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IS SYMPTOMATOLOGY A RELIABLE TOOL FOR PLUM POX VIRUS MONITORING?

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Abstract

Sharka disease, caused by Plum pox virus (PPV), represents an important economically issue of stone fruits growers in Romania. Establishing plum orchards with PPV-free planting material, followed by their virus monitoring and removing infected trees, can contribute to PPV containment. Although PPV monitoring based on symptoms developed combined with serological or molecular assays is recommended for accurate virus detection, such approach is not costly effective in orchards. Therefore, there is under question whether a well recognizing of PPV symptoms developed by infected plum trees can be an acceptable tool for virus monitoring in orchards. To get this information, twenty-seven plum orchards comprising a large assortment of cultivars were surveyed. A total of 540 samples were tested by DAS-ELISA and compared with results of visual observation. Overall results revealed a high coincidental data of PPV infection established by serological detection and virus-based symptoms, suggesting that a good knowledge of PPV symptoms developed by infected trees on leaves could be a reliable tool for virus monitoring large areas of plum orchards.

Key words*: DAS-ELISA, plum, PPV symptoms, Sharka disease, survey.*

INTRODUCTION

Symptoms represent the effect of viruses on growth and development of plants, and are defined as perceptible changes in the functions of plant host (Bos, 1970). In the present, viral pathogens can be identified very precisely by many high sensitivity techniques. However, in cases of some viruses, external symptoms represent the first sign of the presence of pathogen into a plant. Obviously, not all viruses express themselves through symptoms; some of them remain latent in plant for a while. When a virus causes significant changes in plants that affect growth or production it is considered economically important (Hull, 2004). This is the case of Sharka disease, caused by Plum pox virus (PPV), an important economically issue of stone fruits in many European countries (Barba et al., 2011; Cambra et al., 2006), including Romania (Minoiu, 1997; Zagrai et al., 2010). Sharka has been spreading for more than a century in different parts of the world, from Bulgaria (Atanasoff, 1932) to Mediterranean basin, Middle East, Western Europe, Asia, Africa (Egypt, Tunisia), North (Canada) and South (Chile, Argentina) America (Roy and Smith, 1994; Barba et al., 2011) becoming a global concern. PPV is one of the ten most widespread plant viruses in the world (Scholthof et al., 2011). Plum is one of the most stone fruit specie affected by Plum pox virus, the trees once infected often produce typical symptoms more or less visible on leaves and fruits. Usually, PPV express symptoms on leaves, fruits and seeds of susceptible stone fruits cultivars. The symptoms of PPV developed on leaves consist in chlorosis (pale rings, spots more or less diffuse), vein yellowing or leaf mottling, necrotic ring patterns, yellowish to olive green spots or bands (Levy et al., 2000; Llácer and Cambra, 2006; Zagrai and Zagrai, 2024). There are some plum cultivars infected by PPV that do not exhibit any symptoms on fruits, such as Blue free, Opal, Stanley (Hamdorf, 1986), Čačanská Rana (Zawadzka et al., 1998), Čačanská Najbolja, Opal, Hanita, Tuleu timpuriu (Paprstein et al., 2007). However, most of the plum cultivars susceptible to PPV are strongly affected by virus infection leading often to premature dropping of fruits, but also to fruit deformation, irregular rings or lines on skin and/or in pulp, scars, necrosis and gummosis, making the fruits

unsuitable for consumption (Kamenova et al., 2010).

To get a profitable plum orchard, farmers must pay a special attention to PPV management. No doubt that the using PPV resistant cultivars is the most efficient strategy for its control (Ravelonandro et al., 2011; Scorza et al., 2013). Since the scarcity of resistant plum cultivars is still an obstacle in implementation of such strategy, the other prevention measures should not be neglected. Thus, PPV requires a special attention starting with the placement of the new orchards as far as possible from potential sources of virus infection. Then, the new established orchards by using PPV-free planting material, followed by their monitoring and removing infected trees in the first 5 years after planting is recommended (Zagrai et al., 2022). All these prevention methods can substantially contribute to reducing the PPV spreading, and consequently its economically impact.

Although PPV monitoring based on symptoms developed combined with serological or molecular diagnosis is known as a reliable tool, such strategy is not costly effective for virus monitoring in orchards and became not accessible for farmers. However, a good knowledge of PPV symptoms, developed especially on leaves, could allow any farmers to take action for limiting the spread and potential increasing damages in the own orchard. Therefore, there is under question whether a well recognizing of PPV typical symptoms developed by infected plum trees can be an acceptable tool for virus monitoring within the orchards.

MATERIALS AND METHODS

Field surveys

Since Sharka disease is considered the main limiting factor in the profitability of plum crops through the significant damages it causes in endemic areas, the field surveys were focused on typical PPV symptoms on leaves that allowed a preliminary assessment of PPV occurrence based on visual observations. Twenty-seven young plum orchards from ten counties of Romania comprising a large assortment of plum cultivars were surveyed during May-June of 2020 to assess the PPV viral status based on typical PPV symptoms on leaves in comparison with serological detection. Two blocks of one hundred trees each were delineated in diagonal within each orchard according to Figure 1, covering the entire range of cultivars.

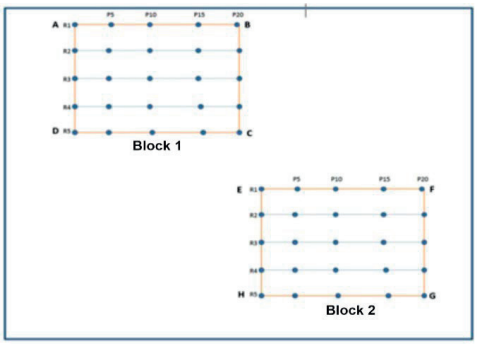

Figure 1. The design of blocks inside of plum orchards

A depth control was carried out within the blocks, each tree being checked for the presence of typical symptoms of PPV that could suggest a potential viral infection (Figure 2). Then, the incidence of PPV was determined based on visual observations of symptoms developed.

Figure 2. Example of typical PPV symptoms on plum leaves

Serological assay

Ten trees from each block were sampled for PPV detection using serological assays, as follows: if the visual incidence of PPV within the block was below 10%, one symptomatic sample and nine symptomless samples were taken; when the visual incidence of PPV was between 10% and 20%, two symptomatic and eight symptomless samples were taken; and so on, if the visual incidence was between 80%

and 90%, nine symptomatic samples and one symptomless sample were taken; and if the visual incidence was between 90% and 100%, only symptomatic samples were taken. Each sample consisted of a minimum ten leaves. The samples were transported under appropriate conditions to the laboratory, kept in the refrigerator and analysed within 1-5 days or stored at minus 24°C for maximum 30 days and subsequently subjected to laboratory testing.

Serological assays for PPV detection were performed by Double Antibody Sandwich - Enzyme Linked Immunosorbent Assay (DAS-ELISA) (Clark and Adams, 1977) using commercial polyclonal antiserum against PPV (Figure 3), according to the manufacturer's instructions (Bioreba, Switzerland).

Absorbance values were measured at 405 nm after 1h substrate hydrolysis. In the cases when absorbance values were more than twice of negative control, samples were considered positive. The samples were also tested for other viruses (data not shown).

Figure 3. Aspects of DAS-ELISA test at FRDS Bistrița

PPV incidence

PPV infections in the plum orchards were determined by correlating the preliminary assessment based of visual observations by the presence of typical PPV symptoms on leaves with the results obtained by serological diagnosis. When PPV infections based on visual monitoring were confirmed by serological diagnosis, PPV infection was established based on visual symptoms. When correlations between visual and serological data were only partially correlated an adjustment was made according to serological results.

RESULTS AND DISCUSSIONS

Field surveys

The orchard number, location, cultivars and the age of plum trees taken in this study are shown in the Table 1. The PPV incidence determined by visual observation revealed that only one out of twenty-seven plum orchards showed no PPV symptomatic tree (orchard no. 22). In the other orchards the PPV incidence based on symptoms developed varied between 0.5% (orchard no. 25) and 78% (orchard no. 20). Thereby, in nine orchards (no. 12, 14, 16, 18, 21, 24, 25, 26, 27) the PPV incidence determined by visual observations was between 0.5-10%, six orchards revealed an incidence of PPV between 11-20% (no. 1, 2, 3, 4, 10, 23), three orchards have recorded a rate of PPV between 21-30% (no. 7, 15, 17), one orchard had PPV incidence between 31-40% (no. 13), other one orchard had a PPV rate between 51-60% (no. 11), three orchards recorded an incidence of PPV between 61-70% (no. 5, 8, 9) and three orchards revealed an infection with PPV between 71-80% (no. 6, 19, 20).

Serological assay correlated with visual monitoring

All PPV symptomatic samples with one exception coming from orchard no. 20 confirmed the presence of PPV by DAS-ELISA. In this orchard, sixteen out of twenty collected samples developed symptoms suggesting a PPV infection, while the other four samples were symptomless. Thus, the incidence of PPV in the orchard no. 20, based on symptoms developed, was determined at 78% (Table 1). DAS-ELISA tests revealed that one symptomatic sample was not the result of PPV infection, but with other virus (data not shown). Consequently, the PPV infection rate in this case was recalculated at 73%. However, it should be noted that in the orchard no. 20 there is concordance of 94% between PPV incidence determined by visual observation of symptoms developed and serological results.

When symptomless trees were tested, only two cases of non-coincidental data were found. Precisely, one symptomless sample collected

from orchard no. 8 and the other one from orchard no. 23 were found infected with PPV, while the others confirmed the PPV-free status. Thus, the PPV incidence in the case of orchard no. 8 was determined at 68.5% when considering visual monitoring, while the adjusted results based on serological assays revealed a PPV incidence at 73%, so the concordance between symptomatology and serological results was calculated at 94%.

Table 1. The incidence of PPV in young plum orchards from Romania

No.	County	Location	Cultivar	Age of trees	PPV incidence (%) based on symptoms	PPV incidence (%) based on DAS-ELISA	Concordance of PPV incidence determined by symptomatology and DAS-ELISA (%)
1	Bihor	Buduslau	Topend plus, Jofela	3	16	16	100
2	Bistrita-Nasaud	Dumitra	Stanley, President, C. Lepotica	1	19	19	100
3	Bistrita-Nasaud	Jelna	Stanley, Topend plus	1	14	14	100
4	Bistrita-Nasaud	Ciceu-Mihaiesti	Stanley, Topend plus	4	16.5	16.5	100
5	Cluj	Cluj-Napoca	Topend	6	61.5	61.5	100
6	Cluj	Cluj-Napoca	Topend	6	77.5	77.5	100
7	Cluj	Tritenii de Sus	Stanley, D'Agen	4	24	24	100
8	Hunedoara	Turdas	Anna Spath, Stanley	3	**68.5**	**73**	**94**
9	Hunedoara	Brad	Tuleu gras, Stanley	3	61	61	100
10	Hunedoara	Ribita	Tuleu gras, Stanley, Anna Spath	1-3	15.5	15.5	100
11	Mures	Reghin	Haganta, President, Blue free	5	50	55	100
12	Mures	Reghin	Tophit, Cacak	5	5	5	100
13	Satu-Mare	Cehal	Stanley, C. Lepotica, Blue free, Centenar	1	39	39	100
14	Satu-Mare	Cehal	Stanley, C. Lepotica	3	9.5	9.5	100
15	Satu-Mare	Sacaseni	Stanley	2	24	24	100
16	Satu-Mare	Sacaseni	Stanley	2	1	1	100
17	Bacau	Itcani	Stanley, Anna Spath, D'Agen, Centenar	4	23.5	23.5	100
18	Bacau	Plopana	Stanley, President	1	0.5	0.5	100
19	Iasi	Scobinti	Stanley, D'Agen, Renclod Althan, Centenar	4	79	79	100
20	Iasi	Iasi-Bucium	Stanley, D'Agen, Centenar	8	**78**	**73**	**94**
21	Iasi	Podu Iloaiei	Tophit, Haganta, Hanita, Topend plus	2	2.5	2.5	100
22	Iasi	Popesti-Padureni	Victoria, Opal	4	0	0	100
23	Neamt	Icusesti	Stanley, Centenar, Dorin, Dambovita	3	**12.5**	**15.5**	**81**
24	Vaslui	Husi	Stanley, President, C. Lepotica	2	6	6	100
25	Vaslui	Husi	Blue free	2	0.5	0.5	100
26	Vaslui	Grumezoaia	Stanley, President, Grossa di Felisio	1	7	7	100
27	Vaslui	Crasna	Stanley, Anna Spath, D'Agen, Centenar	3	6	6	100

In a similar way, 81% concordance between PPV incidence determined by visual symptoms and serological results was calculated at orchard no. 23. It should be highlighted that a perfect coincidental PPV incidence determined by visual observation of typical PPV symptoms and virus detection by DAS-ELISA was established in most of the surveyed orchards (24 orchards out of 27). Moreover, the average rate of PPV infection in all 27 plum orchards (Figure 4) settled by visual inspections (27.6%) was similar with that determined considering DAS-ELISA test (27.7%).

Although there were sporadically cases when PPV infections were not found by both methods, it can be highlighted that the differences between PPV incidences assessed by visual observations and serological tests are insignificant. It should be mention that the field observations were made just one time during the vegetative period and hence a possible missing of symptoms developed by some infected cultivars in such period. Therefore, by increasing the number of surveys, it could reduce even such discrepancy.

Overall results revealed a high coincidental data of PPV infection established by visual monitoring of typical PPV symptoms developed on leaves and considering serological detection (Figure 5). This suggests that a good knowledge of PPV symptoms developed by infected plum trees on leaves could be used as a reliable tool for virus monitoring of large areas of plum orchards. Such monitoring can be very useful and accessible to any farmers interested to limit the PPV impact in their orchards by getting knowledge to virus symptoms expressed by plum cultivars. However, such approach is not at all recommended in activities related to plum propagation.

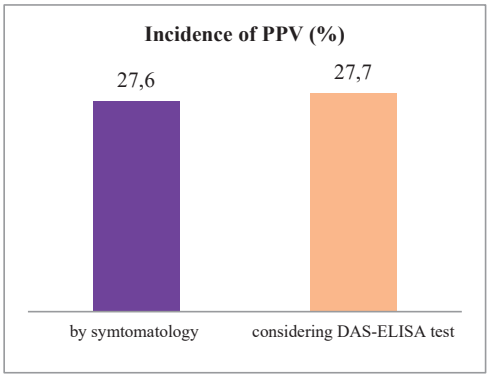

Figure 4. The average incidence of PPV (%) based on symptomatology versus taking into account serological results

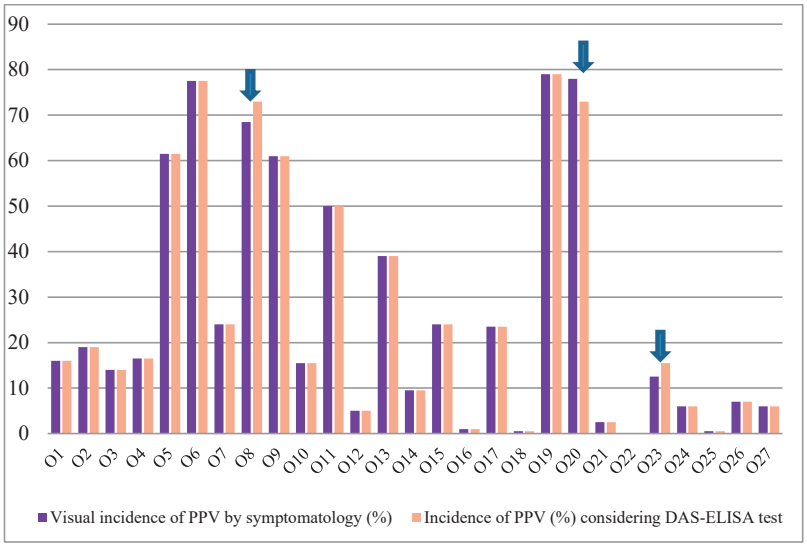

Figure 5. Comparison of PPV infection rate based on symptomatology versus serological results within each surveyed orchard

CONCLUSIONS

A high coincidental data of PPV infection was determined by serological detection and virus-based symptoms. Consequently, a well recognizing PPV symptoms developed by infected plum trees could be a reliable tool for PPV monitoring within the orchards, especially by farmers. If properly trained, they are the first that could identify potential issues of orchards, and to take proper measures in order to limit the spread of this economical important virus inside of orchard and out of it, to other proximal trees.

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