Zagrai I and Zagrai LA (2023)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 51, Issue 4, Article number 13526
DOI:10.15835/nbha51413526
Research Article

Are D and Rec strains of *Plum pox virus* similar or different in terms of competitiveness and symptomatology?

Ioan ZAGRAI, Luminița A. ZAGRAI*

*Fruit Research & Development Station Bistrița, 3 Drumul Dumitrei Nou, 420127, Bistrița, Romania; ioan.zagrai@scdp-bistrita.ro; luminita.zagrai@scdp-bistrita.ro (*corresponding author)*

Abstract

Plum pox virus (PPV) is considered the most detrimental viral pathogen of stone fruits, causing important economic losses. Exhaustive studies revealed that D, M and Rec are the prevalent strains of PPV in Europe. While different experimental reports revealed that PPV-M is more aggressive than PPV-D, limited information is available about the competitiveness of PPV-Rec under field conditions. Also, symptoms developed by PPV-Rec infection are scarcely documented. To increase this knowledge, we selected and monitored an experimental plum orchard already contaminated by both PPV-D and PPV-Rec. The disease spread was monitored by visual observation and ELISA testing each year along five consecutive vegetative periods. The dynamic of single and mixed infections was assessed by molecular strain typing (IC-RT-PCR) of all newly and single-infected trees. While the disease prevalence increased almost linearly, the number of single PPV infected trees (either by PPV-Rec or by PPV-D) remained relatively stable during the five-year period of monitoring, which is explainable by the continual increase of the number of trees infected with both strains together. No significant difference in the progression rate between D and Rec strains was found when considering their presence in both single and mixed infections. Although the types of PPV symptoms developed on leaves and fruits varied among cultivars, no clear-cut D or Rec strain-specific symptoms were developed within the same cultivar. Overall results revealed that PPV-D and PPV-Rec strains had similar behaviour in terms of competitiveness and symptoms developed under our experimental field conditions.

Keywords: molecular typing; plum; serological detection; strains; symptoms

Introduction

Sharka, caused by plum pox virus (PPV) is considered the most devastating disease of the stone fruits in the world. PPV is originated in Eastern Europe being observed for the first time in Bulgaria in 1917 (Atanassoff, 1932). Since then, the disease has progressively spread not only to the most part of European continent (Roy and Smith, 1994; Barba *et al*., 2011) but also it was reported in Asia, Africa (Egypt, Tunisia), North (Canada) and South (Chile, Argentina) America, becoming a huge issue of stone fruits production. There are some countries, such as United States of America, Sweden, Finland, and Estonia where PPV has been eradicated, while in others (Lebanon and New Zealand) never has been confirmed (EPPO, 2023). In Romania, an endemic

Received: 10 Nov 2023. Received in revised form: 30 Nov 2023. Accepted: 18 Dec 2023. Published online: 19 Dec 2023.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

area of PPV, no plum orchard was found PPV free (Zagrai *et al*., 2010a), Sharka causing important yield losses especially in susceptible cultivars (Minoiu, 1997; Sestraș *et al*., 2007).

Sharka disease has agronomic and political consequences because it causes serious economic losses (Cambra *et al*., 2006). Different prevention measures recommended to limit the spread of PPV, like PPV monitoring in nurseries, the use of certified planting material, the removal of infected plants as part of quarantine measures, treatments against aphids, etc., proved to be ineffective for an efficient containment of PPV in endemic areas (Zagrai *et al*., 2022).

A good knowledge of the properties, geographical distribution, dynamics, and variability of the PPV population is a prerequisite for the efficient control of disease. Grace of many international efforts, there is now a relatively good understanding of PPV diversity and of the geographic distribution of PPV strains (Candresse *et al*., 2013).

Current knowledge on nucleotide sequence allowed PPV isolates to be grouped into ten distinct strains (D, M, EA, C, Rec, T, W, An, CR, and CV). PPV-D (Dideron) was originally isolated from apricot, while PPV-M (Marcus) was originally characterized on peach (Kerlan and Dunez, 1979). Based on their serological and molecular properties, the two isolates have been confirmed to represent two distinct strains (Candresse *et al*., 1998). PPV-Rec (Recombinant) is recognized as the third major PPV strain resulting from recombination between PPV-D and PPV-M (Glasa *et al*, 2002). The other seven molecularly distinguished PPV strains are geographically or host-limited. El Amar (EA) was originally isolated from Egypt (Wetzel *et al*., 1991), Winona (W) was originally isolated in Canada (James and Varga, 2004) but its origin was later established in Eastern Europe (Glasa *et al*., 2011; Mavrodieva *et al*., 2013). PPV-T is another recombinant group of PPV that was isolated from apricot in Turkey (Serce *et al*., 2009). Ancestor Marcus (An) is an atypical Albanian PPV isolate that is supposed to be the progenitor of PPV-M (Palmisano *et al*., 2013). The other three PPV strains are cherry-adapted: Cherry (C) was found in sour cherry in Moldova (Kalashyan *et al*., 1994) and Croatia (Kajic *et al*., 2012), and in sweet cherry in Italy (Crescenzi *et al*., 1995), and Romania (Maxim *et al*., 2002); Cherry Russian (CR) an unusual isolate able to infect cherry (Glasa *et al*., 2013); Cherry Volga (CV) a cherry-adapted strain (Chirkov *et al*., 2018), the last two were detected only in Russia (Chrikov *et al*., 2022). Among the ten PPV strains, only three (D, M and Rec) are widespread (Candresse and Cambra, 2006). Interestingly, although Romania is well known as a PPV-endemic area, only D and Rec strains have been found in plum orchards (Zagrai *et al*., 2010b). However, as an effect of the permissive barrier of the intra-community trade, PPV-M has been recently introduced in Romania in a new plum orchard established by using plum material from abroad (Zagrai *et al*., 2022).

Recombination event is considered a major source of variation (Hull, 2004). It can provide some selective advantage to the recombinant isolate over parental variants to become established in nature (Lai, 1992; Aaziz and Tepfer, 1999). Therefore, is a really need to increase the knowledge about PPV-Rec behaviour in infected natural hosts under field conditions. The main objective of our work was to evaluate the competitiveness between PPV-Rec and PPV-D by monitoring the dynamic of spread in an experimental plum orchard already infected by both strains.

Materials and Methods

Experimental orchard plot

An experimental plum orchard, surrounded by a buffer of apple trees, already contaminated with PPV-D and PPV-Rec (Zagrai *et al*., 2008) was selected for trial. The experimental plot belonging to Fruit Research & Development Station Bistrița (Romania), contains more than 50 plum cultivars and hybrids with different numbers of trees. Plum trees grafted onto myrobalan rootstock were planted at a spacing of 4 m between trees and 5 m between rows. A total of 577 plum trees, grown in the field during five vegetative periods, were subjected to PPV monitoring at the age of 12-16 years old. The source of inoculum within the experimental orchard was easily secured by already infected trees with large volumes of canopy.

PPV monitoring

The monitoring of PPV spreading in the experimental plot was processed along five vegetative periods. PPV infection was visually monitored by symptom development and tested using serological diagnosis and molecular discrimination. All trees from the experimental orchard were visually inspected three times and tested once at the beginning of the experiment to establish the initial PPV infection level. In the following four years, all trees found uninfected in the previous year were visually inspected and retested in the same way to establish the dynamic of PPV infection. Positive samples were then strain typed.

Sampling procedure

For detection and discrimination of PPV, a minimum of 10 leaves per tree were collected randomly throughout the canopy. In symptomatic trees, only symptomatic leaves were collected. If symptoms were limited to particular branches, leaves were only sampled from the symptomatic branches. If only a few symptomatic leaves were visually detected, the size of the sample was reduced to symptomatic leaves. If a new infection occurred in different parts of the canopy (on the same tree), sampling was separately made. Infected trees found with a single D or Rec strain in the previous year were resampled to detect the potential for over-infections.

PPV detection

For PPV detection, serological tests were performed by Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) (Clark and Adams, 1977) using a commercial polyclonal antiserum against PPV according to the manufacturer's instructions (Bioreba, Switzerland). Absorbance values were measured at 405 nm after 1 h of substrate hydrolysis. Samples were considered positive if their absorbance values were more than twice those of the negative control.

Strain typing procedure

Molecular typing was performed to establish the proportion of PPV-Rec and PPV-D at the beginning of the experiment and to assess the temporal spread of D and Rec strains within the experimental orchard along with the next four vegetative periods. Strain status of each PPV isolate identified at the beginning of experiment was determined by Immunocapture-Reverse Transcription-Polymerase Chain Reaction (IC-RT-PCR) targeting three genomic regions corresponding to: (i) (Cter) CP using specific primers PD and PM (Olmos *et al*., 1997) which allows only a preliminary discrimination due to the overlapping of PPV-Rec and PPV-M in this genomic section (Dallot *et al*., 2008); (ii) (Cter) NIb-(Nter)CP using the primer pairs mD5/mM3 (Subr *et al*., 2004) that directly detects PPV-Rec; (iii) 6K1-CIP using CIP-M/CIP-MR and CIP-D/CIP-DR primer sets to confirm the presence of PPV-Rec (Kamenova *et al*., 2011). For immunocapture, PPV was trapped with PPV polyclonal antibodies (Bioreba, Switzerland). Qiagen one-step kit (Qiagen, Germany) was used for RT-PCR. An aliquot of the amplified products (10µl) was fractionated onto 1.5% agarose gel electrophoresis in 1 x TAE buffer. Bands were visualized by staining with ethidium bromide under UV light.

During the next four vegetative periods, molecular strain typing (IC-RT-PCR) was performed for every new PPV infection case and for all single-strain infections detected in the previous year in order to check for possible over-infection with the other strain.

Symptoms of trees infected with D or Rec strains

To check if PPV-D or PPV-Rec infections produce similar or distinct symptoms, single infected D and single infected Rec trees of four cultivars were evaluated for the development of symptoms on leaves and/or fruits. Thus, symptoms developed under PPV-D and PPV-Rec infections were compared on the cultivars 'Centenar', 'Iulia', 'Reine Red' and 'Stanley'.

Statistical analysis

PPV dynamics and modelling of the temporal spread of the two strains were analysed by linear regression in order to assess whether or not infection rate of two strains increased in similar way. The specific potential of the progressive increase of infections caused by each of the two strains was further analysed by considering only new single and new mixed infections.

The XLSTAT programme (Addinsoft, New York, USA), ANOVA modelling data, Duncan multiple range test, 95% confidence interval, was used in order to evaluate the significance of differences between the spreading of PPV-D and PPV-Rec.

Results

Initial PPV infection level and strains status

Using DAS-ELISA, 328 out of 577 plum tree were found infected by PPV at the beginning of the experiment, which represents an almost 57% rate of infection. Molecular typing of those 328 isolates of PPV revealed that Rec strain (189 infected trees) was four times more numerous than D strain (44 infected trees), respectively 58% versus 13% when considering their presence in single infection, while mixed infections represented 29% (95 trees infected by D+Rec) (Figure 1A). Considering the presence of the two strains in both single and mixed infections, a double rate of PPV-Rec (67%) in comparison with PPV-D (33%) was ascertained at the beginning of experimentation (Figure 1B). At that time the distribution of D, Rec and mixed infections showed random dispersion within the experimental orchard (spatial pattern not shown).

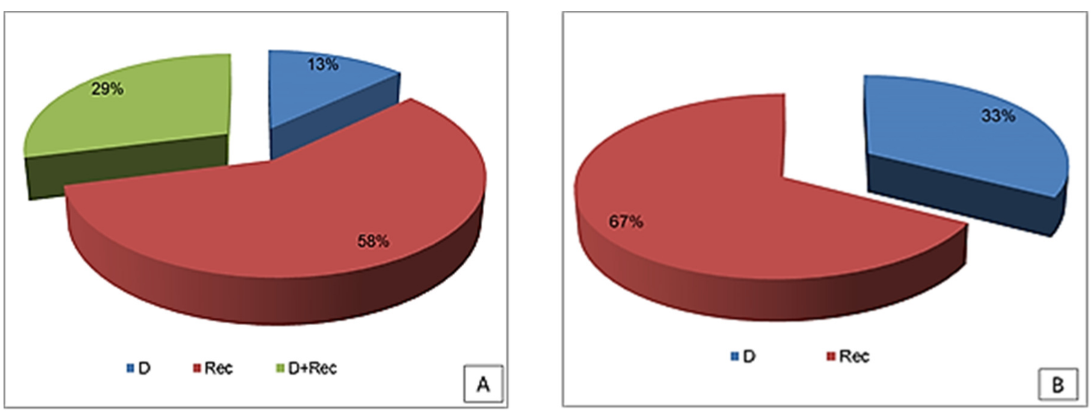

Figure 1. Initial status of infected trees by PPV-D and PPV-Rec before (A) and after (B) splitting of mixed infections

PPV dynamics and temporal spread of D and Rec strains within the orchard

The dynamics of PPV revealed a continual increase of infection throughout the next four vegetative periods. During this time, the disease prevalence increased almost linearly, from about 57% to 75%. A significant linear relationship between the year and the infection rate of PPV was recorded (Figure 2).

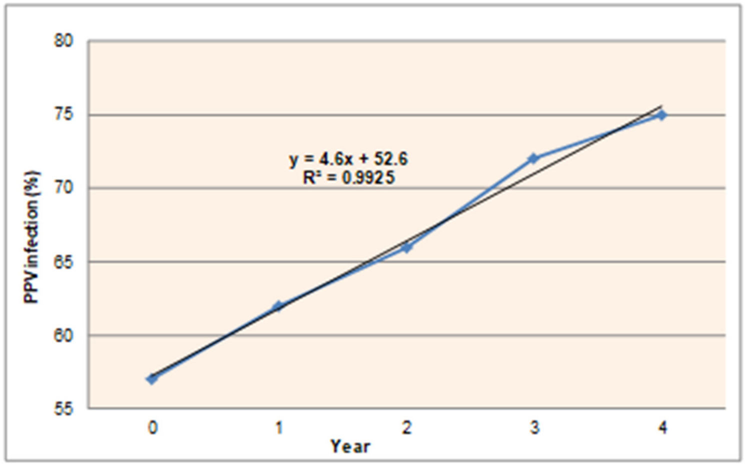

Figure 2. The dynamic of PPV during five consecutive years

The occurrence of the two strains in newly infected trees throughout four vegetative periods showed that PPV-Rec infections were more frequent than those with PPV-D in three of the four years of virus monitoring. Overall results along the four years revealed that the occurrence of PPV-Rec in newly infected trees was more frequent than PPV-D (66 cases versus 37 cases, Figure 3A), keeping thus a similar rate as was recorded at the beginning of investigation. This can be explained by the initial double rate of PPV-Rec and hence a higher chance to infect the remaining healthy trees in comparison with PPV-D.

Both types of over-infection were detected. However, the number of PPV-Rec trees over-infected by PPV-D was higher than opposite each year along the four vegetative periods. During the experimental period, 26 PPV-D infected trees were over-infected by PPV-Rec whereas 66 PPV-Rec infected trees were over-infected by PPV-D (Figure 3B). This difference is explained by the fact that the number of single PPV-Rec infected trees was much higher than the PPV-D infected trees, and hence there is a less chance of a PPV-D tree being over-infected by PPV-Rec than the contrary. Thus, the new PPV-D cases were mostly associated with mixed infections (60 to 70% of new PPV-D cases), as the majority of new PPV-D infections were related to over-infections.

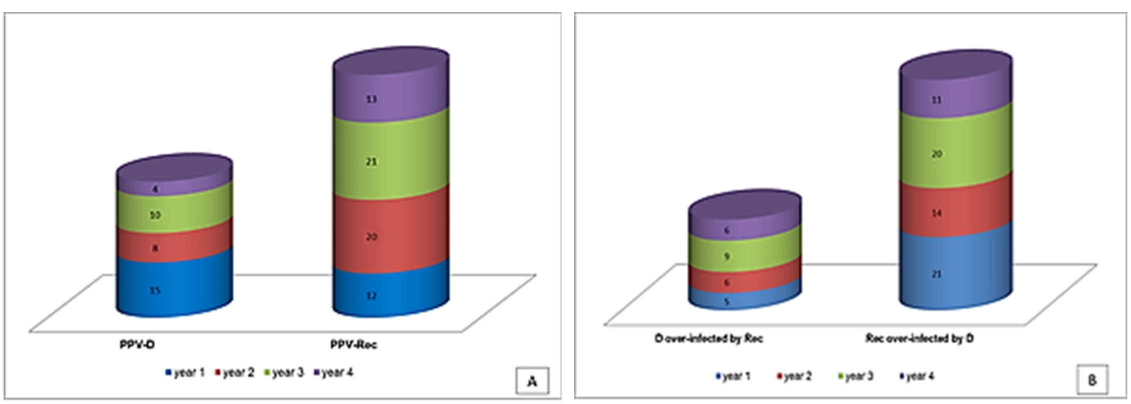

Figure 3. Occurrence of PPV-D or PPV-Rec in newly (A) and co-infected (B) trees

Strains status of infected trees at the end of investigation

Results based on molecular discrimination performed after five years of monitoring showed that the rate of infections by a single D strain remained stable (13%), while infections with a single PPV-Rec recorded a similar rate with mixed infections (44% vs. 43%) - Figure 4A. However, when considering the presence of each strain in both single and mixed strains infection, all D (39%) and all Rec (61%) infected trees (Figure 4B) kept a relatively close rate of infection as was recorded at the beginning of the experiment.

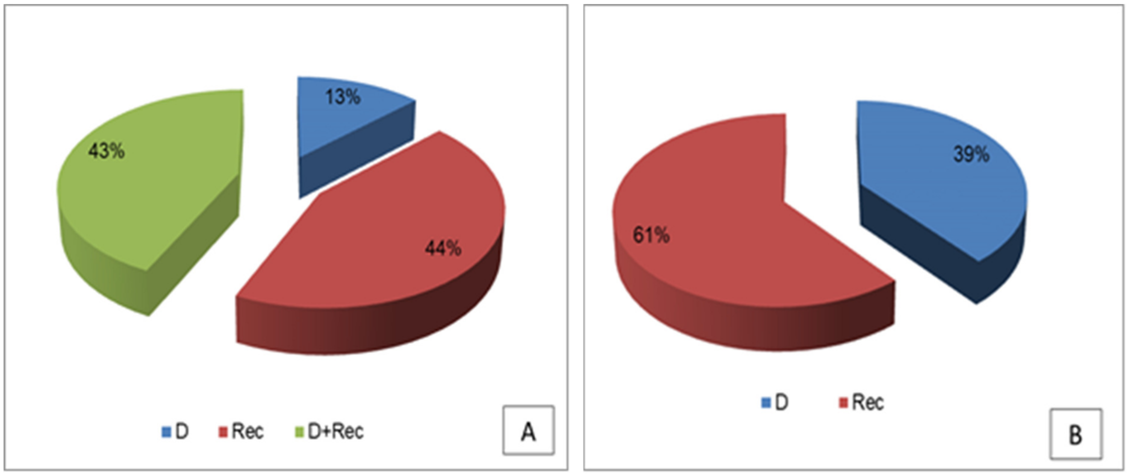

Figure 4. The status of infected trees with PPV-D and PPV-Rec before (A) and after (B) splitting of mixed infections at the end of investigation

The number of trees infected by a single PPV strain (either PPV-D or PPV-Rec) remained relatively stable during the five-year period of monitoring while the number of trees infected with both strains (D and Rec) revealed a continuous significant linear increase (Figure 5).

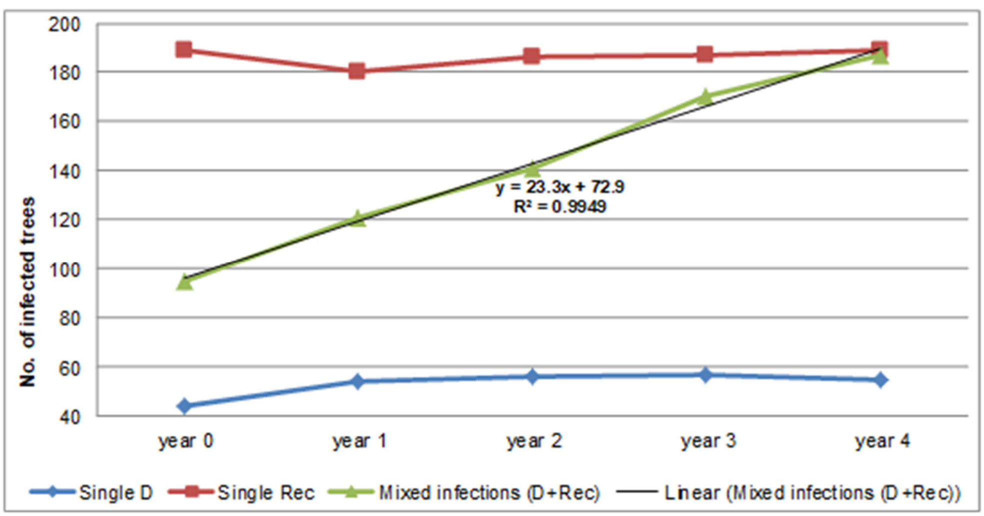

Figure 5. Temporal spread of D and Rec strains in single and mixed infections

Results of the linear regression indicated that there was a very strong collective significant effect between the PPV-D, PPV-Rec, and time (years). The progressive increase of infections with all new PPV-Rec and PPV-D was very close when considering new single infections and also the presence of D or Rec in new mixed infections, confirmed by coefficients of determination of PPV-D (r^2 = 98.5%) and PPV-Rec (r^2 = 99.2%). Moreover, the adjusted slopes showed a similar progression of new PPV-D and PPV-Rec infections (Figure 6).

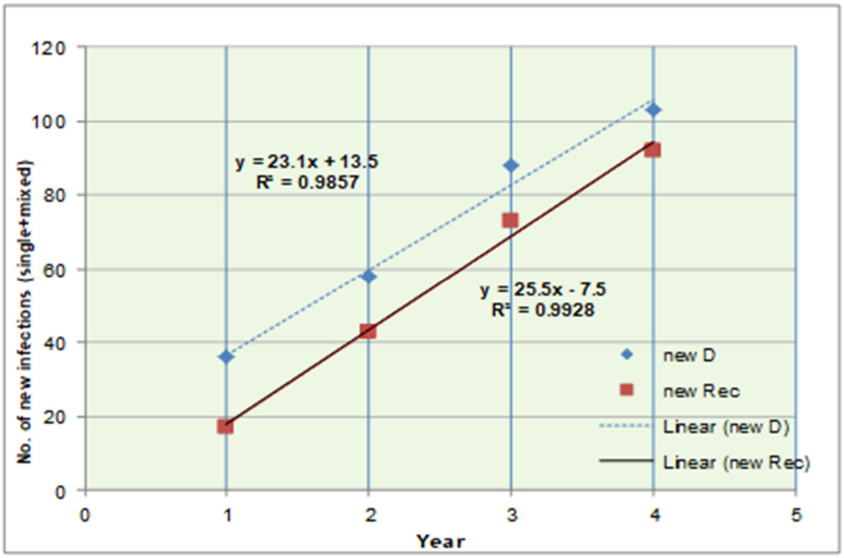

Figure 6. Yearly progression of all new PPV-D and PPV-Rec infections

This similar behaviour is also supported by ANOVA (analysis of variance) and Duncan's Multiple Range Test, which revealed no significant differences between the presence of all PPV-D or all PPV-Rec in the yearly new infected trees and over infections along the four vegetative periods (Table 1).

Table 1. ANOVA (analysis of variance) and Duncan's Multiple Range Test on the presence of all PPV-D or all PPV-Rec in the yearly new infected trees and over infections

PPV strains	New infected trees with D or Rec strains and their presence in new over infections				The average of yearly increasing of infected trees with D or Rec strains
	Year 1	Year 2	Year 3	Year 4	
PPV-D	36	22	30	15	25.75[a]
PPV-Rec	17	26	30	19	23.00[a]
				Pr>F(model)	0.635
				Significant	NO

*Different letters indicate differences at $p < 0.0001$ according to Duncan`s Multiple Range Test.

Symptoms of trees infected with D or Rec strains

Yellowish rings, diffuse spots and vein clearing have been observed on leaves infected either with PPV-D or PPV-Rec on plum 'Centenar' (Figure 7A). The fruits of infected trees were severely deformed, with engraved rings and gum leaks for both strains (Figure 7B). Another example of similarity of symptoms was noted on 'Iulia' infected by PPV-D or PPV-Rec. Rings and spots, more or less diffuse, were observed on leaves with no possibility to distinguish between D and Rec strains (Figure 8A). Skin discoloration, engraved rings and gum leakage were observed on both D and Rec affected fruits of 'Iulia' (Figure 8B). Light green and yellow mottling was developed on leaves of 'Reine Red' infected with PPV-D or PPV-Rec with no possibility for visual distinguishing (Figure 9A). Yellow rings and diffuse spots, vein clearing and sporadically leaf deformation were observed on leaves of 'Stanley' infected with either PPV-D or PPV-Rec (Figure 9B). Thereby, similar symptoms were developed in both PPV-D and PPV-Rec infected trees on the same cultivars.

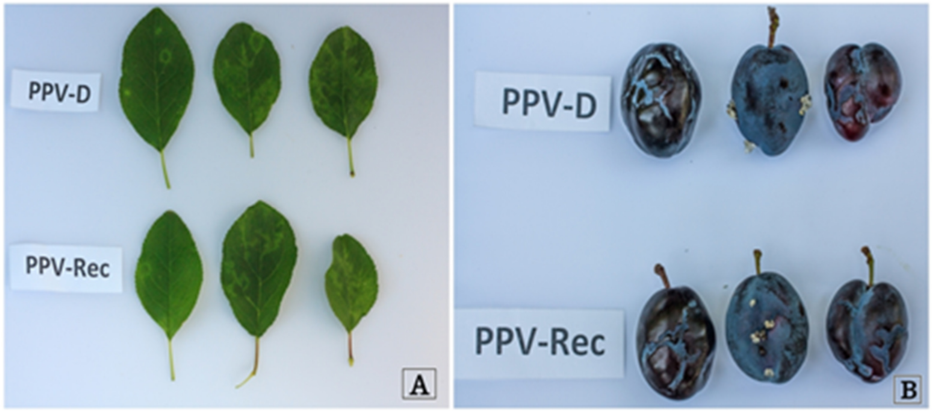

Figure 7. Similarity of PPV-D and PPV-Rec symptoms on leaves (A) and fruits (B) on 'Centenar'

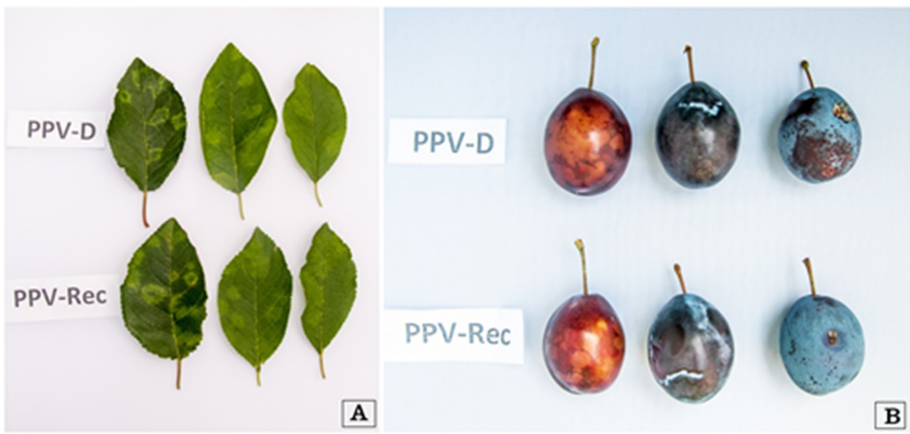

Figure 8. Similarity of PPV-D and PPV-Rec symptoms on leaves (A) and fruits (B) on 'Iulia'

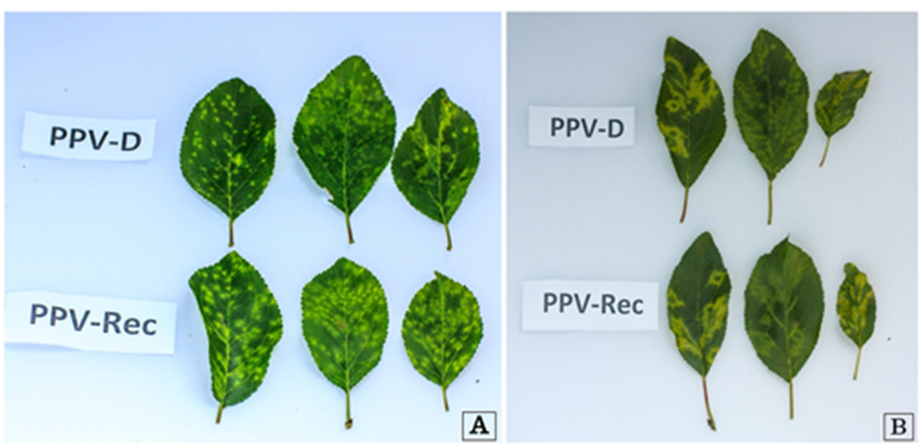

Figure 9. Similarity of PPV-D and PPV-Rec symptoms on leaves 'Reine Red' (a) and 'Stanley' (b)

Discussion

The initial incidence of PPV in the experimental plot (about 57%), combined with the disease prevalence of 75% determined after five years of experimentation, confirmed the problematic situation with

PPV in plum orchards in Romania previously reported (Zagrai *et al*., 2010a). A similar situation of PPV wide presence in plum orchards was also reported in other European countries such as Bulgaria (Kamenova *et al*., 2019) and Bosnia and Herzegovina (Okić *et al*., 2022).

Prevalence of PPV-D, PPV-M and PPV-Rec is different in stone fruits orchards in Europe where PPV is widespread. Thus, surveys on different stone fruits (plum, apricot and peach) orchards from seven European countries revealed that PPV-Rec strain was prevalent in Slovakia, PPV-D in Czeck Republic, while PPV-M in Bulgaria, France, Serbia, Slovenia (Dallot *et al*., 2008), and Hungary (Ádám *et al*., 2015). In plum orchards, PPV-Rec was the prevalent strain in Serbia (Jevremović, 2014), and Bulgaria (Kamenova *et al*., 2015, 2019), while PPV-M, in Croatia (Mikec *et al*., 2008). A recent survey performed in Bosnia and Herzegovina (Okić *et al*., 2022) revealed the occurrence of the same strains of PPV in plum orchards as in Romania, respectively D and Rec, in both single and mixed infections. The presence of two strains in mixed infections, especially D and Rec or D and M, are frequent events in orchards where more than one PPV strain is present, as reported in Serbia (Jevremović, 2012) or Bulgaria (Kamenova *et al*., 2019), and supported by different reports in Romania (Zagrai *et al*., 2006, 2009, 2010b) including the present study.

While PPV-D and PPV-Rec are the two strains widely spread within plum orchards in Romania, we have tried to gain information about the competitiveness of PPV-Rec under field conditions. Thus, the investigation was focused mainly on the temporal spread of D and Rec strains in single and mixed infections within an experimental plum orchard. The typing of PPV performed at the beginning and along the period of study revealed that the strains occurring (PPV-D and PPV-Rec) were in accordance with those previously reported in Romania (Zagrai *et al*., 2006, 2009). The difference was the prevalence of PPV-Rec strain, while a previously performed large survey in a lot of plum orchards in Romania revealed that D was the more frequent strain (Zagrai *et al*., 2010b). The prevalence of PPV-Rec in the present investigation could be explained by the initial source of infection with a possible prevalence of PPV-Rec, and subsequently, within the orchard, a higher number of trees infected by PPV-Rec facilitated new infections mediated by aphid vectors with the same strain to the neighbouring trees.

The number of single PPV-infected trees (either by PPV-Rec or by PPV-D) remained relatively stable during the five-year period of monitoring, while the trees infected with both strains together increased almost linearly. These results confirmed that over-infection is a frequent event in plum as already shown during different previous epidemiological surveys (Jevremović, 2012; Kamenova *et al*., 2019; Zagrai *et al*., 2006, 2009, 2010a).

Even if the initial rate of infection with D strain was lower than those with Rec strain, the progression rate of each strain was almost linear, demonstrating the capacity of each strain to be competitive with the other one, under natural conditions. A continual increasing of mixed strains infection (D+Rec) was expected during the experiment since the number of trees infected by only one strain was high. It is interesting that none of the newly infected trees acquired a simultaneously mixed infection, although there were plenty of plum trees infected with both strains (D and Rec) scattered within the orchard (data not shown). This may suggest that the potential of aphids to transmit both strains simultaneously is extremely limited or less feasible.

Our investigation revealed that the behaviour of Rec and D was quite similar when considering the presence of the two strains in both single and mixed strain infections. This is because although PPV-Rec was more prevalent at the beginning of the study, the progressive increase of infections with the two strains was similar along the next four consecutive vegetative periods. This similar behaviour is supported both by regression analysis of the yearly progression of all new PPV-D and PPV-Rec and by Duncan's Multiple Range Test.

Overall results revealed the epidemic capacity of the two strains, but no clear-cut of PPV-D or PPV-Rec specific behaviour was found in term of competitiveness between them under field conditions. This finding is partly in accordance to a previous report showing that PPV-Rec is competitive in plum hosts under field conditions (Kamenova *et al*., 2017). However, our investigation did not reveal any specific behaviour of PPV-

Rec showing that it is more competitive than PPV-D, but rather similar. No clear-cut strain-specific behaviour of PPV in term of competitiveness between D and Rec was also showed when a non-natural host of herbaceous plants (*Nicotiana benthamiana)* was used for artificial inoculation with the two strains (Glasa *et al*., 2010).

Several types of PPV symptoms on leaves and fruits were observed in different plum cultivars grown in our experimental orchard. However, it should be highlighted that the behaviour of PPV-D and PPV-Rec is very similar in terms of plant response to virus infection. Thus, similar PPV typical symptoms were developed by the same cultivars infected with single PPV-D or PPV-Rec, which also supports a similar behaviour of the two strains in terms of aggressiveness.

Conclusions

Both PPV-D and PPV-Rec were able to cause new single and over infections. No clear-cut definition of PPV-D or PPV-Rec specific behaviour was observed in terms of their competitiveness and symptoms developed by infected plum cultivars under field conditions. These findings may suggest that PPV-Rec had no selective advantage from an epidemic point of view when compared with PPV-D under our experimental condition.

Authors' Contributions

I.Z. concept and designed the experiment; I.Z. and L.A.Z. collected the samples from plum orchard; L.A.Z. prepared samples for serological tests and performed the serological analyses; I.Z. performed molecular analyses. L.A.Z. and I.Z. made data interpretation and wrote the paper. Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This work was supported by European Community's Seven Framework Programme (FP7/2007-2013) under Grant Agreement n°204429, SharCo project, and also by the Romanian Research Ministry, contract 34/2009. We are grateful to Dr. Sylvie Dallot and Dr. Gerard Labonne (INRAE Montpellier, France) for their suggestions in some data processing. The authors also thank to eng. Ioan Baias and eng. Angela Feștilă for technical assistance.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Aaziz R, Tepfer M (1999). Recombination in RNA viruses and in virus-resistant transgenic plants. Journal of General Virology 80:1339-1346. *https://doi.org/10.1099/0022-1317-80-6-1339*

Ádám J, Palkovics L, Tóbiás I, Almási A. (2015). Presence of sharka disease in the north-Hungarian counties. Acta Horticulturae 1063:55-60. *https://doi.org/10.17660/ActaHortic.2015.1063.6*

Atanasoff D (1932). Plum pox. A new virus disease. Yearbook University of Sofia, Faculty of Agriculture and Silviculture 11:49-69.

Barba M, Hadidi A, Candresse T, Cambra M (2011). Plum pox virus. In: Hadidi A, Barba M, Candresse T, Jelkmann W (Eds). Virus and Virus-like Disease of Pome and Stone Fruits. APS Press, St. Paul, MN, USA, pp 185-197.

Cambra M, Capote N, Myrta A, Llácer G (2006). Plum pox virus and the estimated costs associated with sharka disease. Bull OEPP/EPPO Bulletin 36:202-204.

Candresse T, Cambra M, Dallot S, Lanneau M, Asensio M, Gorris MT, Revers F, Macquaire G, Olmos A, Boscia D, Quiot JB (1998). Comparison of monoclonal antibodies and polymerase chain reaction assays or the typing of isolates belonging to the D and M serotypes of plum pox potyvirus. Phytopathology 88(3):198-204. *https://doi.org/10.1094/PHYTO.1998.88.3.198*

Candresse T, Cambra M (2006). Causal agent of sharka disease: historical perspective and current status of Plum pox virus strains. Bull OEPP/EPPO Bulletin 36:139-146.

Candresse T, Glasa M & the SharCo consortium (2013). A global vision of Plum pox virus (PPV) diversity: things we know and things we don't (yet) know. 2nd International Symposium on Plum Pox Virus, Olomouc, Czech Republic, September 3-6, 2013. Book of abstracts, pp 12.

Chirkov S, Sheveleva A, Ivanov P, Zakubanskiy A. (2018). Analysis of genetic diversity of Russian sour cherry Plum pox virus isolates provides evidence of a new strain. Plant Disease 102(3):569-575. *https://doi.org/10.1094/PDIS-07-17-1104-RE*

Chirkov S, Sheveleva A, Gasanova T, Kwon D, Sharko F, Osipov G (2022). New cherry-adapted Plum pox virus phylogroups discovered in Russia. Plant Disease 106:2591-2600. *https://doi.org/10.1094/PDIS-01-22-0006-RE*

Clark M, Adams AN (1977). Characteristic of the microplate method of enzyme linked immunosorbent assay (ELISA) for detection of plant viruses. Journal of General Virology 34:475-483. *https://doi.org/10.1099/0022-1317-34-3-475*

Crescenzi A, Nuzzaci M, Piazzolla P, Levy L, Hadidi A (1995). Plum pox virus (PPV) in sweet cherry. Acta Horticulturae 386:219-225. *https://doi.org/10.17660/ActaHortic.1995.386.28*

Dallot S, Kamenova I, Glasa M, Pittnerova S, Kominek P, Virscek-Marn M, Mavric Plesko I, Paunović S, Jevremović D, Milusheva S (2008). Prevalence and genetic structure of PPV-M in six European countries. Acta Horticulturae 781:227-234. *https://doi.org/10.17660/ActaHortic.2008.781.34*

EPPO Global Database (2023). Plum pox virus. *https://gd.eppo.int/taxon/PPV000/distribution*

Glasa M, Veronique MJ, Labonne G, Subr Z, Kudela O, Quiot JB (2002). A natural population of recombinant Plum pox virus is viable and competitive under field conditions. European Journal of Plant Pathology 108(9):843-853. *https://doi.org/10.1023/A:1021294221878*

Glasa M, Predajna L, Šubr Z (2010). Competitiveness of different Plum pox virus isolates in experimental mixed infection reveals rather isolate-than strain-specific behaviour. Journal of Plant Pathology 92(1):267-271. *http://dx.doi.org/10.4454/jpp.v92i1.42*

Glasa M, Malinowski T, Predajňa L, Pupola N, Dekena D, Michalczuk L, Candresse T (2011). Sequence variability, recombination analysis, and specific detection of the W strain of Plum pox virus. Phytopathology 101:980-985. *https://doi.org/10.1094/PHYTO-12-10-0334*

Glasa M, Proichhodko Y, Zhivaeva T, Shneider Y, Predajna L, Subr Z, Candresse T (2013). Complete and partial genome sequences of the unusual Plum pox virus (PPV) isolates from sour cherry in Russia suggest their classification to a new PPV strain. Petria 22(3):234.

Hull R (2004). Mathews' Plant Virology. 4th Edition. Elsevier Academic Press.

James D, Varga A (2004). Preliminary molecular characterization of Plum pox potyvirus isolate W3174: Evidence of a new strain. Acta Horticulturae 657:177-182. *https://doi.org/10.17660/ActaHortic.2004.657.24*

Jevremović D (2012). Distribution of PPV-D and PPV-REC strains of Plum pox virus in Serbia and the dynamics of their spread in plum orchard. Ph.D. Thesis, University of Belgrade, Belgrade, Serbia.

Jevremović D, Paunović D (2014). Plum pox virus strains: Diversity and geographical distribution in Serbia. Pesticidi i Fitomedicina (Belgrade), 29(2): 97-107. *https://doi.org/10.2298/PIF1402097J*

Kajic V, Cerni S, Skoric D (2012). Plum pox virus on sour cherry in Croatia. Petria 22(3):253.

Kalashyan JA, Bilkey ND, Verderevskaya TD, Rubina EV (1994). Plum pox virus on sour cherry in Moldova. Bull. OEPP/EPPO Bulletin 24:645-649.

Kamenova I, Dallot S, Bozkova V, Milusheva S (2011). First report of the Plum pox virus Recombinant strain on peach in Bulgaria. Plant Disease 95(10):1320. *https://doi.org/10.1094/PDIS-05-11-0405*

Kamenova I, Borissova A, Dragoyski K, Milusheva S, Stefanova B, Dallot S, Glasa M (2015). *Plum pox virus* strains in Bulgaria. Acta Horticulturae 1063:47-54. *https://doi.org/10.17660/ActaHortic.2015.1063.5*

Kamenova I, Tasheva-Terzieva E, Dragoyski K, Stefanova B (2017). Spread and competitiveness of Plum pox virus: Rec and -D strains in experimental plum orchard. Journal of Phytopathology 165:602-609. *https://doi.org/10.1111/jph.12598*

Kamenova I, Borisova A (2019). Update on distribution and genetic variability of Plum pox virus strains in Bulgaria. Plant Pathology Journal 35(3):243-256. *https://doi.org/10.5423/PPJ.OA.09.2018.0189*

Kerlan C, Dunez J (1979). Biological and serological differentiation of strains of Sharka virus. Annales de Phytopathologie 11:241-250.

Lai MMC (1992). RNA recombination in animal and plant viruses. Microbiological Reviews 56:61-79. *https://doi.org/10.1128/mr.56.1.61-79.1992*

Mavrodieva VA, James D, Williams KL, Negi S, Varga A, Mock R, Levy L (2013). Molecular analysis of a Plum pox virus W isolate detected in plum germplasm hand carried into the USA from the Ukraine shows a close relationship to a Latvian isolate. Plant Disease 97(1):44-52. *https://doi.org/10.1094/pdis-01-12-0104-re*

Maxim A, Zagrai I, Isac M (2002). Detection of Plum pox with sweet cherry in Romania. Plant's Health Magazine 6:48-51.

Mikec I, Kajić V, Krajacic M, Škorić D (2008). Occurrence and distribution of Plum pox virus in Croatia. Acta Horticulturae 781:193-197. *https://doi.org/10.17660/ActaHortic.2008.781.28*

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

Okić A, Wetzel T, Nourinejhad Zarghani S, Massart S, Grahić J, Gaši F, Konjić A, Vončina D (2022). Occurrence and distribution patterns of plum tree viruses and genetic diversity of sharka isolates in Bosnia and Herzegovina. Horticulturae 8(9):783. *https://doi.org/10.3390/horticulturae8090783*

Olmos A, Cambra M, Dasi MA, Candresse T, Esteban O, Gorris MT, Asenio M (1997). Simultaneous detection and typing of plum pox potyvirus (PPV) isolates by heminested-PCR and PCR-ELISA. Journal of Virology Methods 68:127-137. *https://doi.org/10.1016/S0166-0934(97)00120-1*

Palmisano F, Boscia D, Minafra A, Myrta A, Candresse T (2013). An atypical Albanian isolate of Plum pox virus could be the progenitor of the Marcus strain. Petria 22(3):224.

Roy AS, Smith IM (1994). Plum pox situation in Europe. Bull OEPP/EPPO Bulletin 24:515-523.

Serce C, Candresse T, Svanella-Dumas L, Krizbai L, Gazel M, Caglayan K (2009). Further characterization of a new recombinant group of Plum pox virus isolates, PPV-T, found in orchards in the Ankara province of Turkey. Virus Research 142:121-126. *https://doi.org/10.1016/j.virusres.2009.01.022*

Sestraș R, Botu M, Mitre V, Sestraș A, Roșu-Mareș S (2007). Comparative study on the response of several plum cultivars in central Transylvania conditions, Romania. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 35(2):69-75. *http://dx.doi.org/10.15835/nbha352225*

Subr Z, Pittnerova S, Glasa M (2004). A simplified RT-PCR - based detection of recombinant plum pox virus isolates. Acta Virologica 48:173-176.

Wetzel T, Candresse T, Ravelonandro M, Delbos RP, Mazyad H, Aboul-Ata AE, Dunez J (1991). Nucleotide sequence of the 3'-terminal region of the RNA of the El Amar strain of the plum pox potyvirus. Journal of General Virology 72:1741-1746. *https://doi.org/10.1099/0022-1317-72-7-1741*

Zagrai I, Gaboreanu I, Ferencz B, Zagrai L, Pamfil D, Popescu O, Ravelonandro M, Capote N, Kovacs K (2006). First detection and molecular characterization of Plum pox virus recombinant strain in Romania. Bulletin UASVM Animal Husbandry and Biotechnologies 62:291-298. *https://doi.org/10.15835/buasvmcn-asb:62:0:2740*

Zagrai I, Zagrai L, Ferencz B, Petricele I, Pamfil D, Octavian P, Briciu A, Feştila A (2009). Serological and molecular typing of Plum pox virus isolates in the Transylvania, Romania. Notulae Botanicae Horti Agrobotanici Cluj Napoca 37(1):265-272. *http://dx.doi.org/10.15835/nbha3713134*

Zagrai I, Zagrai L, Preda S, Isac M, Cardei E (2010a). Incidence of Plum pox virus in Romanian plum orchards. Bulletin UASVM, Horticulture 67(1-2):488. *https://doi.org/10.15835/buasvmcn-hort:4939*

Zagrai I, Zagrai L, Kelemen B, Petricele I, Pamfil D, Popescu O, Preda S, Briciu A (2010b). Typing and distribution of Plum pox virus isolates in Romania. Julius-Kuhn-Archiv 427:342-346.

Zagrai I, Zagrai LA, Moldovan C, Guzu GM, Roșu-Mareș SD, Plopa C, Butac M (2022). Managementul integrat în prevenirea bolilor virotice la speciile prun și cireș. Ghid practic (Integrated management in the prevention of viral diseases in plum and cherry species. Practical guide). Editura Născut Liber, Bistrița.

Zagrai L, Zagrai I, Ferencz B, Gaboreanu I, Kovacs K, Petricele I, Popescu O, Pamfil D, Capote N (2008). Serological and molecular typing of Plum pox virus isolates in North of Romania. Journal of Plant Pathology 90(1):41-46.