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VIRUS INFECTIONS IN NEW PLUM ORCHARDS FROM MOLDOVA, ROMANIA

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Abstract

A survey to assess the phytoviral status of eleven new plum orchards established in Moldova region from Romania was carried out in 2020. Sampling trees were tested by DAS-ELISA for the presence of six viruses: Plum pox (PPV), Prune dwarf (PDV), Prunus necrotic ring spot (PNRSV), Apple chlorotic leaf spot (ACLSV), Apple mosaic (ApMV) and Myrobalan latent ringspot (MLRSV). Ten out of eleven surveyed plum orchards proved to be infected by at least one virus. PPV infections have been present in ten orchards, with a rate between 0.5-79%, which reflects a rather serious situation if we take into account that the orchards are young. Infections with PDV were present in two orchards with a rate between 5-15%, while PNRSV was confirmed in other two orchards with a rate between 15-30%. Overall, the average of infection rate of PPV in the surveyed orchards from Moldova was 19.4%, of PDV was 1.8% and of PNRSV was 4.1%. No infection with ACLSV, ApMV and MLRSV was found in the surveyed orchards.

Key words*: plum, propagation material, survey, DAS-ELISA, viruses.*

INTRODUCTION

The plum has an important economically impact in Romania due to its domination between fruit species (FAOSTAT, 2018).

In most European countries, including Romania, plum is highly affected by Sharka disease, produced by *Plum pox* virus (PPV), known as the most detrimental viral pathogen that affect stone fruits (Barba et al., 2011; Cambra et al., 2006). That because it reduces the quality of the fruits and causes premature dropping, being the most significant factor that limits the plum production (Dunez, and Sutic, 1988; Nemeth, 1994, Stoev et al., 2004). Sharka disease was described for the first time in Bulgaria at the beginning of the 20th century (Atanasoff, 1932), and since then, has progressively spread to a large part of the European continent, being found in America (Chile, Argentina, USA and Canada) and Asia (India, China, Pakistan, Kazakhstan, Iran and Japan) (Capote et al., 2006; García, and Cambra, 2007). In Romania, PPV was found in all plum-growing areas causing serious yield losses (Zagrai et al., 2010).

Other viruses, such as *Prune dwarf* (PDV), *Prunus necrotic ring spot* (PNRSV), *Apple chlorotic leaf spot* (ACLSV), *Apple mosaic* (ApMV) and *Myrobalan latent ringspot* (MLRSV) also might cause direct or indirect damages, as growth reduction, loss of plant vigour, a decrease of quality values, and overall with a negative effect on productive parameters of the crops (Hadidi and Barba, 2011).

A proper management of virus diseases represents a priority in any strategy to limit their damages on the fruit yield. In case of infection with viruses, trees can no longer be treated in the orchard. Therefore, the prevention measures are very important to control virus diseases, such as using resistant cultivars and rootstocks, planting material with virus free status, establishing the new orchards far away from sources of infection, applying treatments against virus vectors. Also, preventing viruses introduction into new area is essential because no eradication by any methods is possible once these pathogens infect an area where trees are growing (Reed, and Foster, 2011). However, there are often situation when the virus infection overcome these prevention measures and escape in the new orchards.

In spite of European regulatory and EPPO standards requested for plant certification, there

are cases with deficiencies in its implementation. In addition, free movement of propagating material within the European Union increases the risk of introduction of new viruses or viral strains in new areas. Thus, new virus outbreaks may occur and can create serious issues in new orchards and, sometimes, even can compromise the investment. Once a virus infection accidentally occurs in the young orchards, removing of the infected trees remain the main measure for limiting the virus spreading. Therefore, early identification of infections in new orchards can sometimes be plum tree life-saving. Thus, the monitoring of viruses in the new orchards, followed by suitable measures for limiting their spreading depending on the specific phytoviral situation observed, may reduce the damage caused by viruses.

New plum orchards were established in the last years by using planting material produced both in Romania and in different European countries. This allowed us to get information about initial virus status of planting material and potential outbreaks by assessing the incidence of the viruses in the young orchards.

MATERIALS AND METHODS

Eleven young plum orchards from Moldova region were the subject of the survey in the summer of 2020. Six of these were established by using propagated material in Romania, and the other five with material from Austria, Czech Republic, Netherlands, Italy and Hungary.

Two blocks with a total of 200 trees (each block of 100 trees) from each orchard were first monitored by visual observation of viral symptoms development. The surveys were mainly focused on typical PPV symptoms on leaves that allowed getting a preliminary evaluation on the incidence of PPV based on the visual observations. Then, ten trees from each block were sampled for virus diagnosis by serological assays, as follow: when PPV incidence based on visual observations was lower than 10%, one symptomatic and nine asymptomatic trees were randomly sampled. When the visual incidence was between 10 and 20%, two symptomatic and eight asymptomatic trees were sampled, and so on, so that when PPV visual incidence was between 90-100%, ten symptomatic trees were sampled. In the case of no symptomatic trees was founded, ten trees were randomly sampled from each block. Because PPV-M, known as the most epidemic strain of PPV was not reported so far in Romania (Zagrai et al., 2010), additional samples with typical PPV symptoms were collected from young orchards established with planting material from abroad in order to check its potential overcoming of the borders (data not show). For virus diagnosis by serological assays a minimum of ten leaves per tree were randomly collected throughout the canopy. In PPV symptomatic trees, only symptomatic leaves were collected. If symptoms were limited to particular branches, leaves were only sampled from symptomatic branches.

A total of 220 trees were sampled for virus diagnosis. Serological tests were performed by Double Antibody Sandwich - Enzyme Linked Immunosorbent Assay (DAS-ELISA) (Clark and Adams, 1977) using a commercial polyclonal antiserum to PPV, PDV, PNRSV, ACLSV, ApMV (Bioreba, Switzerland), and MLRSV (Sediag, France) according to the manufacturer's instructions. Absorbance values were measured at 405 nm after 1 h substrate hydrolysis. Samples were considered positive if their absorbance values were more than twice those of the negative control. Then, a rate of infection was established for each virus.

If was the case, the nearby plum orchards (1-200 m) were visually checked and has been established the incidence of PPV based on the observed typical symptoms in order to check the potential presence of nearby outbreaks/sources of infection (data not show).

RESULTS AND DISCUSSIONS

The results of plum young orchards surveyed for the presence of viruses by visual monitoring and serological assays are show in Table 1.

The data collected revealed that ten out of eleven orchards have viral infections with at least one virus, *Plum pox virus* infections being the most troublesome.

Although the level of infection rate was different from one orchard to another, in some cases it was quite similar. Thus, six orchards showed a PPV infection rate between 0.5-10%,

two orchards between 11-20%, and the other two between 71-80%. The serological analyses against ACLSV, ApMV and MLRSV revealed that these viruses were not present in none of the samples tested.

Plum orchards established with planting material produced in Romania (no. 1, 3, 4, 7, 8 and 11). PPV was visually observed by symptoms development, and then infection was confirmed by serological tests in all six plum orchards, with an incidence between 6% and 79%. PDV was found in two out of six surveyed orchards, with an incidence between 5% and 15%, and PNRSV in one orchard, with an incidence of 30%.

A high rate of PPV infection (73% and 79%, respectively) founded in two orchards (no. 3. Scobinti and no. 4. M. Bucium) correlated with the young age of the orchards, and also a virus spreading throughout the canopy suggests that most part of the planting material was provided as infected from the nurseries. This hypothesis is also supported by the fact that an external source of the inoculum was observed at a distance of over 200 m, and it was represented only by isolated trees in family gardens that could not contribute to such critical situation. These orchards represent an outbreak and a source of PPV for new potential orchards set up around it. Applying a PPV eradication strategy in such cases would mean the destroying of such orchards. This radical measure is not in agreement with the orchard owners' management expectation because of the missing of governmental compensation. Thus, this kind of recommendation is not acceptable and remained just theoretically.

Table 1. Incidence rate of viruses based on visual observation and DAS-ELISA assay on eleven plum orchards from Moldova region

Orchard no./ location (county code)	Provenance of plant material	Visual rate of PPV (%)	Virus incidence (%) based on serological tests (DAS-ELISA)					
			PPV	PDV	PNRSV	ACLSV	ApMV	MLRSV
1. Itcani (BC)	Romania	23.5	23.5	0	0	0	0	n/a
2. Plopana (BC)	Italy	0.5	0.5	0	15	0	0	n/a
3. Scobinti (IS)	Romania	79	79	0	0	0	0	0
4. M. Bucium (IS)	Romania	78	73	5	0	0	0	0
5. Podul Iloaiei (IS)	Austria /Czech Rep.	2.5	2.5	0	0	0	0	0
6. Padureni (IS)	Netherlands	0.0	0	0	0	0	0	0
7. Icusesti (NT)	Romania	12.5	15.5	15	0	0	0	n/a
8. Husi (VS)	Romania	6	6	0	0	0	0	n/a
9. Husi (VS)	Hungary	0.5	0.5	0	0	0	0	n/a
10.Grumezoaia (VS)	Hungary	7	7	0	0	0	0	n/a
11. Crasna (VS)	Romania	6	6	0	30	0	0	n/a
Average		19.6	19.4	1.8	4.1	0	0	0

Two other orchards (no. 7. Icusesti and no. 1 Itcani) revealed a rate of PPV of 15.5% and 23.5% respectively. In both situations, the overwhelming proportion of trees with widespread infections throughout the canopy suggests a high probability that part of the planting material has been infected since the plum nurseries. Also, the presence of trees with partly infections revealed the potential occurring PPV both from infected trees within the orchard, and from its vicinity. In these cases, the removing of infected trees in order to limit the impact of PPV is not an economical solution for the owners. Given that the orchards have varieties that tolerate the PPV damages on fruits, the plantation can be economically profitable, but its profitability will be greatly diminished.

The lower rate of PPV infection was recorded only on two orchards established with planting material propagated in Romania, with an infection rate of 6% (no. 8 Husi and no. 11. Crasna). The occurred PPV infections throughout the canopy of infected trees,

correlated with the absence of nearby sources of infection and the young age of the plantations (2-3 years), suggests that the planting material has been infected since the plum nurseries. In these cases, it was recommended to extend the monitoring of orchards, to eliminate the trees that show typical symptoms of the *Plum pox virus* and to replace them with healthy trees. Monitoring these orchards in the following years to eliminate any subsequently infected trees and applying additional treatments to control aphid vectors could significantly contribute to the containment of PPV spreading and its impact in these young plum orchards.

Plum orchards established with planting material produced in other European countries (no. 2, 5, 6, 9 and 10). Four out of five orchards were found to be infected by PPV with an incidence between 0.5% and 7%, and one orchard was founded with a rate of 15% infection by PNRSV.

Two of these orchards (no.2 Plopana and no. 5. Podu Iloaiei) are located in the proximities of trees infected by PPV. Thus, the infections in the new two orchards were most likely caused by the presence of scattered trees in the vicinity that have facilitated the transmission of the virus especially because they did not receive any treatments to control aphid vectors. This is also supported by the fact that the infected trees in the two orchards developed PPV sporadical symptoms, just on a few shoots, the most part of the canopies remaining symptomless.

The other two orchards (no. 9. Husi and no. 10. Grumezoaia) are very well isolated for external PPV sources being located far away from any potential host for this virus. The fact that one or two years old trees showed PPV symptoms throughout canopy, since no infection sources in the vicinity of orchards were present, suggests that the infected trees acquired the virus before planting.

Overall results revealed that ten out of eleven young plum orchards surveyed in Moldova region were found infected by at least one virus. One orchard (no. 6. Padureni) did not express any symptoms and did not confirm any virus by serological tests, thus proved to be virus free (Figure 1).

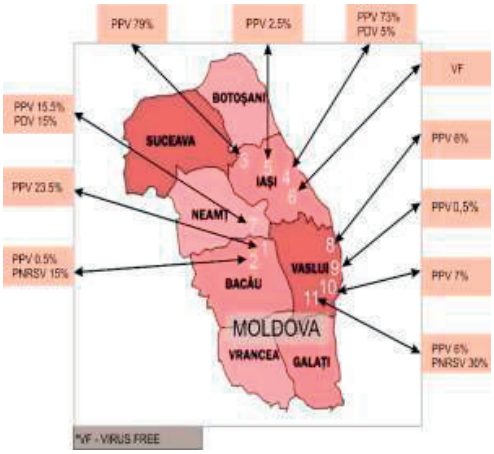

Figure 1. The map of virus incidence on young plum orchards from Moldova region

The average virus incidence at the Moldova region level in the surveyed plum orchards were as follow: 19.4% of PPV, 1.8% of PDV, and 4.1% of PNRSV. ACLSV, ApMV and MLRSV was not confirmed in any young plum orchards surveyed in Moldova region (Figure 2).

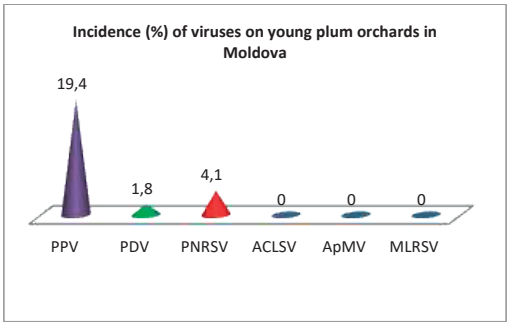

Figure 2. The incidence of viruses in young plum orchards from Moldova

Interestingly, the comparative analysis of the results revealed insignificant differences between the results obtained based on visual observation and serological assays related to *Plum pox virus* in young plum orchards (19.6%, respectively 19.4%). This finding has a practical importance because the owners can proceed themselves periodically surveys by visual monitoring of PPV with the condition to know how the virus express typical symptoms especially on leaves.

Given that PPV is a virus that causes a disease with a major economic impact, farmers' awareness of personal involvement in the early identification of this virus in orchards can significantly contribute to limiting of PPV, of course alongside of other measures depending of the situation.

CONCLUSIONS

Ten out of eleven plum new orchards from Moldova region proved to be infected by at least one virus by using serological assay. PPV was the prevalent virus, followed by PNRSV and PDV. No infections with ACLSV, ApMV and MLRSV were found.

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REFERENCES

Atanasoff, D. (1932). Plum pox. A new virus disease. *Yearbook University of Sofia, Faculty of Agriculture and Silviculture,* 11: 49- 69.

Barba M., Hadidi A., Candresse T., Cambra M. (2011). *Plum pox* virus. In A. Hadidi (Ed.), *Virus and Virus-Like diseases of Pome and Stone Fruits*. (pp. 185-197). St. Paul, Minnesota: The American Phytopathologycal Society.

Cambra M., Capote N., Myrta A., Llacer G. (2006). *Plum pox* virus and the estimated costs associated with sharka disease. *EPPO Bull*., 36:202-204.

Capote, N., Cambra, M., Llácer, G., Petter, F., Platts, L.G., Roy, A.S., Smith, I.M. (2006). A review of Plum pox virus/Une revue du Plum pox virus. *Bulletin OEPP/EPPO Bull.,* 36(2): 201-349.

Clark, M.F & Adams, A.N. (1977). Characteristic of the microplate method of enzyme linked immunosorbent assay (ELISA) for detection of plant viruses. *J. Gen. Virology,* 34: 475-483.

Dunez, J. and D. Sutic. (1988). Plum pox virus. In: *European handbook of plant disease*, (pp. 44-46). Eds. I.M.Smith, J. Dunez, R.A.Eliot, D.H.Phillips, S.A.Arches. Blackwell, London, UK.

García, J.A. & Cambra, M. (2007). Plum pox virus and sharka disease. *Plant Viruses,* 1: 69-79.

Hadidi A. & Barba M. (2011). Economic impact of pome and stone fruit viruses and viroids. In A. Hadidi (Ed.), *Virus and Virus-Like diseases of Pome and Stone Fruits.* (pp. 1-7). St. Paul, Minnesota: The American Phytopathologycal Society.

Nemeth, M. (1994). History and importance of plum pox in stone-fruit production. *EPPO Bull*., 24: 525-536.

Reed, P.J. & Foster, J.A. (2011). Exclusion of Pome and Stone Fruit Viruses, Viroids, and Phytoplasmas by Certification and Quarantine. In A. Hadidi (Ed.), *Virus and Virus-Like diseases of Pome and Stone Fruits.* (pp. 381-388). St. Paul, Minnesota: The American Phytopathologycal Society.

Stoev, A., P. Iliev, M. Milenkov (2004). Sharka (plum pox) disease-an eternal challenge. *European Meeting '04 on Plum Pox, Skierniewice, Poland, Book of Abstracts,* 2004:14.

Zagrai I., Zagrai L., Kelemen B., Petricele I., Pamfil D., Popescu O., Preda P., Briciu A. (2010). Typing and distribution of *Plum pox* virus isolates in Romania. *Julius-Kuhn-Archiv*, 427: 342-346.

FAOSTAT, 2018. http://www.fao.org/faostat/en/#data/QC.

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