VIRUS-VECTOR RELATIONSHIP BETWEEN POTATO LEAFROLL
VIRUS  PLRV AND MYZUS PERSICAE SULZER

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Abstract


Experiments were carried out to determine the periods of acquisition, latent period, inoculation and retention of Potato leaf roll virus when transmitted by the green peach aphid. Trials were set up with the indicators Physalis floridana L. and Datura stramonium L., and the method DAS-ELISA was used to determine the latent carriers of the virus. It was established that after acquisition of PLRV, M. persicae remains viruliferous for its lifetime. The optimal period for acquisition of PLRV by the green peach aphid is determined to be 6 hours, and the minimal is 30 minutes. The latent period of the virus in the vector is 6 hours, whereas the optimal period for inoculation of PLRV by the green peach aphid is 120 minutes and the minimal is 30 minutes. The statistical analysis of the obtained results includes one-way analysis of variance (ANOVA) and Student-Newman-Keuls test for multiple comparisons (SNK).

Key words: PLRV, Myzus persicae, acquisition, latent period, inoculation, retention

Introduction

Potato leafroll virus is a member of the family of Luteoviridae and is a typical representative of genus Pollerovirus. It is distributed in all potato producing regions of the world. PLRV spreads mainly by means of infected tubers and by aphid vectors (Robert, 2000). Transmission does not occur by mechanical inoculation, seeds or pollen.

Vectors transmit PLRV in a persistent manner (Kennedy et al., 1962), whereas the mechanism of transmission includes the following four stages: acquisition of virus particles in the stylet; absorption of virus particles in the chaemolymph; latent period; inoculation of the virus (Radcliffe et al., 1993; Ragsdale et al., 1994; Gildow, 1999).

Research in Bulgaria is mainly directed towards the development of methods for diagnostics of the virus in leaf material, tubers and vectors (Atanasov, 1930; Krastev, 1950; Bajlova-Jankulova, 1961; Janculova et al., 1983; Kotzampigikis and Hristova, 2006). Investigations were performed on the influence of different agrotechnical procedures on potato seed fields (Matacov et al., 1986a; b). Studies focused on the relationship virus-vector-host have not been carried out yet.
The aim of the present study is to investigate the virus-vector-host relationship with an accent on the duration of the periods for acquisition, latent period, inoculation and retention of Potato leafroll virus when transmitted by the green peach aphid.

Material and Methods

Green peach aphids from a virus-free laboratory population were used in the experiments to determine the relationship between Potato leafroll virus and its vector *M. persicae*.

Wingless forms of a standardized population of the green peach aphid (Ilieva, 2003) were placed on tobacco leaves on moist filter paper in glass Petri dishes. Newborn larvae were separated and the virus free population of *M. persicae* was then reared under controlled laboratory conditions: constant photoperiod (16/8 h), temperature 24°C, relative humidity of 75-85% and host *Nicotiana tabacum* L. cv. „Nevrokop 1146”.

Potato tubers with PLRV infection, confirmed by DAS-ELISA, were provided by the Station of Seed Production and Maintainance of Varieties near Plovdiv. Subsequently, indicator plants were inoculated with PLRV and used as sources of infection in the course of the experiments. *Ph. floridana* and *D. stramonium* plants grown under controlled laboratory conditions (constant photoperiod 16/8 h, temperature 24°C and relative humidity of 75 85%) were used as indicator plants.

Transmission of PLRV depending on the number of aphids per plant

For the needs of the experiments to determine virus transmission depending on the number of aphids per plant, wingless adult females were left to feed on the source of infection for 6 hours. Subsequently, different numbers of viruliferous insects were moved to healthy test plants following the pattern of 1, 3, 5, 10 and 20 viruliferous aphids per test plant. Each variant was set in 3 repetitions with 10 test plants per repetition.

Acquisition of PLRV

In order to determine the time for acquisition of the virus, adult wingless forms of the laboratory population of the green peach aphid were collected with a brush, distributed on infected plants (*Ph. floridana*) and left to feed on them for different periods of time: 20 minutes, 1, 2, 4, 6, 12 and 24 hours, respectively. Each variant was set in 3 repetitions with 10 test plants per repetition. The insects were then moved on healthy *Ph. floridana* and *D. stramonium* test-plants and left to feed on them for 48 hours. At the end of the experiment, the aphids were mechanically destroyed.

Latent period of PLRV in the vector

Wingless adult forms from a laboratory population of *M. persicae* were left to feed on a source of infection for 6 hours. After the period for acquisition of the virus, the insects were moved in batches of 5 to healthy indicator plants and left to feed on them for 4, 6, 12, 18, 24, 30 and 36 hours respectively. Each variant of feeding time on healthy plants was set in 3 repetitions with 10 plants per repetition.

PLRV inoculation

In order to determine the inoculation period of the virus, aphids were left on infected plants for 30 hours. Subsequently, the insects were moved to healthy test plants and left on them for 30, 60, 120, 180 and 360 minutes, respectively. Each variant was set in 3 repetitions with 10 plants per repetition.

Persistence of PLRV in the vector

In order to determine the retention period of PLRV in the vector, i.e. the retention of its ability to infect new plants, individuals from the laboratory population of the green peach aphid were left to feed for 6 hours on a source of infection. After the period for viral acquisition, the aphids were repeatedly moved for intervals of 3, 6, 12, 24 and 48 hours to new healthy indicator plants of the respective species. Five wingless female forms were distributed on each test plant. Negative control was set up with 10 indicator plants on which non-infected aphids have been fed.
The indicators were monitored for development of symptoms for 21 days. After this period, the plants with visible symptoms were recorded and asymptomatic plants were analysed using DAS-ELISA (Clark and Adams, 1977) with kits of LOEWE Biochemica GmbH Sauerlach, Germany. Positive control was set up with plants manifesting infection. In the cases when infection was established after immunological testing of asymptomatic plants, the number of these plants was added to the number of visibly infected ones.

**Statistical analysis**

The experimental data were analyzed using one-way analysis of variance (ANOVA) and Student-Newman-Keuls test for multiple comparisons (SNK).

**Results and Discussion**

Transmission of PLRV depending on the number of aphids per plant

The results of the experiment showed that the number of vectors is a significant factor for PLRV transmission to the indicator plants *Ph. floridana* (F = 38.4; p<0.001) and *D. stramonium* (F = 38.6; p<0.001). The increase in number of viruliferous aphids per test-plant led to an increase in the number of infected indicators. The greatest numbers of infected plants resulted from feeding of 5, 10 and 20 aphids per plant. For the variants of 1 and 3 vectors per plant for both indicators, it was established that the number of latent carriers determined by DAS-ELISA was greater than the number of plants showing visible symptoms. In the case of *Ph. floridana*, 5 out of 30 tested plants were latent carriers of infection, and for *D. stramonium* 4 out of 30. For the variants of 1 and 3 viruliferous aphids per plant, the number of symptomatic *Ph. floridana* plants was greater, compared to *D. stramonium*. The comparative analysis of the different variants for both indicators (Table 1) did no show statistically significant differences in the cases of 5, 10 and 20 aphids per plant. Therefore, 5 viruliferous aphids are able to infect the maximum number of plants.

As it is obvious from Table 1, there are differences in the sensitivity of the two indicators. For *D. stramonium*, the difference in the numbers of infected plants for the variants of one and three vectors per plant are significant at level of significance $\alpha=0.001$. For *Ph. floridana*, the difference between these two variants is not significant. It was also established that the number of *Ph. floridana* plants showing visible symptoms of PLRV infection was greater than that for *D. stramonium*, which is a piece of evidence that *Ph. floridana* is more sensitive to the virus.

The experiment showed that a single aphid is able to transmit PLRV to a healthy plant. This result confirms the data of Curpetino (1995) who reports that after a single aphid acquires the virus from a potato

<table>
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</tr>
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</tbody>
</table>

* significant differences at a level of significance $\alpha=0.05$

** significant differences at a level of significance $\alpha=0.01$

*** significant differences at a level of significance $\alpha=0.001$
plant, it is able to transmit it to a healthy *Ph. floridana* test plant.

**Acquisition of PLRV**

The local reaction of the indicator *D. stramonium* to PLRV developed 8 days after removal of the aphids from the infected plants. It was characterized by local chlorotic spots and yellowing. The greatest percentage of infected test plants was observed for the variants in which aphids had been feeding on the sources of infection for 6, 12 and 24 hours. Systemic symptoms usually developed 12 days after removal of the vectors. These were initially manifested with interveinal yellowing and later with development of interveinal necrosis and rolling and cupping of leaves.

The results from the observations of *Ph. floridana* showed that the percentage of infected plants was greatest when vectors were left to feed on the plants for 4, 6, 12, and 24 hours. Infected plants exhibited local chlorotic spots and later (15 days after removal of the aphids) developed systemic vein necrosis and epinasty, accompanied by leaf deformation and stunted growth.

The DAS-ELISA results showed that after an acquisition period of 30 minutes, two *Ph. floridana* plants and one *D. stramonium* plant had become latent carriers of infection. The virus was also detected in 1 plant of each species after a PLRV acquisition period of 1 hour.

The results from ANOVA showed that there are

### Table 2

Multiple Comparisons (SNK) between variants with different time for PLRV acquisition by green peach aphids with indicators *Ph. floridana* and *D. stramonium*

<table>
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<td>0.33</td>
<td>-</td>
</tr>
</tbody>
</table>

* significant differences at a level of significance $\alpha = 0.05$

** significant differences at a level of significance $\alpha = 0.01$

*** significant differences at a level of significance $\alpha = 0.001$

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![Fig. 1](image1.png)

**Fig. 1.** Relation between the number of infected *Ph. floridana* plants and the duration of the period of acquisition of PLRV

![Fig. 2](image2.png)

**Fig. 2.** Relation between the number of infected *D. stramonium* plants and the duration of the period of acquisition of PLRV
significant differences in the average number of infected plants per variant - for Ph. floridana $F = 79.3$ at $p<0.001$ and for D. stramonium $F = 126.8$ at $p<0.001$. The results from the Student-Newman-Keuls test for significance of the differences between

The data presented on Figures 1 and 2 show that feeding of aphids on the infection source for periods of 30 min, 1 hour and 2 hours results in a lower transmission rate of the virus. With increase of the feeding period on the infection source, the percentage of infected test plants is also increased.

Comparative analysis of data obtained for the two indicators did not show significant differences between variants with times for acquisition of 6, 12 and 24 hours respectively. Therefore, feeding of aphids on the infection source longer than 6 hours does not influence PLRV acquisition. For the following trials, the period of 6 hours was accepted as optimal for PLRV acquisition.

The acquisition of the virus is a complex process which depends on the type of infection that PLRV causes (chlorosis). In such infection, the virus is localized in the vascular system of the leaves and long time feeding of the vector is necessary in order for PLRV to reach the cells of the phloem. This process is also influenced by the source of infection, the vector, the viral isolate and the environmental conditions. Tamada and Harrison (1981) reported that PLRV is more efficiently transmitted by M. persicae when acquisition takes place at 30°C, rather than at 15°C. Robert (1971) established that separate geographic populations of the green peach aphid differ in their efficiency of PLRV transmission. According to Taliantsky et al. (2003), the minimal period for acquisition of the virus is 1 hour. The authors showed that longer periods of PLRV acquisition lead to more efficient transmission of the virus.

The DAS-ELISA method used in the experiment allowed for precise detection of latent carriers of infection. PLRV was detected in two Ph. floridana plants and one D. stramonium plant in the variant with minimal 30-minute feeding time of the vector on the infection source. This could be attributed to the high virulence of the isolate used in the experiment, as well as to the greater sensitivity of Ph. floridana, compared to D. stramonium.

In conclusion it can be stated that the optimal time for PLRV acquisition is 6 hours and the minimal is 30 min.

**Latent period of PLRV in the vector**

The reaction of D. stramonium to PLRV infection was manifested with local chlorotic spots visible 9 days after inoculation which gradually increased in size. The highest number of symptomatic plants was observed after viruliferous aphids fed on healthy indicator plants for periods of 24, 30 and 36 hours. Systemic symptoms usually appeared 14 days after the vectors were removed.

Ph. floridana developed local chlorotic spots 8 days after removal of the viruliferous aphids. More pronounced symptoms were observed when aphids were left to feed for periods of 24, 30 and 36 hours. Systemic symptoms appeared 17 days after removal of the vectors and were manifested by vein necrosis, epinasty, slight rolling and cupping of leaves and stunted growth.

No plants were infected in the variant with feeding period of 4 hours, which was confirmed by DAS-ELISA tests. Therefore, the latent period is longer than 4 hours. With aphid feeding periods of 6 and 12 hours, a total of 7 Ph. floridana plants and 5 D. stramonium plants, which were asymptomatic, tested positive for PLRV in the subsequent DAS-ELISA tests. The lowest number of infected plants was observed after feeding of 6 hours. This time was therefore defined as the minimal duration of the latent period of the virus.

The results of ANOVA showed significant differences in the number of infected plants at different periods of feeding of the aphids for Ph. floridana $F = 137.7; p<0.001$ and for D. stramonium $F = 123.4; p<0.001$. Table 3 presents the results from the Student-Newman-Keuls test for duration of the latent period. The two indicators have identical reactions. This is probably due to the fact that the latent period reflects the virus-vector relationship, while the peri-
ods of acquisition and transmission characterize the virus-vector-host relationship.

The statistical analysis showed no significant difference between feeding periods of 6 and 12 hours. For these variants, the number of infected plants was very low (Figures 3 and 4).

In their studies Sugawara et al. (1974) established that the latent period of PLRV is 12 hours, but this conclusion was based only on observation of visible symptoms on indicator plants. Garret et al. (1996) reported that the duration of the latent period is 8 hours. For their trials, the authors used a field isolated of PLRV designated as PLRV L18 and a French population of the green peach aphid. Our experiment showed that the latent period is 6 hours.

**PLRV inoculation**

Chlorosis was observed on the fifth day after feeding of the aphids on the indicator *Ph. floridana* plants. The greatest percentage of infected plants was recorded after feeding periods of 120, 180 and 360 minutes. During subsequent observations it was established that the apical leaves of infected test-plants were undersized and with shorter internodes. On the 13th day the plants were severely stunted and the older leaves were rolled and cupped. Later, systemic ne-
crosis and epinasty developed.

*D. stramonium* developed local symptoms later than *Ph. floridana*. Eight days after removal of the aphids, chlorotic spots and yellowing of the leaves were observed. Again, the number of plants symptomatic for PLRV infection was greatest when time for inoculation was in the interval from 120 to 360 minutes. Yellowing was observed 15 days after removal of the vectors, followed by interveinal necrosis and rolling and cupping of leaves.

The results from the serological analysis showed that after a 30-minute period of inoculation, one plant of each indicator species was a latent carrier of the disease. With 60-minute inoculation time, the virus was confirmed in 3 *Ph. floridana* plants and 4 *D. stramonium* plants.

The results from ANOVA showed that the duration of the period for viral inoculation has a significant influence on the number of infected plants - *F* = 114.8; *p* < 0.001 (for *Ph. floridana*) and *F* = 210.9; *p* < 0.001 (for *D. stramonium*). The results from the Student-Newman-Keuls test for multiple comparisons are presented on Table 4. Significant differences were established between variants with periods of inoculation of 30 and 60 min and the rest of the variants. These differences were established at the strictest level of significance (*α* = 0.001), which is evidence for the strong influence of the duration of this period on PLRV transmission. The statistical analysis of data showed that the differences between the groups with 120, 180 and 360 minute periods for inoculation of PLRV are not significant for the indicator *Ph. floridana* and are

<table>
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<th>180</th>
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<td>-</td>
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<td>-</td>
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<td>4.67 ***</td>
<td>1</td>
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<td>-</td>
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</tbody>
</table>

* significant differences at a level of significance *α* = 0.05
** significant differences at a level of significance *α* = 0.01
*** significant differences at a level of significance *α* = 0.001

Fig. 5. Relation between the number of infected *Ph. floridana* plants and the period of inoculation of PLRV

Fig. 6. Relation between the number of infected *D. stramonium* plants and the period of inoculation of PLRV
significant at a level of \( \alpha = 0.05 \) for *D. stramonium*.

The graphic representation of the results (Figures 5 and 6) shows that with periods of inoculation of 120, 180 and 360 min, there are a high numbers of infected plants for both indicators.

The results of the experiment showed that at the minimal time for inoculation of 30 min, there are infected indicator plants. Differences were established in the sensitivity of the two indicators: *Ph. floridana* was more sensitive to PLRV, compared to *D. stramonium*.

Leonard and Holbrook (1978) established that several hours of feeding are necessary for maximum efficiency of PLRV inoculation without specifying the exact period. Fereres et al. (1999) reported that the ability of vectors to infect healthy plants increases with the time of acquisition of the virus. The longer period necessary for successful PLRV inoculation is explained by the persistent manner of transmission of the virus, where prolonged feeding of vectors on the healthy plant is needed for infection (Hull, 2002). Therefore, there is enough evidence to define the optimal period for PLRV inoculation by the vector as 120 minutes and the minimal as 30 min.

**Retention of PLRV in the vector**

*Ph. floridana* reacted with chlorosis 8 days after the feeding of aphids on the indicators and symptoms were first visible in the variants where vectors were moved to the plants 12, 24 and 48 hours after feeding on the infection source. During the subsequent monitoring, severely stunted growth and undersized apical

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**Table 5**

Multiple comparisons (SNK) between the mean values of the separate variants for time of retention of PLRV in aphids with indicators *Ph. floridana* and *D. stramonium*

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</table>

* significant differences at a level of significance \( \alpha = 0.05 \)
** significant differences at a level of significance \( \alpha = 0.01 \)
*** significant differences at a level of significance \( \alpha = 0.001 \)

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![Fig. 7. Relation between the number of infected *Ph. floridana* plants and the period of PLRV retention in the vector](image)

![Fig. 8. Relation between the number of infected *D. stramonium* plants and the period of retention of PLRV in the vector](image)
leaves were observed. Later, the older leaves started rolling and cupping, and manifested systemic necrosis and epinasty.

*D. stramonium* reacted with local chlorotic spots one week after the infestation. The number of infected plants was higher when aphids were moved to the indicators at intervals of 24 and 48 hours. The symptoms gradually became more pronounced and on the 14th day after the feeding, systemic infection was observed. The peripheral leaf blade was slightly rolled inwards.

The immunological test showed that when aphids were moved at interval of 3 hours, 2 *Ph. floridana* and 3 *D. stramonium* plants had become latent carriers of infection.

The results from ANOVA showed significant differences between the separate variants: $F = 72.1; p<0.001$ for *Ph. floridana* and $F = 56.1; p<0.001$ for *D. stramonium*. The data presented in Table 5 show significant differences between the variants with intervals of moving the vectors of 3, 6 and 12 hours. At intervals of 3 and 6 hours, the number of infected plants was lower, and the first plants to which the vectors were moved remained uninfected. The SNK method determined the differences between intervals of 24 and 48 hours as statistically not significant. On the other hand, the number of infected plants for these variants was high. Figures 7 and 8 represent the results of the experiment for retention of the ability of the vector to transmit PLRV to the two indicators.

After moving viruliferous aphids to new plants at intervals of 48 hours, it was established that they are able to infect healthy plants for a period of 20 days, which is equal to their lifetime.

In conclusion, the vector retains its ability to infect healthy plants for its lifetime. This confirms the results of Tamada and Harrison (1981).

**Conclusions**

The results from the present study lead to the following conclusions:

- The optimal time for acquisition of the persistent PLRV by the green peach aphid is 6 hours and the minimal is 3 hours. The latent period of the virus in the vector is 6 hours.
- The optimal time for successful PLRV inoculation by the green peach aphid is 120 min and the minimal is 30 min.
- After PLRV acquisition, *M. persicae* retains its ability to infect new plants for its lifetime.

**Acknowledgements**

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**References**


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