Scientific Papers. Series B, Horticulture. Vol. LXX, No. 1, 2026
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

NATURAL PLUM POX VIRUS INFECTION IN PLUM: FIVE-YEAR FIELD ASSESSMENT OF GENOTYPIC RESPONSE

Luminița Antonela ZAGRAI, Ioan ZAGRAI, Georgeta Maria GUZU, Claudiu MOLDOVAN

Fruit Research & Development Station Bistrița, 3 Drumul Dumitrei Nou Street, Bistrița, Romania

Corresponding author email: izagrai@yahoo.com

Abstract

Thirty-four plum genotypes were monitored over a five-year period (2021-2025) to evaluate their response to natural infection with plum pox virus (PPV), the most damaging viral pathogen of stone fruit species. The experimental orchard was established at FRDS Bistrița in spring 2020 using virus-free planting material. Two to six trees per cultivar were planted in the field and visually inspected during each growing season. During the first three vegetative periods, trees showing PPV symptoms were removed, destroyed, and replaced with healthy planting material. In 2024 and 2025, symptomatic trees were no longer removed in order to avoid large age differences within the orchard and to maintain comparable conditions among genotypes. Visual monitoring for PPV symptoms continued during these years, and in 2025 all trees were additionally tested serologically using DAS-ELISA. The elimination of PPV sources during the first three growing seasons limited virus spread, resulting in a low PPV incidence of 2.7% in 2023. After this practice was discontinued in 2024-2025, virus spread increased sharply, with PPV incidence reaching 14.1% by 2025. Nevertheless, thirteen cultivars remained symptomless throughout the five-year period, and PPV infection was not detected by DAS-ELISA.

Key words*: cultivars, DAS-ELISA, sharka, survey, visual monitoring.*

INTRODUCTION

Plum is one of the most widespread and economically important fruit species in temperate regions, mainly due to the extensive cultivation of *Prunus domestica* L. (Martínez-Gómez et al., 2026). It is used both for fresh consumption and for processed products such as dried plums, jams, juices, and traditional drinks, which increases its economic value in Eastern and Central Europe and supports rural development and agro-biodiversity in traditional growing areas (Wangchu et al., 2021). Despite these advantages, plum growing is often severely affected by viral diseases, among which *Potyvirus plumpoxi* (plum pox virus, PPV), the causal agent of sharka disease, is recognized as the most economically important viral pathogen of stone fruit species (García et al., 2024). Moreover, PPV is considered one of the ten most important plant viruses worldwide (Scholthof et al., 2011), highlighting its global economic impact and the major threat it poses to stone fruit production.

Plum pox virus causes substantial losses in yield and fruit quality (García et al., 2014; Barba et al., 2015). Its spread is facilitated by nearby infection sources from other *Prunus* species, including *P. spinosa*, *P. cerasifera*, *P. salicina*, and *P. domestica* (Llácer and Cambra, 2006; Barba et al., 2011).

The virus is efficiently transmitted by aphids in a non-persistent manner (Labonne et al., 1995), which allows rapid dissemination within and between orchards. The impact of PPV is particularly significant in countries where plum represents a major fruit crop, such as Romania. Plum ranks first among fruit species in the country in terms of production, exceeding apple production, and Romania currently ranks second worldwide in plum production after China (FAOSTAT, 2026). A survey conducted in Romanian plum orchards showed a PPV incidence of approximately 69%, indicating that the virus has an endemic presence in these growing areas (Zagrai et al., 2010).

A recent survey at the national level conducted in young plum orchards (1 to 5 years old), reported a PPV infection rate of about 23.5%, with values varying widely between 0.5% and 77.5%. (Zagrai I. et al., 2022). These findings confirm that PPV remains a persistent problem in Romania and underline the importance of systematic monitoring, as well as the use of

certified pathogen-free planting material in accordance with EU directives (Zagrai L. et al., 2022). In susceptible cultivars, yield losses can be severe, occasionally reaching total crop failure under high PPV infection pressure (Minoiu & Lefter, 1987; Minoiu, 1997; Zagrai et al., 2001). In addition to quantitative losses, fruit quality deterioration further reduces market value and processing suitability. Cambra et al. (2024) estimated that sharka disease caused losses of about €2.4 billion between 1995 and 2023, while total cumulative losses since its emergence may exceed €13 billion. Therefore, sharka disease is not only a plant health problem but also a serious economic threat to stone fruit production in Europe and in other affected regions, such as Romania.

The response of plum cultivars to PPV represents an essential component for selecting cultivars suitable for a given fruit-growing area (Zagrai et al., 2001; Butac, 2020). When infection sources are present in the surrounding environment, the genotypic response of cultivars becomes a decisive factor influencing orchard productivity and long-term sustainability. The use of PPV-resistant or highly tolerant plum cultivars, combined with virus-free planting material when establishing new orchards, represents one of the most effective preventive strategies to limit virus impact, since removing infected trees and controlling vectors alone do not provide sufficient protection. For this reason, breeding programs increasingly focus on the development of new resistant plum cultivars (Zhang et al., 2024). However, the practical value of genetic improvement must be confirmed through long-term field evaluations under natural infection pressure, which allow a realistic assessment of cultivar behaviour and the identification of genotypes with stable and reliable tolerance or resistance to PPV.

MATERIALS AND METHODS

Experimental trial

A total of 34 plum cultivars, comprising 19 Romanian (Tuleu gras, Diana, Matilda, Elena, Delia, Gras ameliorat, Zamfira, Iulia, Agent, Flora, Andreea, Jubileu 50, Stanley, Ivan, Minerva, Doina, Centenar, Carpatin and Zandra), and 15 foreign genotypes (French improved, Joganta, Jofela, Tophit, Topper, Topend plus, Top five, Jojo, Anna Spath, President, Blue free, Victoria, Empress, d'Agen, and Black Sun), grafted on Myrobalan 29C, were assessed over five consecutive years (2021-2025) to evaluate their response to natural PPV infection.

The experimental trial (Figure 1) was established at Fruit Research & Development Station Bistrița (FRDS Bistrița) in spring 2020, with two to six trees per cultivar included in the study. Other *Prunus* species scattered in the surrounding area of the plum orchard provided favourable conditions for natural PPV transmission by aphids.



Figure 1. The experimental plum orchard with 34 cultivars (FRDS Bistrița)

Visual monitoring

Each year during the vegetative period, all trees were inspected for typical PPV symptoms (Figure 2) particularly in spring (May - June), when symptom expression on young leaves is most pronounced.

Given that accurate recognition of PPV symptoms in infected plum trees can serve as a practical tool for orchard monitoring (Zagrai et al., 2024), visual assessments of the trees were conducted during the first four years. In the first three growing seasons after planting, symptomatic trees were removed and destroyed to eliminate potential sources of infection within the orchard, thus limiting the spread of the virus. They were subsequently replaced with healthy planting material in order to preserve the integrity of the the experimental design. After that, the removal of symptomatic trees was discontinued to prevent excessive age differences within the orchard and to ensure relatively uniform agronomic conditions for comparative evaluation of the studied genotypes.



Figure 2. Typical symptoms of PPV infection on *P. domestica* leaves

Serological analyses

In 2025, all trees were additionally subjected to serological testing using the DAS-ELISA method to detect latent or asymptomatic PPV infections, ensuring a more accurate assessment of the overall infection status within the orchard. A minimum of 10 leaves per tree were randomly collected from different parts of the canopy to ensure representative sampling. In the case of symptomatic trees, only leaves showing typical PPV symptoms were sampled. When symptoms were confined to specific branches, samples were collected exclusively from those affected branches. If only a limited number of symptomatic leaves were present, the sample size was adjusted accordingly, consisting exclusively of the available symptomatic leaves. Leaf samples were homogenized at a ratio of 1:20 (w/v) and analyzed using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) method described by Clark and Adams (1977). PPV detection was carried out with a PPV-specific commercial kit (Bioreba, Switzerland), following the manufacturer's instructions.

Samples exceeding twice the negative control absorbance were considered positive, with kit controls included in each assay.

RESULTS AND DISCUSSIONS

PPV monitoring by visual observation

During the first three growing seasons after planting, the incidence of natural PPV infections remained low (0.5-2.7%) due to the systematic removal of trees visually identified as infected (Table 1). When the removal of symptomatic trees was discontinued, the PPV incidence increased to 13.7%. Visual monitoring for PPV symptoms was continued, and in 2025 all trees were additionally tested using DAS-ELISA to detect latent or symptomless PPV infections.

DAS-ELISA assay

Serological testing of all trees (Table 1) showed that PPV incidence had increased to 14.1%, reflecting greater virus spread once infection sources were no longer controlled. Nine Romanian plum cultivars (Matilda, Elena, Delia, Gras ameliorat, Agent, Jubileu 50, Minerva, Carpatin, and Zandra), and four foreign plum cultivars (Joganta, Jofela, Top Five, Jojo) did not display typical PPV symptoms over the five-year period, despite continuous infection pressure. Furthermore, serological analyses did not detect PPV in these genotypes, suggesting a favourable response under natural infection conditions, although longer-term observations are required to confirm this behaviour.

Temporal spread of PPV

The removal of PPV infection sources during the first three growing seasons clearly mitigated virus dynamics (Figure 2). Once this practice was discontinued, PPV incidence increased sharply. The second-degree polynomial model captures this change, reflecting an accelerated rather than linear trend over time. The model's coefficient of determination ($R^2 = 0.868$) indicates that approximately 87% of the observed variability is explained, supporting its adequacy. The data indicate an initial phase of low PPV incidence, followed by a rapid increase in infections after 2023, when infected trees were no longer removed from the orchard. The upward trend can be attributed both to the presence of infection sources within the orchard and to external inoculum pressure from surrounding *Prunus* species.

Infected trees acted as local inoculum sources until removal, while asymptomatic infections likely contributed to viral persistence. During aphid flight periods, the latency between transmission and symptom expression allowed recently infected trees to remain symptomless but infectious, facilitating further spread to neighbouring trees.

Table 1. Visual monitoring followed by serological analyses of plum cultivars to PPV

Cultivar	No. of PPV infected trees/No. of total trees				
	Visual monitoring of PPV/year				DAS-ELISA
	2021	2022	2023	2024	2025
President	0/6	0/6	0/6	1/6	1/6
Tuleu gras	0/6	0/6	0/6	1/2	1/2
Diana	0/6	0/6	0/6	1/6	1/6
French improved	0/6	0/6	0/6	3/6	5/6
Joganta	0/6	0/5	0/6	0/5	0/5
Jofela	0/6	0/6	0/6	0/5	0/5
Tophit	0/6	0/6	1/6	2/5	2/5
Topper	0/6	1*/6	0/5	1/5	0/6
Topend plus	0/6	0/6	0/6	0/6	1/6
Top five	0/6	0/6	0/6	0/6	0/6
Jojo	0/6	0/6	0/6	0/6	0/6
Anna Spath	1*/6	0/6	0/6	1/6	1/6
Matilda	0/6	0/6	0/6	0/5	0/5
Elena	0/6	0/5	0/5	0/6	0/6
Delia	0/6	0/6	0/6	0/6	0/6
Gras ameliorat	0/6	0/6	0/6	0/6	0/6
Zamfira	0/6	0/6	0/6	1/6	1/6
Iulia	0/6	0/6	0/6	1/6	1/6
Agent	0/6	0/6	0/6	0/6	0/6
Flora	0/6	0/6	0/6	1/6	1/6
Andreea	0/6	0/6	0/6	1/6	1/6
Jubileu 50	0/6	0/6	0/6	0/6	0/6
Stanley	0/6	0/6	0/6	1/6	1/6
Ivan	0/6	1*/6	0/5	2/6	1/6
Minerva	0/6	0/6	0/6	0/6	0/6
Doina	0/6	0/6	0/6	1/6	1/6
Centenar	0/6	0/6	1/6	1/6	1/6
Carpatin	0/6	0/6	0/6	0/6	0/6
Blue free	-	0/4	3*/4	0/4	0/4
Victoria	-	0/4	0/4	2/4	2/4
Empress	-	0/4	0/4	1/4	1/4
Zandra	-	0/2	0/2	0/2	0/2
d'Agen	-	0/2	0/1	1/6	1/6
Black Sun	-	0/4	0/4	2/4	2/4
PPV	1/168 (0.5%)	2/186 (1.1%)	5/184 (2.7%)	25/183 (13.7%)	26/184 (14.1%)

*Trees infected with PPV were removed during the first three vegetative seasons and replaced with healthy trees when planting material of the respective cultivars was available. The spaces in the table marked with "-" indicate the absence of the respective cultivars in 2021, as the trees were planted in autumn 2021 or spring 2022.

As no curative treatments are available for viral diseases under field conditions, management depends on preventive strategies, including the use of pathogen-free planting material, the removal of infection sources to limit PPV spread, complemented by physical barriers and resistant cultivars that reduce virus transmission (Cambra & Vidal, 2017).

The small difference between the incidence estimated in 2024 (13.7%, based on visual symptoms) and that determined in 2025 by DAS-ELISA testing (14.1%) can be explained by several biological and epidemiological factors. Most trees infected in 2024 were already symptomatic and detected through visual inspection.

Under relatively stable infection pressure in the orchard, the proportion of additional latent or asymptomatic infections was likely low. The serological testing performed in 2025 confirmed nearly the same number of infected trees, indicating that visual monitoring was effective in identifying most cases.

Within a single vegetative season, virus spread was probably limited. Plum pox virus is primarily transmitted by aphids, and the infection rate depends on vector abundance, climatic conditions, and cultivar susceptibility.

An additional factor that may have contributed to the limited increase in PPV incidence between 2024 and 2025 was the severe hailstorm on June 4, 2024, which almost completely defoliated the trees. By the time the foliage recovered, the peak aphid flight period had passed, and the population dynamics of aphid vectors capable of transmitting the virus were likely affected, reducing opportunities for new infections. If these factors did not favor intensive transmission during 2024-2025, newly infected trees would remain few. Additionally, the short interval between assessments may have limited detection of recent infections, as newly infected trees might not yet accumulate sufficient viral load or develop visible symptoms.

Overall, the close agreement between visual assessment and serological testing suggests that careful visual monitoring provided a reasonable reliable representation of the orchard's epidemiological status, with DAS-ELISA identifying only a small number of additional latent or weakly symptomatic infections, thereby supporting our previous finding (Zagrai et al., 2024).

The differences observed among cultivars under natural PPV infection highlight the importance of cultivar selection for both breeding programs and orchard management. Cultivars that remained symptomless over five-year experiment and tested negative in serological assays may be of interest for further investigation, although longer-term studies are needed to confirm their performance under continued PPV exposure. At the same time, the use of less susceptible cultivars in commercial orchards may be considered in regions where PPV occur, but primarily when these cultivars offer significant advantages in fruit quality, yield, or other agronomic traits.

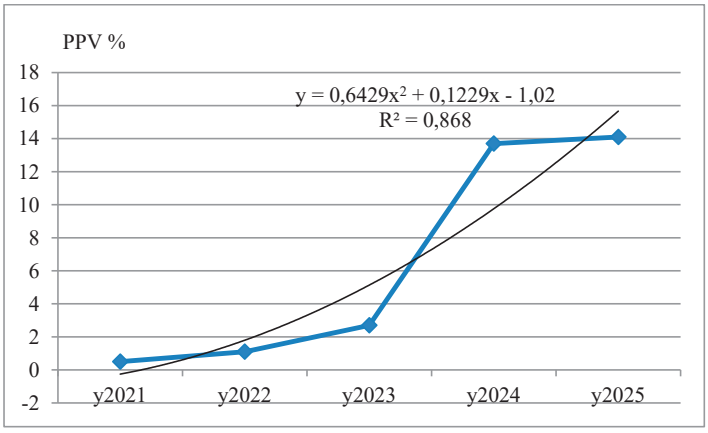

Figure 2. Dynamics of PPV infections in a plum orchard (comparative trial with 34 varieties) where infected trees were removed up to the third year after planting (2023)

CONCLUSIONS

A five-year field evaluation clarified PPV dynamics and the epidemiological factors influencing virus spread within the experimental trial.

Several plum cultivars, developed both through Romanian breeding programs, including Zandra, Matilda, Elena, Delia, Gras ameliorat, Agent, Jubileu 50, Minerva and Carpatin, as well as foreign cultivars such as Joganta, Jofela, Top Five and Jojo, remained symptomless and tested negative for PPV by DAS-ELISA under continuous infection pressure. These findings suggest that these cultivars may be valuable for further studies, although longer-term evaluations are needed to draw definitive conclusions regarding their response to PPV. At the same time, the removal of infected trees during the first three years likely helped limit local inoculum sources, contributing to the low infection levels determined throughout this period.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Ministry of Agriculture and Rural Development, ADER 6.3.4/2023 Project and also with the support of the Academy of Agriculture and Forest Science "Gheorghe Ionescu-Șișești", CDI-ASAS No. 2183/2023.

REFERENCES

Barba, M., Hadidi, A., Candresse, T. & Cambra, M. (2011). *Plum pox* virus. In *Virus-Like Diseases of Pome and Stone Fruits.* The American Phytopathological Society, St. Paul, Minnesota. Hadidi A., Barba M., Candresse T. and Jelkmann W. (eds.) pp. 185-197.

Barba, M., Ilardi, V., & Pasquini, G. (2015). Control of Pome and Stone Fruit Virus Diseases. *Advances in Virus Research*, *91*, 47-83.

Butac, M. (2020). Plum breeding. In: *Prunus*. IntechOpen, http://dx.doi.org/10.5772/intechopen.92432

Cambra, M., & Vidal, E. (2017). Sharka, a vector-borne disease caused by Plum pox virus: vector species, transmission mechanism, epidemiology and mitigation strategies to reduce its natural spread. *Acta Hortic*., *1163*, 57–68.

Cambra, M., Madariaga, M., Varveri, C., Caglayan, K., Morca, A.F., Chircov, S. & Glasa, M. (2024). Estimated costs of plum pox virus and management of sharka, the disease it causes. *Phytopathologica Mediterranea, 63*(3), 343-365.

Clark, M., & Adams, A.N. (1977). Characteristic of the microplate method of enzyme linked immunosorbent assay (ELISA) for detection of plant viruses. *J. Gen. Virology*, *34*: 475-483.

FAOSTAT, (2026). https://www.fao.org/faostat/en/#rankings/countries_by_commodity

García, J.A., Glasa, M., Cambra, M. & Candresse, T. (2014). Plum pox virus and sharka: a model potyvirus and a major disease. *Mol Plant Pathol. 15(3):*226-41. doi: 10.1111/mpp.12083

García, J.A., Rodamilans, B., Martínez-Turino, S., Valli, A.A., Simon-Mateo, C., & Cambra, M. (2024). Plum pox virus: An overview of the potyvirus behind sharka, a harmful stone fruit disease. *Ann Appl Biol*., 1-27, doi: 10.1111/aab.12958

Labonne, G., Yvon, M., Quiot, J., Avinent, L. & Llácer, G. (1995). Aphids as potential vectors of Plum pox virus: comparison of methods of testing and epidemiological consequences. *Acta Hortic. 386*:207-218. https://doi.org/10.17660/ActaHortic.1995.386.27

Llacer, G. & Cambra, M. (2006). Host and symptoms of Plum pox virus: fruiting *Prunus* species. *Bull OEPP, 36*(2):219-221. 10.1094/APSnetFeature-2000-0300.

Martínez-Gómez, P., Korićanac, A., Nicolás-Almansa, M., Ruiz, D., Farcuh, M., Stănică, F., Dardick, C., DeJong, T. (2026). Plum (*Prunus salicina*) and Prune (*Prunus domestica*), Ed. Manganaris, G. In: *Temperate Tree Fruits and Nuts*, Academic Press, Pages 295-321, ISBN 9780443291371, https://doi.org/10.1016/B978-0-443-29137-1.00007-4.

Minoiu N. & Lefter Gh. (1987). *Bolile și dăunătorii speciilor sâmburoase*. Editura Ceres, București.

Minoiu, N. (1997). Bolile și dăunătorii prunului (Plum Diseases and Pests). In: *Prunul (The Plum)*, Cociu, I., Botu, I., Minoiu, N., Modoran, I. (eds.) Editura Conphys, Pitesti, Romania. pp. 343-374.

Scholthof, K.B., Adkins, S., Czosnek, H., Palukaitis, P., Jacquot, E., Hohn, T., Hohn, B., et al. (2011). Top 10 plant viruses in molecular plant pathology. *Molecular Plant Pathology*, 12: 938-954.

Wangchu, L., Angami, T., & Mandal, D. (2021). Plum. in temperate fruits. *Apple Academic Press*. 297-331.

Zagrai, I., Ardelean, M., Maxim, A. & Zagrai, L. (2001). Researchers regarding the influence of *Plum pox virus* on production at different plum cultivars, clone and hybrids. *Jubilee session of Horticulture Faculty from Iasi. Horticulturae, 44*:150-151.

Zagrai, I., Zagrai, L., Preda, S., Isac, M., & Cardei, E. (2010). Incidence of Plum Pox Virus in Romanian Plum Orchards. *Bulletin UASVM, Horticulture 67*(1-2):488. ISSN 1843-5254.

Zagrai, I., Zagrai, L.A., Moldovan, C., Guzu, G., Roșu-Mareș, S., Plopa, C. & Butac, M. (2022). *Managementul integrat în prevenirea bolilor virotice la speciile prun și cireș. Ghid practic.* Editura Născut Liber, Bistrița. ISBN 978-606-95507-1-7; 155 pg.

Zagrai, L.A., Zagrai, I., Roșu-Mareș, S. D., & Moldovan, C. (2022). Assessment of the virus infections occurrence in new established plum and sweet cherry orchards in Transylvania, Romania. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 50(2), 12734-12734.

Zagrai, L., Zagrai I., Guzu G., Roșu-Mareș S. & Moldovan C. (2024). Is symptomatology reliable tool for plum pox virus monitoring. *Scientific Papers. Series B, Horticulture. Vol. LXVIII*(1), 238-243.

Zhang, L., Chen, C., Li, Y., Suo, C., Zhou, W., Liu, X., & Yang, X. (2024). Enhancing aphid resistance in horticultural crops: a breeding prospective. *Horticulture Research*, 11(12), 275.


EX·TERRA·AVRVM

VITICULTURE AND OENOLOGY


EX·TERRA·AVRVM