

EVALUAREA STĂRII FITOVIRALE A UNOR LIVEZI DE PRUN DIN REGIUNEA MUNTENIA

ASSESSMENT OF THE PHYTOVIRAL STATUS OF SOME PLUM ORCHARDS IN THE MUNTENIA REGION

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Abstract

To reduce the damage caused by plum virus infections, were evaluated phytoviral, plantations aged between 1 and 13 years in different areas of Muntenia: Breasta-Dolj, Dobrețu-Olt, Nicolae Bălcescu-Vâlcea, Cepari and Bălilești-Argeș, Bucov-Prahova, Săhăteni/Istrița-Buzău, Sărulești-Călărași, Pietroșani-Teleorman, in order to identify viruses with special economic implications for this species: *PPV*, *PDV*, *PNRSV*, *ACLSV* and *ApMV*. The varieties, included in the 10 orchards analyzed were: 'Anna Spath', 'd'Agen', 'Stanley', 'Cacanska Lepotica', 'Centenar', 'President', 'Tepend' and 'Tophit'. They were followed: the symptomatology, the highlighting by the serological method DAS-ELISA as well as the etiology of the viruses. The results of the diagnosis by the DAS-ELISA test, highlighted the presence of positive samples: 14.7% *PPV*, 0.3% *ApMV* and 0.05% *PDV*. The cause of infection of the plants found positive was established as depending on circumstances both in the nursery and occurred during life in the orchard.

Cuvinte cheie: virus, metoda ELISA, plantatie, prun.

Key words: virus, ELISA method, orchard, plum tree.

1. 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). *Plum pox virus (PPV)*, is considered the most harmful virus that attacks the stone species and causes losses of up to 80-100% (Nemeth, 1986; Cambra et al., 2006). *PPV* is transmitted by vegetative and natural propagation through vector aphid species. In addition to *PPV*, *Prunus necrotic ringspot virus (PNRSV)*, *Prune dwarf (PDV)* and *Apple mosaic virus (ApMV)* also, cause significant damage with different distribution worldwide (Matic et al., 2008). *Apple chlorotic leaf spot (ACLSV)* is also an economically important virus due to its high prevalence and effects even if the infection caused by this virus is not externalized by symptoms in most varieties. All these viruses can seriously affect the yield of production and the development of trees (Uyemoto et al., 1992). The use of infected plant material for propagation is the main cause of long-distance spread, as at short distances, the spread is mediated by a number of aphid species in a non-persistent manner (Labonne et al., 1995; Isac et al., 1998). Control measures depend on the identification of diseases and their etiological agents. Diagnosis is the most important aspect of controlling fruit plant viruses. Early detection of viruses in fruit trees or in propagating material is a prerequisite for their control and to guarantee a sustainable agriculture (Barba et al., 2014). Vector control plays an important role in the management of systemic diseases, but should be used in conjunction with other control measures such as the eradication of infected plants and the use of certified propagating material.

2. Material and methods

Ten plum orchards in the Muntenia-Oltenia Region (map 1) were evaluated in June 2020. Each plantation was coded with a number from 1 to 10, for each being recorded data on: location / assortment / age of the plantation / biological category of the propagating material used to establish the plantation / no. phytosanitary treatments applied until the time of evaluation and sampling viral analysis:

- 1/ Breasta-Dolj district/ Anna Spath, D'Agen, Stanley, Cacanska Lepotica, Centenar/4/ CAC/3;
- 2/ Dobrețu, Olt district/ Stanley/2/CAC/-;
- 3/ Nicolae Bălcescu, Vâlcea district / Centenar, Stanley, Anna Spath, D'Agen/3/CAC/3;
- 4/ Cepari, Argeș district / President, Stanley, Anna Spath/7/ CAC/5;
- 5/ Bălilești, Argeș district / Tepend, Tophit/2/CAC/-;
- 6/ Bucov, Prahova district / Stanley, Anna Spath, Centenar/1/ Certificat/2;
- 7/ Săhăteni- Istrița, Buzău district / Anna Spath, Stanley/13/ Certificat/4;

8/ Sărulești Călărași district / Stanley/2/ Certificat/8;

9/ Pietroșani, Teleorman district / Anna Spath, Stanley/8/ Certificat/-;

10/ Pietroșani, Teleorman district / Stanley/8/ Certificat/-;

In every plantation, for evaluation and sampling of leaves, were delimited 2 blocks of 100 trees, containing all the varieties from the plantation. Another criterion in the location of the blocks was to include areas with symptomatic trees (PPV, ACLSV, PDV, PNRSV, ApMV), if any. Visual observations were made in each block and 10 samples were collected randomly: one sample with symptoms in 10 symptomatic trees and one sample without symptoms in 10 asymptomatic trees. Nearby plum orchards (1-200 m) were visually checked and the incidence of PPV was established based on symptoms to check for the potential presence of outbreaks / sources of infection nearby.

As evaluation methods used in addition to the visual observations performed was laboratory testing by DAS-ELISA serological technique (Cark & Adams, 1977), using a commercial polyclonal antiserum for *PPV*, *PDV*, *PNRSV*, *ACLSV*, and *ApMV* viruses (Bioreba, Switzerland). The absorbance values were measured at 405 nm after 30-120 minutes of hydrolysis of the substrate. The samples were considered positive if the absorbance values were more than 2.5 times higher than those of the negative control. Then, an infection rate was set for each virus.

3. Results and discussions

A. Identification of viral infections

Visual observations effected in plantations revealed the presence of viral symptoms for 301 plants out of 2,000 evaluated (Table 1).

Plantation 1, were established 2 blocks of 4 rows, of 25 trees each row. Visually were identified with symptoms of *PPV*: 'Centenar' 25 trees from 25 trees analyzed, 'd'Agen' 6 trees from 50 trees analyzed, 'Stanley' 7 trees from 50 trees evaluated, 'Cacanska Lepotica' 5 trees from 50 analyzed, 'Anna Spath' 3 trees from 25 analyzed (Fig.1).

Plantation 2, were established 2 blocks of 4 rows of 25 trees each row, of the 'Stanley' cv. Visually, were identified *PPV* symptoms on the leaves of a shoot on a single tree.

Plantation 3, those two blocks established had 10 rows of 10 trees each row. Visually were identified *PPV* symptoms, trees from variety: 'Centenar' 30 trees from 30 trees analyzed, 'Stanley' 6 trees from 70 trees evaluated 'd'Agen' 5 trees from 30 trees analyzed, 'Anna Spath' 5 trees from 70 analyzed. For 'd'Agen' variety, were identify on the leaves of a one tree, symptoms that were confirmed to be caused by the *ApMV* virus (Fig. 2).

Plantation 4, those two blocks had 5 rows of 20 trees each row. Visually, they were identified with symptoms of *PPV*, trees from varieties: 'Stanley' 14 trees from 110 trees analyzed and 8 tree with symptoms from 80 evaluated of 'Anna Spath' cv. The viral symptoms not observed on 'President' cv. (Fig. 3)

Plantation 5, were established 2 blocks of 4 rows of 25 trees each row. On the assortment formed by the 'Topend' and 'Tophit' cvs., a single tree was registered with symptoms, which following the serological ELISA test was established that they were produced by the *PDV* virus.

Plantation 6, were established 2 blocks of 4 rows of 25 trees each row. The trees from Anna Spath, 'Stanley' and 'Centenar' cvs., in the 1st year after planting don't showed symptoms on the leaves.

Plantation 7, were established 2 blocks of 4 rows of 25 trees each row, represented by 50 trees by 'Anna Spath' cv. and 150 trees by 'Stanley' cv. Of the 'Stanley' cv., were identified 6 trees sporadically distributed with *PPV* infection.

Plantation 8, were established 2 blocks of 4 rows of 25 trees each row, represented by 200 trees of 'Stanley' cv. Didn't showed the viral symptoms.

Plantation 9, every block has 4 rows of 25 trees with 'Stanley' cv. On leaves were observed typical symptoms of *PPV* of 115 tree and other symptoms that could be attributed of some viral infections to 3-tree, which were determined by DAS-ELISA test, to be caused by *ApMV* (Figure 4).

Plantation 10, every block has 4 rows of 25 trees with 100 trees 'Stanley' cv. and 100 trees 'Anna Spath' cv. On leaves were observed typical symptoms produced by *PPV* to 39 trees 'Stanley' cv. and 20 trees 'Anna Spath' cv. and other symptoms that could be attributed of some viral infections to 2-tree by Anna Spath, which were determined by DAS-ELISA test, to be caused by *ApMV* (Figure 5).

Analyzing the incidence of viruses detected after visual observations and tests by serological method DAS-ELISA, which confirmed the symptoms, it is found that most infections were caused by *PPV* virus, 294 (14.7%) positive trees, followed by *ApMV* 6 (0.3%) positive trees and one tree (0.05%), found positive with *PDV*.

B. Identification of viral infection sources -etiology.

The etiology of most viruses in fruit trees is still unknown. In plum, although some of the viruses with incidence are transmitted by grafting: *PPV* and *ApMV* or vector insects (especially aphids), the case of *PPV*

virus, pollen or seed *PDV* virus, the success of transmission also depends on various factors: concentration in the virus, temperature, etc. Gilmer (1961; 1963) explains the very poor spread of pollen compared to the seed transmission of *PDV*, in that in some varieties, eggs pollinated with infected pollen die before the virus invades neighboring cells.

The strategies for managing infection and the spread of orchard infections are based on the factors that lead to this risk. Thus, after viral evaluation performed, by analyzing the influencing factors of the spread of viruses in the 10 plantations studied, was established the cause / origin of the disease of the trees.

Plantation 1. The plum plantation has *PPV* infections. Infection rate by 23% with *PPV*, generalization of infection in the crown of trees, plantation age (4 years), use of CAC planting material, untested virologically, the existence of old apricot and plum plantations nearby, indicates a high probability that the trees, was infected from the nursery as well as in the orchard with a high rate of spread in the orchard.

Plantation 2. The virotic condition is very good, it shows infections with *PPV*, on a single tree, isolated on the leaves of a shoot. Considering the age of the plantation (2 years), the use of CAC seedlings, it can be concluded that the source of infection is from the nursery.

Plantation 3. The plum plantation is infected with *PPV* in a proportion of 20%, the presence of both generalized and isolated infections and the infection with *ApMV* in a proportion of 0.5%, the age of the plantation (4 years), the presence of scattered plums in the family gardens, use of CAC planting material, without viral evaluation, indicates that the material was mainly infected in the nursery.

Plantation 4. The plum plantation is 11% infected with *PPV*. The infections in the crown of the trees are mostly generalized, the age of the plantation (7 years), the use of planting material CAC, suggests that some of the material was infected in the nursery, but can not be excluded and infections that occurred after the establishment of the plantation, provided that, there are scattered plums in family gardens that have *PPV* infections.

Plantation 5. The infection rate (0.5%) is low, indicates a good phytovirotic status, but the *PDV* infection most likely occurred in the nursery, provided that, this virus is transmitted through pollen and seed and the plantation is only 2 years old.

Plantation 6. Very good phytovirotic status. The 1-year-old plum plantation, established with planting material, biological category Certificate, has no viral infections.

Plantation 7. Good phytoviral status. The 13-year-old plum plantation, established with planting material, biological category Certificate, shows only sporadic infections with *PPV* (3%).

Plantation 8. Very good phyto-virotic status. The 2-year-old plum plantation, established with planting material, biological category Certificate, does not show viral infections.

Plantation 9. The plum plantation established with planting material, biological category Certificate, is massively infected with *PPV*. Provided that partial infections predominate in the crown of trees and there is a source of inoculation nearby, there is a high probability that within 8 years since the plantation was established, most of the infection has occurred. However provided that, high infection rate of 57.5%, there is also the probability that another part of the infections occurred in the nursery.

Plantation 10. The plum plantation established with planting material, biological category Certificate, is massively infected with *PPV*. Provided that partial infections predominate in the crown of trees and there is a source of inoculation nearby, there is a high probability that within 8 years since the plantation was established, most of the infection has occurred. However provided that, high infection rate of 29,5 %, there is also the probability that another part of the infections occurred in the nursery.

4. Conclusions

In the case of very young plantations with a low infection rate, the main measure to limit the spread of the virus is to eliminate of the infected trees and extend monitoring throughout the plantation and in the event of infections to replace diseased trees with healthy trees;

In the case of medium and high rates of *PPV* infection, eliminating infected trees to limit the impact of *PPV* is not an economical solution, although profitability will be affected. The maintenance of the plantation is based on the fact that there are also tolerant varieties in the plantation: Stanley, Anna Spath, Cacanska lepotica and it is recommended to respect the cultivation technology, insisting on phytosanitary treatments to limit / slow down the propagation of *PPV* to healthy plants;

Assessing the spread of plum viruses will promote the rationale for the effectiveness of the monitoring system.

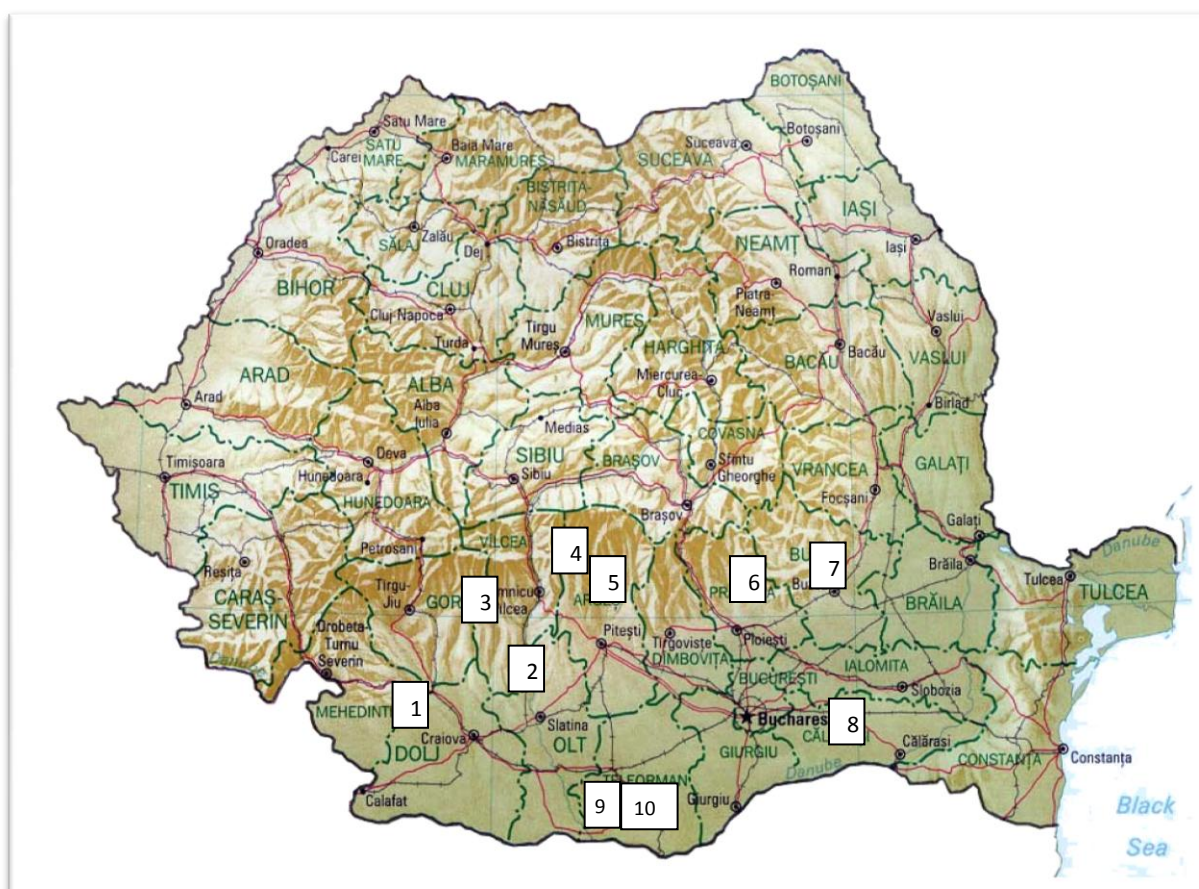
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Tables and Figures



Map 1. Location of evaluated plantations in Romania

Table 1.The results of the visual assessment

Plantation	Nr. evaluated plants / no. plants with visual symptoms	Description of symptoms
1	200/46	Light green spots, or diffuse rings on the leaves, generalized in the crown of trees (typical <i>PPV</i>), to 46 trees
2	200/1	Light green spots on the leaves of a shoot (typical <i>PPV</i>), on 1 tree.
3	200/46	Light green spots, or diffuse rings on the leaves, generalized in the crown of trees to 45 trees (typically <i>PPV</i>) and 1 tree with symptoms on the leaves that would suggest viral infections.
4	200/22	Light green spots, or diffuse rings on the leaves, generalized in the crown of trees to 22 trees (typically <i>PPV</i>).
5	200/1	1 tree with symptoms on the leaves that would suggest viral infections.
6	200/-	The trees have no symptoms.
7	200/6	Light green spots, or diffuse rings on the leaves, generalized in the crown of trees to 6 trees (typically <i>PPV</i>).
8	200/-	The trees have no symptoms
9	200/118	Light green spots, or diffuse rings on the leaves, generalized in the crown of trees to 115 trees (typically <i>PPV</i>) and other symptoms that could be attributed to viral infections in 3 trees
10	200/61	Light green spots, or diffuse rings on the leaves, generalized in the crown of trees to 59 trees (typically <i>PPV</i>) and other symptoms that could be attributed to viral infections in 2 trees.
Total	2.000/301	

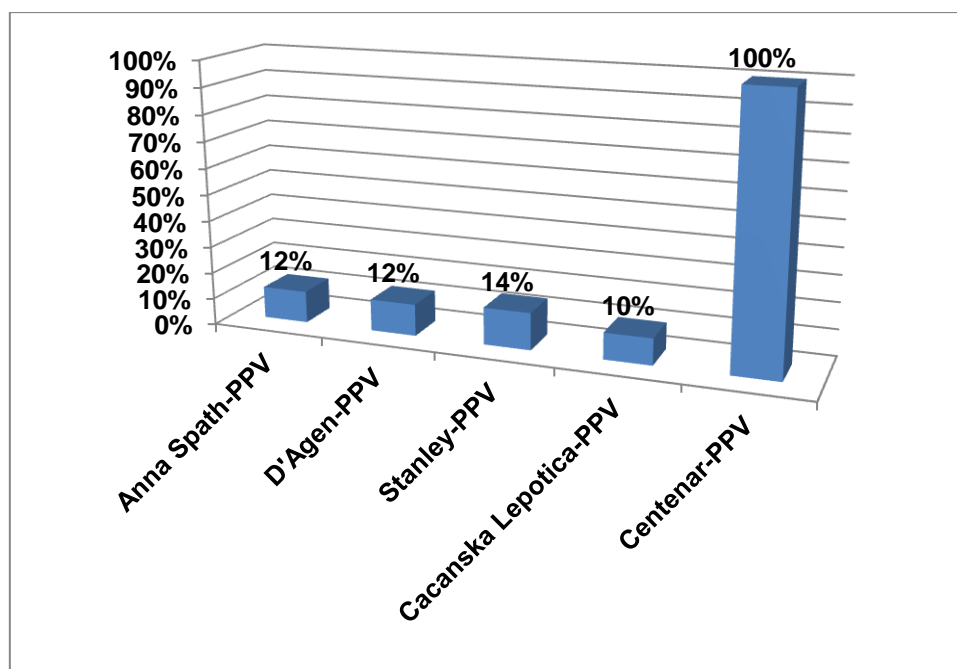


Fig. 1. The degree of infection of the varieties in the blocks evaluated in Plantation 1

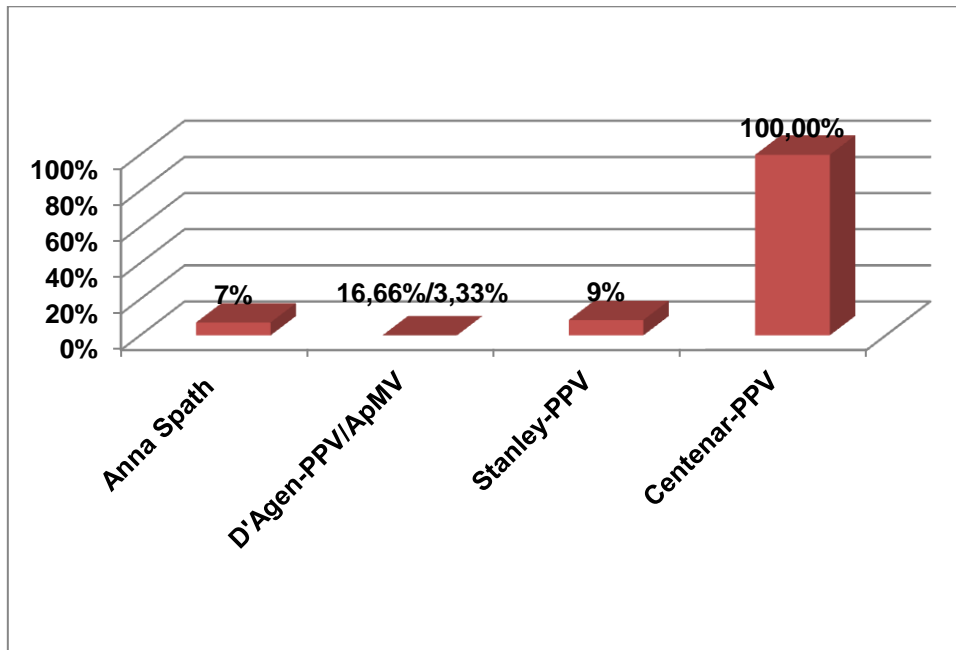


Fig. 2. The degree of infection of the varieties in the blocks evaluated in Plantation 3

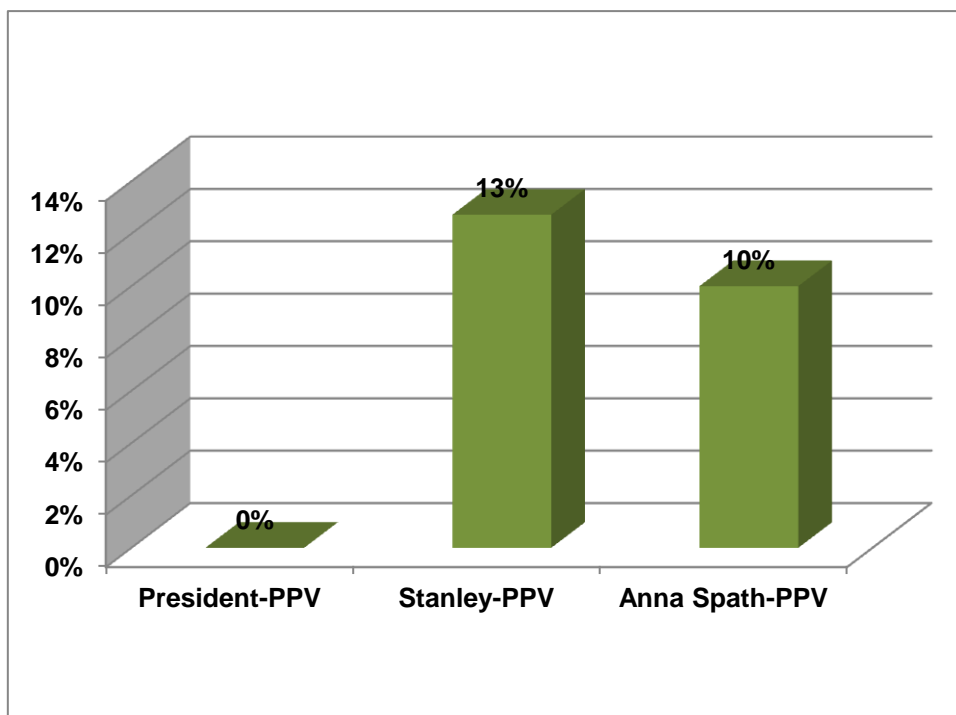


Fig. 3. The degree of infection of the varieties in the blocks evaluated in Plantation 4

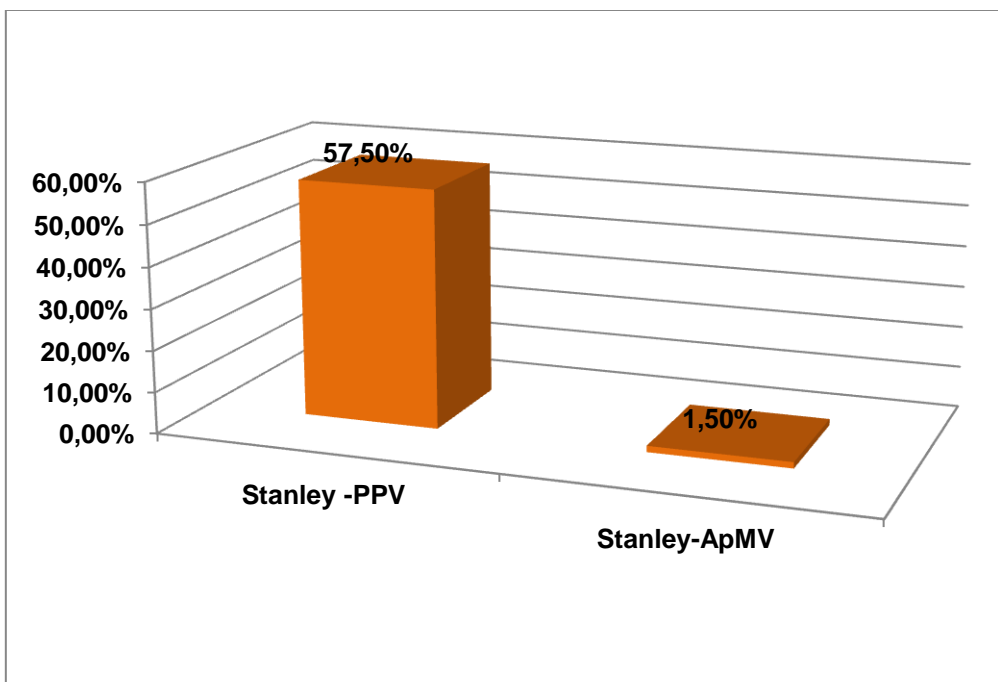


Fig. 4. The degree of infection of the varieties in the blocks evaluated in Plantation 9

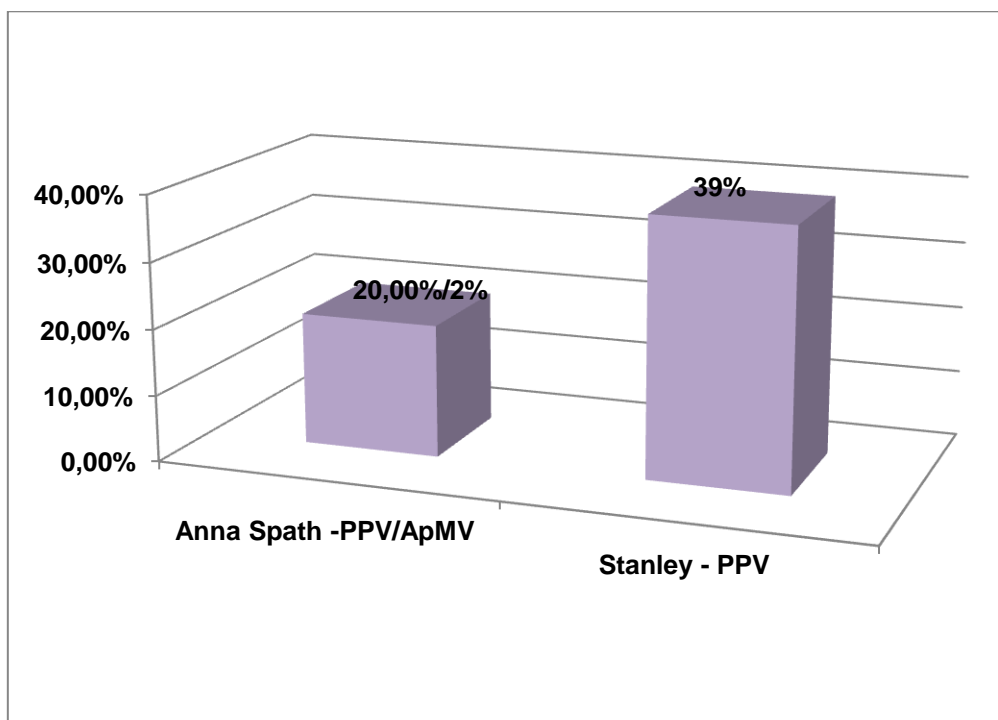


Fig. 5. The degree of infection of the varieties in the blocks evaluated in Plantation 10

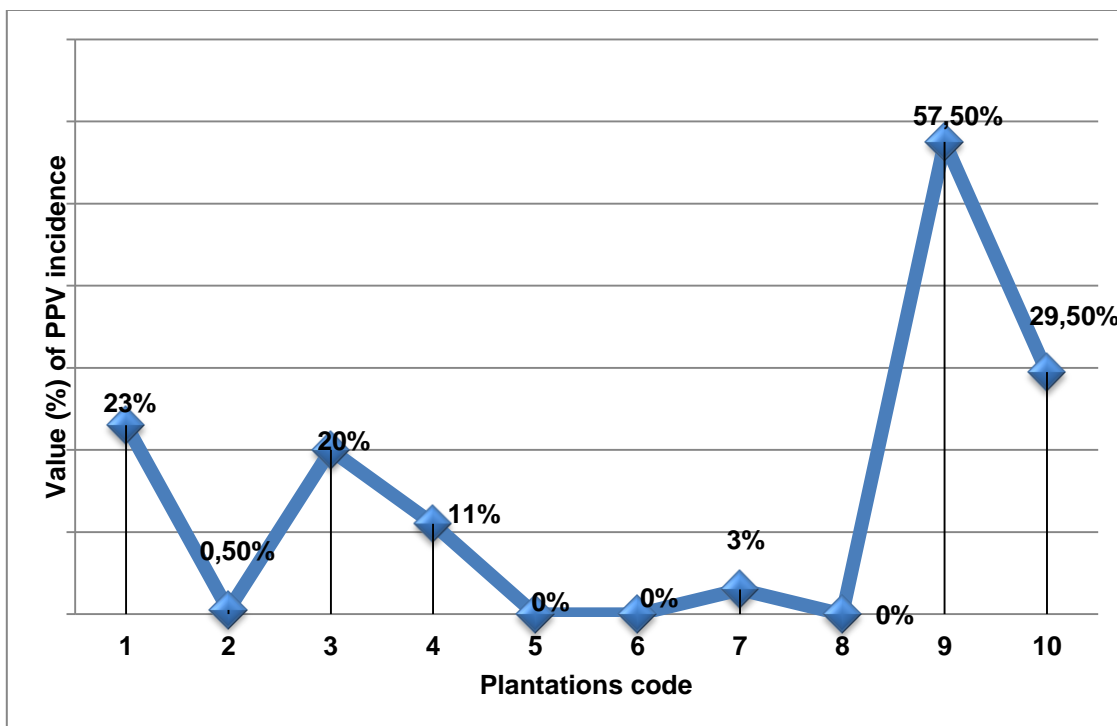


Fig. 6. Incidence of *PPV*

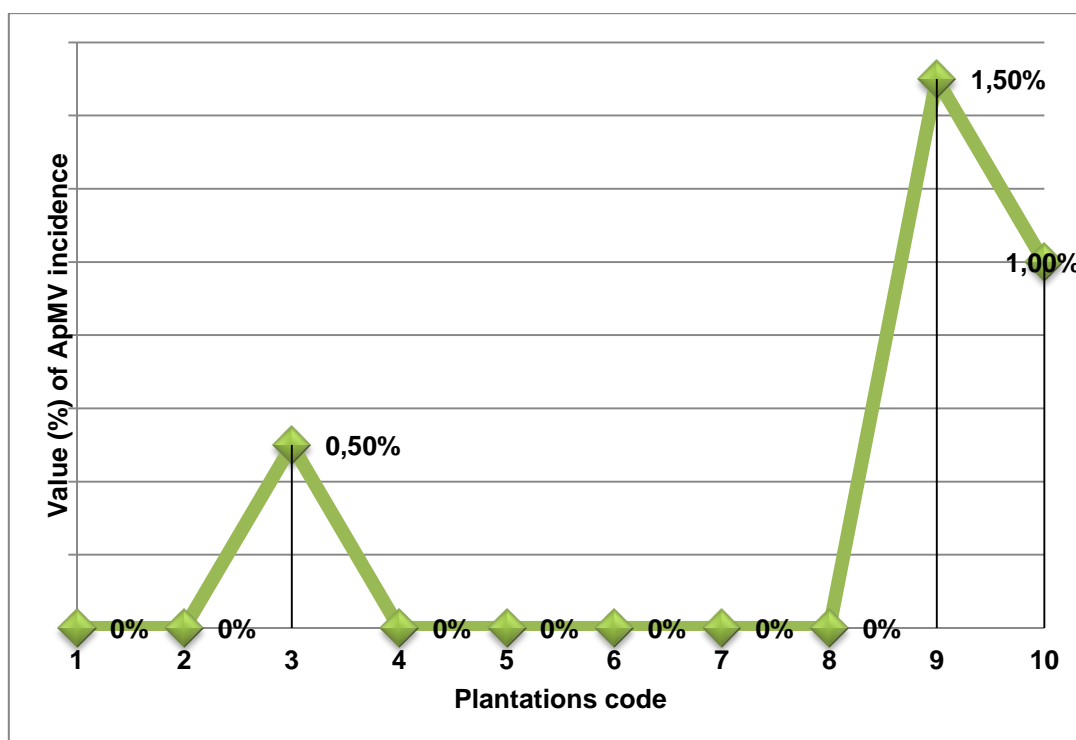


Fig. 7. Incidence of *ApMV*

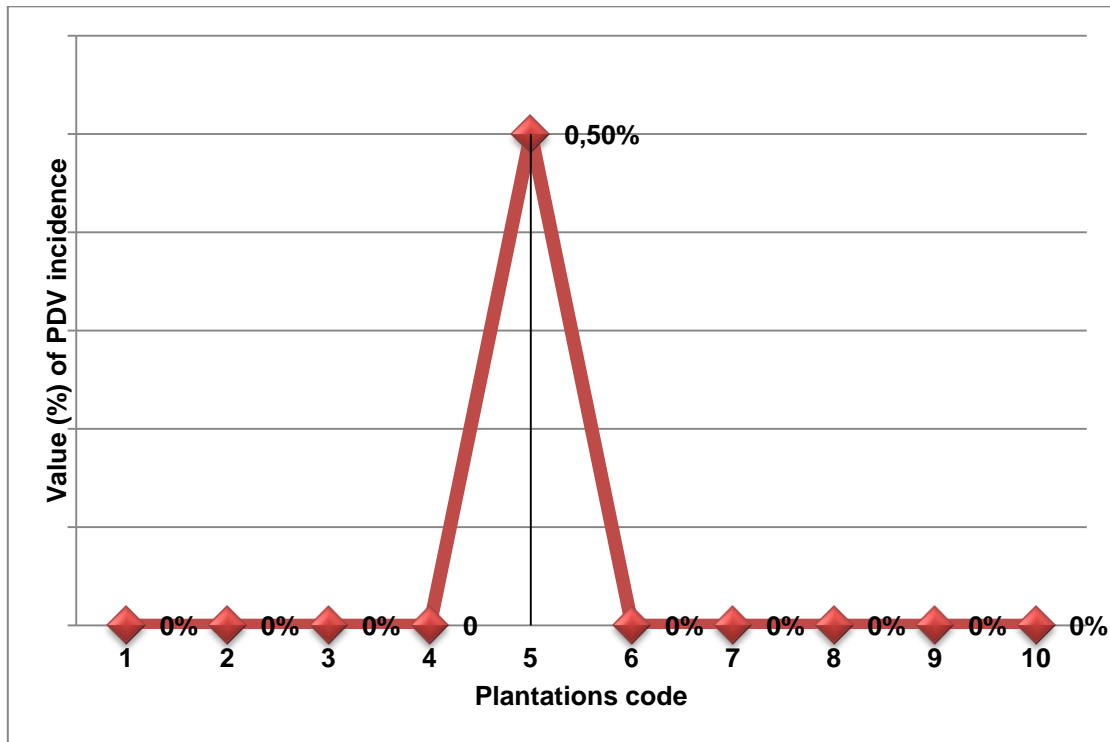


Fig. 8. Incidence of *PDV*