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DISTRIBUTION OF POTATO LEAFROLL VIRUS – (PLRV) AND POTATO VIRUS Y – (PVY^N) IN A FIELD EXPERIMENT

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Abstract

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The aphid activity flight and the distribution of PLRV and PVY^n were studied in an experimental potato field.

The field experiment was carried out with three varieties of potatoes: Concorde - early, Arinda – medium early, Desiree – late, in an artificially created infectious background of 5% for each virus. Monitoring was carried out to identify viruliferous aphids through a biological test, for PLRV ELISA was also performed. Infection of potato plants was determined using DAS-ELISA.

The following aphid species vectors of both viruses were present: *Myzus persicae* Sulzer, *Aphis gossypii* Glover, *Aphis fabae* Scop., *Aphis nasturtii* Kalt., *Aulacorthum solanii* Kalt., *Macrosiphum euphorbiae* Thomas. It was monitored that *M. persicae* is a dominating vector with a peak in flight during the 27th week (4 – 10 July). A week after that, it was observed the highest percentage of plants infected with PVY^n. The highest number of plants infected with PLRV was observed during the 30th week (25-31 July), three weeks after the peak in flight of aphids and a week after the observation of the highest number of wingless individuals.

PLRV causes a three-fold decrease in yield from the late and medium early variety. PVY^n causes most severe damages to the early variety, reducing the yield four times. Mixed viral infection has the highest impact on yield with decrease in yield ranging from 4 to 5-fold, depending on the variety.

Key words: PLRV, PVY, dynamics of spread of infection, aphids as virus vectors, potatoes, yield

Introduction

Potato leafroll virus - PLRV and *Potato virus Y* – PVY are the most wide spread viruses in potatoes (Weidemann, 1988; Salazar, 1996; Boiteau et al., 1998, Robert, 2000), also in Bulgaria (Kovachevsky; 1945; Krastev, 1950; Baylova – Jankulova, 1961; Braikova et al., 1981; Krachanova et al., 1978; Jankulova et al., 1983). These viruses cause both yield quantity and quality decrease. (Webley et al., 1972; Harper et al., 1975; Crosslin et al., 2006).

The epidemiology of PLRV and PVY is widely studied. The spread of PVY depends mainly on winged aphids (Ioannou, 1989; Ragsdale et al., 1994; Katis et al.. 1998), and for PLRV it depends on the population density of both winged and wingless mor-

phs (Ribbands, 1965). A major role for the spread of the two viruses is also attributed to the period of appearance of the first viruliferous morphs (Basky, 2002; Mehle et al., 2004; Zhu et al., 2006). The transmissibility of different PVY strains is different (Katis et. al., 1986; McDonald and Sight, 1996; Kostiw, 2004). The spread of viral infection on varieties with different sensitivity has been researched (Cockerhan, 1970; Braikova et al., 1981; Mehle et al., 2004). The role of different agrotechnical practices in connection to the spread of the two viruses has been studied (Burt et al., 1964; Matakov et al., 1986; Mistri, 2003; Whitworth et al., 2006).

A disadvantage of most existed epidemiological studies is the lack of presenting a complete research paper that combines data on the initial quantity of viral inoculum as a source of infection in the field, the tracing of the lethal infection, and especially of mixed infections with both viruses present in plants; at the same time, to identify virulence of vectors in the process of the epidemiological study.

The aim of the present study is to investigate the dynamics of spread of PLRV and PVY ^{n} and the host-virus-vector relationship in a field experiment.

In order the above aim to be achieved, the following objectives have been outlined:

1. Monitoring of the aphids
2. Diagnostics of the viruses in the aphids using ELISA and biotest
3. Observation of the spread of the viral infection in plants
4. Investigation of the reaction of each variety towards infection with PVY ^{n} and PLRV
5. To examine the impact of viral infection on yield

Material and Methods

The studies were carried out during the vegetation period of 2005 (May-September) on the experimental field of Entomology department of Agricultural University, Plovdiv. Virus free planting material of the following varieties was used: Concorde – early, Arinda – medium early, and Desiree – late, produced in the Laboratory of Tissue Cultures in Proslav (Plovdiv region).

Planting was performed on May 2 (at the beginning of the18th calendar week). We set up the experiment in a 150 m^2 fields with 120 tubers of each variety, planted in 11 rows, 12 tubers in each row. In the middle of the plot tubers infected with PLRV and PVY^n were positioned (Schepers, 1972; Dusi et al., 2000) in order to create an infectious background of 5% for each virus. There were no Solanacea plants or any other plants to act as sources of infection in the area.

Cultivation of the potatoes was performed in accordance with the biological and agro technical requirements of the plant. The control of pests and weed species was carried out mechanically. The vegetation of the early variety Concorde finished by the end of the 30th week, of the medium early variety Arinda – 34th week, and of the late variety Desiree – 36th week.

The observations on the flight of aphids begun immediately after planting of the tubers, using traps of type "Moericke" (Moericke, 1951). The traps were initially placed on the surface of the soil and then moved at the height of the potato plants after germination. The trapped aphids were preserved in tubes containing 95% ethanol and 75% lactic acid in a ratio of 2/1 (Eastop and van Emden, 1972). The keys of Taylor (1984) and Shaposhnikov (1964) were used for identification of the species.

After the appearance of winged aphids, samples of them were analyzed using DOT-ELISA in order to identify the first viruliferous individuals that transmit PLRV; DAS-ELISA was used during vegetation (Kotzampigikis and Hristova, 2006). At the same time, a biological test on *Physalis floridana* L. for identification of PLRV and PVY ^{n} in vectors was performed. For the confirmation of the viral infection in the indicator species, the plants were analyzed using DAS-ELISA.

In order to detect infection with PLRV and PVY ^{n}

in potato plants, DAS-ELISA (Clark and Adams, 1977) was performed weekly, following the appearance of the first viruliferous vectors and until the end of vegetation. Yield was measured per variety, tubers per plant were counted and then separated in the following fractions, depending on their weight: >200g, 150g – 200g, 100g – 150g, and under 100g. DAS-ELISA was also used to analyze sprouting eyes from the tubers of each plant. Yield from infected plants was compared to this of healthy plants i.e. plants in which viruses were not immunologically detected in leaf samples and tubers.

Statistical analysis included dispersion analysis of variance and the Kruskal-Wallis test, correlation and regression analysis, z-test, and *chi* square test.

Results

Monitoring of aphids

Winged morphs were registered during the planting of tubers in the 18th calendar week (May 2 – 8). The total number of trapped aphids during the period of vegetation was 417. Their quantitative distribution versus time in weeks is represented in Figure 1.

The highest number of aphids occurred during the 27th week, at the beginning of July, and the lowest – during the 33rd week, in mid-August.

From the species so far reported in literature to vector PLRV and PVY, the following ones were present: *Myzus persicae* Sulzer, *Aphis gossypii* Glover, *Aphis fabae* Scop., *Aphis nasturtii* Kalt., *Aulacorthum solanii* Kalt., *Macrosiphum euphorbiae* Thomas.

The highest number of trapped aphids was *M. persicae* – a total of 164 alate viviparous females with a peak in flight during the 27th week (July 4 – 10). Apterous females of this species were present in the period from 27th to 30th week (July 4 – 31). Their density was highest during the 29th week (July 19 – 24), reaching 15 individuals per 100 leaves with colonization established for 5% of the plants.

Diagnostics of PLRV and PVY in aphids using ELISA and biological test

The first viruliferous individuals transmitting PLRV were diagnosed using the method DOT-ELISA at the beginning of the 21st week (May 23) i.e. 22 days after planting, when the plants were in the leaf-formation stage. The results from the subsequent analysis with DAS-ELISA showed a gradual increase in the percentage of viruliferous aphids versus the total number of analyzed samples, reaching its highest value during the 28th week (48%) (Figure 2).

The results of the biological test, which was used to identify PLRV and PVY^n in alate parthenogenic female individuals, are presented in Figure 3.

Winged aphids were more likely to carry PVY^n, rather than PLRV. The number of individuals transmitting PVY^n was highest in the 26th week (65% of all analyzed samples) and remained high during the 28th and the 30th week (53%). The results of the biological test, used to identify PLRV in vectors, were very similar to the ones obtained using ELISA.

Serological analysis of apterous females during the 28th and the 30th week showed that 83% and 90% of the aphids transmitted PLRV respectively. The biological test for PVY^n in apterae was negative, with the exception of aphids isolated from plants infected with the virus.

Dynamics of the spread of infection in plants

PLRV

The first symptoms of PLRV infection on potatoes were observed during the 20nd week in plants which were grown from infected tubers.

On the other hand, in the plants which were grown from healthy tubers symptoms of infection were observed as early as the leaf-formation stage, during the 22nd week, or 14 days after the appearance of the first viruliferous aphids. The plants exhibited chlorosis of younger leaves and funnel shaped leaf roll. Consecutively, the lower leaves became hard and leathery to the touch. In some of the infected plants, the

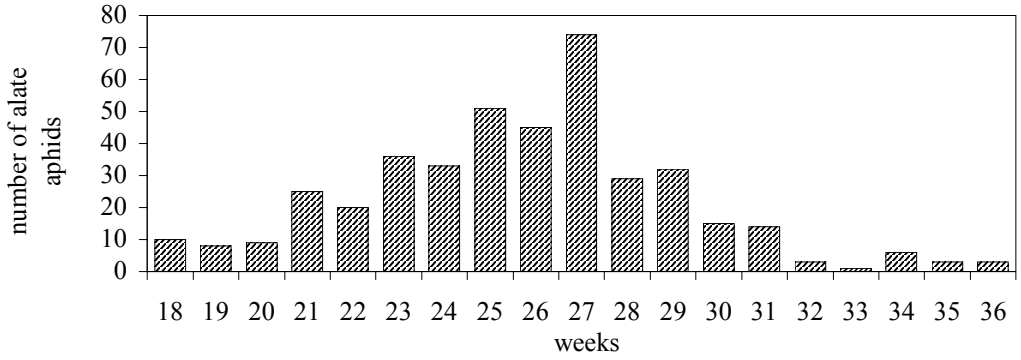

Fig. 1. Winged parthenogenic aphids trapped during the vegetation period

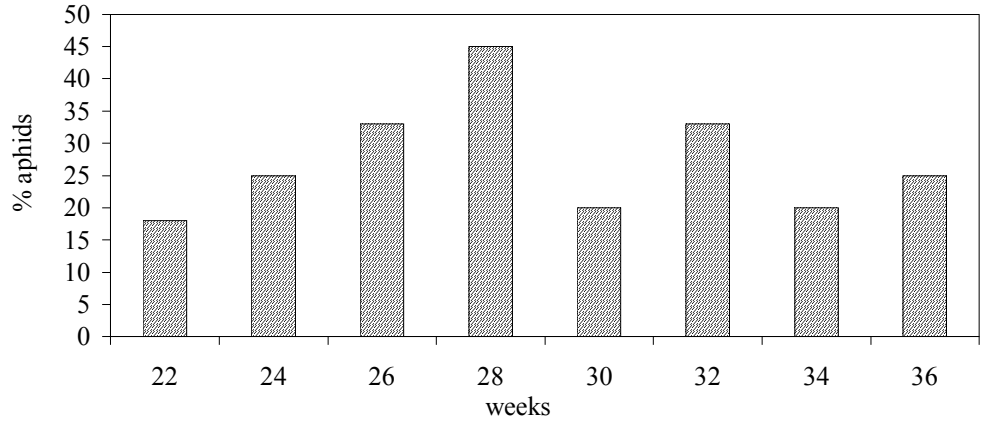

Fig. 2. Distribution of aphids transmitting PLRV identified using DAS-ELISA during the vegetation period

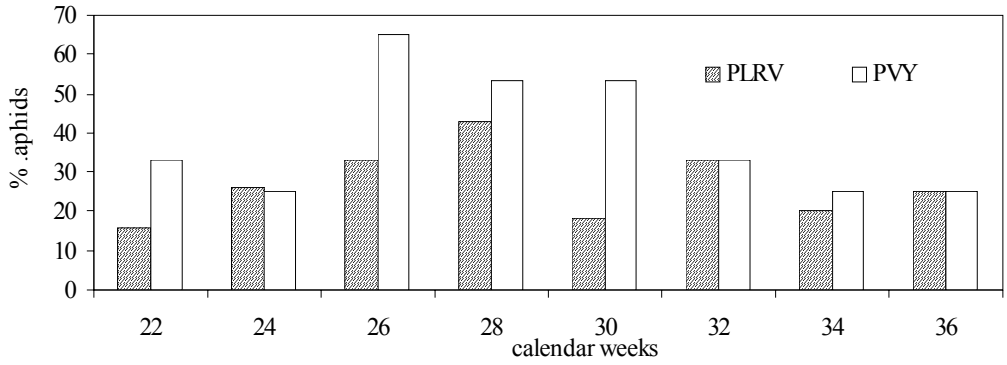

Fig. 3. Distribution of aphids transmitting PLRV and PVY ^{n} during vegetation, identified through a biological test with *Ph. floridana*

petioles bended near the next to the stem, withered and remained hanging. In addition, withering and dying away of stem branches or whole plants was observed.

The first plants infected with PLRV were observed in close proximity to the sources of infection. Moreover, areas of infected potato plants formed around the sources. The dispersion analysis showed significant differences in spread of the viral infection versus time during the vegetation period ($F=9.8$; $p=0.004$).

The highest percentage of plants of varieties Arinda and Desiree infected with PLRV was established during the 30th week (25% and 31% respectively), and of variety Concorde – during the 28th week (14%) (Figure 4).

Correlation analysis showed a high degree of correlation between the number of infected plants and the number of viruliferous aphids trapped during the previous week. The correlation coefficient for variety Desiree was $r=0.76$ ($p=0.03$), and for variety Arinda - $r=0.79$ ($p=0.03$).

PVY

The first symptoms of PVY ^{n} infection were also observed during the 20nd week in plants which were grown from infected tubers.

In the plants which were grown from healthy tubers symptoms of infection were observed in the leaf-formation stage, during the 22nd week, or 12 days after the appearance of the first viruliferous aphids. The symptoms were represented by mosaic on foliage and crinkled leaves. Consecutively, plant growth slowed down, the internodes were shortened and the apical leaves were undersized.

In contrast to PLRV, the spread of PVY infection in the field was scattered.

During the leaf-formation period, the percentage of infected plants was monitored to be increasing, with highest number of infections observed for the early variety Concorde (Figure 5). The changes in the spread of infection during the whole period were examined using dispersion analysis ($F=14.6$; $p=0.001$).

The highest percentage of infected plants was observed during the 28th week: 23% for Desiree, 20% for Arinda, and 30% for Concorde. During the following weeks, the percentage of infected plants decreased.

The relation between percentage of PVY-infected plants and number of vectors in the previous week was investigated (Figure 6).

The spread of infection is closely related to the number of alate females. This was confirmed by the extremely high values for the correlation coefficient for all three varieties: Desiree – $r=0.98$ ($p<0.001$), Arinda – $r=0.95$ ($p=0.001$), and Concorde – $r=0.95$ ($p=0.01$).

Mixed infection

Plants with mixed infection (PLRV and PVY ^{n}) differed from the ones with monoinfection by the expression of symptoms. They were underdeveloped and usually died away prematurely. Variety Concorde predominantly showed symptoms, characteristic of PVY^{n} (mosaic and crinkled leaves), and the symptoms of PLRV appeared later, also they were difficult to differentiate. In varieties Desiree and Arinda, the symptoms of mixed infection were differently exhibited, depending on the time of infection. In plants, infected early in the vegetation period, mosaic symptoms and deformation of the leaf blade were present and later on during vegetation browning of the leaf and stem veins was observed. In plants, infected during late vegetation, PLRV-induced symptoms were more visible. Apart from the symptoms, caused by PVY ^{n}, was also observed funnel-shaped leaf roll which is characteristic for PLRV.

Mixed infection was first detected in plants of the early variety Concorde, during the 24th week, for the late variety Desiree and the medium early variety Arinda mixed infection was monitored two weeks later, during the 26th week (Figure 7).

The number of plants with mixed infection was highest for Desiree, followed by Arinda and Concorde. The highest percentage of plants with mixed infection for all three varieties was observed during the 30th week. For Concorde, the percentage remained high until the end of the vegetation period, and for Arinda

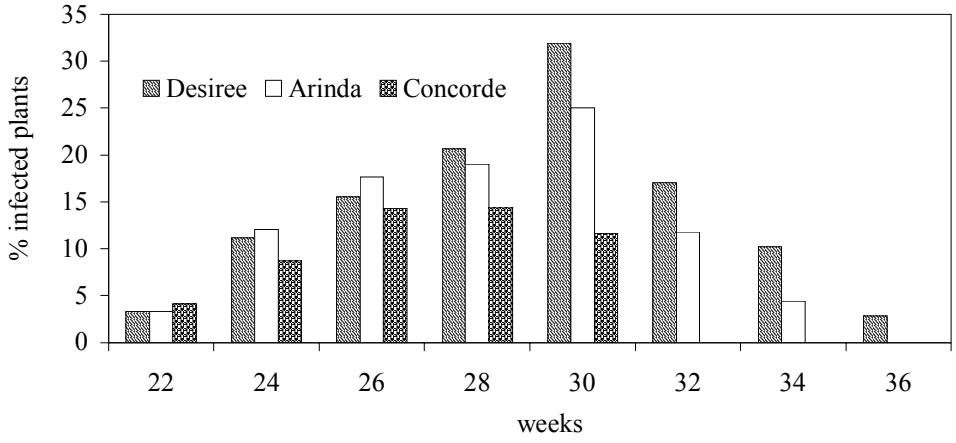

Fig. 4. Dynamics of spread of PLRV infection in potato plants

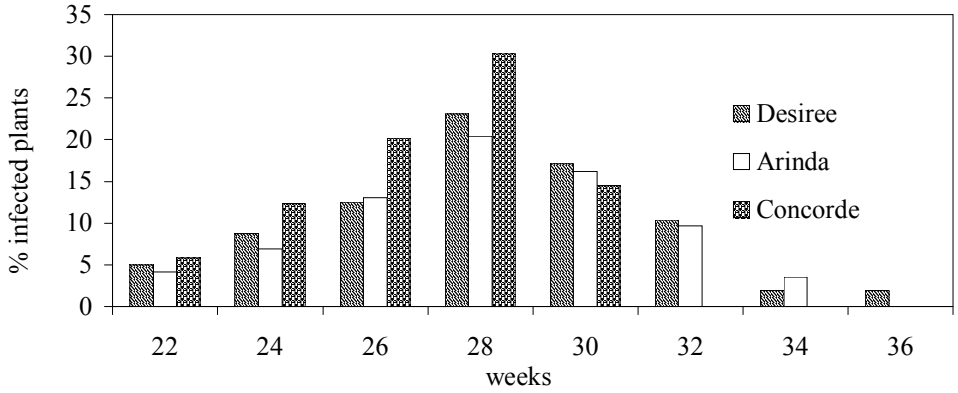

Fig. 5. Dynamics of spread of PVY infection in potato plants

and Desiree the percentage decreased. No plants of variety Desiree with mixed infection were monitored during the 36th week.

Behaviour of varieties towards viral infection

Spread of the viral infection in plants

The distribution of the four groups of plants – infected with PLRV, infected with PVY ^{n}, with present mixed infection, and healthy plants - for the whole vegetation period is represented in Figure 8. The *chi* square test showed significant differences between the three varieties studied: C^2=25.6; d.f.=6.

The early variety Concorde proved to be the most susceptible to PVY ^{n}. Presence of the virus in 42.5% of plants was detected. Regarding the share of plants infected with PVY ^{n}, significant differences were observed in relation to the other two varieties Desiree (z=3.93; p<0.001) and Arinda (z=2.88; p=0.004).

The late variety Desiree is the most resistant to PVY ^{n} and the most susceptible to mixed viral infection. The percentage of plants with detected mixed infection reached 40%.

The ratios between the different groups for the medium early variety Arinda are similar to those for Desiree. The highest percentage of plants infected with PLRV (33.3%) was found in the variety Arinda.

Spread of viral infection in the tubers

Plants infected during the leaf-formation stage showed higher concentrations of PLRV and PVY in the tubers. This was demonstrated by the higher ex-

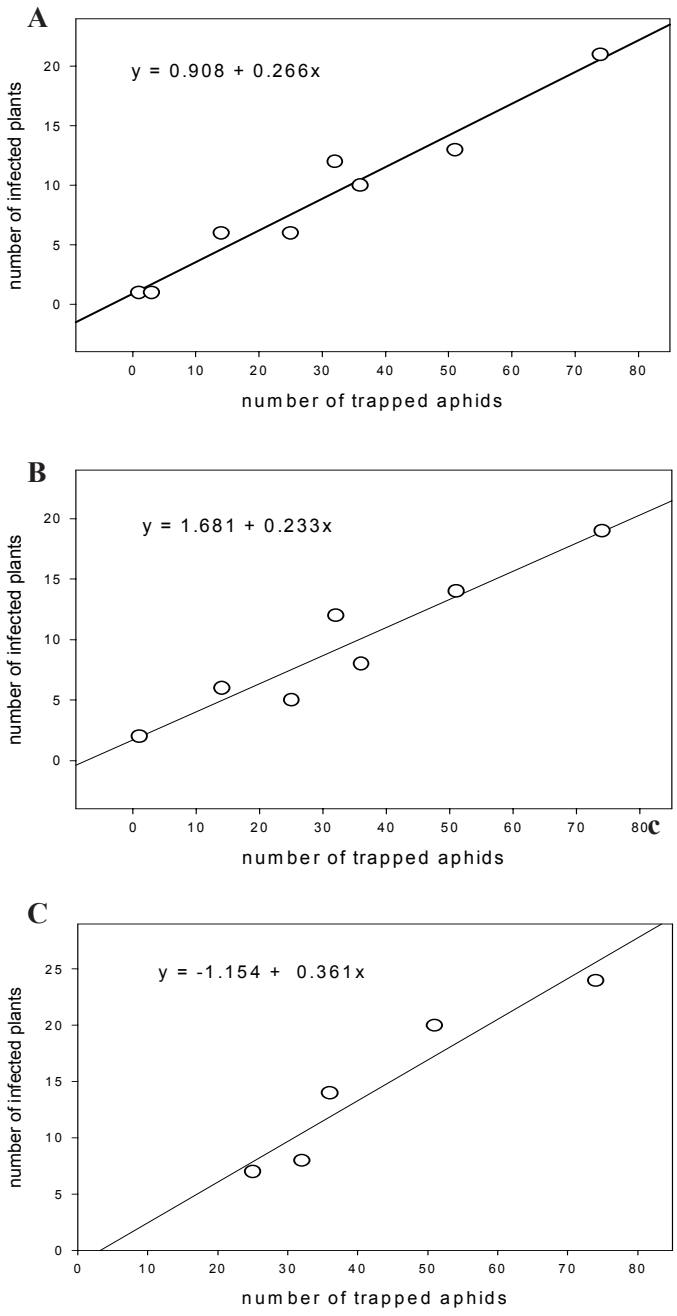

Fig. 6. Relation between the number of plants infected with PVY, and the number of aphids trapped during the previous week in varieties
A/ Desiree;
B/ Arinda, and
C/ Concorde

tinction values obtained after analysis of their tubers, compared to the values obtained for plants which were infected at later growth stages (data is not presented).

Using the *chi* square test, it was observed that the three varieties show significant differences in distribution of PLRV- and PVY ^{n} -infected tubers, tubers with mixed infection and healthy tubers (C^2=27.33; d.f.=6). That distribution (Figure 9) follows the general patterns, established for the spread of viral infections in plants. The highest number of tubers, infected with PVY ^{n} was observed in variety Concorde. The Z-test showed significant differences between Desiree (z=3.40; p<0.001) and Arinda (z=2.77; p=0.006).

Some plants of the three varieties, even though testing negative for leaf infection, had tuber infection. In some cases of mixed infection in plants, only one of the viruses was detected in the tubers. This can be explained by the fact that infection with the second virus has occurred at a later stage in development.

Influence of viral infection on yield

In the experiment, we obtained values for average yield per one healthy plant: for variety Desiree - 555g, Arinda – 499g, and Concorde – 481g. The influence of viral infections on the total weight

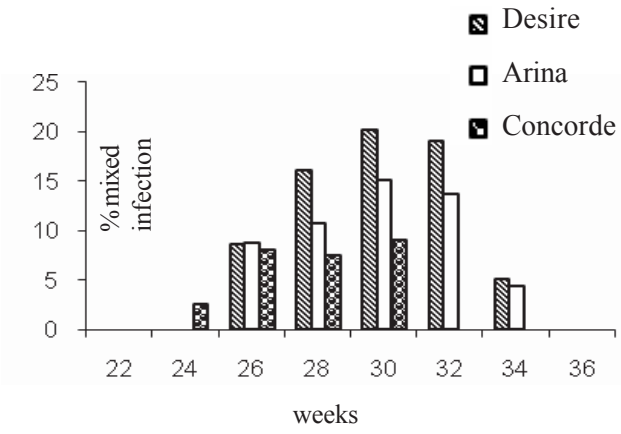

Fig. 7. Dynamics of spread of mixed infection in potato plants

of tubers from one plant was also investigated (Figure 10).

The Kruskal-Wallis test showed significant differences in yield between plants infected with PLRV, PVY^n, or with mixed infection and healthy plants for all three varieties: Desiree ($H=49.79$; $p<0.001$), Arinda ($H=31.32$; $p<0.001$) and Concorde ($H = 40.79$; $p < 0.001$). Multiple comparisons after the method of Dunn were performed. The control group showed much higher values for weight of tubers, compared to the other groups.

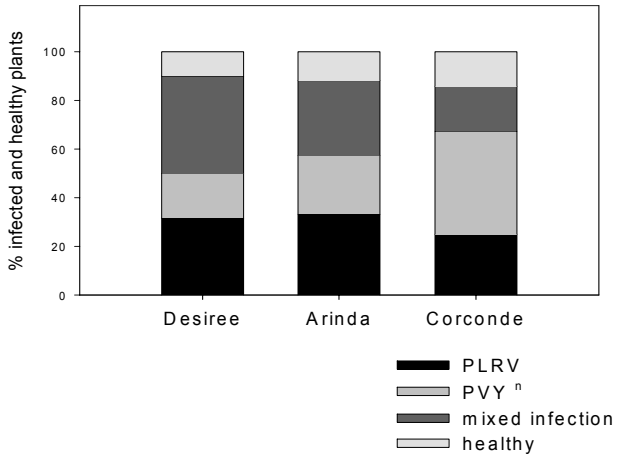

Fig. 8. Distribution of infection in plants

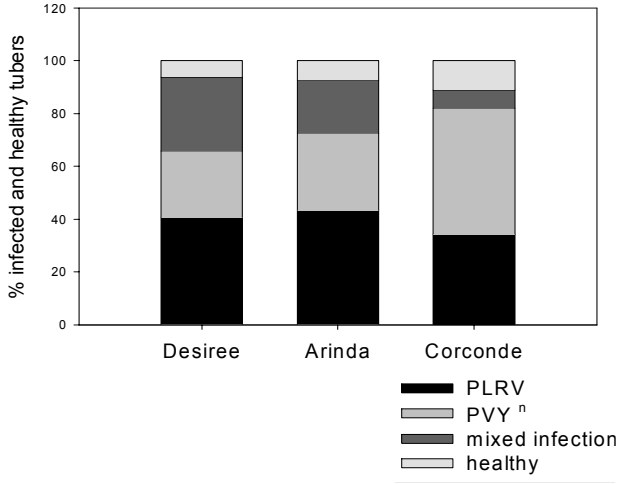

Fig. 9. Distribution of viral infection in tubers

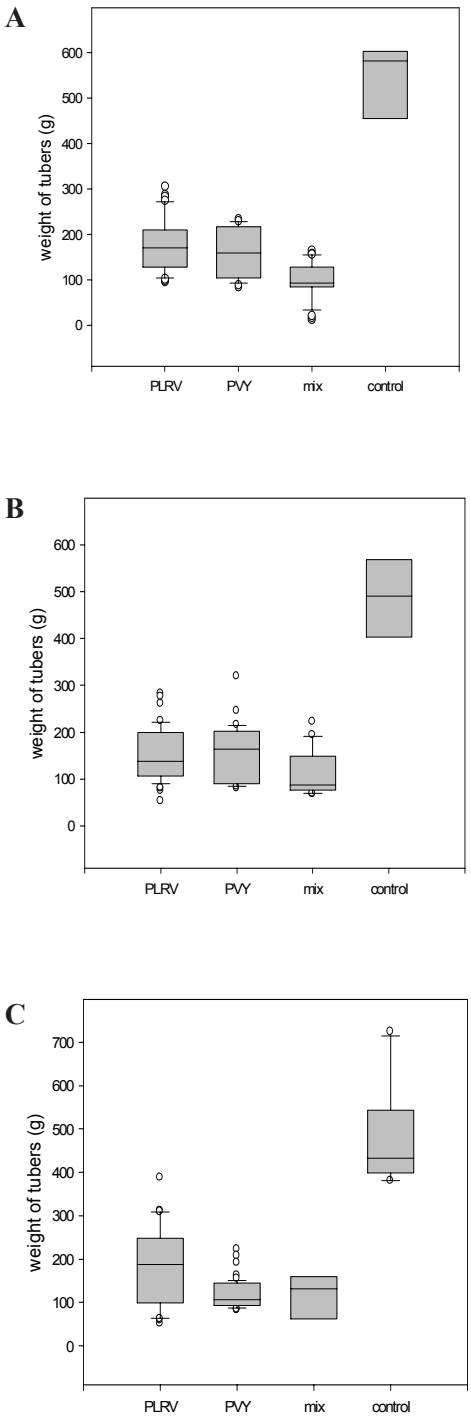

Fig. 10. Influence of viral infections on yield for potatoes of varieties Desiree (10A), Arinda (10B) and Concorde (10C)

Yield from varieties Desiree and Arinda, obtained from plants with mixed infection was lower and significantly different from the yield obtained from plants with monoinfections with PLRV and PVY^n for Desiree and with PLRV for Arinda. For the early variety Concorde, the lowest yield was obtained from plants, infected with PVY^n.

Yield from PLRV-infected plants of the late and medium early variety decreased 3 times. PVY^n is most damaging to the early variety, causing a 4-fold decrease in yield. Yield was lower for plants with mixed infection of all three varieties; the yield decreased 4-5 times, depending on the variety.

Furthermore, PLRV caused a 2-fold decrease in the quantity of tubers for the medium early and the late variety, and PVY^n caused a decrease in the number of tubers for the early variety.

Viral infection not only influences the quantity of yield, but also causes a decrease in the quality of production. The low-weight fraction of tuber with weight under 100g was predominant for infected plants, and no tubers weighing more than 200g were obtained.

Discussion

The monitoring of aphids performed showed that *M. persicae* is the dominant vector of PLRV and PVY^n. The sensitive method ELISA and the biological test allowed us to diagnose the first viruliferous aphids from their appearance and to monitor the virulence of insects through the course of the vegetation period.

The artificially created 5% infectious background, the screening of all plants during the experiment and the serological establishment of latent and mixed infection with the two viruses in plants accounted for precise observation of the dynamics of spread of PLRV and PVY^n.

A strong correlation between the number of trapped alate females from the previous week and the number of infected plants was also observed. Alate females are most essential to the transmission of PVY^n and therefore PVY^n infection in the field is scattered. The above outcomes seem to agree with the ones obtained from Ioannou (1989), Ragsdale et al. (1994) and Katis (1998). Both winged and wingless morphs, participate in the transmission of PLRV. Therefore, plants in close proximity to the source of infection are infected. These results also come to an agreement with the results found by Ribbands, (1965) who proved that PLRV distribution depends on population density of both winged and wingless aphids.

In the course of the vegetation period, the percentage of PLRV-infected plants increased and reached its highest values for varieties Arinda and Desiree during the 30th week. This is due, on one hand, to the high degree of virulence of apterous viviparous aphids, and on the other hand, to the age of plants, which were in the blossoming stage and were therefore preferred by aphids. Meanwhile, the early variety Concorde ended active vegetation and plants exhibited the so called mature resistance. This variety has the shortest vegetation period. Compared to Arinda and Desiree, it develops lower leaf biomass and winged morphs were more rarely observed on it. This leads to the result PLRV-infected plants belonging in this particular variety to be less in number. The maximum of PVY^n infection was monitored during the 28th week for all three varieties. After this period, the percentage of infected plants decreased. This is due to the limited number of vectors, regardless of their high degree of virulence and the preference of winged aphids to feed on infected plants, being attracted by the brighter color of their leaves.

The initial artificially created 5% infectious background for both viruses exhibited 5-fold increase for PLRV on Concorde and more than 6-fold increase for this virus on Arinda and Desiree by the end of the experiment. The early variety Concorde is most susceptible to PVY^n infection. The increase in the number of infected plants was more than 8-fold.

Our studies allowed a precise definition of the in-

fluence of viral infection on yield, which depends on the virus and the variety. Most damages were observed on production from plants with mixed infection, resulting in 4-5 times reduction in yield compared to the yield from healthy plants.

Regarding the existing literature it needs to be mentioned that publications in relation to the influence of mixed infection on yield of potatoes are few. Loebenstain et al. (2001), demonstrated that viral diseases cause greater economic losses in potato production if plants are infected at early growth stages or with mixed infections. Pourrahim et al. (2007), showed that viruses are widespread in Iran and that they are often found in mixed infections.

Conclusion

The outcomes of this specific study enable us to draw the following important conclusions.

In the experimental field of Entomology department of Agricultural University, Plovdiv the main vector of PVY was *M. persicae*. The pick of aphid alates was recorded in the 27th calendar week (4-10 July). The greater percent of infected plants was observed during 28th week.

The number of PLRV-infected plants was greater in the 30th week (25-31 July), three weeks after the pick of alate aphids or a week after the observation of the greater number of wingless morphs.

The initial infectious background of 5% for each virus at the end of vegetation period infection of PLRV is getting greater by 5 fold referring to the variety of Concorde and more than 6 fold referring to medium early Arinda and late Desiree. The early variety Concorde is more susceptible towards infection of PVY - the number of infected plants was more than 8 fold.

PLRV is getting lesser by 3 folds the yield of late variety Desiree and the same happens in the medium early Arinda.

PVY influences more the early variety Concorde. It lessens the yield 4 fold. Mixed infection has been found to influence the yield more than the others, the reduction of the yield is 4-5 fold depending on the variety. Viral infection influences both the quality and quantity of the potato production. Moreover, in infected plants fraction of tubers with less than 100g was greater and tubers with mass more than 200g were absent.

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