IMPACT OF FEEDING TIME ON PVY^{N} AND PVY^{NTN} TRANSMISSION BY *MYZUS PERSICAE* (SULZ.)

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Received: February 25, 2011
Accepted: August 4, 2011

Abstract: In potato seed production, virus diseases especially Potato virus Y (PVY) are of great economic importance. This virus is transmitted by many aphid species in a non-persistent manner, while *Myzus persicae* (Sulz.) is its most important vector. The first aim of our research was to find out the dependence of the aphid feeding time, both in terms of acquisition feeding time and inoculation feeding time on PVY^{N}W and PVY^{NTN} transmission. The second aim was to compare the retention of both strains of the virus in the body of *M. persicae*. The research was carried out in a laboratory and in a glasshouse. It was found out that the dependence between feeding time and the effectiveness of PVY^{N}W and PVY^{NTN} transmission was similar. Along with the prolonging of the acquisition feeding time from up to 7 s, the effectiveness of the initial transmission of both strains increased. After transmission effectiveness reached the optimum, transmission clearly decreased, but within the extent of the applied feeding time (7 and 30 s; 2, 8, 32 and 60 min) it did not lower down to zero. The highest infection of test plants *Physalis floriana* Rydb. by both strains was recorded during a 2-minute-long feeding of aphids. The percentage of infected plants amounted to 50% with PVY^{NTN} transmission, and 30% with PVY^{N}W transmission. However, the prolonging the inoculation feeding time of aphids also initially increased the transmission effectiveness of both virus strains. Having reached the optimum, which took place as a result of a 30-second-long feeding (PVY^{NTN}) and a 30-second to 2-minute-long feeding (PVY^{N}W), the share of plants infested by these strains was 30% and 15%, respectively. Continuous prolonging of the feeding time caused a slow decrease in the effectiveness of transmission. During a 60-minute-long feeding, the share of infested plants was 15% (PVY^{NTN}) and 10% (PVY^{N}). The retention of PVY^{N}W and PVY^{NTN} in aphids which were starved following the acquisition of the virus was similar and lasted less than 2 hours. However, in relation to aphids feeding after the acquisition of the virus, the retention of PVY^{NTN} was much shorter (aphids could effectively transfer the virus as far as the 4th plant out of 10 consecutively inoculated) than that of PVY^{N}W (in which the 7th plant was also infected). In total, PVY^{NTN} was more effectively transmitted than PVY^{N}W.

Key words: PVY^{N}W, PVY^{NTN}, *Myzus persicae*, transmission, retention

INTRODUCTION

Poland is still the leading producer of potatoes in Europe and all over the world. This is true, even though for several years now, due to a lowered market demand, the crops area is decreasing every year.

Potato is particularly susceptible to dysgenics. For this reason, a periodical exchange of seed-potatoes is very important. The frequency of dysgenics depends on virus pressure in the region of the crops as well as on the cultivar's resistance to viruses. Efficient seed production is thus the basic condition for effective potato production.

The greatest economic importance in potato seed production is attributed to virus diseases. Potato virus Y (PVY) is the major cause for the seeds to become disqualified. In a wider production, the most harmful effect of reproduction of the infected tubers in consecutive years is their smaller growth and as a result of this their progressing degeneration, *i.e.* dysgenics which consequently leads to a decrease in the amount of crops (Singh *et al*. 2008).

PVY is present in all potato crop regions all over the world. It belongs to the *Potyvirus* genus, within the *Potyviridae* family (Brunt 2001). Apart from potato, it can also infect other plant species of the *Solanaceae* (De Box and Huttinga 1981; Brunt *et al*. 1996), and among others, tobacco (*Nicotiana tabacum* L.), tomato (*Lycopersicum esculentum* Mill.) and pepper (*Capsicum frutescens* L.).

PVY isolates are traditionally classified as belonging to 3 major groups of strains: PVY^{0}, PVY^{C} and PVY^{N} (Chikh Ali *et al*. 2010). As a result of the recombination phenomenon, there are also some changes in the population of PVY strains. Particularly strong changes took place in the last 30 years (Chrzanowska 2009). In 1983, the author discovered in Poland a new strain PVY^{N}, which was moderate on potatoes, and which was named

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$PVY^{N}W$ (deriving from Wilga on which it was registered). The strain was found to be different than the others of the so far described necrotic strains. $PVY^{N}W$ was formed as a result of recombination between strains PVY^{N} and PVY^{0}. It is highly infectious and spreads out rapidly, but on potatoes $PVY^{N}W$ cause milder symptoms than PVY^{N} (Chrzanowska 2009).

According to Chrzanowska and Doroszewska (1997), PVY^{NTN} is a recombinant. In Poland, it was discovered for the first time in 1994 (Chrzanowska 2009). This strain is marked by a greater pathogenesis than $PVY^{N}W$. On several potato cultivars it causes strong, often necrotic symptoms. The economic importance of PVY^{NTN} isolates, however, is connected mainly with the possibility of being able to provoke symptoms of potato tuber necrotic ring diseases – PTNRD. Infected tubers are not even suitable for food processing. According to Golnik *et al*. (2007), both discussed strains dominate on potato crops in Poland at a ratio of about 80% (PVY^{N} W) to 20% (PVY^{NTN}), while the significance of PVY^{NTN} increases. The so-called common or zero strain PVY^{0} which used to be popular some years ago, is only occasionally found in Poland.

In natural conditions, aphids which are both colonizing and uncolonizing the potato crop, are the major vectors of PVY. All experts agree that *Myzus persicae* (Sulz.) a world-popular green peach aphid, is the most effective PVY vector. The virus is transmitted in a non-persistent manner, on an aphid's stylet. In practice, it means that both the acquisition of virus from a plant which is infected, and transmission onto a healthy plant take place during the probing of the plant by the insect's stylet. Probing lasts only for several seconds. The transmission of PVY by selected aphid species was studied by many researchers (Bradley 1954, 1956, 1964; Kostiw 1973, 1976a; Manoussopoulos 2001; Kotzampigikis *et al*. 2009). With respect to acquisition feeding time from an infected plant, Kostiw (1973, 1976) recorded that the longer the feeding time, ranging from 7 to 30 s, the quicker was the increase of the effectiveness of virus transmission. Following the optimum of transmission (as a result of a 30-minute-long feeding), effectiveness decreased equally fast. One can also claim, that as far as the inoculation feeding time is concerned the general model of virus transmission may be quite similar to the one described, only that the initial prolonging of feeding time caused a slow increase in the effectiveness of transmission, and after reaching the optimum an equally slow decrease was observed. The existing differences mainly concerned optimal feeding times. Wingless *M. persicae* forms transmitted PVY most effectively during feeding which lasted from 4 to 16 minutes, whereas the winged ones 1–2 minutes. As a result of further prolongation of feeding, the effectiveness of virus transmission decreased very slowly.

The possibility of virus transmission already during the vectors' very short feeding time, substantially hampers potato protection against viruses transmitted by aphids in a non-persistent manner. It has been known for a long time that chemical control of vectors is not very effective. This is especially true if it concerns cultivars that are susceptible to PVY (with their level of resistance 5 or lower, in a 9-grade-scale) and in conditions of numerousness of winged specimens. None of the available insecticides can destroy aphids immediately following the treatment, and until the insects are paralyzed they continue to transmit viruses actively.

In epidemiology of viruses transmitted by aphids in a non-persistent manner (stylet borne), a large degree of significance is attributed to virus retention, *i.e.* the time in which the insect having acquired the virus can become its active vector. On the basis of the available research results, it is known that the retention of PVY in aphids feeding after virus acquisition was about 2 hours (Bradley 1959; Kostiw 1987; Nault 1997), and in the case of starved aphids – about 4 hours. Occasionally retention lasting up to 16 or even slightly longer than 17 hours has been recorded (Bradley 1954; Kostiw 1987). After this time period, aphids lose the virus and cease being its vectors. So far in papers concerning PVY transmission by aphids, in most experiments, no strains of the virus were distinguished and a normal strain (PVY^{0}) or a necrotic one (PVY^{N}) were applied. Only to a limited extent was PVY^{NTN} the subject of research with the aim to assess the effectiveness of this strain's transmission by vectors (Kaliciak and Syller 2009; Verbeek *et al*. 2010).

The first aim of the research was to compare the dependence between *M. persicae* feeding time, concerning both acquisition feeding time and inoculation feeding time on $PVY^{N}W$ and PVY^{NTN} transmission by these aphids. The second aim was to compare the retention of these strains in *M. persicae* organisms.

MATERIALS AND METHODS

The research was carried out in a laboratory and in a glasshouse. The virus vector rearing with *M. persicae* aphids was carried out on healthy plants of *Brassica rapa* subsp. *pekinensis* kept in a well isolated insectaria, in artificial light about 3,000 lux. Day and night length was respectively 16 and 8 hours, while the temperature ranged between 20–25°C. Air humidity was not regulated.

Ph. floridana was the testing plant. Seedlings grown from seeds, were put into 8-cm-long diameter pots. The pots containing the seedlings were filled with an evaporated mixture of soil and peat, and fertilized adequately. Pots were placed in an insect-free glasshouse chamber on parapet lined with a thin layer of peat. Temperature ranged between 18 and 27°C. The research started when test plants reached a stadium of 2–4 leaves.

The source of $PVY^{N}W$ and PVY^{NTN} were secondarily infected potatoes of Vital and Kolia cultivars, respectively. Plants were kept in separate isolators in glasshouse chambers.

Before the start of the experiment, *M. persicae* aphids which were used as virus vectors, were starved for about 2 hours, and kept at a temperature of 21–23°C, in the same room in which inoculation of test plants was carried out later. During starving, insects were kept in 6-cm-long and 2-cm-wide testing tubes protected with bolting-cloth in order to ensure free air flow. Only winged aphid morphs were used. After a 2-hour-long starving, insects were gently taken by their wings using tweezers and placed onto the source of the virus. Following the assumed vi-

rus acquisition feeding time, aphids were transferred in the same way directly onto the test plant in order to launch the inoculation feeding time. Acquisition and inoculation feeding times were measured while the insect's stylet was monitored under a 5 times or more, if necessary, magnifying glass. For the start of the feeding, the moment of contact of the final part of the rostrum with the leaf surface was considered. Only the insect's factual and continuous feeding time was taken into account. The first brief piercings were not considered, as almost every aphid does it before it begins a longer feeding. Therefore, only a single aphid per test plant was used to transmit the virus. Following the inoculation, feeding time aphids were removed from the test plant also using tweezers, and were destroyed mechanically. In order to eliminate the impact of the environment on the obtained results, on one day the same number of plants were infected for each of the applied virus strain and the assumed feeding time. This constituted one series of the experiment. The impact of six different acquisition and inoculation feeding times on $PVY^{N}W$ and PVY^{NTN} transmission by aphids was measured. They were the following times: 7 and 30 s; 2, 8, 32 and 60 min. When the acquisition feeding time was altered (as above), then the inoculation feeding time on the test plant was constant and amounted to 1 hour (aphids remained on the plant for 1 hour), whereas when the inoculation feeding time was altered (as above), then the virus acquisition feeding time was persistent and lasted 2 minutes. For an altering acquisition feeding time, one series of the experiment was concerned with 5 plants for each of the 6 applied feeding times (30 plants in total). Four series of the experiment for each of the virus strain were made (240 plants were inoculated in total). In relation to the changing inoculation feeding time, the schema of the experiment as well as the number of inoculated plants, were the same as for the changing acquisition feeding time.

In the experiment concerning the retention of the studied PVY strains in aphids starved following the virus acquisition, individuals of *M. persicae* were placed on the plant – the source of the virus for a 2-minute-long acquisition feeding. When the feeding was over, the aphids were placed in a glass test tube protected with bolting-cloth. There the insects were kept according to the assumed starving time. The following times were considered: 1, 4, 16, 128 and 1024 minutes. When the estimated starving time passed, the aphid was placed on a test plant (*Ph. floridana*) in order to initiate the inoculation feeding time. The time was constant and took 2 minutes. In one series of the experiment 5 plants were inoculated for each of the 5 applied starving times (25 plants in total). Four series of the experiment for each of the strains were made (in total 100 plants were inoculated). The total number of inoculated plants for both strains was 200.

In the experiment assessing the retention of strains in aphids feeding after the acquisition of the virus, the aphid was first placed on the plant – the virus source for a 2-minute-long pathogen acquisition feeding, after which it was transferred onto subsequent test plants in such a way that the same aphid subsequently inoculated 10 test plants. On each of them the inoculation feeding time lasted for 2 minutes. This constituted 1 series of the experiment. For each of the strains, 5 series of the experiment were conducted. In total, for both strains, 100 test plants were inoculated. For each series of the experiments, and each of the applied aphid starving times, one extra control plant *Ph. floridana* was added and it was not inoculated.

Detecting the virus in test plants was carried out by means of a visual method, *i.e.* 3–4 observations of symptoms of test plant infection. The first observation was carried out following the record of the first infection symptoms on inoculated plants, *i.e.* 7–10 days after inoculation. Subsequent observations were made in 2–3 day intervals, and they were made until it was ensured that no more plants were infected.

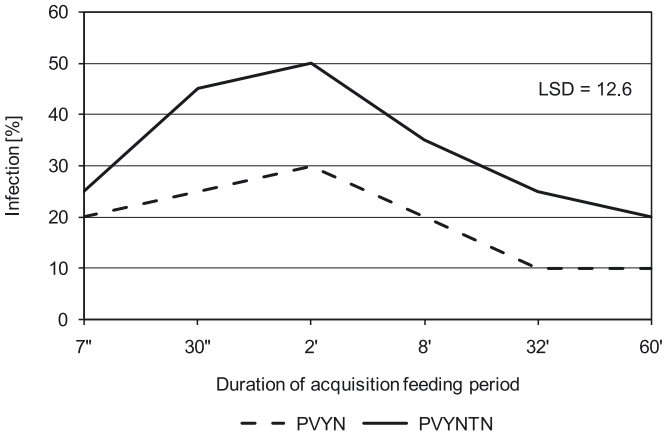
Symptoms of diseases caused by the two strains took the form of a mosaic and deformation of leaves. After some time, plants became infected by PVY^{NTN} since the infection additionally reacted with a strong dwarfish and descent of leaves, and the resulting drying out of shoots. In the case of PVY^{N}, the infected plants did not react with such strong symptoms.

The results of the experiments concerning the impact of aphid feeding time on the transmission of the applied PVY strains, were statistically analyzed. For calculations, an average of all the series of the experiment percentages of plants infected within the applied feeding times was taken. Calculations were made separately for each strain of the virus. Variance analysis was made with a single classification using the Statystyka program designed by the Agricultural and Technical Academy in Bydgoszcz, Poland. The results of the research concerning the retention of $PVY^{N}W$ and PVY^{NTN} were not analyzed statistically because their interpretation is explicit.

RESULTS

Results concerning the impact of $PVY^{N}W$ and PVY^{NTN} acquisition feeding time by *M. persicae* on the transmission effectiveness of these strains is presented in figure 1. Both strains were transmitted in a very close manner. Along with the prolonging of the feeding time from 7 s, initially the effectiveness of transmission increased and, having reached the optimum, it clearly decreased. But within the extent of the applied feeding times, the transmission effectiveness did not lower to zero. The highest infection was recorded during a 2-minute-long aphid feeding. The percentage of infected plants was 50% during PVY^{NTN} transmission, and 30% during $PVY^{N}W$ transmission. A significantly higher effectiveness of PVY^{NTN} transmission in comparison with $PVY^{N}W$ transmission was recorded. These differences were registered during feedings which lasted for 30 s, 2, 8, and 32 minutes.

The impact of inoculation feeding time of *M. persicae* on test plants, on the transmission of the researched PVY strains was presented in figure 2. It was found out that the initial prolonging of the feeding time increased the transmission effectiveness of both virus strains. The optimum was reached during a 30-second-long (PVY^{NTN}) and a 30s-2-min-long ($PVY^{N}W$) feeding time (share of plants infected by these strains was respectively 30 and 15%).



LSD – least significant difference

Fig. 1. The impact of PVY^{N} and PVY^{NTN} acquisition feeding time by *M. persicae* on the effectiveness of transmission of these strains

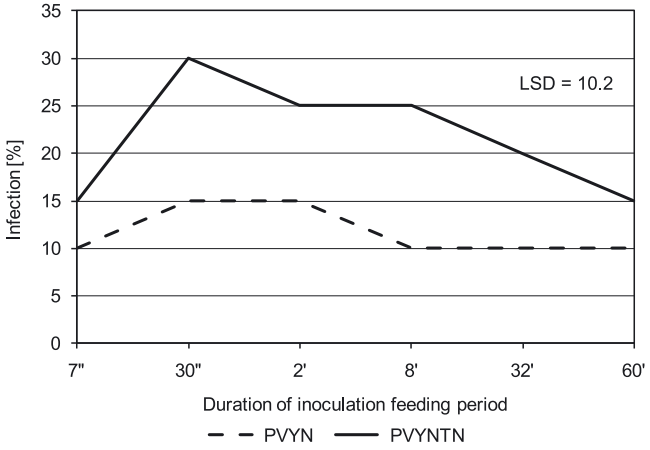


Fig. 2. The impact of inoculation feeding time of *M. persicae* on test plants on the transmission of PVY^{N} and PVY^{NTN}

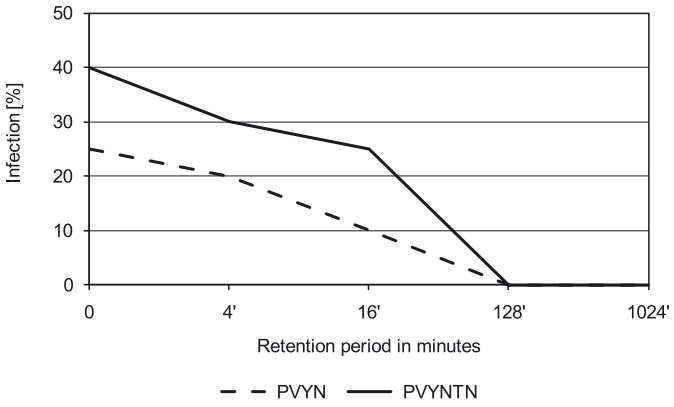


Fig. 3. The retention of PVY^{N} and PVY^{NTN} in *M. persicae* that were starved following the virus acquisition

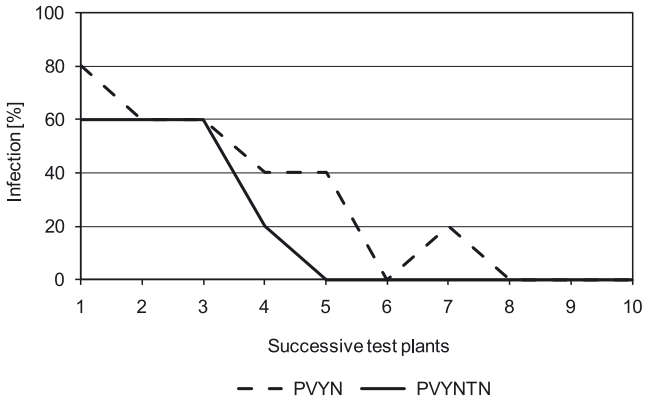


Fig. 4. The retention of PVY^{N} and PVY^{NTN} in *M. persicae* feeding following the virus acquisition

A further prolonging of the feeding time caused a slow decrease of transmission, especially in case of PVY^{NTN} and $PVY^{N}W$ (15 and 10% share of infected plants respectively, at a 60-minute-long feeding). In our research, the PVY^{NTN} strain was also significantly more effectively transmitted than $PVY^{N}W$, during the feeding lasting for 30 s; 2, 8, and 32 minutes.

Figure 3 presents the results concerning the retention of the researched PVY strains in aphids that were starved following the virus acquisition. The results are explicit. Along with the prolonging of aphid feeding time following their acquisition of $PVY^{N}W$ and PVY^{NTN}, the transmission effectiveness of these virus strains clearly decreased. The greatest percentage of infected plants was recorded when aphids were transferred directly from the virus source (without starving) onto test plants on which they had inoculation feeding. The share of plants infected with $PVY^{N}W$ and PVY^{NTN} was then 25 and 40%, respectively. A four-and sixteen-minute-long starvation caused a significant decrease in virus transmission effectiveness and as a result the percentage of infected plants amounted to 20 and 10% ($PVY^{N}W$), respectively and 30 and 25% (PVY^{NTN}), respectively. During a 128-minute-long starvation, aphids "lost" the virus and entirely lost the ability to transmit both of the strains.

$PVY^{N}W$ and PVY^{NTN} retention in aphids feeding following the virus acquisition, is presented in figure 4. PVY^{NTN} retention was shorter than that of $PVY^{N}W$. Aphids were able to successfully transmit the virus as far as the fourth plant out of 10 consecutively inoculated. However, at $PVY^{N}W$ transmission, the 7th plant was also infected. Aphids kept the greatest ability to transmit both strains as a result of plant inoculation in the first, second and third rounds done consequently. The share of plants infected with $PVY^{N}W$ was 80, 60 and 60%, respectively, and with PVY^{NTN} it was the same (60%) for all three rounds of inoculation.

DISCUSSION

Research results showed that dependency between the feeding time and the effectiveness of $PVY^{N}W$ and PVY^{NTN} transmission by *M. persicae* winged specimens was very similar. The similarity was true, both in terms virus acquisition feeding time from infected plants as well as inoculation feeding time of infected aphids on healthy plants. It was observed that in both cases, as the feeding time prolonged from 7 s to 60 minutes, the ability to transmit viruses initially increased, and having reached the optimum (during feeding lasting from 30 s to 2 minutes, depending on the researched strain) it decreased. In relation to the acquisition feeding time, the results are close to those obtained by the author in earlier research (Kostiw 1976a, 1987) in which, however, PVY was used without determining the strain (Kostiw 1976) or it was a normal strain PVY^{0} (Kostiw 1987). Nevertheless, in terms of inoculation feeding time in previous research, the optimum of transmission also lasted longer than 2 minutes. In fact, it was registered during aphid feeding lasting from 2 to 16 minutes, after which the decline of infection was much milder. In previously published research, there is no information on the dependence between feeding time and the effectiveness of PVY^{NTN} transmission by aphids. Thus, there is no possibility to compare results.

It was also observed that PVY^{NTN} was transmitted by *M. persicae* with a significantly higher effectiveness than $PVY^{N}W$. This may testify even more to the possibility of the virus spreading easily in natural conditions since *M. persicae*, the most effective PVY vector (Sigvald 1984; Verbeek *et al.* 2010), is very popular in Poland and it occurs on potato crops every year, at times in very large numbers (Wisłocka and Kostiw 1978; Kostiw and Robak 2009, 2010).

A similar $PVY^{N}W$ and PVY^{NTN} retention in aphids starved for less than 2 hours after acquiring the virus, may testify to the fact that in natural conditions the possibility to transmit these strains by *M. persicae* to further distances is limited. This kind of retention is, nevertheless, long enough for the virus to spread within the plantation. This is especially true, if virus sources may be found. Thus, it is of huge importance in the seed production of this plant, that a thorough negative selection is carried out, and to make sure the seed plantation remains isolated spatially from other potato crops. Both, proper isolation as well as negative selection are essential in the technology of seed production. In the papers concerned with the topic, there is no information concerning PVY^{NTN} retention.

Also, a difference was marked in retention between the researched strains, in aphids feeding following the acquisition of the virus in consecutive healthy plants.

At $PVY^{N}W$ transmission, the same aphid was capable of transmitting the virus onto 5 out of 10 consecutively inoculated plants. In one case, even the 7th plant became infected. Meanwhile, PVY^{NTN} retention was shorter. The same aphid transmitted the virus as far as the fourth plant. This means that aphids "lost" PVY^{NTN} earlier than $PVY^{N}W$. This data points to the fact that in natural conditions $PVY^{N}W$ may spread more easily than PVY^{NTN}. The lack of data on this topic in earlier papers means that there was no possibility to compare results.

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