# Variation among aphid vectors of *Potato leafroll virus*

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*Potato leafroll virus* (PLRV) and its main vector, the peach-potato aphid, *Myzus persicae*, have been studied extensively at SCRI. The mechanisms involved in the transmission of PLRV are both scientifically interesting (transmission is a highly specialised process), and economically important because infection with PLRV is a serious problem for seed potato production. Current measures to prevent the spread of PLRV in Scotland involve the intensive use of insecticides to prevent the build-up of potential vector populations. Understanding more about epidemiological aspects of PLRV biology and, in particular, the transmission process of PLRV, will enable us to pinpoint those aphid populations responsible for spreading the virus. This is a prerequisite for developing novel, more environmentally sensitive control methods.

PLRV is mainly confined to phloem tissues of infected plants. Only aphid species that colonise potato plants are natural vectors because they must feed from the phloem to acquire the virus. Transmission involves the passage of ingested virus particles from the gut to the haemocoel and their subsequent transport through the accessory salivary glands and into saliva, where they can infect a new host. Most attention has been focused on properties of the virus particles that are recognised by aphid tissues. Several studies suggest that proteins on surfaces of virus particles play a key role in transmission. Early progress in this area was made with the discovery of poorly aphid-transmissible (PAT) isolates of PLRV that lack epitope(s) found on the surface of particles of highly transmissible (HAT) isolates. Work at SCRI has also suggested that changes in the amino acid sequence of the readthrough protein might account for alterations in efficiency of transmission. However, more recent transmission experiments with virus-like particles that lacked readthrough protein (*Ann. Rep. 1996/97*, 164), led us to revise this hypothesis, and to consider the role of vector components in transmission. Less is known about vector proteins involved in transmission, but clones of *M. persicae* that are inefficient vectors of PLRV would be a useful model for comparative experiments.

At least 10 aphid species colonise potato foliage and ingest phloem contents, but they do not all transmit PLRV efficiently. For example, the potato aphid, *Macrosiphum euphorbiae*, is usually the most numerous species in Scottish potato crops, but it transmits PLRV less efficiently than *M. persicae*. Moreover, in addition to differences in the efficiency with which PLRV is transmitted by aphid species, there are reports of differences in transmission efficiency for individual clones of *M. persicae*. Distinguishing such clones is difficult because they show remarkably little morphological or biochemical variation. However, the recent development of a DNA fingerprinting method enables us to characterise clones by variability in the lengths of the IGS regions between ribosomal genes (*Ann. Rep. 1997/98*, 126). We have therefore been able to compare the transmissibility of PLRV using distinct aphid clones, and to re-examine the transmission of PAT isolates of PLRV. Collaborative work with colleagues at the Institut National de la Recherche Agronomique (INRA) at Le Rheu in Brittany, France has enabled us to extend these comparisons by including a French PAT isolate of PLRV, as well as additional aphid clones.

Modern techniques for characterising aphid populations have led to surprising conclusions about the tax-

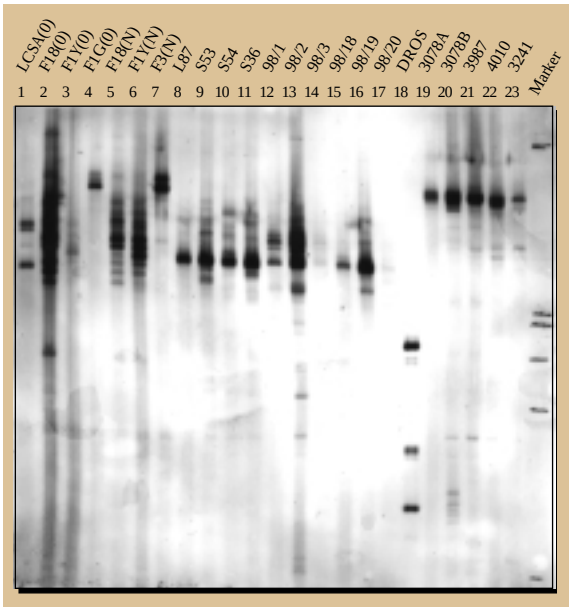


**Figure 1** IGS fingerprinting of *Myzus* clones. Clones of *M. antirrhinii* from France (lanes 4,7) and England (lanes 19-23) have one or a few large bands, whereas clones of *M. persicae* have smaller band patterns that can be distinguished by number, position and intensity. LCSA (lane 1) shows a pattern that is similar by these criteria to those found in MP1S (lane 8) and other clones of from Scotland (lanes 9-17) and France (lanes 2,3,5,6).

onomy of *M. persicae*. Two distinct populations have been designated as separate species. A dark green form, separated as *Myzus antirrhinii*, which we have detected in Scottish potato crops (*Ann. Rep. 1997/98*, 126), was clearly distinguished from *M. persicae* clones, using the IGS fingerprinting method. *M. antirrhinii* clones showed much larger bands than were seen in clones of *M. persicae* (Fig.1). However, clone LCSA, a red aphid representative of the recently named species *Myzus nicotianae*, could not be distinguished from *M. persicae* by this method as it exhibited polymorphic bands of the same type as those found in *M. persicae*. To assess variation in transmission efficiency, we compared the standard *M. persicae* clone (Mp1S), which has been used for many years at SCRI, with other *M. persicae* clones, and several clones of *M. antirrhinii*, and with LCSA.

Aphids need to feed for several hours to acquire PLRV from infected plants, and the amount of virus

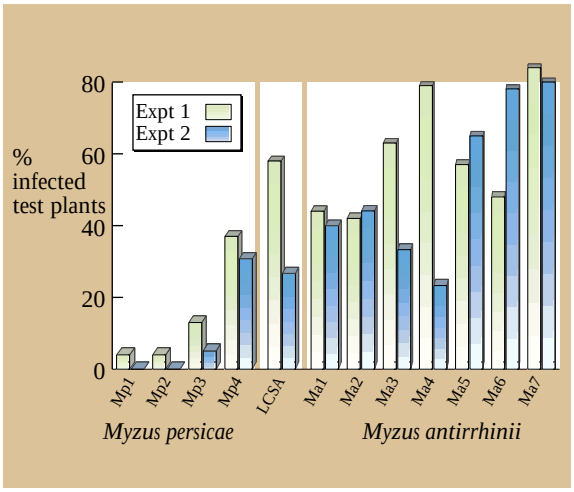


**Figure 2** Aphid clonal variation in transmission efficiency after a 24h Acquisition Access Period (AAP) on *Physalis floridana* infected with PLRV-C.

acquired, and probability of transmission, increases with the duration of the acquisition feed. Long 'acquisition access periods' (AAP) on infected plants, and 'inoculation access periods' (IAP) on test plants are useful to find out if an aphid species can transmit. In previous studies with other species, we gave aphids AAP and IAP of 3-6 days, but we used shorter AAP to demonstrate variations in vector efficiency among *Myzus* clones. Fig. 2 shows the variation in the efficiency of 12 aphid clones in transmitting PLRV-C in two series of experiments, 9 months apart. Aphids were given a 24h AAP on excised leaves of PLRV-infected *Physalis floridana*, and then transferred, in groups of three, for an IAP of 3-5 days. There were consistent differences between clones. LCSA, and all of the *M. antirrhinii* clones, transmitted PLRV-C to 40% or more test plants, but only one of the *M. persicae*

| Clone | % infected test plants | |
|---|---|---|
| | PLRV-V | PLRV-C |
| Mp1 | 8 | 53 |
| LCSA | 43 | 95 |

**Figure 3** Clone LCSA transmits both PLRV-C and the 'poorly aphid-transmitted' isolate, PLRV-V, more efficiently than does Mp1S.

| PLRV isolate | % transmission | |
|---|---|---|
| | Mp1S | LCSA |
| C 80 µg/ml | 30 | 81 |
| C 20 µg/ml | 17 | 32 |
| V 80 µg/ml | 30 | 75 |
| V 20 µg/ml | 0 | 43 |

**Figure 4** Clone LCSA transmits purified PLRV more efficiently than Mp1S in membrane feeding experiments, but the difference in transmissibility between the two PLRV isolates is detected only when virus is presented at lower concentration.

clones approached this efficiency. Transmission rates increased when the aphids were given a 48h AAP, but similar clonal differences were found. The poor efficiency of clone Mp1S in these tests was surprising because these aphids had been efficient vectors of PLRV-C in earlier experiments. If Mp1S had lost its ability to transmit PLRV efficiently, it would no longer be possible to discriminate between HAT and PAT isolates of PLRV with this clone.

In earlier tests when aphids had been given an AAP of 6 days on infected potato plants, Mp1S transmitted PLRV-V much less efficiently than PLRV-C. Repeating these tests more recently with Mp1S and LCSA, we still found that PLRV-V was transmitted less efficiently, but that transmission also depended on the aphid clone. LCSA transmitted both isolates more efficiently than did Mp1S, causing infection with PLRV-C in almost twice as many test plants, and five times as many with PLRV-V (Fig. 3). Moreover, tests in France showed that other aphid clones could also transmit PLRV-V more efficiently than did Mp1S, although never as efficiently as they transmitted HAT isolates. LCSA was also an efficient vector of the French isolate, 14.2, which could not be transmitted, or only poorly transmitted, by several clones of *M. persicae*, including Mp1S. These effects are not thought to result from lower concentrations of virus in plants infected with PAT isolates because their virus contents in ELISA tests were similar, but they could depend on differences in aphid feeding behaviour, or differences in the availability of the different isolates at aphid feeding sites. Indeed, differences in aphid feeding behaviour might well account for the large variations in the rates at which different clones transmitted PLRV-C (Fig. 2).

To examine these differences between virus isolate and aphid clone in more detail, and eliminate variations in virus content in leaves, aphids were fed purified virus through stretched Parafilm-M® membranes. In these tests, Mp1S and LCSA were confined for a 24h AAP on sachets of 20% sucrose containing PLRV-C or PLRV-V at two concentrations. Again, LCSA was a more efficient vector than Mp1S for both isolates, but transmission rates depended on virus concentration. It was only possible to distinguish the transmissibility of the isolates when they were presented at lower concentrations (Fig. 4). When aphids were fed through membranes on PLRV at 80mg/ml, both isolates were transmitted efficiently by LCSA, and moderately efficiently by Mp1S, but at

20mg/ml, Mp1S transmitted PLRV-C, but not PLRV-V, whereas LCSA transmitted both isolates equally efficiently.

These results show that the ability of aphid vectors to transmit PLRV depends not only on the structure of the virus coat protein, but also on biological factors contributed by the aphids themselves. There were large interspecific differences between aphids in vector competency for PLRV-C when sensitive assays were used. The tested clones of *M. antirhinii* came from geographically widespread sites, and comprised two distinct karyotypes, but all of them were more efficient vectors than was Mp1S. There were also intraspecific differences in transmission efficiency between the clones of *M. persicae.*

It is premature to conclude that qualitative or quantitative differences between recognition sites for PLRV could account for these differences in transmission rates. The feeding behaviour of the clones may differ, even when they are exposed to virus in sucrose diets. Current experiments are being made to compare feeding rates of efficient and inefficient vector clones during AAP on virus preparations. Mp1S is unable to reproduce sexually, and has been kept in parthenogenetic culture at SCRI since 1977. Interestingly, the two least efficient French clones of *M. persicae* had also been cultured at Le Rheu for many years. Although this may indicate some long term effect of laboratory culture on transmission efficiency, recent evidence from Scotland suggests that the low vector competency of the Mp1S genotype is not confined to the laboratory culture. In 1996, we found that the IGS pattern characterising Mp1S was found in some 30% of *M. persicae* samples from eastern Scotland. In 1998, almost all clones of *M. persicae* that were derived from migrant alatae colonising potatoes and brassicas at Invergowrie gave an identical IGS fingerprint to that of Mp1S (Fig. 1). The vector competency of three of these 1998 clones that have been tested is no greater than that of the standard Mp1S. If this result proves to be typical for other samples of the Mp1S genotype, it could have important implications for the epidemiology of PLRV in Scotland. Aphids with this IGS fingerprint have been found in Scottish samples over several years and, at present, it appears to be the predominant clone. Amongst UK populations of *M. persicae*, Mp1S is unusual in being susceptible to insecticides. Careful monitoring should ensure the early detection of any changes in the clonal composition of *M. persicae* or closely related species on seed potato crops that could herald the arrival of more efficient vectors and increase the risk of spread of PLRV.

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