

## EVALUAREA STĂRII VIROTICE A PLANTAȚIILOR MAMĂ CATEGORIA BAZA ȘI CERTIFICAT LA PRINCIPALELE SPECII SÂMBUROASE ÎN ZONA ARGEȘ EVALUATION OF VIRAL STATUS OF MOTHER PLANTATIONS BASE AND CERTIFICATE CATEGORY TO THE MOST IMPORTANT STONY SPECIES IN ARGEȘ AREA

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### Abstract

Fruit trees can be infected with numerous viruses that affect production, fruit quality, and tree decline. Against viral diseases, the best solution is to monitor their presence through different diagnostic methods and to produce viral healthy planting material. The researches were carried out during the period 2010-2016, and they targeted the mother plantations in two locations: Leordeni Argeș for the graft branches mother plantations of Certificate category, the plum species, and in Marăcineni Argeș for the graft branches mother plantations the biological categories Basic and Certificate for plum, sweet cherry, sour cherry, peach, apricot, and rootstock mother plantations for plum, peach, apricot, sour and sweet cherry species. The serological diagnosis of ACLSV, ApMV, PPV, PDV, PNRSV, ArMV, TBRV, SLRSV, RpRSV, and CLRV was done by the DAS-ELISA and TAS-ELISA methods. Biological tests were also carried out on the biological indicators 'GF 305' and 'Vânăț de Italia' in the field and biological tests on herbaceous *Chenopodium quinoa* and *Cucumis sativus* herbs were applied in the greenhouse.

**Cuvinte cheie:** virus, plantație mamă, prun, piersic, cais, cireș, vișin.

**Key words:** virus, mother plantation, plum, peach, apricot sweet cherry, sour cherry.

### 1. Introduction

In Romania, the Research Institute for Fruit Growing Pitești maintains the largest mother plantations in the Base and Certificate biological categories.

Fruit trees can be infected with numerous viruses that affect fruit production and quality, ultimately resulting in significant losses.

Plants infected with the virus are more susceptible to fungal and bacterial diseases and various environmental stresses (Zawadzka, 1989). Of the most dangerous viral diseases in the stone species are: *Apple chlorotic leaf spot trichovirus virus (ACLSV)*, on plum, apricot, peach, sweet and sour cherry; *Apple mosaic (ApMV)* on plum, apricot, peach, cherry, and *Plum pox virus (PPV)*, on plum, peach, apricot; *Strawberry latent ringspot nepovirus (SLRSV)* on peach, sweet and sour cherry; *Apricot chlorotic leafroll (ACLRV)*, on plum, apricot, peach; *Prune dwarf virus (PDV)* and *Prunus necrotic ringspot (PNRSV)*, on plum, peach, apricot, sweet and sour cherry, and *Arabidopsis mosaic nepovirus (ArMV)*, *Cherry leaf roll nepovirus (CLRV)*, *Tomato black ring nepovirus (TBRV)*, *Raspberry ringspot nepovirus (RpRSV)*, on sweet and sour cherry.

Against viral diseases, the best solution is to monitor their presence (Nemeth, 1986), and produce viral healthy planting material. For this an important role is represented by the health of the mother plantations.

The most commonly used methods of viral diagnosis are serologic methods, because they reduce the time required for testing, allows rapid diagnosis of the virus and immediate elimination of infected plants (Minoiu, 1990; Nemeth, 1986; Pop, 1988; Stankienė, J. et. al., 2012, Plopa C. et al., 2014).

But, often, testing by biological methods comes to complete with added safety the laboratory diagnostic methods.

The present paper aims at presenting aspects of viral disease monitoring as a measure to prevent infections in mother plantations for grafting branches and rootstocks, in order to produce healthy propagating material free of viral pathogens.

### 2. Material and method

The researches were carried out between 2010 and 2016, in two locations Leordeni Argeș, in the mother plantations of grafting branches, Certificate biological category, established in 1995, for plum species: 'Stanley', 'Pescăruș', 'Anna Spăth' and 'Tuleu timpuriu' varieties and Marăcineni Argeș in the mother plantations of grafting branches Base biological category founded in 2008 with the plum varieties:

'Stanley', 'Pescăruș', 'Anna Späth', 'Tuleu gras', 'Agent', 'Centenar', 'Andreea', 'Record', 'Roman', 'Gras românesc', 'd' Agen' varieties, peach 'Redhaven', 'Cardinal', 'Collins', 'Southland' varieties, apricot 'Olimp' variety, sweet cherry with the varieties of 'Daria', 'Germersdorf', 'George', 'Stella', 'Van', 'Lucia', 'Radu', 'Maria', 'Stefan', 'Marina', 'Bucium', sour cherry with varieties 'Schattenmorelle', 'Ilva', 'De Botosani', and rootstocks mother plantations Certified biological category, for plum 'Mirobolan dwarf', 'Mirobolan C5', 'BN 4 Kr', peach 'Adaptabil', apricot 'Apricor', sour and sweet cherry 'Portavium', 'Semavium', 'IPC-2', 'IPC-3' and 'IPC-5', established in 1992-2009.

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

The serological diagnosis of the *ACLSV*, *ApMV*, *PPV*, *PDV*, *PNRSV*, *ArMV*, *TBRV*, *SLRSV*, *RVRSV*, *CLRV*, viruses was performed by: DAS-ELISA (Clark MF, Adams MF, 1977) and TAS-ELISA et al., 1994). The collected samples consisted of leaves (6-8 / tree).

For the identification of some viruses and phytoplasmas (*PPV*, *PDV*, *PNRSV*, *ACLSV*, etc) was applied, the field biological testing on 'Vânăț de Italia' and 'GF 305' biological indicators.

The identification of *ACLRV*, *ApMV*, *PNRSV*, *PDV* and Nepo viruses in the stone trees; *ArMV*, *CLRV*, *SLRSV*, *TBRV*, *RpRSV* was also done on the herbaceous indicators: *Cucumis sativus* and *Chenopodium quinoa*, in greenhouse.

For ELISA tests were used kits from BIOREBA and SEDIAG. The extracts from the young leaves of the tested plants were analyzed according to the protocol provided by the producer.

### 3. Results and discussion

Following evaluation made between 2010-2016, by visual observation on *PPV* virus, whose symptoms are evident in case of infection at plum, apricot and peach species, diseased plants have been identified (Table 1). At plum have been identified with symptoms 38 mother plants, out of which 10 of the 'Anna Späth' variety, 6 of the 'Tuleu gras', 8 of the 'Centenar', 4 of the 'Pescăruș' and 10 of the 'd' Agen' variety. To apricot have been identified with typical symptoms of *PPV* two mother plants of the 'Olimp' variety. There have been no symptoms of *PPV* in peaches.

Laboratory tests for viral diagnosis (Table 2) have revealed during the years of research on plum, peach and cherry samples individual infections but also complex infections.

Of the 56 positive findings 3 were *PNRSV* and 6 with *PDV*, the remaining 47 being infected with *PPV*. Were positive until 2016, mother plants from the mother plantation established in 1995 (Leordeni) as follows: 'Stanley' 5 trees, 'Pescăruș' 3 trees, 'Anna Späth' 9 trees and 'Tuleu timpuriu' 7 trees. From the plum mother plantation established in 2008, in Mărăcineni, until 2016, 4 parent plants of the 'Anna Späth' variety, 6 of the 'Tuleu gras', 5 of the 'Centenar', 3 'Gras românesc', 1 'd' Agen', 2 'Pescăruș' were found positively in plum tree plantations; *PPV* and *PDV* infections prevailed, but to a lesser extent, being reconfirmed the increased incidence of *PPV* virus. It can also be noticed that the younger mother plantation from Mărăcineni site is less infected with *PPV* compared to the Leordeni mother plantation where several *PPV* infected 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.

On peach, the presence of the *ACLSV* virus was found to be dominant, virus which was also found in cherry. But at cherry the highest incidence had the *PDV* virus with 7 mother plants being positive. Also were identified infected 7 mother plants with *SLRSV*, 4 with *ArMV*, 3 with *ACLSV* and 1 with *TBRV*. In the Mărăcineni Certified mother plantation, cherry rootstocks, established in 1992, have been diagnosed with virus infections: *ACLSV* and neviruses *ArMV* and *SLRSV* in a mother plant of the 'Semavium' rootstock and *ACLSV*, *PDV*, *SLRSV* and *ArMV* in a mother plant from 'Portavium'. In cherry, even younger 'IPC-2' and 'IPC-5' rootstocks have been identified infected.

The observations made on the biological test for plums, cherries and peaches (Table 3) indicate that the 'GF 305' indicator on the 'Tuleu gras', 'Centenar' and 'Pescăruș' plums 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. For the same mother plants, biological tests performed on the 'Vânăț de Italia' indicator, did not reveal symptoms. Also, testing of mother plants from: 'Collins', 'Southland' and 'Redhaven' did not induce symptoms on the 'GF 305' indicator.

In the evaluation of tests carried out on greenhouse indicators for 'Tuleu gras', 'Gras românesc' and 'Centenar', no changes were noted on the leaves of the *Cucumis sativus* indicator (foto1) and the *Chenopodium quinoa* indicator on which the biological testing of some mother plants of cherry varieties: 'George', 'Bucium', 'Maria' and 'Stella' was carried out.

### 4. Conclusions

It is reconfirmed the increased viral incidence with *PPV* in plum.

In a cherry rootstock mother plantation, a plant of 'Semavium', infected with *ACLSV*, *SLRSV* and *ArMV*, was identified and a plant of 'Portavium' infected with *ACLSV*, *PDV*, *SLRSV* and *ArMV*, was identified.

The cherry rootstock studied proved to be more susceptible to viral diseases than the plum rootstocks studied, the year of setting up the mother plantations for both plum rootstocks and cherry rootstocks 'Portavium' and 'Semavium' being the same. Also, were detected viral infections to 'IPC-5' and 'IPC-2' cherry rootstocks, plantations younger than the two previously mentioned.

Tests performed on biological indicators revealed the presence of symptoms that could have been associated with viral diseases only on 'GF 305' wood indicator. On the 'Vânăț de Italia' and the herbaceous indicators *Cucumis sativus* and *Chenopodium quinoa*, no symptoms.

## 5. Acknowledgement

This paper was published under the project ADER 3.3.1/2015.

## References

1. 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.
2. Clark M.F., Adams M.F., 1977. Characteristics of microplate method of Enzyme linked immunosorbent assay for the detection of plant viruses. *Journal of general virology* 34: 475-483.
3. Jelkmann W., 2004, Detection of virus and virus-like diseases of fruit trees-laboratory assay, bioassay and indicators, *Acta Horticulturae* (657): 575-596.
4. Minoiu N, Lefter Gh., 1990. Bolile și dăunătorii speciilor sâmburoase, Editura CERES.
5. Németh M., 1986. Virus, Mycoplasma and Rickettsia Diseases of Fruit Trees, Akademia Kiado, Budapest.
6. Plopa Catita, Preda Silvia, 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.
7. Pop, 1988. Virusurile plantelor horticole și combaterea lor. Editura CERES, ISBN 973-40-0034-9.
8. 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.
9. Zawadzka B., 1989. The influence of virus and mycoplasma diseases on frost damage of apple trees. *Acta Horticulturae*, vol. 235, p. 59–67.

## Tables and Figures

**Table 1. Typical PPV visual symptoms**

Species	Plum	Apricot	Peach
No. evaluated plants / no. infected plants during 2010-2016	1.813/38	280/2	290/0
Infected varieties / no. plants with visual symptoms	Anna Späth /10 Tuleu gras/6 Centenar/8 Pescăruș/4 D'Agen/10	Olimp/2	-

**Table 2. Results of the ELISA serological test, study period 2010-2016**

Location / year of establishment	Species	Variety	No. of plants annually tested	Virus detected	No. infected plants
Mărăcineni /2008	Plum	Anna Späth	25	PPV	4
		Tuleu gras	10	PPV	6
		Centenar	10	PPV	5
		Gras românesc	10	PPV	3
		D'Agen	10	PPV	1
		Pescăruș	10	PPV	2
		Agent	20	PDV	3
		Andreea	18	PDV	1
		Stanley	20	-	-
		Record	10	-	-
Mărăcineni/1992	Plum	Mirobolan dwarf	20	PPV	2
		Mirobolan C5	20	PNRSV	3
		BN 4Kr	15	-	-
Mărăcineni /2008	Peach	Collins	10	PDV	1
		Redhaven	10	ACLSV	1
		Cardinal	10	ACLSV	1
		Southland	10	-	-
		Adaptabil	30	-	-
Mărăcineni /2008	Apricot	Olimp	20	-	-
	Sweet cherry	George	7	SLRSV	1
		Bucium	10	SLRSV	1
		Stella	10	SLRSV	1
		Maria	5	PDV	1
		Ștefan	5	PDV	1
		Daria	10	-	-
		Germersdorf,	10	-	-
		Radu	5	-	-
		Van	10	-	-
		Lucia	5	-	-
		Marina	5	-	-
		Bucium	5	-	-
		Sour cherry	Schattenmorelle	5	-
	Ilva		5	-	-
De Botoșani	5		-	-	
Mărăcineni/1992	Sweet and sour cherry	Semavium	20	SLRSV	1
			20	ACLSV	1
			20	ArMV	2
			20	PDV	1
		Portavium	20	SLRSV	2
			20	ACLSV	1
			20	ArMV	2
Mărăcineni/2009	Sweet and sour cherry	IPC 5	15	ACLSV	1
			15	PDV	2
			15	TBRV	1
		IPC2	15	PDV	1
			15	-	-
			15	-	-
Apricot	Apricor	5	-	-	
Leordeni/1995	Plum	Stanley	18	PPV	5
		Pescăruș	10	PPV	3
		Anna Späth	20	PPV	9
			20	PDV	2
		Tuleu timpuriu	10	PPV	7

**Table 3. Results of the biological test**

Mother plantation / year of establishment	Species	Biological indicator	Variety tested	Health status
Mărăcineni /2008	plum	Vânăț de Italia	Tuleu gras	absence of symptoms
		Vânăț de Italia	Gras românesc	absence of symptoms
		Vânăț de Italia	Centenar	absence of symptoms
		Vânăț de Italia	Anna Späth	absence of symptoms
		Vânăț de Italia	Pescăruș	absence of symptoms
		GF 305	Tuleu gras	Symptoms that could have been associated with viral diseases
		GF 305	Gras românesc	Symptoms that could have been associated with viral diseases
		GF 305	Centenar	Symptoms that could have been associated with viral diseases
		GF 305	Pescăruș	Symptoms that could have been associated with viral diseases
		GF 305	Anna Späth	absence of symptoms
Mărăcineni /2008	peach	GF 305	Collins	absence of symptoms
		GF 305	Southland	absence of symptoms
		GF 305	Redhaven	absence of symptoms
Mărăcineni /2008	plum	<i>Cucumis sativus</i>	Tuleu gras	absence of symptoms
		<i>Cucumis sativus</i>	Gras ameliorat	absence of symptoms
		<i>Cucumis sativus</i>	Centenar	absence of symptoms
Mărăcineni /2008	cherry	<i>Chenopodium quinoa</i>	George	absence of symptoms
		<i>Chenopodium quinoa</i>	Bucium	absence of symptoms
		<i>Chenopodium quinoa</i>	Maria	absence of symptoms
		<i>Chenopodium quinoa</i>	Stella	absence of symptoms

**Fig. 1. Biological testing in greenhouse- herbaceous indicator: *Cucumis sativus***