissue of susceptible and T-MR clones (data not shown).

Distribution of PLRV in phloem tissue of grafted stem sections

The distribution of PLRV was examined in the grafted stems from rootstock-inoculated (reflecting upward movement) and scion-inoculated (reflecting downward movement) graft constructs. Susceptible clones Maris Piper and Pentland Squire and H-MR clones Pentland Crown and G7445(1) were selected for this experiment and similar results were obtained for both pairs of susceptible and resistant clones. Data are shown for only Maris Piper and Pentland Crown (Fig. 5). When rootstock-inoculated plants were examined at 21 days after grafting, both internal and external phloem bundles of the grafted test stems of susceptible clones were infected (Fig. 5*c*), but there were few infected cells in the external phloem of stem pieces from resistant clones (Fig. 5*a*). It is interesting to note that in plants containing resistant stem sections, fewer cells of the external phloem of the (susceptible) receptor scion became infected (position 6, Fig. 5*a*) than in plants with susceptible test stem segments (position 6, Fig. 5*c*). At 28 days after grafting, many more cells of the external phloem in the resistant segments had become infected (Fig. 5*b*), although the majority of infected cells were in internal phloem bundles in comparison with susceptible stem segments (Fig. 5*d*) where the majority of the infected cells were in the external phloem.

Scion-inoculated plants were sampled at 7 and 14 days after grafting. The majority of infected cells in resistant stem segments were in internal phloem bundles (Fig. 5*e,f*) compared with the susceptible stem segments in which many internal and external phloem cells were infected. As with the rootstock-inoculated plants, these differences were reflected in fewer cells of the external phloem of the (susceptible) receptor rootstocks (position 1, Fig. 5*e,f*) becoming infected than with plants with susceptible stem segments (position 1, Fig. 5*g,h*).

Discussion

In potato, there are several lines of evidence suggesting that a mechanism operates in resistant clones to restrict PLRV movement in phloem tisue (Golinowski *et al*., 1987; Derrick & Barker, 1992; Wilson & Jones, 1992). Likewise, restriction of virus movement in vascular tissue has been shown to be a factor in pepper lines that are resistant to cucumber mosaic virus (Dufour *et al*., 1989). Despite their artificial nature, grafting experiments provide an excellent means of examining long-distance movement of PLRV and the effects of the host plant on this process. In our experiments, the movement of PLRV through two graft unions began around 7 days after grafting and was generally complete by about 14 to 16 days, though in some experiments, virus movement was slightly slower. Very similar estimates were made by Wilson & Jones (1992). Movement of virus through grafts occurred soon after acquiring functional phloem continuity across grafts as demonstrated by 6(5)-carboxyfluorescein tracing (around 7 days after grafting; Table 1). Most of the delay between the time of grafting and detection of virus in scions was probably the result of the time necessary to develop new phloem strands across the graft and PLRV movement throughout stems was, therefore, very rapid. It is indeed possible that PLRV movement along phloem sieve elements is an essentially passive process. It is, after all, known that the movement of PLRV, and many other viruses, is strongly dependent on metabolite fluxes and that movement of the virus in potato plants can be manipulated by adjusting sink strength (Mowry, 1995).

The rate of movement of PLRV through test stem pieces was similar whether test stems were taken from H-MR, T-MR or susceptible potato clones (Tables 1, 2 and 3). It is, therefore, likely that neither the H-MR nor T-MR mechanisms present in the tested clones cause a slowing of PLRV movement along phloem strands. In similar experiments, Wilson & Jones (1992) observed that test stem pieces of potato cv. Bismark did reduce the rate of PLRV movement and suggested that this was possibly the result of the severe necrosis associated with PLRV infection in this cultivar. Similarly, potato cv. Apta is resistant to PLRV because infected sieve elements respond to infection by intense callose deposition followed by necrosis of the phloem which results in inhibition of further spread of the virus (Golinowski *et al*., 1987). Two lines of evidence suggest that none of the resistant clones we tested develops a hypersensitive type of response to PLRV infection. Firstly, there is no necrotic response in phloem tissue following PLRV infection, even in clone G7445(1) in which virus movement from foliage to tubers can be impeded (Barker, 1987*b*). Secondly, transformation of H-MR clones with the PLRV coat protein gene in a translatable form does not induce a hypersensitive response (Barker *et al*., 1994).

The expression of H-MR has many similarities with the T-MR phenotype (Barker *et al*., 1992). Derrick & Barker (1992) concluded that one of the underlying mechanisms of these forms of resistance in secondarily infected tissue operated by reducing the number of cells that became infected in the external phloem bundles. The roles of internal and external phloem bundles in plants are not, however, well understood and so the physiological significance of this restriction of PLRV invasion is unclear. We have now shown that the same mechanism also operates in primarily infected tissue. Thus, when grafted stem sections were inoculated using either infected rootstocks or scions, the distribution of infected cells

was different in resistant and susceptible stems with primary PLRV infection, with a greater proportion of infected cells in resistant stems tending to occur in internal phloem (Fig. 5). Thus, this resistance mechanism is effective in restricting the movement of primarily infecting PLRV into companion cells of external phloem bundles, despite a very heavy inoculum pressure from infected rootstocks and scions. Another important feature of the resistance mechanism is that it becomes less effective in stem tissue of plants with either primary or secondary infection as the plants age. Thus, in old tissue of resistant clones, many cells of both the internal and external phloem become infected (Figs 4 and 5).

In the potato clones we have studied, the ability of the coat protein (CP) transgene to inhibit virus accumulation and spread in the external phloem tissue is more likely to represent a function of the CP RNA transcript rather than the CP itself (Barker *et al*., 1993). As we have not been able to find any differences in the distribution of PLRV in H-MR and T-MR clones (Derrick & Barker, 1992), nor in the ability of stem pieces to retard movement of virus through them (this study), it seems possible that a similar, if not identical, mechanism of virus suppression occurs in both genotypes; naturally occurring and active in the H-MR clones and switched on in the T-MR clones by the presence of the transgene.

Given the present state of knowledge about PLRV, we have developed the following model of the movement of PLRV which is based partly on the results reported here in addition to published work (Shepardson *et al*., 1980; Barker & Harrison, 1986; Tacke *et al*., 1991, 1993; Barker *et al*., 1992; Derrick & Barker, 1992; Wilson & Jones, 1992) and is partly hypothetical. In susceptible hosts, virus moves long distances through sieve elements, carried passively in assimilate flow. The short distance movement between sieve elements and companion cells may be mediated by the PLRV-encoded 17K protein which could function to gate plasmodesmata and act as a chaperone to viral RNA. Long distance movement through sieve elements of resistant potato clones, with either H-MR or T-MR, seems to occur at the same rate as in tissue from susceptible clones. However, in resistant plants, short distance movement from sieve elements to companion cells is dramatically impaired, with the result that fewer cells in the external phloem become infected. The product of the H-MR genes may act directly against (or interact poorly with) the 17K protein, possibly inactivating it. The mRNA from the CP transgene may inhibit the expression of the viral gene encoding the 17K protein which is translated by internal initiation of an open reading frame within the CP gene. H-MR and T-MR may be more effective in the external phloem because of greater gene expression than in internal phloem bundles or because greater amounts of the 17K protein are required to gate plasmodesmata in this tissue. As the tissue ages, the differential distribution of virus declines, possibly coincident with a decline in the expression of the product of the H-MR gene or a change in the nature of the plasmodesmata.

Clearly, there are untested hypotheses in the above model but it may provide a framework for future work to test the mechanism of PLRV movement. Study of the interaction between the 17K protein and H-MR gene products, and possible inhibition of the 17K protein by the CP transgene, may provide clues to enable the mechanism underlying PLRV host resistance to be elucidated.

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