Fruit Growing Research, Vol. XXXIV, 2018 DOI 10.33045/fgr.v34.2018.24
http://publications.icdp.ro/index.php

# EVALUAREA STĂRII VIRALE PENTRU PRINCIPALELE VIRUSURI ÎN PLANTAŢIA MAMĂ DE CAIS MENŢINUTĂ LA SCDP CONSTANŢA

# EVALUATION OF THE VIRAL STATUS FOR THE MAIN VIRUSES, OF APRICOT MOTHER PLANTATION, MENTAINED AT THE RSFG CONSTANŢA

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**Abstract**

**The viruses with the highest incidence of apricot are: *Apple chlorotic leaf spot (ACLSV), Apple mosaic virus (ApMV), Plum pox potyvirus (PPV), Prune dwarf ilarvirus (PDV)* and *Prunus necrotic ringspot ilarvirus (PNRSV)*. The annual retesting at the RSFG Constanţa, during the 2010-2016 periods, in the mother plantation of the apricot, BASIC biological category, established in 2008, showed the infected plants. The mother plants were represented by the varieties: 'Admiral', 'Augustin', 'Tudor', 'Goldrich', 'Sirena', 'The Best of the Hungary', 'Sulina', 'Mamaia', 'Elmar', 'Olimp', 'Ovidius', 'Auras' and 'Umberto'. Laboratory tests were done each time after visual evaluations from the mother plantation. In order to establish a more accurate diagnosis, several methods were applied: DAS-ELISA for all target viruses, and for the detection of *PPV*, the AgriStrip immune-chromatographic test was also applied. Also, for the detection of *PDV* and *PNRSV* viruses, the mother plants were also tested by the biological method using the 'GF 305' indicator. During that the mentioned period, were visually identified 9 mother plants with *PPV* symptoms on the leaves, the infections being confirmed by both methods laboratory testing, 2 infections with the *PNRSV* virus, 2 infections with the *PDV* virus and 1 positive sample with the *ACLSV* virus by the ELISA serological method. In a mother plant the infection was mixed: *PDV* + *ACLSV*. Biologically tested plants did not show symptoms on leaves that could have been associated with viral infections, as confirmed by the serological test.**

**Cuvinte cheie:** plantaţie mamă, cais, viruşi, metode de testare
**Key words:** mother plantation, apricot, viruses, test methods

## 1. Introduction

In Constanta area, Romania, the apricot is among the most cultivated tree species. Besides the fruit-producing plantations, the production of propagation material has also been developed lately, the starting point being Constanta, at Research Station for Fruit Growing, which owns the mother plantations of superior biological categories.

In apricots, like most of the stone species, *Plum pox virus (PPV), Prune dwarf virus (PDV) and Prunus necrotic ringspot (PNRSV*) are the viruses with the highest incidence.

PPV is currently the most common and serious disease in Europe for all species of the genus Prunus (Dosba et al., 1994), influencing production, fruit quality and tree decline. The disease-causing pathogen, Sharka, is classified as a quarantine organism in the European Union, but also in countries on other continents.

As there are no curative methods of protection against viral diseases, a solution is the monitoring of their presence (Nemeth, 1986), because of the mother plantations plays an important role is the health status. Also, virus-infected plants are more susceptible to fungal and bacterial diseases and environmental stresses (Zawadzka, 1989).

The diagnostic serological methods used in virology are most preferable because it reduces the time required for testing, allows for rapid diagnosis of the virus and the immediate elimination of infected plants (Minoiu, 1990; Pop, 1988; Isac, et al., 2002).

The present paper aims at presenting some aspects regarding the diagnosis of the virus as a measure of infection prevention in order to produce healthy propagating material free of viral pathogens in apricot.

## 2. Material and methods

The researches were carried out during the period 2010-2016, in the mother plantation BASIC biological category, established in 2008, at RSFG Constanţa, which includes: 'Admiral', 'Augustin',

'Tudor', 'Goldrich', 'Sirena', 'Hungary's Best', 'Sulina', 'Mamaia', 'Elmar', 'Olimp', 'Ovidius', 'Auraș' and 'Umberto' apricot varieties.

Field assessment: visual inspections and sampling were performed from the beginning of the spring until the high temperatures arrived in the first half of June. The collected samples consisted of leaves (6-8 / tree).

The serological diagnosis of *Plum pox virus* (PPV), *Prunus dwarf virus* (PDV), *Prunus necrotic ring spot virus* (PNRSV), *Apple chlorotic leaf spot virus* (ACLSV) and *Apple mosaic virus* (ApMV) was applied DAS-ELISA method (Clark and Adams, 1977).

For the identification of the PPV virus, was applied the immune-chromatographic AgriStrip test and for the PDV and PNRSV viruses, the biological tests were performed on the 'GF 305' indicator.

For the ELISA and AgriStrip tests, the kits used were purchased from BIOREBA. The extracts from the young leaves of the tested plants were analyzed according to the protocol provided by the manufacturer and the OEPP Bulletin, 2015, PM 7/125 (1).

## 3. Results and discussions

During field visual inspections, 370 mother plants were evaluated in every year.

Symptoms produced by the PPV virus could be evident on the leaves in a discrete way. However, in some monitored plants, have been observed several symptoms that may have been associated with viral diseases, such as chlorotic and yellow spots.

The results of the DAS-ELISA serological test presented in Table 1 showed the number of mother plants found positive to the viruses tested. A number of 9 mother plants were identified as infected with the PPV virus: 'Amiral' 1 plant, 'Goldrich' 2 plants, 'Sirena' 2 plants, 'Mamaia' 3 plants and 'Olimp' 1 plant.

Infections to the PDV virus have been detected in two 'Amiral' and 'Sulina' apricot mother plants. Serological analysis of apricot samples revealed in addition to individual infections and mixed infections, so in addition to the PDV virus in the 'Amiral' variety, it was found in the same plant and the ACLSV virus.

The serological analyzes performed also identified two plants infected with PNRSV, from 'Goldrich' and 'Elmar' variety.

Also, the diagnosis of PPV virus by the DAS-ELISA method confirmed the result determined by the AgriStrip method (Table 2), the positive findings were coincident with the diagnosis performed by the two methods.

The biological method performed with the GF 305 indicator, which targeted the diagnosis of mother plants of 'Augustin', 'Hungary's Best', 'Mamaia' and 'Umberto' varieties, did not reveal symptoms that could have been associated with viral infections (Table 3). The ELISA test performed on the same parent plants for PDV and PNRSV viruses also had negative results.

By making a general analysis of the viral status of the apricot mother plantation at RSFG Constanţa (Figure 1), it can be observed that in 2016, the mother plants with which the mother plantation was established in 2008, the number of plants remained unchanged 'Augustin', 'The Best of Hungary', 'Ovidius', 'Auras' and 'Umberto'. Of the 'Sulina' and 'Elmar' varieties there are 96% virally healthy mother plants, 95% 'Olimp', 94% 'Amiral', 93% 'Goldrich', 92% 'Tudor' and 'Sirena' and 91% 'Mamaia'.

Mother plants with positive diagnosis have been removed from mother plantations.

## 4. Conclusions

During the 2010-2016 periods, the highest incidence of PPV virus was found in 9 mother plants, followed by PDV and PNRSV viruses identified in 2 mother plants and ACLSV in 1 mother plant. Not have been identified infections with the ApMV virus.

The DAS-ELISA test method confirmed the AgriStrip immune-chromatographic test for PPV virus and the biological testing method for PDV and PNRSV viruses of apricot.

## Acknowledgement

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

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***Tables and Figures***

**Table 1. Identification of the viruses to apricot using the DAS-ELISA method**

| Variety | Mother plants Year 2010 | Number of mother plants positive : | | | | |
|---|---|---|---|---|---|---|
| | | ***PPV*** | ***PDV*** | ***PNRSV*** | ***ACLSV*** | ***ApMV*** |
| Amiral | 50 | 1 | 1 | - | 1 | - |
| Augustin | 32 | - | - | - | - | - |
| Tudor | 25 | 2 | - | - | - | |
| Goldrich | 15 | - | - | 1 | - | - |
| Sirena | 25 | 2 | - | - | - | - |
| Cea mai bună de Ungaria | 57 | - | - | - | - | - |
| Sulina | 30 | - | 1 | - | - | - |
| Mamaia | 37 | 3 | - | - | - | - |
| Elmar | 26 | - | - | 1 | - | - |
| Olimp | 22 | 1 | - | - | - | - |
| Ovidius | 23 | - | - | - | - | - |
| Auraș | 22 | - | - | - | - | - |
| Umberto | 6 | - | - | - | - | - |
| **TOTAL** | **370** | **9** | **2** | **2** | **1** | **0** |

**Table 2. Comparative analysis use different laboratory methods for *PPV* virus identification**

| Variety | Number of samples identified positiv | |
|---|---|---|
| | **DAS-ELISA** | **AgriStrip** |
| Amiral | 1 | 1 |
| Augustin | - | - |
| Tudor | 2 | 2 |
| Goldrich | - | - |
| Sirena | 2 | 2 |
| Cea mai bună de Ungaria | - | - |
| Sulina | - | - |
| Mamaia | 3 | 3 |
| Elmar | - | - |
| Olimp | 1 | 1 |
| Ovidius | - | - |
| Auraș | - | - |
| Umberto | - | - |

**Table 3. Results of identification test of *PDV* and *PNRSV* virus using the DAS-ELISA serological method and the biological method of some apricot mother plants**

| Variety | *PDV infections* | | *PNRSV infections* | |
|---|---|---|---|---|
| | **GF 305** | **DAS-ELISA** | **GF 305** | **DAS-ELISA** |
| Augustin | - | - | - | - |
| Cea mai bună de Ungaria | - | - | - | - |
| Mamaia | - | - | - | - |
| Umberto | - | - | - | - |

DOI 10.33045/fgr.v34.2018.24

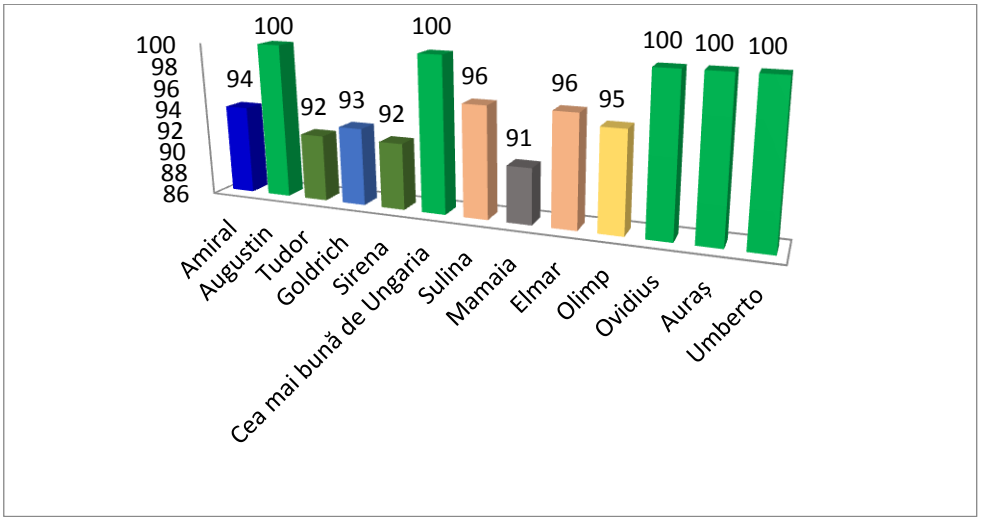


**Fig.1. Proportion (%) of mother plants health from of viral point of view in the apricot mother plantation in 2016**


**Photo 1, 2, 3. *Plum pox virus*, symptoms on leaves and stone of apricot**

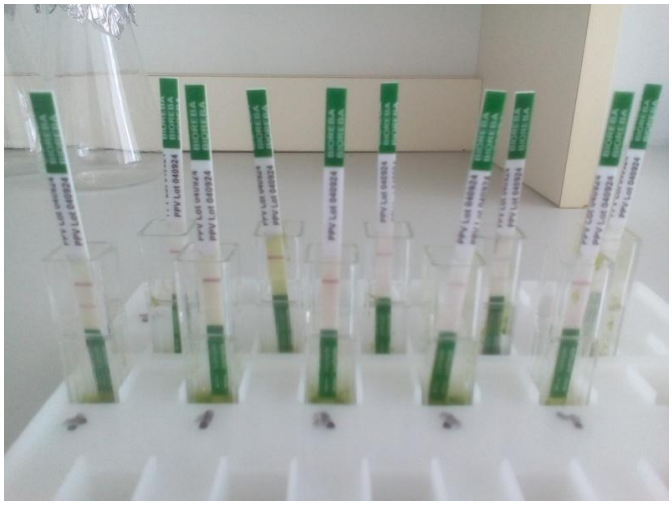

**Photo 4. The AgriStrip immune-chromatographic test, performed for rapid identification of the *PPV* virus**