

A new Romanian *Prunus domestica* L. cultivar exhibiting high tolerance to natural infections to plum pox virus

L.A. Zagrai and I. Zagrai*

Fruit Research and Development Station Bistrita, Romania

*Corresponding author email: izagrai@yahoo.com

ABSTRACT

This study evaluates the long-term behaviour of plum pox virus (PPV) infections within a genetically diverse plum orchard established in 1998 at the Fruit Research & Development Station Bistrița, Romania. The experimental plot comprises six hybrids, including the recently homologated 'BN 4/1' hybrid as 'Zandra' cultivar, and ten plum cultivars, with a total of 534 trees. Over 20 years (2003–2024), the temporal progression of PPV was monitored through visual inspections and serological tests (DAS-ELISA), revealing a consistent increase in virus incidence from 11.4% to 58.4%. Significant differences in PPV susceptibility were found among the genotypes. Highly susceptible genotypes ('Diana', 'Geta', 'HAS 2G', 'Tuleu timpuriu') exhibited infection rates exceeding 83%, while susceptible genotypes showed incidence levels over 60%. Tolerant cultivars such as 'Stanley' and 'Čačanska Najbolja', displayed moderate infection rates ranging from 40% to 60%. Remarkably, the hybrid 'BN 4/1' ('Zandra' cultivar) displayed the lowest PPV incidence at just 20%. Infected 'Zandra' trees exhibited localized symptoms, confined to one or two shoots per tree, with no evidence of systemic infection throughout the canopy. Detailed serological analyses confirmed the restricted viral movement in symptomatic 'Zandra' trees. Moreover, 80% of the trees remained uninfected despite long-term exposure to high inoculum pressure from surrounding infected trees, indicating a high level of tolerance to natural PPV infection. In addition to its viral tolerance, 'Zandra' exhibits superior organoleptic qualities, highlighting its potential as a valuable cultivar for sustainable plum production systems.

Keywords: plum, sharka, high tolerance, serological diagnosis, visual monitoring

INTRODUCTION

European plum (*Prunus domestica* L.) represents the most important fruit species in Romania, ranking second globally in plum production, after China, and holds the top position in Europe (FAO, 2025). Despite its economic importance, viral infections severely impact both productivity and fruit quality. Sharka disease, caused by the *Potyvirus plumipoxi* (plum pox virus, PPV), is one of the most damaging diseases affecting stone fruit trees worldwide, and has had a significant economic impact for over a century (Barba *et al.*, 2011; Garcia *et al.*, 2024). First identified in Bulgaria at the beginning of the 20th century (Atanasoff, 1932), the virus has since spread across Europe, Asia, and parts of North and South America. It causes severe damage, particularly in susceptible cultivars (Minoiu, 1997), and is ranked among the top ten plant viruses globally due to its broad distribution and serious effects (Scholthof *et al.*, 2011). Cambra *et al.* (2024) estimated the economic losses caused by Sharka at €2.4 billion over the last 28 years (1995–2023); when considering the entire period since its emergence, total losses may exceed €13 billion. PPV drastically reduces the commercial value of stone fruit crops by causing fruit deformation, necrosis,

and premature drop, often rendering the fruit unsuitable for fresh consumption or industrial processing.

Cultivar resistance to viral diseases, particularly to PPV, is recognized as the most efficient strategy for virus control, especially in endemic areas (Ravelonandro *et al.*, 2011; Scorza *et al.*, 2013). Cultivars exhibiting high levels of tolerance can also substantially contribute to mitigating the economic losses associated with viral diseases. Therefore, breeding and promoting the use of such cultivars represent a key strategy for viral disease control, preserving fruit quality, and ensuring stable production.

Conventional breeding efforts aimed at conferring PPV resistance in *Prunus domestica* have achieved limited progress over the past century, primarily constrained by the scarcity of resistance genes within the cultivated germplasm (Zagrai *et al.*, 2021). Nevertheless, breeding for hypersensitivity has led to cultivars like 'Jojo' (Hartmann and Neumüller, 2006, 2013), which respond to PPV infection with a hypersensitive reaction. While such genotypes appeared to have a field resistance, studies indicate this resistance may be strain- or isolate-dependent (Polák and Jarošová, 2011, 2012; Rodamilans *et al.*, 2023).

As an alternative or complement to conventional breeding, pathogen-derived resistance (PDR) led to the development of the RNAi-based transgenic plum clone C5, later named 'HoneySweet', carrying PPV-CP transgenes, which has demonstrated high and durable resistance to PPV (Scorza *et al.*, 1994, 2013; Ravelonandro *et al.*, 1997; Malinowski *et al.*, 2006; Zagrai *et al.*, 2011; Polak *et al.*, 2017). Unfortunately, 'HoneySweet' plum is not available for cultivation in the European Union (EU), where PPV remains a major threat to stone fruit production. Given these considerations, the breeding and use of highly tolerant cultivars continues to be a pertinent and valuable strategy for reducing the economic impact of PPV. Important progress has been made in developing highly tolerant plum cultivars, especially through targeted breeding programs in South-Eastern Europe. In Serbia, results from the breeding program have identified several plum hybrids exhibiting resistance or a high level of tolerance to specific PPV strains, as demonstrated through both artificial inoculation and natural infections (Milošević *et al.*, 2019; Glišić *et al.*, 2021).

Butac *et al.* (2021) evaluated the performance of several Serbian plum cultivars under Romanian ecological conditions, including their response to PPV. The results indicate good adaptability and enhanced tolerance to PPV, highlighting the potential of these cultivars for successful cultivation in regions affected by the virus. A more recent study (Butac *et al.*, 2024) has demonstrated that certain Bulgarian plum cultivars exhibit very low susceptibility to PPV when evaluated under natural infection conditions. This low susceptibility suggests a form of tolerance that enables these cultivars to sustain both productivity and fruit quality in the presence of PPV infection.

In line with regional advancements, the plum breeding program at Bistrița aims not only to develop cultivars resistant to plum pox virus but also to identify and assess genotypes exhibiting a high level of tolerance to the virus. Accordingly, the present study was specifically designed to identify and assess such tolerant cultivars.

MATERIALS AND METHODS

Experimental orchard

An experimental orchard comprising six plum hybrids ('HAS 1 I', 'HAS 2 G', 'HAS 2/5', 'HAS 2/VI', 'HAS 3/4' and BN 4/1 – homologated as 'Zandra' in 2025) and ten plum cultivars ('Diana', 'Geta', 'Tuleu timpuriu', 'Centenar', 'Early Rivers', 'Matilda', 'Piteștean', 'Stanley', 'Čačanska Najbolja' and 'Anna Späth') with a total of 534 trees, was established in 1998 at Fruit Research & Development Station Bistrița (FRDS Bistrița). The cultivars were also planted in variable numbers, ranging from 5 to 188 trees, planted at a spacing of 5/3 meters. Each hybrid was represented by 12 to 60 trees, and interspaced among cultivar rows. The

experimental orchard is located near potential sources of PPV infection, including various *Prunus* species, such as *P. cerasifera*, *P. spinosa* and *P. domestica*, which are commonly found in the surrounding area and may serve as virus reservoirs.

Pest and disease management in an experimental orchard

The maintenance of the experimental orchard involves the application of 8–10 phytosanitary treatments per season to control diseases and pests. These treatments include targeted interventions against aphids, which are known vectors of the PPV, in order to reduce the risk of PPV spread within the orchard.

PPV spreading in the orchard

In addition to assessing the agronomic performance of the hybrids under the local climatic and soil conditions, the trees were also monitored to evaluate their susceptibility, tolerance or resistance to plum viruses, with a focus on natural infections with PPV. Beginning in 2003, systematic monitoring of PPV spread within the orchard was carried out every three years. This included visual inspections during the growing season (minimum twice per year) for characteristic symptoms on leaves and/or fruits, as well as serological testing conducted in 2003 and 2018. It is worth noting that a recent study demonstrated that accurate recognition of PPV-specific symptoms in infected plum trees can serve as a reliable tool for monitoring the presence and spread of the virus within the commercial orchards (Zagrai *et al.*, 2024).

PPV spreading within the canopy

To assess the internal spread of PPV within the tree canopy, selected hybrid trees exhibiting localized PPV infections were sampled and tested in various canopy zones. This approach was applied in the case of the 'BN 4/1' hybrid ('Zandra' cv.). The aim was to determine the extent of virus movement from infected shoots to adjacent branches. The spread of PPV was assessed on three out of twelve trees showing localized PPV symptoms. Both symptomatic and symptomless shoots (leaves) located below, above and beside the infected areas were collected for comparative serological testing. Additionally, symptomless leaves were also sampled from the opposite part of the canopy in order to evaluate the potential latent infections. Where applicable, samples from root suckers emerging near the trunk were also collected and tested serologically. Moreover, all sixty trees of 'BN 4/1' hybrid ('Zandra' cv.) were serologically tested in 2024.

Serological detection

A minimum of ten leaves per sample were collected for serological tests. Fresh leaf samples were homogenized in extraction buffer at a 1:20 (w/v) ratio using a HOMEX 6 homogenizer. Serological testing was conducted using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), following the method described by Clark and Adams (1977). A commercial kit from Bioreba (Switzerland), containing polyclonal antiserum specific to PPV, was used in accordance with the manufacturer's instructions. Samples were considered positive if absorbance values exceeded twice those of the negative control. Both positive and negative controls supplied with the kit were included in each assay to ensure reliability.

Statistical analysis

XLSTAT software (Addinsoft, New York, USA) was used to perform statistical interpretation. Analysis of variance (ANOVA) by Duncan's Multiple Range Test was conducted in order to verify the differences between cultivars/hybrids regarding the PPV incidence. The threshold

for statistical significance was set at $p < 0.0001$, indicating a very high level of confidence in the observed differences.

RESULTS AND DISCUSSIONS

Incidence and temporal spread of PPV in the experimental plum orchard

Visual assessments and serological testing conducted over a 20-year monitoring period revealed a progressive and continual temporal spread of PPV within the plum orchard. The overall incidence increased from 11.4% in 2003 to 58.4% (267 infected trees out of 457 total trees) in 2024 (Table 1). Over this timeframe, losses of plum trees due to various causes were recorded; of the 534 trees initially planted in 1998, a total of 457 remained in the orchard by 2024. The annual increase in PPV incidence varied between 3.5% and 9%, highlighting a gradual but consistent expansion of the infection across the orchard.

Table 1. Temporal spread of PPV within the experimental plum orchard during the period 2003-2024 (by visual monitoring and/or serological tests)

Year	PPV infected trees/total trees	Incidence of PPV (%)	The annual increase of PPV incidence (%)
2003*	58/511	11.4	-
2006	75/502	14.9	+3.5
2009	97/491	19.8	+4.9
2012	126/480	26.2	+6.4
2015	155/476	32.5	+6.3
2018*	191/469	40.7	+8.2
2021	229/461	49.7	+9.0
2024	267/457	58.4	+8.7

*Years in which all trees were tested serologically using DAS-ELISA; in the remaining years, PPV incidence was determined only based on visual monitoring.

The temporal spread of PPV within the orchard, assessed at three-year intervals over 20 years, revealed a nearly linear progression in virus incidence. A statistically significant linear relationship was observed between the increase in PPV incidence and passage of time (Figure 1), suggesting a steady and predictable pattern of virus dissemination under the given management conditions.

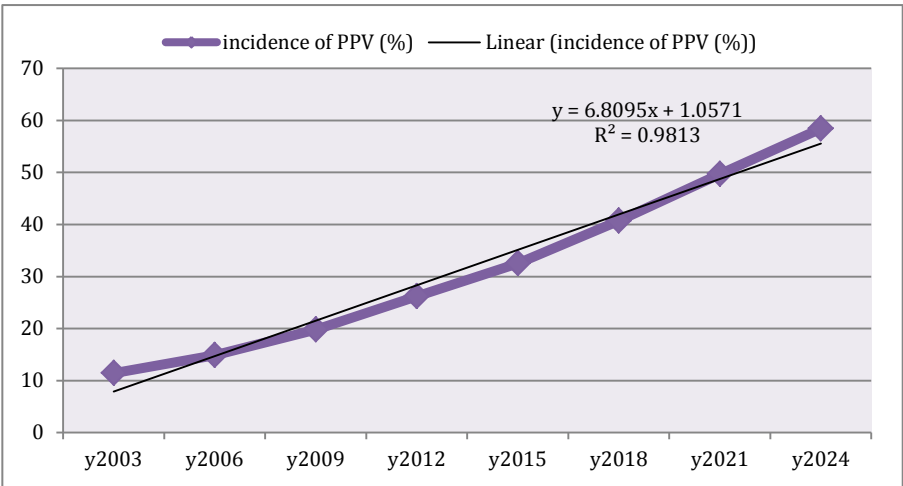


Figure 1. Temporal spread of PPV within the experimental plum orchard during 2003-2024

The continual rise in PPV infection rates reflects sustained and increasing pressure from infections on the remaining uninfected trees, highlighting the persistent potential for virus dissemination within the orchard.

Pattern of PPV symptoms within the canopy of infected plum trees

In 2024, all trees belonging to ten cultivars and six hybrids grown in the orchard were visually inspected to assess the extent of PPV infections within the canopy. Among the 267 PPV-infected trees, 244 (91.4%) exhibited generalized infections affecting the entire canopy, while only 23 trees (8.6%) showed localized PPV infections (Figure 2), typically confined mainly to a single branch or shoot. These results indicate a predominance of systemic virus spread within infected trees. However, the limited infection to particular branches observed in a small subset of trees raised attention due to its potential implications for early-stage infection or partial resistance. Such localized symptoms may reflect initial infection foci before systemic spread or could be associated with host genotype-related responses that restrict viral movement.

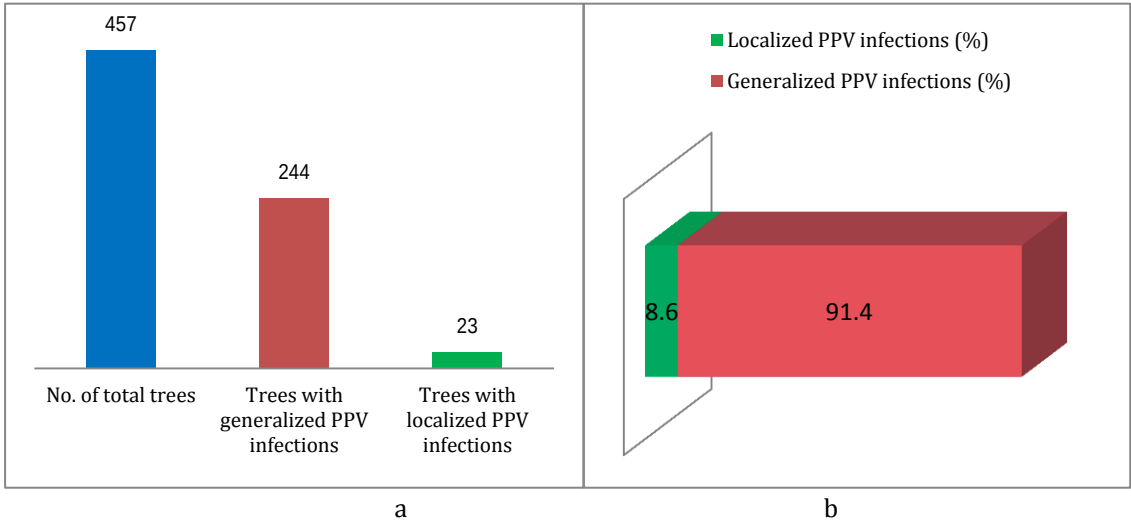


Figure 2. Generalized versus localized PPV infections in trees in 2024 (a. trees; b. percentage)

The results obtained from a long-term study spanning over two decades on the behaviour of various plum cultivars and hybrids to natural PPV infections have enabled the classification of genotypes based on their response (Table 2).

High infection rates, exceeding 83%, were recorded in genotypes such as ‘Diana’, ‘Geta’, ‘HAS 2G’ and ‘Tuleu timpuriu’ with no statistically significant differences among them. These genotypes were thus classified as highly susceptible to natural PPV infection.

Moderate infection levels, ranging between 60% and 80%, were observed in e genotypes including ‘Centenar’, ‘Early Rivers’, ‘Matilda’, ‘HAS 2/VI’, ‘HAS 2/’5 and ‘Piteştean’, which were accordingly classified as susceptible.

In contrast, genotypes such as ‘Stanley’, ‘Čačanska Najbolja’, ‘Anna Späth’, ‘HAS 3/4’ and ‘HAS 1I’, showed lower infection rates, between 40% and 60%, and were therefore designated as tolerant to PPV.

Among all genotypes evaluated only ‘BN 4/1’ (‘Zandra’) consistently exhibited the lowest PPV incidence over the two-decade study period, with just 20% of trees affected, as confirmed by serological tests performed in 2024. Notably, all infections in this hybrid were localized to one or two shoots, and none of the sixty observed trees developed generalized symptoms. This suggests at least a high level of tolerance to natural PPV infection, even under high inoculum pressure within the orchard. Furthermore, statistical analysis revealed

significant differences in PPV incidence between ‘BN 4/1’ hybrid (‘Zandra’ cv.) and all the other genotypes included in the study.

Table 2. PPV incidence and statistical interpretation of the results (2024)

Cultivar/hybrid	PPV-infected trees/no. of trees			PPV incidence (%)
	generalized	localized	total	
‘Diana’	5/5	0/5	5/5	100.0 ± 0.0 a
‘Geta’	4/4	0/4	4/4	100.0 ± 0.0 a
‘HAS 2G’	13/20	3/20	16/20	84.23 ± 5.66 ab
‘Tuleu timpuriu’	35/43	1/43	36/43	83.83 ± 3.32 ab
‘Centenar’	14/17	0/17	14/17	80.23 ± 0.21 b
‘Early Rivers’	6/8	0/8	6/8	77.76 ± 19.25 b
‘Matilda’	7/9	0/9	7/9	77.76 ± 19.25 b
‘HAS 2/VI’	13/19	0/19	13/19	68.20 ± 2.77 bc
‘HAS 2/5’	21/31	0/31	21/31	67.86 ± 3.70 bc
‘Piteștean’	12/18	0/18	12/18	66.6 ± 16.65 bc
‘HAS 3/4’	5/12	2/12	7/12	58.86 ± 8.36 cd
‘Stanley’	87/160	3/160	90/160	56.33 ± 3.18 cd
‘Čačanska Najbolja’	7/16	1/16	8/16	50.00 ± 10.00 cd
‘Anna Späth’	10/23	1/23	11/23	47.63 ± 4.10 d
‘HAS 1I’	5/12	0/12	5/12	41.66 ± 14.43 d
‘BN 4/1’	0/60	12/60	12/60	20.00 ± 5.00 e
Pr>F(Model)				<0.0001
Signification				YES

**Different letters between cultivars/hybrids denote significant differences (Duncan test, $p < 0.0001$). Different letters between PPV incidences of cultivars/hybrids denote significant differences.*

Localized PPV Infections in ‘BN 4/1’ (‘Zandra’)

Out of a total of sixty observed trees of the ‘BN 4/1’ (‘Zandra’) genotype, twelve exhibited localized PPV infections, predominantly confined to the northern part of the canopy. This spatial distribution may be partly attributed to a higher inoculum pressure of PPV in the northern section of the orchard compared to the southern exposure (Figure 3). Additionally, most of the infected shoots originated from dormant buds located near the trunk, suggesting limited virus movement within the canopy. In certain cases, although the suckers arising from the rootstock displayed typical PPV symptoms, the trees of ‘BN 4/1’ scion remained uninfected over time. Notably, despite being surrounded by plenty of PPV-infected trees, 80% of the ‘BN 4/1’ trees remained symptom-free over a period exceeding 25 years. These findings indicate a high degree of field tolerance to natural PPV infections under conditions of permanent and intense inoculum pressure.

Given that ‘BN 4/1’ (‘Zandra’ cv.) exhibited the lowest PPV incidence over 25 years among all studied cultivars and hybrids, shoots displaying localized PPV symptoms were closely monitored, and additional shoots were sampled from adjacent areas surrounding the symptomatic ones. Thus, in the case of some trees exhibiting localized PPV symptoms, four distinct canopy areas were subjected to serological analysis, with the results summarized in Table 3.

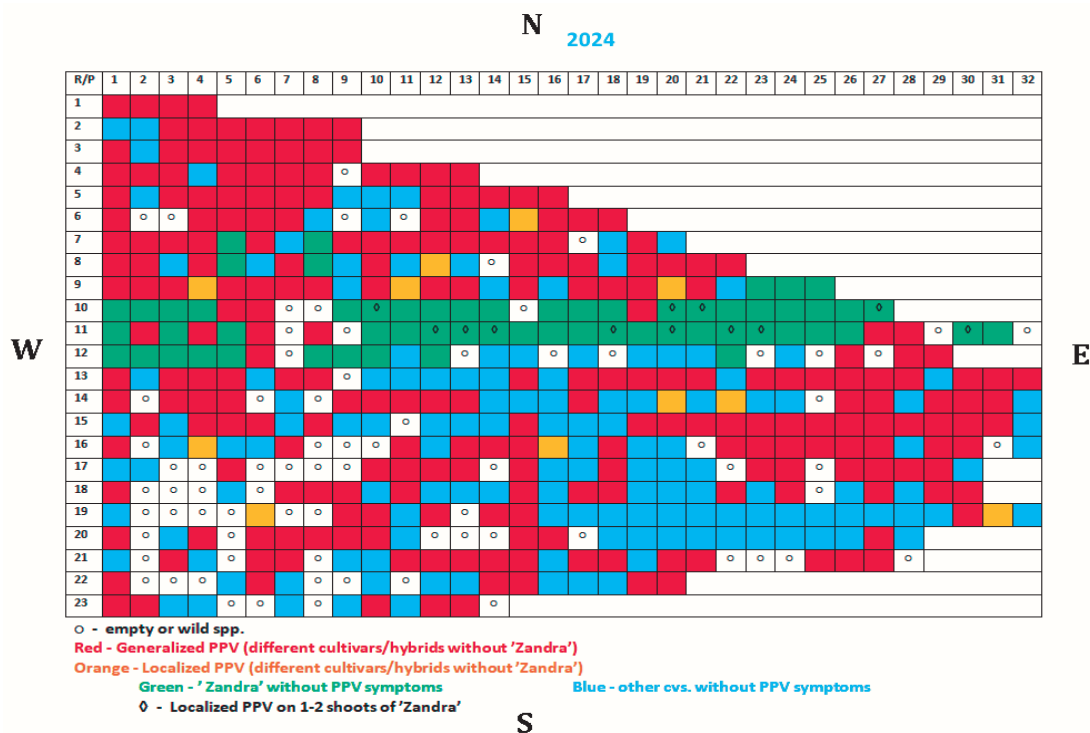


Figure 3. The map of PPV infections in plum trees (2024)

Table 3. The serological results of DAS-ELISA test (2024) of 'Zandra' samples

Part of canopy	Localization of shoots	The visual presence of PPV	DAS-ELISA test results
1	A - symptomatic shoot	Yes	+
	B - above A	No	-
	C - beside A	No	-
	D - below A	No	-
2	E - symptomatic shoot	Yes	+
	F - above E	No	-
	G - above left of E	No	-
	H - above right of E	No	-
	I - below E	No	-
	J - next to E	No	-
3	K - middle of the canopy	No	-
	M - upper part	No	-
	N - lower part	No	-
4	P - symptomatic suckers	Yes	+
	R - lower part of canopy	No	-
	S - average sample from the rest part of canopy	No	-

Figure 4 illustrates the canopy zones of 'BN 4/1' ('Zandra') that were analysed by DAS-ELISA. In the first case, PPV infection was confirmed in the symptomatic shoot (zone A) but was not detected above (zone B), in lateral (zone C) or basal (zone D). This pattern indicates that there was no movement of the virus from the initial infection site to the rest of the canopy.

In the second case, involving another tree of 'BN 4/1' ('Zandra'), PPV was detected exclusively in the symptomatic shoot (zone E), with no detectable virus presence in the surrounding canopy zones (F, G, H, I, and J). In the third case, which examined the opposite side of the canopy in the same tree (case 2), none of the sampled shoots (zones K, M, N) tested positive for PPV. These findings suggest either a true absence of infection in these zones or a viral concentration below the detection threshold of the DAS-ELISA assay.

In the fourth case, involving another 'BN 4/1' ('Zandra') tree, PPV was detected exclusively in the symptomatic suckers (zone P), while no virus was detected in the lower part of the canopy (zone R), or in the rest of the canopy (zone S). This distribution pattern suggests that the infection in the suckers most likely originated from the rootstock which became infected as a result of its susceptibility to the virus, whereas the scion-derived shoots in the canopy remained uninfected.

Overall results indicate that the hybrid 'BN 4/1' ('Zandra' cv.) possesses the ability to restrict PPV infection, keeping the virus localized close to the initial infection site and preventing its systemic spread throughout the tree canopy over time. This behaviour confers it a high level of field of tolerance to natural PPV infections.

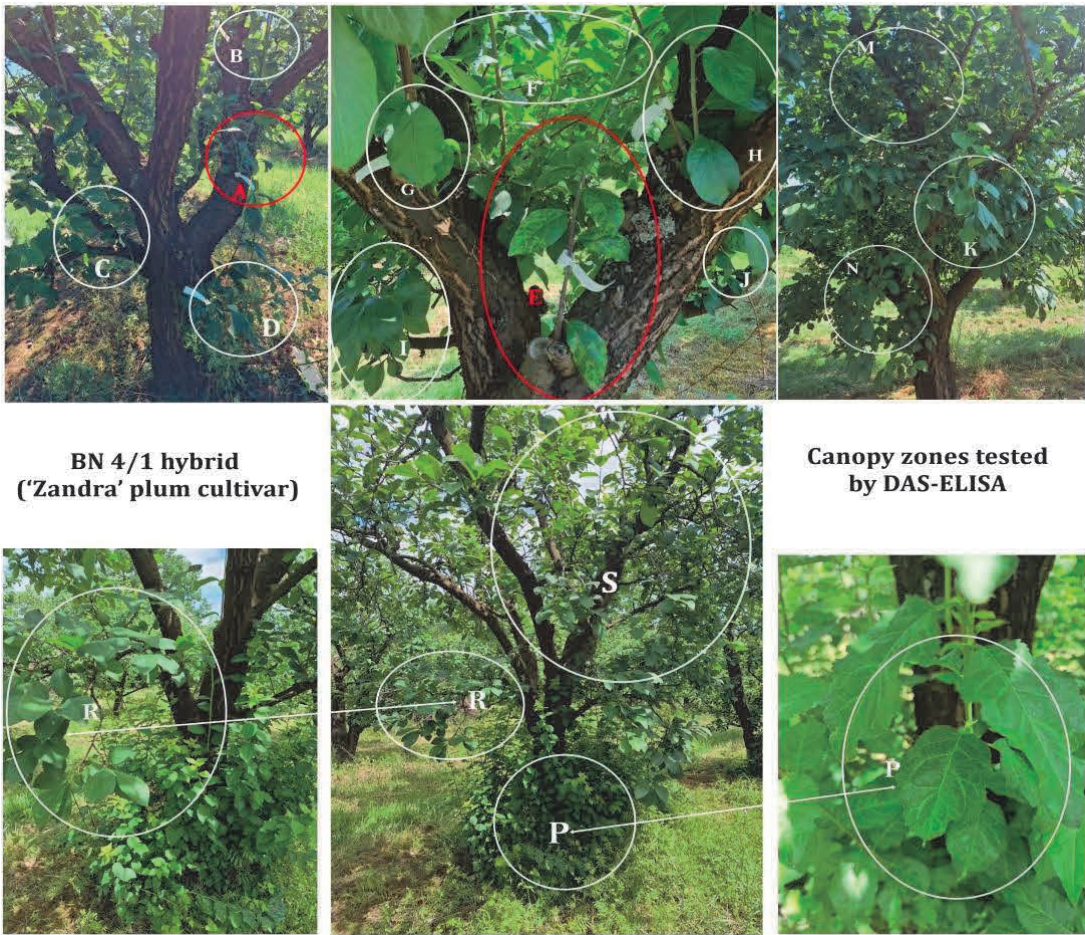


Figure 4. Canopy zones of 'BN 4/1' ('Zandra' cv.) analysed by DAS-ELISA

In addition to its pronounced tolerance to natural PPV infections, the 'Zandra' plum cultivar (Figure 5) exhibits superior organoleptic qualities - including flavour, fruit shape, and desirable size - positioning it as a valuable candidate for integration into the current plum production system.



Figure 5. Fruits of hybrid 'BN 4/1' ('Zandra' cv.)

CONCLUSIONS

The incidence of plum pox virus in the studied plum orchard increased steadily over two decades, reflecting a consistent and progressive spread of the virus under natural conditions. Among the evaluated genotypes, the hybrid 'BN 4/1' ('Zandra' cv.) exhibited the lowest PPV incidence with only 20% of trees affected over the 25 years. Notably, infections in this genotype remain localized to one or two shoots, indicating a high level of tolerance to natural PPV infection, even under conditions of high inoculum pressure. Serological analysis of 'BN 4/1' in different canopy zones confirmed restricted movement of the virus within the tree, further supporting the cultivar's ability to limit systemic viral dissemination.

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