ANNALS OF THE UNIVERSITY OF CRAIOVA

Series: ✔*Biology*
✔*Horticulture*
✔*Food products processing technology*
✔*Environmental engineering*

Vol. XXVIII (LXIV) - 2023

DETECTION OF PLUM AND CHERRY VIRUSES IN THE SOUTHERN PART OF ROMANIA

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Keywords: *virus, fruit, tree, ELISA*

ABSTRACT

Plum plantations which included 'Anna Späth', 'd'Agen', 'Stanley', 'Čačanska Lepotiča', 'Centenar', 'President', 'Topend plus' and 'Tophit' cv. and sweet cherry plantations with the 'Kordia', 'Skeena', 'Ferrovia', 'Sweetheart', 'Regina', 'Prime Giant', 'Stella', 'Van', 'Maria', 'Bigarreau Burlat', 'Vanda', 'Karina', 'Lapins', 'Penny', 'Summit' cv., from the areas of Muntenia and Oltenia, Romania, were evaluated to identify the main viruses ACLSV, ApMV, PPV, PDV, PNRSV for plum and ACLSV, ApMV, ArMV, PPV, PDV, PNRSV, CLRV, TBRV, SLRSV, RpRSV for sweet cherry. The diagnosis was established by the DAS-ELISA technique, PPV being also identified by the AGRISTRIP method. The degree of viral infection was different, thus, in plum, PPV was identified between 0.00% and 57.50%, ApMV between 0.00% and 1.50% and PDV between 0.00% and 0.5% and in sweet cherry plantations TBRV between 0 and 20.00%, ArMV 0.00% and 15.00% and ACLSV 0.00% and 5.00%.

INTRODUCTION

The stone fruit species are hosts for a large number of viruses that can cause substantial economic losses (Nemeth 1986; Desvignes 1999; Myrta et al. 2003). The diagnosis is the most important aspect of the control of the fruit trees viruses.

Early detection of viruses in fruit trees or propagation material is a prerequisite for their control and to guarantee a sustainable agriculture (Barba et al. 2014).

For health safety, current legislation (O.M. 784/2016, O.M. 119/2020, O.M. 40/2023) requires testing for the diagnosis of an important number of plum viruses: *Apple chlorotic leaf spot virus (ACLSV), Apple mosaic virus (ApMV), Myrobalan latent ringspot virus (MLRSV), Plum pox virus (PPV), Prune dwarf virus (PDV), Prunus necrotic ringspot virus (PNRSV), European stone fruit yellows phytoplasma (ESFYP)* and for sweet cherry: *Apple chlorotic leaf spot virus (ACLSV), Apple mosaic virus (ApMV), Arabis mosaic virus (ArMV), Cherry green ring mottle virus (CGRMV), Cherry leaf roll virus (CLRV), Cherry mottle leaf virus (CLMV), Cherry necrotic rusty mottle virus (CNRMV), Little cherry virus 1 (LCHV 1), Little cherry virus 2 (LCHV 2), Plum pox virus (PPV), Prune dwarf virus (PDV), Prunus necrotic ringspot virus (PNRSV), Raspberry ringspot virus (RPRSV), Strawberry latent*

ringspot virus (SLRSV), Tomato black ring virus (TBRV), Candidatus phytoplasma prunorum (ESFYP). All these viruses can seriously affect production yield and tree development (Uyemoto et al. 1992).

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 & Foster 2011).

MATERIALS AND METHODS

Ten plum plantations aged between 1-13 years and ten sweet cherry plantations aged between 2-12 years from Muntenia and Oltenia areas were the subject of the survey in the summer of 2020 respectivelly 2021.

Plum included the cultivars 'Anna Späth', 'd'Agen', 'Stanley', 'Čačanska Lepotiča', 'Centenar', 'President', 'Topend plus' and 'Tophit' and sweet cherry plantations consisting of the cultivars 'Kordia', 'Skeena', 'Ferrovia', 'Sweetheart', 'Regina', 'Prime Giant', 'Stella', 'Van', 'Maria', 'Bigarreau Burlat', 'Vanda', 'Karina', 'Lapins', 'Penny', 'Summit'.

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.

For plum the tested were for *Apple chlorotic leaf spot virus (ACLSV), Apple mosaic virus (ApMV), Myrobalan latent ringspot virus (MLRSV), Plum pox virus (PPV), Prune dwarf virus (PDV), Prunus necrotic ringspot virus (PNRSV), European stone fruit yellows phytoplasma (ESFYP)* and for sweet cherry: *Apple chlorotic leaf spot virus (ACLSV), Apple mosaic virus (ApMV), Arabis mosaic virus (ArMV), Cherry green ring mottle virus (CGRMV), Cherry leaf roll virus (CLRV), Cherry mottle leaf virus (CLMV), Cherry necrotic rusty mottle virus (CNRMV), Little cherry virus 1 (LCHV 1), Little cherry virus 2 (LCHV 2), Plum pox virus (PPV), Prune dwarf virus (PDV), Prunus necrotic ringspot virus (PNRSV), Raspberry ringspot virus (RPRSV), Strawberry latent ringspot virus (SLRSV), Tomato black ring virus (TBRV), Candidatus phytoplasma prunorum (PHYPPR).*

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. If symptoms were limited to particular branches, leaves were only sampled from symptomatic branches.

Serological tests were performed by Double Antibody Sandwich - Enzyme Linked Immunosorbent Assay (DAS-ELISA) (Clark & Adams 1977) using a

commercial polyclonal antiserum (Bioreba, Switzerland), and according to the manufacturer's instructions.

The intensity of the color reaction, which measures the amount of specific antibodies bound to the antigens present in the serum to be investigated, was determined photometrically. Positive samples were those that had the value of extinction at least 2.5 times higher than the average of negative witnesses. The absorbance and cut-off value are measured in nanometers. The readings were made at MICROPLATE READER. 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 obtained from the evaluation of the 10 plum plantations in the Oltenia and Muntenia regions highlighted the presence of the *PPV* virus in 7 plantations with values ranging from 0.5% to 57.5% infected trees (Table 1).

From the data obtained through different diagnostic methods, it can be seen that the methods used confirmed the existence of viral infections, the identification through visual observations being confirmed in the case of the *PPV* virus by the ELISA serological method and the AgriStrip immunochromatographic method. A low presence of 0.5% was diagnosed for *ApMV* viruses in the Vâlcea plantation and *PDV* in the Argeș 2 plantation.

If we analyze the situation of plum plantations from the point of view of factors: age, no. administered treatments, biological category and source of planting material (Table 2), it is observed that the lack of phytosanitary treatments had a very large influence on the degree of infection. The vector insects in this case led to the diagnosis of a percentage of 30.50% - 59.00% in the Teleorman 1 and Teleorman 2 plantations where no treatments were done for several years.

The two plantations were 8 years old compared to 13 years for the plum plantation in Buzău, where 4 phytosanitary treatments were administered in the evaluation year until the time of sampling and the proportion of virally infected trees was only 3%. On the other hand, the biological category and the origin of the planting material had a visible influence. With the exception of the previously analyzed Teleorman 1 and Teleorman 2 plantations, an increase in the health quality of the material analyzed in the Certified category compared to CAC and that produced in authorized nurseries can be observed, which is in line with both the current legislation and the recommendations of specialists (Coman M. et al. 2022; Zagrai I. et al. 2022).

The Vâlcea plantation and the Dolj plantation have infections of 20.50% and 23.00%, being established with CAC material at an age of only 4 years. The planting material from the Vâlcea plantation being produced by an amateur, the classification as CAC being even excessive.

Likewise, PDV was detected in the Argeș 2 plantation, the plantation being in year 2, the *PDV* virus is transmitted through the seed, which indicates that the planting material was infected from the nursery.

Table 1

Results of viral evaluation in plum orchards

Location cod	Orchard age (years)	Assortment	Visual symptoms	DAS-ELISA confirmation	AGRISTRIP confirmation of *PPV*
Dolj	4	Anna Späth, d'Agen, Stanley, Čačanska Lepotiča, Centenar	23.00% typical *PPV*	23.00% *PPV*	23.00% *PPV*
Olt	2	Stanley	0.50% typical *PPV*	0.50% *PPV*	0.50% *PPV*
Vâlcea	4	Centenar, Stanley, Anna Späth, d'Agen	20.00% typical *PPV*; 0.50% other symptoms	20.00% *PPV;* 0.50 % *ApMV*	20.00% *PPV*
Argeș 1	7	President, Stanley, Anna Späth	11.00% typical *PPV*	11.00% *PPV*	11.00% *PPV*
Argeș 2	2	Topend plus, Tophit	0.50% other symptoms	0.50% *PDV*	0.00 %
Prahova	1	Stanley, Anna Späth, Centenar	0.00	0.00	0.00 %
Buzău	13	Anna Späth, Stanley	3.00% typical *PPV*	3.00% *PPV*	3.00% *PPV*
Călărași	2	Stanley	0.00	0.00	0.00 %
Teleorman 1	8	Anna Späth, Stanley	57.50% typical *PPV;* 1.50 % other symptoms	57.50% *PPV*	57.50% *PPV*
Teleorman 2	8	Stanley	29.50% typical *PPV*; 1.00 % other symptoms	29.50% *PPV*	29.50% *PPV*

Figure 1. Nunc MaxiSorp plate preparation with antigen

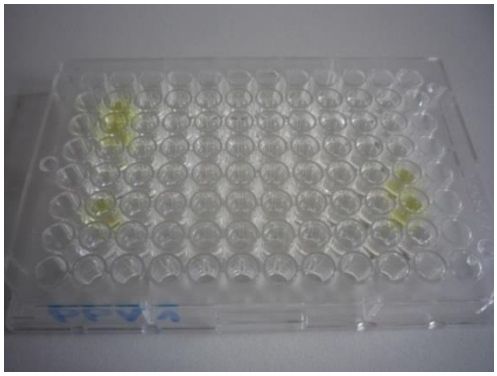

Figure 2. Highlighting the blank and positive samples by the appearance of yellow color

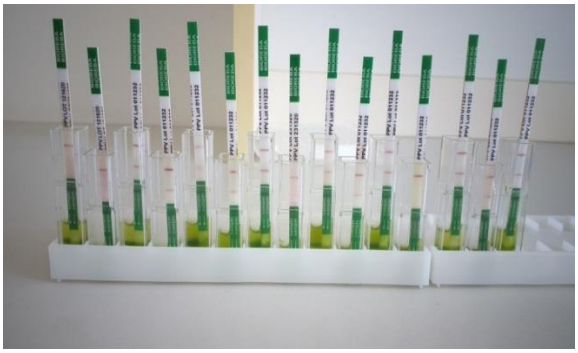

Figure 3. Confirmation of positive samples with *PPV* by immunochromatography AgriStrip test based on lateral flow

Table 2

The incidence of viral infections according to the age of the plantation, the number of treatments performed and the origin of the plum planting material

Location cod	Orchard age (years)	No. of treatments	Biological category	Source of planting material	% viral infected plants
Dolj	4	3	CAC	Authorized nursery RO	23.00 %
Olt	2	-	CAC	Authorized nursery RO	0.50 %
Vâlcea	4	3	CAC ?	Particular person (amateur) RO	20.50 %
Argeș 1	7	5	CAC	Authorized nursery RO	11.00 %
Argeș 2	2	3	CAC	Authorized nursery RO	0.50 %
Prahova	1	2	Certificate	Authorized nursery RO	0.00 %
Buzău	13	4	Certificate	Authorized nursery RO	3.00 %
Călărași	2	8	Certificate	Authorized nursery RO	0.00 %
Teleorman 1	8	-	Certificate	Authorized nursery RO	59.00%
Teleorman 2	8	-	Certificate	Authorized nursery RO	30.50 %

Regarding the state of health from the viral point of view of the sweet cherry plantations (Table 3) evaluated, the presence of 3 viruses *TBRV, ArMV* and *ACLSV* was found. *TBRV* was identified in 2 plantations in a percentage of 20.00% in the Argeș plantation (A) and 5.00% in the Ilfov plantation, a plantation in which the viruses *ArMV* 15.00% and *ACLSV* 5.00% were also detected. Symptoms did not appear in all diseased plants.

Table 3

Results of viral evaluation in cherry orchards

Location cod	Orchard age	Assortment	Visual symptoms	DAS-ELISA confirmation	AGRISTRIP confirmation of *PPV*
Dolj 1	2	Kordia, Regina	without symptoms	-	-
Dolj 2	2	Regina	without symptoms	-	-
Argeș (A)	12	Kordia, Skina, Ferrovia	without symptoms	20.00% *TBRV*	-
Argeș (B)	4	Burlat, Vanda, Karina, Lupins, Penny, Kordia	without symptoms	-	-
Argeș (C)	10	Summit, Lupins, Karina, Kordia, Regina	without symptoms	-	-
Dâmbovița	2	Regina, Kordia, Van, Maria	without symptoms	-	-
Buzău	9	Kordia, Sweetheart	without symptoms	-	-
Călărași	3	Regina, Kordia	without symptoms	-	-
Ilfov	3	B.Burlat, Sweetheart, Kordia	5,00 % somes symptoms	15.00% *ArMV;* 5.00% *TBRV;*5.00% *ACLSV;*	-
Ialomița	3	Prime Giant, Regina	without symptoms	-	-

The incidence of cherry virus infections related to the age of the plantation, the number of treatments and the source of planting material (table 4), it can be observed that the number of treatments or the biological category of CAC material cannot be a cause of the infections, being only in two plantations. The most likely source of infection in this case, given the fact that the *TBRV* and *ArMV* viruses are present, which are transmitted by nematodes, is the land infected with this type of viral vectors and/or the origin of the planting material, an assumption based on the published information by Pop, 1988 and present in EPPO PM 4/29 (1) and EFSA, 2013.

Table 4

The incidence of viral infections according to the age of the plantation, the number of treatments carried out and the source of the cherry planting material

Location cod	Orchard age (years)	No. of treatments	Biological category	Source of planting material	% viral infected plants
Dolj 1	2	3	CAC	Authorized nursery RO	0.00
Dolj 2	2	5	Certificate	Authorized nursery RO	0.00
Argeș (A)	12	6	Certificate	Authorized nursery GR	20.00%
Argeș (B)	4	6	Certificate	Authorized nursery NL	0.00
Argeș (C)	10	4	CAC	Authorized nursery RO	0.00
Dâmbovița	2	5	Certificate	Authorized nursery RO	0.00
Buzău	9	4	Certificate	Authorized nursery IT	0.00
Călărași	3	5	Certificate	Authorized nursery IT	0.00
Ilfov	3	4	Certificate	Authorized nursery IT	25.00%
Ialomița	3	5	Certificate	Authorized nursery RO	0.00

CONCLUSIONS

In the assessed plum plantations, the *PPV* virus had the highest incidence, which requires more rigorous monitoring and the need to apply phytosanitary treatments against vector insects to limit/slow down the spread of *PPV* in healthy plants. Removing infected trees to limit the impact of *PPV* is not an economical solution for orchard owners where the incidence is low. Orchards may be economically profitable, but profitability will be greatly diminished. Maintenance is based on the fact that there are varieties tolerant to *PPV* in the plantation.

The added viral health safety benefit of using Certified propagating material over CAC propagating material has been noted due to the requirement of viral assessments for Certified propagating material.

In the monitored cherry plantations, the viruses *ArMV, TBRV* and *ACLSV* were identified in a 12-year-old plantation and a 3-year-old plantation, which raises the suspicion of two causes: the land infested with the nematodes *Xiphinema diversicaudatum, Longidorus attenuatus* and *Longidorus elongatus* and/or the health of the planting material used.

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