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INCIDENȚA BOLILOR VIRALE ÎN PLANTAȚIILE MAMĂ DE PRUN DIN ROMÂNIA
THE OCCURRENCE OF VIRAL DISEASES IN PLUM MOTHER PLANTATIONS FROM ROMANIA

Plopa Catița[1], Preda Silvia[2], Zagrai Ioan[3], Isac Valentina[1], Zagrai Luminița[3], Dragomir Iosif[1], Butac Mădălina[1]
[1]Research Institute for Fruit Growing Pitești, Romania
[2]Research Station for Fruit Growing Vâlcea, Romania
[3]Research Station for Fruit Growing Bistrița, Romania

Abstract

A solution for obtaining quality planting material is monitoring the presence of viral diseases in mother plantations using different diagnostic methods. In this regard in three centers for the maintenance and production of BASIC and CERTIFICATE biological categories plum propagation material, were performed viral tests on the following cultivars: 'Stanley', 'Pescăruș', 'Anna Späth', 'Tuleu timpuriu', 'Tuleu gras', 'Agent', 'Centenar', 'Andreea', 'Record', 'Roman', 'Grase românesti', 'd' Agen', 'Mirobolan dwarf', 'Mirobolan C5', 'BN 4Kr' from the Research Institure for Fruit Growing Pitești; Iulia, Matilda, Geta, Zamfira, Ivan, Dani, Doina, Romaner, Elena, Jubileu 50, Flora, Renclod Althan, Stanley, Anna Spath, Carpatin, Agent, Gras ameliorat, Centenar, Minerva, Andreea and Delia from the Research Station for Fruit Growing Bistrița); Rival, Oltval, Miroval, Pinval, Otești 11, Otești 8, Corval, Carpatin, Tuleu gras, Andreea, Stanley, Diana, Vânăt românesc, Tita, Centenar from the Research Station for Fruit Growing Vâlcea). For identification of the viruses: *PPV, PDV, PNRSV, ACLSV, ApMV* and *MLRSV*, different diagnostic methods have been used: DAS-ELISA and TAS-ELISA was used for *PPV, PDV, PNRSV, ACLSV, ApMV MLRSV*; the AgriStrip immunochromatographic assay, was applied for *PPV* identification; the biological test using the GF 305 indicator was performed for *PPV, PDV* and *PNRSV*. During the yearly evaluations were identified *PPV, PNRSV, ACLSV* and *PDV* viruses.

Cuvinte cheie: virus, metoda ELISA, portaltoi, ramură.
Key words: virus, ELISA method, rootstocks, branch.

1. Introduction

The serious consequences of viral diseases require rigor and increased demand in the chain production of *virus free* planting material. This is because the production of high quality fruit and the productive potential of plum varieties is closely correlated with the planting material.

In Romania, the main centers for the maintenance of the plum fruit tree plantations are: Research Institute for Fruit Growing Pitești (RIFG Pitesti), Research Station for Fruit Growing Bistrita (RSFG Bistrița) and University of Craiova-Research Station for Fruit Growing Vâlcea (RSFG Vâlcea).

Fruit trees can be infected with numerous viruses that affect production and fruit quality, finally resulting significant losses. Usually viruses are transmitted by different vectors, including seedling, pollen, scion, stock, seed insects (Amari, et al. 2009; Fulton, et. al, 1952; Uyemoto, et. al 1992) thus making the virus easily spread among countries and regions. Virus infected plants are more susceptible to fungal and bacterial diseases and to environmental stresses (Zawadzka, 1989). Also, mixed infection between viruses in stone fruits is common (Liebenberg, 2013). Against viral diseases, an important solution is to monitor their presence (Nemeth, 1986; Kapoor, et al., 2018) and the production of healthy planting material, from a viral point of view. For this an important role is represented by the health of the mother plantations.

The most common method of viral diagnosis for propagation material from mother plantations is the ELISA serological method, because the time required for testing is low, allows rapid diagnosis of the virus and thus the immediate elimination of infected plants (Minoiu, 1990; Nemeth, 1986; Pop, 1988; Stankienė, et al., 2012; Plopa et al., 2014). Often, however, biological testing helps to detection of viral infections.

The aim of present paper is to present some aspects regarding viral disease monitoring as a measure to prevent infections in mother plantations, in order to produce healthy propagating material free of viral pathogens.

2. Material and methods

The researches were carried out during 2010-2017 at RIFG Piteşti in two locations of Argeş county: Leordeni where there is a mother plantation for grafting branches, CERTIFICATE biological category with *virus tested (VT)* status, established in 1995 with the varieties: 'Stanley', 'Pescăruş', 'Anna Späth' and 'Tuleu Timpuriu' and the second location: Mărăcineni where there are: mother plantation for grafting branches, biological category Basic, with *VT* status, established in 2008, for: 'Stanley', 'Pescăruş', 'Anna Späth', 'Tuleu gras', 'Agent', 'Centenar', 'Andreea', 'Record', 'Roman', 'Grase românești', 'd 'Agen' pum variety, and mother plantation for grafting branch, *VT* status, Certification biological category, established in 2014 with the varieties: 'Stanley', 'Anna Spath', 'Centenar', 'Pescăruş', 'Agent', 'Andreea', 'Tuleu gras'. Also in the Maracineni location are the Basic mother plantations, for seedling plum rootstocks: 'Mirobolan dwarf', 'Mirobolan C5' and 'BN 4Kr', established in 1992 and 'Mirobolan dwarf' and 'Mirobolan C5' established in 2011, both with VT status.

At the RSFG Bistriţa the mother plantations to be referred to are: mother plantation Basic biological category under vector protection conditions from 2015 for the: 'Iulia', 'Matilda', 'Geta', 'Zamfira', 'Ivan', 'Dani', 'Doina', 'Romaner', 'Elena', 'Jubileu 50', 'Flora', 'Renclod Althan', 'Stanley', 'Anna Späth', 'Carpatin', 'Agent', 'Gras ameliorat', 'Centenar', 'Minerva', 'Andreea', 'Delia' plum varieties, and mother plantation Basic biological category, set up in 2014 in Baţa Mihăeşti with the varieties: 'Iulia', 'Matilda', 'Geta', 'Zamfira', 'Ivan', 'Elena', 'Jubileu 50', 'Flora', 'Renclod Althan', 'Stanley', 'Anna Spath', 'Carpatin', 'Agent', 'Gras ameliorat', 'Centenar', 'Minerva', 'Andreea', 'Delia'. The initial Basic material used to establish those twoo mother plantation was obtained by satisfying EPPO recomanded certification standards for *virus free (VF)* status. Research took place between 2016 and 2018.

At University of Craiova - RSFG Valcea, the assessments were carried out in Certificate mother plantation biological category, established in 2000, represented by the rootstocks: 'Rival', 'Oltval', 'Miroval', 'Pinval', 'Oteşani 11', 'Oteşani 8', 'Corval' and in the mother plantation for grafting branches established in 1997 with the: 'Carpatin', 'Tuleu gras', 'Andreea', 'Stanley', 'Diana', 'Vinete românești', 'Tita' and 'Centenar' varieties. The mother plantations existing at RSFG Vâlcea have *VT* status. Virological observations and analyzes refer to the period 2010-2017.

Field assessment: visual inspections and sampling were performed from the beginning of spring to the arrival of high temperatures, the first half of June each year.

The serological diagnosis of *Apple chlorotic leaf spot trichovirus (ACLSV*), *Plum pox virus (PPV), Prune dwarf virus (PDV)* and *Prunus necrotic ringspot (PNRSV), Mirobalan latent ringspot (MLRSV), Apple mosaic virus (ApMV),* was performed by DAS-ELISA (Clark and Adams, 1977) and TAS-ELISA (Cambra, et al., 1994) according to the working protocol recommended by reagent manufacturers (BIOREBA and SEDIAG) and Bulletin OEPP, 2015, PM 7/125(1). The collected samples consisted of leaves (6-8/tree). For *PPV* diagnosis was also used the AgriStrip immunochromatographic and field testing on the GF 305 indicator, for 'Tuleu gras', 'Grase românești', 'Centenar', 'Pescăruș' and 'Anna Spath' varieties.

3. Results and discussions

At RIFG Pitesti, after *PPV* symptoms evaluation by visual observations, were found diseased plants (Table 1). From a number of 3,133 mother plants evaluated during 2010-2017, there were identified with leaf symptoms, 42 mother plants, 12 of the 'Anna Späth', 6 of the 'Tuleu gras'', 10 of the 'Centenar', 4 of the 'Pescăruș' and 10 mother plants of the 'd'Agen'.

At the RSFG Bistrița in three years (2016-2018), were not identified typical symptoms of the *PPV* virus and other symptoms suggesting potential viral infections in the two plum mother plantations.

During the 2010-2017 period, mother plum tree plantation at RSFG Vâlcea was evaluated visually, to a number of 1,124 mother plants, and 37 showed *PPV* symptoms on the leaves of the varieties: 'Carpatin' 3 plants, 'Tuleu gras' 7, 'Stanley' 4, 'Tita' 5, 'Centenar' 7 and 'Oltval' rootstocks 6 plants and 'Pinval' 5 plants.

Plants with symptoms have been eliminated.

Laboratory tests for viral diagnosis (Table 2) have revealed during the years of research on plum samples, individual infections but also complex infections. In the mother plantations of RIFG Piteşti, a number of 58 infected with *PPV, PNRSV* and *PDV* were detected by the DAS/TAS-ELISA method.

In the mother plantation of grafting branches the Basic biological category, founded in 2008 in Maracineni area, the diagnosed infections were with *PPV* to 'Anna Späth' 4 plants, 'Tuleu gras' 6 plants, 'Centenar' 5, 'Grase românești' 3, 'd'Agen' 2, 'Pescăruş' 2 and with *PDV* 'Agent' 3 and 'Andreea' 1. In the Basic mother plantation, rootstocks, established in the Maracineni location in 1992, were found positive by the ELISA test, *PPV*, 2 plants from the 'Mirobolan dwarf', 3 plants infected with *PNRSV* from the 'Mirobolan C5' rootstock. In the mother plantation for rootstocks Basic biological category, established in 2011, infected plants were not founded.

Tests conducted during the 2010-2017 period for the mother plantation set up in 1995 at Leordeni area showed that *PPV* infections were predominantly: 'Stanley' 5 trees, 'Pescarus' 3 trees, 'Anna Späth' 9 trees and 'Tuleu timpuriu' 7 trees. The 'Anna Spath' variety has 2 of the samples infected with *PDV*. In the mother plantation established in 2014, was detected one positive PPV sample at 'Anna Spath' cultivar.

In the mother plantation Certificate biological category from SCDP Vâlcea, viral evaluation during the period 2010-2017, were found positive 17 plants with *PPV*: 5 in the 'Centenar', 4 in the 'Tuleu gras', 4 at the 'Pinval' rootstock, 4 at the 'Oltval' rootstock. An 'Oltval' plant showed mixed infection of *PPV* and *ACLSV*.

At the SCDP Bistrita (Table 3), the serological tests carried out between 2016 and 2018 in both the mother plantation and the biologic category Base, under vector protection, as well as in the mother plantation with basic biological category from Baţa-Mihăeşti field conditions, did not confirm the presence of any virus, the status fitoviral of the plants remaining *virus free.*

Observations recorded to biological test (Table 4) on 'GF 305'' indicator for 'Tuleu gras', 'Grase românești', 'Centenar', and 'Pescăruș' varieties showed symptoms that could have been associated with viral diseases. On the same indicator used for testing the 'Anna Späth' variety, no symptoms were reported. The use of the DAS-ELISA and AgriStrip test used to reconfirm the diagnosis by the biological testing method determined that the virus infection was present only in two of the tested varieties 'Tuleu gras' 2 plants from 20 tested and 'Pescăruș' with 1 plant from 20 tested. To 'Grase românești' and 'Centenar' varieties it was established that the symptoms on the leaves of the indicator plants were not caused by *PPV* virus. By applying the AgriStrip test to RIFG Piteşti in 2015, evaluation results were relatively consequent (photo 1), indicating the high and average concentrations of the virus. The effectiveness of this type of testing was appreciated by Malinowski, 2009, in his research.

In the plum tree mother plantations, *PPV* infections prevailed, thus confirming the increased incidence of *PPV* virus, as mentioned by other authors as a result of researches conducted in other countries. The incidental occurrence of *PPV* in nurseries cannot be excluded in countries and areas with the endemic presence of *PPV* (Musa A, et al., 2010; Polak et al., 2016).

4. Conclusions

During 2010-2017 period at RIFG Pitești-Mărăcineni, 49 mother plants were found PPV positive, 6 plants positive for *PDV* and 3 for *PNRSV*. At RSFG Vâlcea were identified 31 plants with *PPV* and 1 plant with *ACLSV*.

It can also be noticed that the younger mother plantation in the Mărăcineni area is less infected with *PPV* compared to the older Leordeni mother plantation where more *PPV* plants have been identified, confirming once again that plants can become infected / reinfected as a result of the influence of factors favoring the spread of viruses, mainly vectors.

These results show, on the one hand, that maintaining a mother plantation under vectors protection provides favorable conditions for avoiding infections and can be recommended in endemic countries. On the other hand, the lack of infections in the mother plantation BASIC in the open field suggests that the planting of a Basic material with VF status and the subsequent implementation of rigorous measures to avoid viral infections (isolation distance, vector control treatments, weed control, periodic monitoring, etc.) may lead to maintaining initial viral status over a certain period of time.

References

1. Amari K., et al., 2009. Vertical transmission of *Prunus necrotic ringspot* virus: hitch-hiking from gametes to seedling. *Journal of General Virology.* 90, 1767–1774.
2. Cambra M., Asensio M., Gorris M.T., Pérez E., Camarasa E., García J.A., Moya J.J., López-Abella D., Vela C., Sanz A., 1994. Detection of *plum pox* potyvirus using monoclonal antibodies to structural and non-structural proteins. EPPO Bull. 24, 569–577.
3. Clark M.F., Adams M.F., 1977. Characteristics of microplate method of Enzyme linked immunosorbent assay for the detection of plant viruses. Journal of generally virology 34: 475-483.
4. Fulton E.W., 1952. Mechanical transmission and properties of *Rose mosaic virus*.*Phytopathology.* 42, 413–416.
5. Liebenberg A., 2013. Influence of latent apple viruses on *Malus sieboldii* derived *Apple proliferation* resistant rootstocks. http://www.ub.uni-heidelberg.de/archiv/1503.
6. Malinowski Tadeusz, 2010. Evaluation of the reliability of lateral flow immunochromatography strips for detection of *Plum pox virus,* Proceedings of the 21st International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops, Neustadt, Germany, Julius-Kühn-Archiv, 427.
7. Minoiu N, Lefter Gh., 1990. Bolile și dăunătorii speciilor sâmburoase, Editura CERES.

8. Musa A., Merkuri J., Milano R., Djelouah K., 2010. Investigations on the phytosanitary status of the main stone fruits nurseries and mother plants in Albania, Proceedings of the 21st International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops, Neustadt, Germany, Julius-Kühn-Archiv, 427.
9. Németh M., 1986. Virus, Mycoplasma and Rickettsia Diseases of Fruit Trees, Akademia Kiado, Budapest.
10. Paunović S., Jevremović D., 2010. Health status of pome and stone fruit planting material imported to Serbia, Proceedings of the 21st International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops, Neustadt, Germany, Julius-Kühn-Archiv, 427.
11. Plopa C., Preda S., 2014. Viral testing using biological and serological assay for most important viruses to plum, Fruit Growing Research, vol XXX/ ISSN 2286–0304,ISSN–L2286–0304.
12. Polák J., Komínek P., 2016. Investigation on the incidence of *Plum pox virus* in fruit nurseries of the Czech Republic. Plant Protect. Sci., 52: 158–163.
13. Pop I., 1988. Virusurile plantelor horticole și combaterea lor. Editura CERES, ISBN 973-40-0034-9.
14. Kapoor S., Sharma A., Shylla B., Handa A., 2018. Ilarviruses and the Importance of Certified Elite Planting Material in Apple Production System-An Overview,Int.J.Curr.Microbiol.App.Sci7(4):2444-2462.
15. Stankienė J.et. al., 2012. Virological assessment of stock planting material of apple and raspberry cultivars, Žemdirbystė=Agriculture Vol. 99, No. 1, ISSN 1392-3196.
16. Uyemoto J. K., Asai W. K. & Luhn C. F., 1992. Ilarviruses: evidence for rapid spread and effects on vegetative growth and fruit yields of peach trees. *Plant Disease* 76, 71.
17. Zawadzka B., 1989. The influence of virus and mycoplasma diseases on frost damage of apple trees / Acta Horticulturae, vol. 235, p. 59–67.
18. OEPP/EPPO Bulletin, (2015), 45(3) PM 7/125(1) ELISA tests for viruses. ISSN 0250-8052.

Tables and figures

Table 1. Typical *PPV* visual symptoms

Species	**RIFG Pitesti** during 2010-2017	**RSFG Bistrița** during 2016-2018	**RSFG Vâlcea** during 2010-2017
No. evaluated plants / no. infected plants	3,113/42 (1,35%)	762/0	1,124/37 (3,29 %)
Infected varieties / no. plants with visual symptoms	Anna Späth /12 Tuleu gras/6 Centenar/10 Pescăruș/4 D'Agen/10	-	Carpatin/3 Tuleu gras/7 Oltval/6 Stanley/4 Tita/5 Centenar/7 Pinval/5

Table 2. Results of the DAS/TAS-ELISA serological test, study period 2010-2017 at RIFG Pitești and RSFG Vâlcea

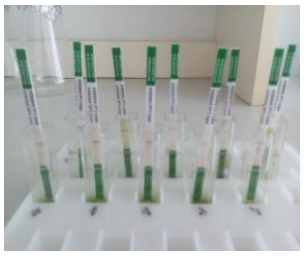
Location / Year of Establishment	Variety	Average no. of plants annually tested	Virus detected	No. infected plants on tested period
Mărăcineni /2008- Basic/grafting branches	Anna Späth	25	*PPV*	4
	Tuleu gras	10	*PPV*	6
	Centenar	10	*PPV*	5
	Gras românesc	10	*PPV*	3
	D'Agen	10	*PPV*	2
	Pescăruș	10	*PPV*	2
	Agent	20	*PDV*	3
	Andreea	18	*PDV*	1
	Stanley	20	-	-
	Record	10	-	-
	Roman	10	-	-
Mărăcineni/1992- Basic/rootstocks	Mirobolan dwarf	20	*PPV*	2
	Mirobolan C5	20	*PNRSV*	3
	BN 4Kr	20	-	-
Mărăcineni/2011- Basic/rootstocks	Mirobolan dwarf	25	-	-
	Mirobolan C5	30	-	-
Mărăcineni /2014- Certificate/ grafting branches	Stanley	20	-	-
	Anna Späth	25	*PPV*	1
	Centenar,	10	-	-
	Pescăruș	10	-	-
	Agent	20	-	-
	Andreea,	20	-	-
	Tuleu gras	20	-	-
Leordeni/1995- Certificate/ grafting branches	Stanley	18	*PPV*	5
	Pescăruș	10	*PPV*	3
	Anna Späth	20	*PPV*	9
			PDV	2
	Tuleu timpuriu	10	*PPV*	7
RSFG-Vâlcea/1997/ grafting branches	Carpatin	10	-	-
	Tuleu gras	10	*PPV*	4
	Andreea	10	-	-
	Stanley	10	-	-
	Diana	10	-	-
	Vânăt românesc	10	-	7
	Tita	10	-	7
	Centenar	10	*PPV*	5
RSFG-Vâlcea/2000/ rootstocks	Rival	10	-	-
	Oltval	10	*PPV*	4
			ACLSV	1
	Miroval	10	-	-
	Pinval	10	*PPV*	4
	Oteșani 11	10	-	-
	Oteșani 8	10	-	-
	Corval	10	-	-

Table 3. Results of the DAS-ELISA serological test, study period 2016-2018 at RSFG Bistrița

Location / Year of Establishment	Variety	No. of total plants tested on 2016-2018 period	Virus detected	No. infected plants on tested period
Bistrița /vectors protection-Basic/ grafting branches	Anna Späth	5	-	-
	Stanley	5	-	-
	Carpatin	3	-	-
	Renclod Althan	3	-	-
	Andreea	3	-	-
	Minerva	2	-	-
	Centenar	2	-	-
	Gras ameliorat	2	-	-
	Flora	2	-	-
	Agent	2	-	-
	Delia	2	-	-
	Matilda	2	-	-
	Jubileu	2	-	-
	Geta	2	-	-
	Ivan	2	-	-
	Zamfira	3	-	-
	Elena	3	-	-
	Romaner	3	-	-
	Dani	1	-	-
	Iulia	5	-	-
	Doina	5	-	-
Bața Mihăești /2014-Basic / grafting branches	Iulia	22	-	-
	Zamfira	16	-	-
	Carpatin	16	-	-
	Elena	7	-	-
	Matilda	7	-	-
	Stanley	32	-	-
	Anna Späth	17	-	-
	Renclod Althan	11	-	-
	Minerva	7	-	-
	Geta	5	-	-
	Flora	3	-	-
	Jubileu	3	-	-
	Ivan	4	-	-
	Centenar	11	-	-
	Agent	11	-	-
	Gras ameliorat	7	-	-
	Andreea	8	-	-
	Delia	8	-	-

Table 4. Results of the biological test RIFG Pitești

Mother Plantation / Year of establishment	Biological method			DAS -ELISA	AgriStrip
	Biological indicator	Variety / no tree tested/no tree with symptoms	Health status		
Mărăcineni / 2008	GF 305	Tuleu gras / 20/2	Symptoms that could have been associated with viral diseases	Positive: *PPV* Negative: *PDV, PNRSV*	Positive: *PPV*
	GF 305	Gras românesc / 20/1	Symptoms that could have been associated with viral diseases	Negative: *PPV, PDV, PNRSV*	Negative: *PPV*
	GF 305	Centenar / 20/1	Symptoms that could have been associated with viral diseases	Negative: *PPV, PDV, PNRSV*	Negative: *PPV*
	GF 305	Pescăruș / 20/1	Symptoms that could have been associated with viral diseases	Positive: *PPV* Negative: *PDV, PNRSV*	Positive: *PPV*
	GF 305	Anna Späth / 30/0	Absence of symptoms	Negative: *PPV, PDV, PNRSV*	Negative: *PPV*



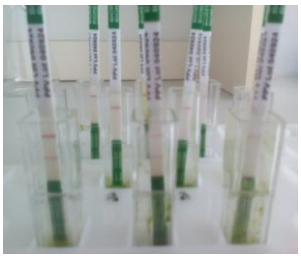


Photo 1. Imunocromatografic AgriStrip test

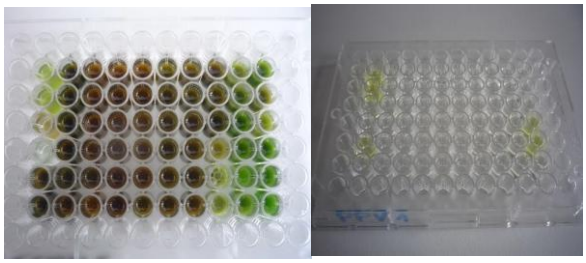


Photo 2. ELISA test