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# VIRUS-VECTOR RELATIONSHIP BETWEEN POTATO VIRUS Y – PVY AND *MYZUS PERSICAE* SULZER

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## Abstract

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Experiments were carried out to determine the periods of acquisition, latent period, inoculation and retention of Potato virus Y when transmitted by the green peach aphid. Trials were set up with indicators *Physalis floridana* L. and *Niccotiana tabacum* L., and the method DAS-ELISA was used to detect the virus in test plants. It was established that a single aphid is able to infect a healthy plant with PVY. The results showed that the optimal period for acquisition and inoculation of PVY by the aphids is 30 seconds and the minimal is 1 second. The period of retention of PVY by *Myzus persicae* Sulzer varies from 2 to 4 hours, depending on the number of healthy plants visited and the duration of feeding on them. With increase in the number of plants that vectors feed on, the retention period decreases i.e. aphids lose their ability to infect new plants more rapidly. The results were statistically processed using one-way analysis of variance (ANOVA) and Student-Newman-Keuls test for multiple comparisons (SNK).

*Key words*: PVY, *Myzus persicae*, acquisition, inoculation, retention

## Introduction

Potato virus Y (PVY) is a type representative of genus *Potyvirus* (family *Potyviridae*). Its host range comprises more than 60 plant species, among which are the representatives of the families *Solanaceae, Chenopodiaceae* and *Leguminosae.*

The virus can be mechanically transmitted with plant sap, by contact with a healthy plant and with seeds. Infected tubers and aphids have the greatest significance for the transmission of the virus in nature. PVY is transmitted by aphids in a non-persistent manner, with *M. persicae* being its most important vector (Kennedy et al., 1962; Sigvald, 1984; Katis и Gibson, 1985; Romancer et al., 1994).

The mechanism of PVY transmission is not fully understood. The hypothesis of "ingestion-salivation" proposed by Martin et al. (1997) is generally accepted. According to it, the virions attach to the frontal parts of the digestive tract (the foregut) and transmission occurs only for viral particles in proximity to the tip of the stylet on its inner surface, where the food canal

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and the salivary canal become confluent. The hypothesis states that viral transmission occurs at the time of salivation.

PVY is a non-persistent virus and it remains in the stylet of the insect for a short period of time. Immediately after acquisition of the virus, the vector is able to infect a healthy plant through several seconds of probing. It has been established for more than half of the aphids that the acquired ability to infect new plants is lost 30 minutes after acquisition. In other cases the vectors may remain infective for several hours (Nault, 1997).

In Bulgaria, studies have been carried out mainly for the causal agent of epsilon virosis on potatos – PVY (Atanasov, 1930; Kovachevski, 1945; Krustev, 1950; Brajkova et al., 1981; Krachanova et al., 1978; Jankulova et al., 1983).

**The aim** of the present study is to investigate the virus-vector-host relationships between PVY, the green peach aphid and the plant hosts, with an accent on the durations of the periods of acquisition, inoculation and retention of the virus by *M. persicae.*

## Material and Methods

In the trials investigation the relationship between the causal agent of epsilon virosis on potato and the vector *M. persicae*, aphids from a virus free standardized laboratory population were used. Initially, potato plants grown from tubers with $PVY^n$ infection confirmed by DAS-ELISA were used as sources of infection. Subsequently, indicator plants were infected and used as sources of infection during the experiments. The test-plants were *Ph. floridana* and *N. tabacum* cv. Nevrokop 1146 at the 3-4 leaf stage, grown under laboratory conditions: photoperiod 16/8h, temperature 24°C, relative humidity 75-85%.

### *Transmission of PVY depending on the number of aphids per plant*

For the trials for dependence of viral transmission on the number of aphids per plant, wingless females were left to feed on the infection source for 30 seconds, which is the experimentally confirmed optimal acquisition period. Subsequently 1, 3, 5, 10 and 20 virulipherous aphids respectively were moved on healthy test plants. The separate variants were set up in 3 repetitions with 10 plants per repetition.

### *Period for acquisition of PVY*

In order to determine the acquisition period from an infected plant, aphids were first left to starve in Petri dishes for 1 hour. Then they were moved to infected *Ph. floridatna* plants using a brush and left to feed for different periods of time. Ten variants of feeding time on *Ph. floridana* were tested: 1, 5, 10, 20, 30, 60, 120, 300, 600 and 900 seconds. The insects were then left to feed on healthy plants for 48 hours. At the end of the experiment the aphids were mechanically destroyed.

### *Period for inoculation of PVY*

In order to determine the period for inoculation of the virus, insects were left to feed for 30 seconds on the infection source (*Ph. floridana*) and acquire the virus. The insects were then moved to indicator plants and left on them for 1, 5, 10, 20, 30, 60, 120 and 300 seconds, respectively.

### *Period of retention of PVY in the vector*

In order to determine the period of retention of PVY 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 on an infected plant for 30 seconds. The viruliferous forms were then moved consecutively at intervals of 12, 30, 60, 120, 180 and 240 minutes on 10 healthy indicator plants for each interval.

The aphids were observed under stereomicroscope and the duration of feeding was recorded with a timer. The time reading started when the tip of the stylet of the vector assumed a perpendicular position to the leaf surface and made contact with the epidermis and the antennae assumed a position parallel to the body. The acquisition of the virus was interrupted by gently touching the vector's abdomen with a brush. The time reading stopped when the antennae of the aphid moved up and the stylet moved away from the leaf

surface.

Five wingless female forms were put on each test-plant. Ten indicator plants on which virus-free aphids had been feeding were used as a negative control group.

The indicators were observed for emergence of symptoms for 21 days. Then the number of plants with visible symptoms was recorded and asymptomatic plants were analyzed using DAS-ELISA (Clark and Adams, 1997) with kits of LOEWE *Biochemica GmbH Sauerlach*, Germany. Symptomatic plants were used as positive control group. The number of plants with confirmed latent infection was added to the number of symptomatic plants. The results were statistically analyzed using ANOVA and SNK test.

## Results and Discussion

### *Transmission of PVY depending on the number of aphids per plant*

The indicators on which 5, 10 and 20 aphids were located manifested local symptoms of infection 2 days earlier than the plants with 3 aphids. The plants reacted to infestation with 5, 10 and 20 aphids with local lesions. For the variant of one aphid, only one plant from *Ph. floridana* developed mosaic 10 days after removal of the vector.

The results from the serological DAS-ELISA test of *Ph. floridana* plants with no visible symptom for the variants with 1 and 3 aphids per plant showed that 1 and 2 plants, respectively, were latent carriers of the virus. For the same variants, 2 and 4 *N. tabacum* plants respectively were established as latent carriers of PVY. The results from ANOVA showed significant differences in the numbers of infected plants depending on the number of vectors: for *Ph. floridana* $F = 171.5$ at $p < 0.001$ and for *D. stramonium* – $F = 174.1$ at $p < 0.001$. The results from the multiple comparisons are presented on Table 1.

The SNK test established a significant difference in transmission of the virus between the variants of 5, 10 or 20 aphids per plant and the variants of 1 or 3 aphids per plant at a very strict level of significance $\sigma = 0.001$, where the probability of a Type I error is less than 0.001. No significant differences where established among the variants of viral transmission with 5, 10 or 20 aphids per plant.

The diagnosis of latent infection using DAS-ELISA for both indicators in the variant of one aphid per plant is of great importance; as such plants may serve as sources of infection in the field. It became clear that a single aphid is able to infect a healthy plant with PVY. Ram et al. (2005) reported that a single aphid is able to transmit Papaya ring spot potyvirus to a healthy plant. Katis et al. (2006) established that another member of genus Potyvirus (Zucchini yellow mosaic virus) can also be transmitted by a single green peach aphid.

**Table 1**

**Results from Multiple Comparisons (SNK) between variants with different numbers of PVY vectors per plant with indicators *Ph. floridana* and *N. tabacum***

| Number of aphids | | *N. tabacum* | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 10 | 20 |
| *Ph. floridana* | 1 | - | 5.67 *** | 8.67 *** | 9.00 *** | 9.33 *** |
| | 3 | 6.67 *** | - | 3.00 *** | 3.33 *** | 3.67 *** |
| | 5 | 8.67 *** | 2.00 *** | - | 0.33 | 0.67 |
| | 10 | 9.00 *** | 2.33 *** | 0.33 | - | 0.33 |
| | 20 | 9.33 *** | 2.67 *** | 0.67 | 0.33 | - |

* 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 $\alpha = 0.001$

***Acquisition period of PVY***

The symptoms observed on *Ph. floridana* were typical of the virus. Eight days after removal of the vector, the test plants reacted with local lesions for the variants of feeding of the aphids on the infection source for 5, 10, 20, 30 and 60 seconds. On the 11th day after infestation, higher percentage of diseased plants was observed due to the reaction of plants from the rest of the variants (120, 300, 600 and 900 seconds). At periods of acquisition ranging from 5 to 60 seconds, plants with strongly manifested symptoms of stunted growth, dwarfing and severe mosaic accompanied by yellow spotting were observed. Older leaves frequently exhibited bending of the petioles. At acquisition time of one second, 5 out of 30 test-plants developed symptoms of viral infection.

The first symptoms of PVY infection on *N. tabacum* were manifested as mosaic 9 days after the infestation. Subsequently, vascular discoloration and spotting of leaves were observed. The first plants to develop visible symptoms were from the variants with 5, 10, 20, 30, 60 and 120-second acquisition periods. The disease progressed relatively quickly and on the 18th day the leaves of the tobacco plants exhibited systemic vein necrosis, typical for infection with the $PVY^n$ strain. The observations also showed that at acquisition periods of 1 second, 2 test-plants out of 30 reacted with weak symptoms 12 days after the infestation and the rest did not develop any symptoms.

The results from the DAS-ELISA tests of plants with no visible symptoms showed that a total of 18 *N. tabacum* plants and 14 *Ph. floridana* plants have latent infection. The ANOVA detected significant differences between all variants of time for acquisition of PVY (for the indicator *Ph. floridana* F=41.2 at $p<0.001$, and for the indicator *N. tabacum* F=18.2 at $p<0.001$). The results from the SNK test are presented on Table 2.

The relation between the number of infected plants and the period of acquisition of the virus is presented on Figures 1 and 2.

The graphs show for both indicators that after 1-second feeding time for viral acquisition, the vector is able to infect healthy plants, even though in very low numbers. A trend of increase in the number of infected plants with the increase of the acquisition period to 30 seconds was established. The highest number of

**Table 2**

**Multiple Comparisons (SNK) between variants with different time for PVY acquisition by green peach aphids with indicators *Ph. floridana* and *N. tabacum***

| Variant, sec. | | *N. tabacum* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 5 | 10 | 20 | 30 | 60 | 120 | 300 | 600 | 900 |
| *Ph. floridana* | 1 | - | 3.33 ** | 4.33 *** | 5.33 *** | 5.67 *** | 3 ** | 1 | 0 | 1 | 1.33 |
| | 5 | 2.33 *** | - | 1 | 2 | 2.33 | 0.33 | 2.33 * | 3.33 ** | 4.33 *** | 4.67 *** |
| | 10 | 3.33 *** | 1 | - | 1 | 1.33 | 1.33 | 3.33 ** | 4.33 *** | 5.33*** | 5.67 *** |
| | 20 | 4 *** | 1.67 ** | 0.67 | - | 0.33 | 2.33 | 4.33 *** | 5.33 *** | 6.33 *** | 6.67 *** |
| | 30 | 4.67 *** | 2.33 *** | 1.33* | 0.67 | - | 2.67 * | 4.67 *** | 5.67 *** | 6.67 *** | 7 *** |
| | 60 | 2.33 *** | 0 | 1 | 1.67 ** | 2.33 ** | - | 2 * | 3 * | 4 ** | 4.333 *** |
| | 120 | 0.67 | 1.67 ** | 2.67 *** | 3.33 *** | 4 *** | 1.67 * | - | 1 | 2 | 2.333 |
| | 300 | 0.33 | 2.67 *** | 3.67 *** | 4.33 *** | 5 *** | 2.67 *** | 1 | - | 1 | 1.333 |
| | 600 | 1.33 * | 3.67 *** | 4.67 *** | 5.33 *** | 6 *** | 3.67 *** | 2 * | 1 | - | 0.333 |
| | 900 | 1.67 * | 4 *** | 5 *** | 5.67 *** | 6.33 *** | 4 *** | 2.33 ** | 1.333 * | 0.333 | - |

* 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 $\alpha = 0.001$

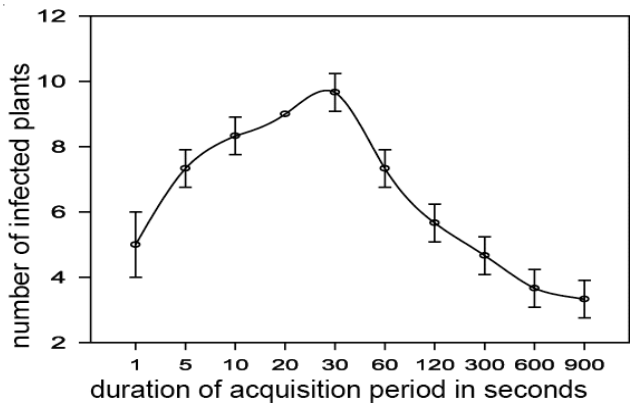


**Fig. 1. Relation between the number of infected *Ph. floridana* plants and the duration of the period of acquisition of PVY**

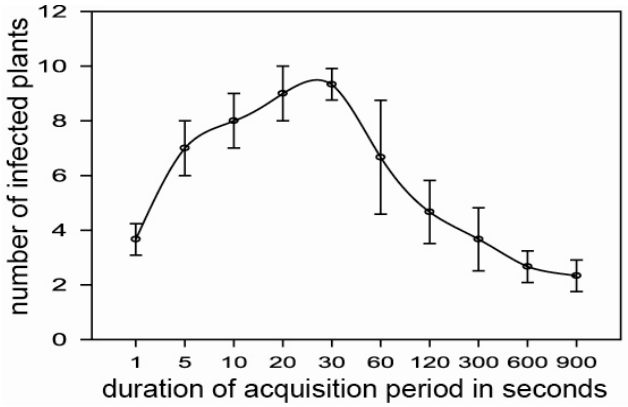


**Fig. 2. Relation between the number of infected *N. tabacum* plants and the duration of the period of acquisition of PVY**

infected plants was recorded after acquisition periods of 20 and 30 seconds. No significant differences were found for these variants.

Acquisition longer than 30 seconds led to rapid decrease in the vector ability of aphids because the virus lost its infectivity under the inactivating effect of the saliva secreted by the aphids. In the following experiments, the period of 30 seconds is regarded as the optimal time for acquisition of PVY by the aphids.

The results from the trials show that with period of acquisition of 1 second, the virus is ingested by the aphids and they are able to immediately infect new healthy plants. The short PVY acquisition period is explained by the character of infection induced – mosaic. When such type of symptoms is present, the virus is localized in the leaf's epidermal cells and the stylet quickly and easily gets in contact with PVY during sampling probes. The vectors acquire the virus from infected cells for a short period of time (Hull, 2002).

The short acquisition period may be attributed to the higher virulence of the necrotic PVY isolate, used in the experiments, and also to the greater sensitivity of indicator plants. De Bokx and Huttinga (1981) reported that the period for acquisition of PVY ranges from 15 to 60 seconds. In trials with the indicator plant *N. tabacum* cv. "White Burley", Gibson et al. (1988) established, that the optimal time for PVY acquisition is 25 seconds. Manoussopoulos (2001) reports that PVY is acquired from an infected plant by *M. persicae* within seconds and is inoculated in a new plant through short sampling probes for a period of 8 seconds. He also defined the first probe of the stylet in the plant surface as the start of acquisition and used a timer to measure the different periods. However, he only took into account visible symptoms on indicator plants when recording the results. For his experiments the author used the $PVY^0$ strain which causes mosaic disease. The isolate used in our experiments is necrotic and more virulent than $PVY^0$. The author also used a different indicator – *N. benthamiana* L. and made conclusions only on the basis of assessment of visible symptoms of the indicator. All of the above mentioned authors did not use the ELISA method to confirm latent infection in plants. The use of sensitive methods such as DAS-ELISA which can give positive results for viral concentration in the range of 1 – 10 ng/ml is a contribution of the present study[1] .

Based on our results, it can be concluded that the optimal period for PVY acquisition by the green peach aphids is 30 seconds, and the minimal – 1 second.

***PVY inoculation period***

In the variants with aphid feeding times of 30, 60, 120 and 300 seconds on *Ph. floridana*, local symptoms were visible after 8 days. The leaves of the indicators manifested local lesions. The number of infected plants for these variants was greater than for the rest

**Table 3**

**Multiple Comparisons (SNK) between variants with different time of inoculation of PVY for green peach aphids with indicators *Ph. floridana* and *N. tabacum***

| Variant, sec. | | *N. tabacum* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 5 | 10 | 20 | 30 | 60 | 120 | 300 |
| *Ph. floridana* | 1 | - | 2 * | 3.33 *** | 5 *** | 7.67 *** | 8.67 *** | 8.67 *** | 9 *** |
| | 5 | 2.67 *** | - | 1.33 | 3 ** | 5.67 *** | 6.67 *** | 6.67 *** | 7 *** |
| | 10 | 4.33 *** | 1.67 ** | - | 1.67 * | 4.33 *** | 5.33 *** | 5.33 *** | 5.67 *** |
| | 20 | 5 *** | 2.33 ** | 0.67 | - | 2.67 ** | 3.67 *** | 3.67 *** | 4 *** |
| | 30 | 8.33 *** | 5.67 *** | 4 *** | 3.33 *** | - | 1 | 1 | 1.33 |
| | 60 | 8.67 *** | 6 *** | 4.33 *** | 3.67 *** | 0.33 | - | 0 | 0.33 |
| | 120 | 9 *** | 6.33 *** | 4.67 *** | 4 *** | 0.67 | 0.33 | - | 0.33 |
| | 300 | 9.33 *** | 6.67 *** | 5 *** | 4.33 *** | 1 | 0.67 | 0.33 | - |

* 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 $\alpha = 0.001$

of the variants. Systemic symptoms developed two weeks after removal of the vectors. Initially, mosaic spots were observed along secondary veins. Then yellowing that gradually encompassed a significant part of the leaf surface appeared.

For *N. tabacum*, we initially observed mosaic on the leaves where aphids were put, followed by vascular discoloration. The highest number of infected plants was established for aphid feeding periods ranging from 30 to 300 seconds. Systemic symptoms such as veinal necrosis developed 8 days after the initial ones. This systemic reaction progressed with time and led to withering of leaves.

The DAS-ELISA results showed that with feeding time of 1 and 10 seconds respectively, one plant from both indicators had become a latent carrier of infection. In the variant of 5 seconds feedin time, the virus was detected in two *Ph. floridana* and three *N. tabacum* plants.

The performed ANOVA confirmd that the duration of the inoculation period has a statistically significant effect on the number of infected plants – for *Ph.*

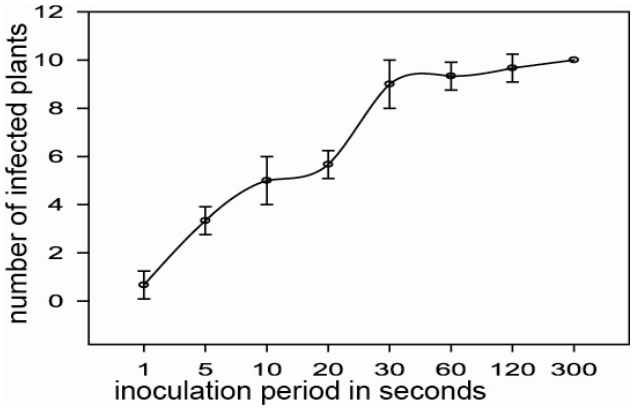


**Fig. 3. Relation between the number of infected *Ph. floridana* plants and the period of inoculation of PVY**

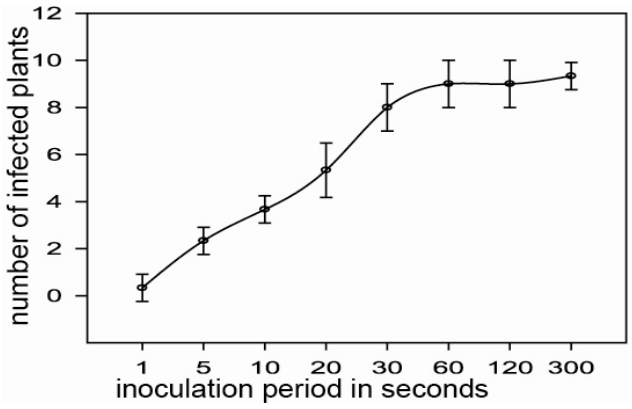


**Fig. 4. Relation between the number of infected *N. tabacum* plants and the period of inoculation of PVY**

*floridana* F=78.1 at $p<0.001$ and for *N. tabacum* F=51.1 at $p<0.001$. We used the SNK test for multiple comparisons of the different variants (Table 3).

The performed statistical analysis showed that there are no significant differences between the variants of 30, 60, 120 and 300 seconds. Also, these variants have the highest values for number of infected plants. The results from the trials to determine the inoculation periods are represented with graphs on Figures 3 and 4. Therefore, the optimal time of inoculation of PVY by viruliferous aphids leading to successful transmission to healthy indicator plants is 30 seconds.

The minimal period for viral inoculation is 1 second (Figures 3 and 4), which results in the lowest rate of infection. The total number of indicators for which PVY infection was confirmed by DAS-ELISA and visible symptoms were present was 2 *Ph. floridana* and 1 *N. tabacum* plants.

De Bokx and Huttinga (1981) established that the period of inoculation for successful PVY transmission ranges from 30 to 60 seconds, and Manoussopoulos (2001) reports that the inoculation period of the virus depends on the environmental conditions and varies from 5 to 15 seconds. Both publications acknowledge that the inoculation period is influenced by the viral strain, host species, vector population and the conditions under which the experiment was carried out.

The DAS-ELISA method used in the present study allowed for detection of latent carriers of PVY infection. Thus, we were able to determine that the optimal period for viral inoculation is 30 seconds and the minimal is 1 second.

***Period of retention of PVY in the vector***

The results of the trials with *Ph. floridana* showed that the number of infected plants is higher when vectors were moved at intervals of 15 and 30 seconds. Ten days after the infestation, the plants reacted with local lesions. Systemic symptoms developed 14 days after feeding of the vectors. The plants manifested mosaic which gradually became more severe and resulted in yellowing of leaves. Infected plants were smaller, compared to the healthy negative control.

Infected *N. tabacum* plants started reacting with 12 days after removal of the vectors, while *Ph. floridana* plants reacted two days later. During the next observations, vascular discoloration was established. Systemic veinal necrosis developed on the 16$^{th}$ day. The number of infected tobacco plants when vectors were moved at intervals of 15 and 30 minutes was lower than this for *Ph. floridana*. Also, at interval of 60 minutes, the virus was detected by DAS-ELISA in two *N. tabacum* and one *Ph. floridana* plants. Therefore, the latter indicator is more sensitive to PVY.

The ANOVA for the duration of the retention period of PVY showed significant differences between

**Table 4**

**Multiple Comparisons (SNK) between the mean values of the separate variants for time of retention of PVY in aphids with indicators *Ph. floridana* and *N. tabacum***

| Variant, min | | *N. tabacum* | | | | | |
|---|---|---|---|---|---|---|---|
| | | 15 | 30 | 60 | 120 | 180 | 240 |
| *Ph. floridana* | 15 | - | 0 | 2.33 ** | 4.67 *** | 5.33 *** | 5.67 *** |
| | 30 | 0.33 | - | 2.33 *** | 4.67 *** | 5.33 *** | 5.67 *** |
| | 60 | 3.67 *** | 3.33 *** | - | 2.33 *** | 3 *** | 3.33 *** |
| | 120 | 6 *** | 5.67 *** | 2.33 *** | - | 0.67 | 1 |
| | 180 | 6.67 *** | 6.33 *** | 3 *** | 0.67 | - | 0.33 |
| | 240 | 7.33 *** | 7 *** | 3.67*** | 1.33 * | 0.67 | - |

* 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 $\alpha = 0.001$

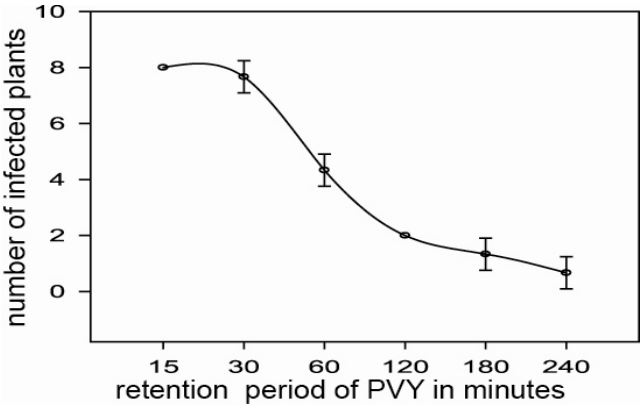


**Fig. 5. Relation between the number of infected *Ph. floridana* plants and the period of PVY retention in the vector**

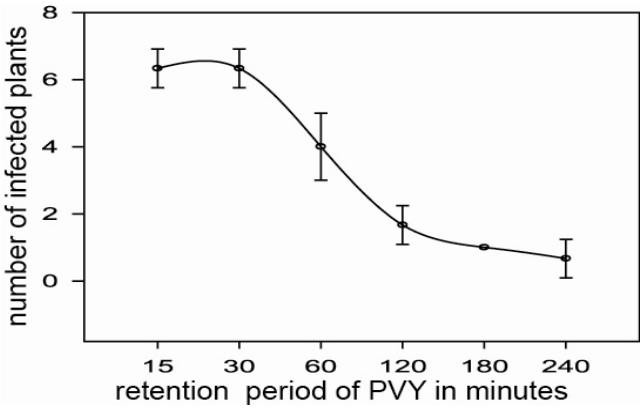


**Fig. 6. Relation between the number of infected *N. tabacum* plants and the period of PVY retention in the vector**

the variants – for *Ph. floridana* $F = 139.8$; $p < 0.001$ and for *N. tabacum* $F = 52.1, 4$; $p < 0.001$. The data obtained from the SNK test for multiple comparisons between the variants are represented on Table 4.

The highest values for the number of infected plants were obtained when virulipherous aphids were moved to new plants at intervals of 15 and 30 minutes (Figures 5 and 6). With increase in the duration of the period above 30 minutes, viral retention decreased. When aphids were moved to indicators at intervals of 120, 180 and 240 minutes, the numbers of infected plants were low. The analysis showed no statistically significant differences between these three variants.

In their studies, de Bokx and Huttinga (1981) establish that most vectors lose their ability to transmit PVY one hour after acquisition. Yuan and Ulman (1996) and Nault (1997) report, that aphids remain viruliferous for a short period of time, usually in the range of 1-2 hours, and then lose their ability to infect healthy plants.

The present experiment established that vectors completely lose their ability to transmit the PVY 4 hours after acquisition and removal from the source of infection and for this period they are able to infect up to 4 plants. It was also shown that if aphids move to 8 different plants for a period of 2 hours after acquisition, they completely lose their ability to transmit PVY. These data suggest that after leaving the infection source, feeding on the next host and movement to other hosts results in a decrease of the possibility of successful viral transmission.

## Conclusions

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

- The optimal period for acquisition and inoculation of the non-persistent PVY with vector *M. persicae* is 30 seconds, and the minimal is 1 second.

- The retention period of PVY in *M. persicae* varies from 2 to 4 hours and depends on the number of healthy plants aphids feed on, as well as on the duration of the feeding. With increase in the number of plants aphids visit, the retention period increases, i.e. the aphids lose their ability to transmit PVY more rapidly.

- A single viruliferous aphid is able to infect healthy plants with PVY.

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