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Plum pox virus as a stress factor in the vegetative growth, fruit growth and yield of plum (*Prunus domestica*) cv. 'Cacanska Rodna'

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Abstract During 2005–2007, under the environmental conditions of Cacak (43°53'N; 20°21'E), western Serbia, the effect of *Plum pox virus* (PPV) on the phenology of vegetative shoot and fruit growth was investigated in a plum orchard of cv. 'Cacanska Rodna' including trees non-infected with PPV and those with infection and clearly visible symptoms, as verified by RT-PCR. The results showed that PPV did not affect the growth phenology of the vegetative shoot (length and thickness) and the fruit growth. However, it caused negative effects on the average length (22.31±0.28 cm in non-infected trees, 18.35±0.07 cm in infected trees) and thickness (4.21±0.03 mm in non-infected trees, 4.01±0.02 mm in infected trees) of vegetative shoots as well as on the average fruit weight (20.43±0.16 g in non-infected fruits, 17.58±0.18 g in infected fruits) and fruit dimensions. The greatest effect of PPV during the trial was the induction of a massive premature fruit drop and, consequently, a 52.94% decrease in total yields per tree (18.0±1.10 kg) and unit area (12.0±0.89 t ha^{-1}) in infected vs non-infected trees.

Keywords Fruits · *Plum pox virus* · Stress · Vegetative shoots · Yield

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Introduction

Plum pox, also called Sharka, was reported in Serbia in 1936 (Josifović 1937). It has caused irrecoverable economic damage for >70 years. The negative effect of PPV is reflected in the stress it causes in diseased cells, tissues and organs of plums, peaches, apricots, sour cherries, sweet cherries, almonds and other plants, particularly the fruit, inducing changes inhibitory to normal metabolic processes (Hernández et al. 2007). Major objectives in the development of new plum *Prunus domestica* cultivars include good cropping, large, high-quality fruits with blue skin, trees as dwarf as possible (Kosina 2004), and in particular resistance or tolerance to the agents of economically major diseases, primarily PPV (Hartmann and Neumüller 2006; Prichodko 2006).

PPV is generally considered one of the key reasons for the onset of a serious crisis in Serbian plum cultivation, as well as in the republics of former Yugoslavia, now independent states. As a result, 'Pozegaca', once a dominant cultivar, has almost completely disappeared from Serbian commercial orchards. Furthermore, PPV constantly threatens to become a limiting factor in the successful cultivation of other newly bred and high-quality plum cultivars and other stone fruit species — peaches, apricots, sweet and sour cherries (Bulatovic-Danilovich et al. 2006) in Serbia and worldwide.

'Cacanska Rodna' is an outstanding Serbian plum cultivar derived from 'Stanley' × 'Pozegaca' characterised by heavy crops, high-quality fruit, and a wide range of uses, among other traits (Paunovic et al. 1975). It is cultivated in many European countries, and sporadically in Australia and New Zealand (McLaren and Glucina 1992). Polák et al. (2005) reported that in the 1960s and 1970s 'Cacanska Rodna' was used in the PPV-resistant plum breeding programme in Cacak, Serbia. However, Ranković (1986) determined the susceptibility of 'Cacanska Rodna' to PPV. As regards the sensitivity to the economically most important plum diseases, Ogašanović et al. (1993) observed that 'Cacanska Rodna' was also susceptible to anthracnose, rust and to PPV, which caused only negligible economic damage. As reported by Prichodko (2006), 'Cacanska Rodna' exhibits susceptibility to PPV in Russia, as well as in Poland (Zawadzka et al. 1994) and Germany (Neumüller et al. 2007; Neumüller and Hartmann 2008). Zagrai et al. (2005) determined that 'Cacanska Rodna' belongs to the group of cultivars that can be easily infected with the sharka virus. In certain studies conducted in the Czech Republic, it was used as a rootstock, i. e. as a biological indicator of the susceptibility of some apricot cultivars to PPV (Polák et al. 2003).

In view of the highly positive agronomic traits, on the one hand (Paunovic et al. 1975) and PPV susceptibility (Ranković 1986), on the other, the main objective of this study was to detect the response of the vegetative and generative growth of 'Cacanska Rodna' plum to PPV per tree and unit area.

Materials and methods

The commercial orchard of the cv. 'Cacanska Rodna' grafted on Myrobalan rootstock (*Prunus cerasifera* Ehrh.) was located in the village of Gornja Gorevnica, 9 km from Cacak (43°53'N; 20°21'E), western Serbia. It was established in 1998 at a 5×3 m spacing (667 trees ha^{-1}). The surface area of the orchard was 0.75 ha. Common cultivation practices were intensively applied during the experiment. Pruning was severe; branches were thinned to less vigorous lateral ones, while vigorous fertile branches were trimmed by one fourth to one third of the total length. No irrigation was applied. Autumn fertilization with 500 kg ha^{-1} of compound NPK (15:15:15) and 400 kg ha^{-1} of calcium ammonium nitrate with 27% of total nitrogen (two thirds prior to the growing season and one third at the beginning of June) was applied. The soil was kept fallow by constant tillage, while disease and pest control included 5–6 treatments on average, as necessary.

The experiments were carried out over 2005–2007. At the end of May 2004, five trees with four replications with clearly visible PPV symptoms (RT-PCR positive for PPV), and as many without visible symptoms (RT-PCR negative for PPV) were marked. Non-infected trees were also registered and checked by RT-PCR throughout the observation period.

After the commencement of the growing season in 2005, upon reaching a length of 5 cm, 20 vegetative shoots in five replications were marked on each of the selected trees in order to monitor the dynamics of growth in length (cm) and thickness (mm). Measurements of fruit weight (g) of a sample of 20 fruits per tree in five replications began after the fruits had reached the size of a wheat grain. Shoot dimensions and fruit weight were measured every 10 days by a ruler, vernier calipers and a Tehnica ET-1111 electronic scale (range of measurement 0.01–120.00 g, measurement precision ±0.01 g). Also, the amount of premature fruit drop on the infected or non-infected trees was measured until 20 and 23 August, irrespective of the study year. An ACS System electronic scale (range of measurement 0.005–30.00 kg, measurement precision ±5.0 g) was used to measure fruit yield per tree. Fruit harvest was followed by measurements of fruit yields per tree (kg) and per hectare (t ha^{-1}).

The data were subjected to an ANOVA analysis using the MSTAT-C (1993) statistical software package. Mean separation was done using the least significant difference test (LSD at $P \leq 0.05$ and $P \leq 0.01$). The Statistica 6.0 (StatSoft®) software for Windows (StatSoft Inc. 2001) was employed to make a graphic representation of the growth dynamics of the vegetative shoots and fruits.

Results

Vegetative shoot growth in length and thickness

The increases in length and thickness of the vegetative shoots were greater in non-infected trees (Fig. 1a, b). These differences became apparent from mid-May to the end of measurements (17 August), when the differences in growth intensity were clearly manifested in the vegetative shoots of the non-infected trees. Whether originating from infected or non-infected trees, the shoots were found to grow most intensively until 25 June, following which their growth noticeably slowed down until 7 July and eventually ceased. The average length of the vegetative shoots of the virus-infected and non-infected trees was 18.35±0.07 cm and 22.31±0.28 cm, respectively (Table 1).

The phenology of the vegetative shoot growth in thickness followed that in length (Fig. 1b) with the vegetative shoots of the non-infected trees growing thicker and more intensively than those of the infected ones. This resulted in the average thickness ranging from 4.01±0.02 mm in the infected to 4.21±0.03 mm in the non-infected trees (Table 1).

Fruit increase in weight

No significant differences in fruit growth were observed throughout the period from 24 April to 4 June (Fig. 1c). From then until harvest conducted on 28 August for the period 2005–2007, fruit growth on the infected trees was reduced. The average fruit weight of 'Cacanska Rodna' over the observation period was 20.43±0.16 g and 17.58±0.18 g in the non-infected and infected trees, respectively (Table 1).

Yield

During the observation period, the fruits were abundantly set on both non-infected and infected trees. The first natural fruit thinning (following flowering) and the second physiological one during the stone lignification stage resulted in equal amounts of fruits that remained on both groups of trees. However, differences between these groups of trees became apparent when the fruit skin changed its basic colour, which took place from 10–15 August, when the fruits of the infected trees began to drop off naturally and at a much higher rate than those of the non-infected trees (Table 2). The final fruit yield of the non-infected trees was 38.2±1.50 kg tree^{-1} or 25.5±0.45 t ha^{-1}, that of the infected ones being 18.0±1.10 kg tree^{-1} or 12.0±0.89 t ha^{-1} (Table 3). The difference in the total yield per tree between the infected and non-infected trees was 20.2±1.12 kg or 13.5±0.70 t ha^{-1}. The above indicates that the infected trees of 'Cacanska Rodna' had a 52.94% lower yield per tree and per hectare.

Discussion

Vegetative shoot growth

Vegetative shoot growth in length starts with leafing, i. e. immediately after flowering, and it is manifested as the elongation of vegetative bud axes. It ends with the formation of the terminal bud (Millington and Chaney 1973) and its entrance into dormancy. There are growth differences between the shoots of purely vegetative character and future bearing shoots. Purely vegetative shoots elongate for two to three months, and the future bearing shoots elongate for a shorter time (Prica 1977). According to Prica (1977) short bearing shoots of 'Pozegaca', a cultivar highly sensitive to PPV, take 10–20 days to elongate. Initial shoot growth and fruit growth coincide, both using the reserve nutrients that have remained from the previous growing season, and a competitive relationship is later developed between them, which was also confirmed by this study, regardless of the fact whether the trees of 'Cacanska Rodna' were infected or non-infected (Fig. 1a, b, c). The shoots of both infected and non-infected trees of 'Cacanska Rodna' grew intensively until 25 June on average, but their length remained unchanged thereafter. 'Cacanska Rodna' is a plum cultivar with an exceptionally high fruit bearing capacity (Paunovic et al. 1975). Consequently, the vegetative shoots exhibited a relatively modest growth in length in the non-infected trees in this study (Table 1). Paunović and Ogašanović (1972) determined that the intensive growth in length of 'Stanley' shoots terminated 10 days before our results. The vegetative shoots of cvs 'Cacanska Rana', 'Cacanska Lepotica', 'Cacanski Secer' and 'Stanley' showed an intensive growth in length, being represented by a sigmoid curve, the tendency thereof being contrary to the fruit growth dynamics until 25 June (Milosevic 1997). The effect of PPV

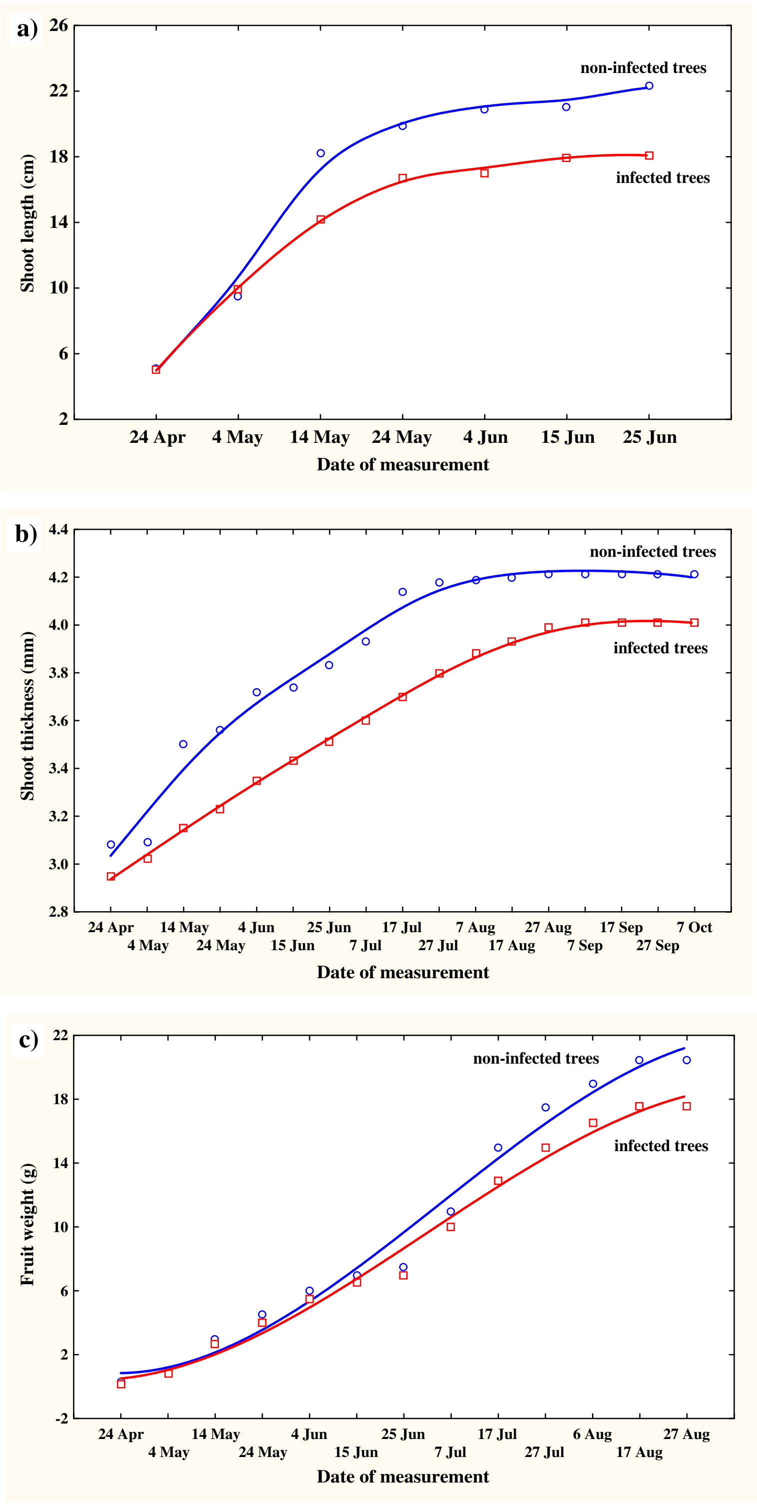

Fig. 1 Phenology of vegetative shoot growth in length **a**, thickness **b** and dynamics of change in fruit weight of cv. 'Cacanska Rodna' plum **c**

Table 1 The length and thickness of vegetative shoots and fruit weight on non-infected and infected trees of cv. 'Cacanska Rodna' (mean±SE)

Parameter	Non-infected trees	Infected trees
Shoot length (cm)	22.31±0.28*	18.35±0.07
Shoot thickness (mm)	4.21±0.03*	4.01±0.03
Fruit weight (g)	20.43±0.16*	17.58±0.18

The asterisks in horizontal columns indicate a significant difference between means at $P \leq 0.05$, $n=100$

on the growth and vigour of infected plum trees is related to the susceptibility of the cultivar with fruits being particularly more affected in susceptible cultivars (Kišpatić 1987). The difference in vegetative shoot length was significant (Table 1), which unambiguously confirmed that the presence of PPV on the 'Cacanska Rodna' tree markedly reduced its vegetative growth and consequently the total vegetative mass, the PPV stress impact being, therefore, clearly visible.

The growth of the vegetative shoots of 'Cacanska Rodna' in thickness was slower in the infected trees (Fig. 1b). The growth of vegetative organs in thickness (secondary thickening) occurs independently of the growth in length. Namely, it commences later than the growth of vegetative shoots in length and lasts until the end of the growing season. It is made possible through the products of photosynthesis and is dependent on environmental factors. It can be represented by a sigmoid curve (Prica 1977). The difference in the average vegetative shoot thickness was significant, PPV having considerably inhibited the secondary thickening of the vegetative shoots of 'Cacanska Rodna'. This may be explained by the fact that PPV induced bark splitting in sensitive plum cultivars (Nemeth 1986). Previous studies have shown that plum shoot thickening is highly intensive until 30 June, after which time the process continues at an increasingly lower rate, until the beginning of October (Paunović and Ogašanović 1972). The vegetative shoots of cv. 'Cacanska Rana', 'Cacanska Lepotica', 'Cacanski Secer' and 'Stanley' grow in thickness until 10 October (Milosevic 1997). Our results are highly consistent with these results.

Fruit growth and yield

While the growth phenology of infected and non-infected 'Cacanska Rodna' fruit was similar, the rate of growth of non-infected trees was greater and non-infected fruit reached a larger final size (Fig. 1c). Jordović and Janda (1963) showed that the fruits of sensitive plum cultivars ('Zimmers', 'Pozegaca', 'Italian Prune', 'Monarch'), while they were still green, developed hollow depressions that enlarged as the fruit matured, as well as wrinkles that deformed the fruit form. The above authors also reported that the deformities were due to the non-uniform growth of infected tissues, as they ceased to grow and eventually underwent necrosis, as opposed to the undisturbed growth of non-infected tissues. According to these authors, the PPV-infected fruits of cvs 'Agen', 'Bon de Bry', 'Crvena Ranka', 'Dobojska Rana', 'Ersingers', 'Giant', 'Green Gage', 'Pearl' and 'Stanley' retain their normal form, but are smaller in size. The results obtained in this study showed that the infected and non-infected fruits of 'Cacanska Rodna' exhibited an almost identical dynamics of change in their weight (rapid, slow and very rapid stage), the dynamics in infected fruits being, however, of lower intensity (Fig. 1c). The above dynamics induced statistically significant differences in the final average fruit weight of 2.85 g or 13.95% (Table 1). Christov (1947) conducted a thorough examination of the morphological changes in fruits of some plum cultivars and determined that the changes resulted in weight losses and reduction in size, as confirmed by

Table 2 The amount of premature fruit drop from non-infected and infected trees of cv. 'Cacanska Rodna' (mean±SE)

Parameter	20 August kg tree^{-1}	23 August kg tree^{-1}	Total yield kg tree^{-1}	Total yield t ha^{-1}
Fruit drop from non-infected trees	5.6±0.09	5.2±0.09	10.8±0.61	7.2±0.18
Fruit drop from infected trees	10.8±0.52**	14.8±0.84**	25.6±1.12**	17.1±0.70**
Differences	5.2±0.09	9.6±0.51	14.8±1.10	9.9±0.14

The asterisks in vertical columns indicate a significant difference between means at $P \leq 0.01$, $n=20$

Table 3 The amount of harvested fruit of cv. 'Cacanska Rodna' (mean±SE)

Parameter	Total yield kg tree^{-1}	Total yield t ha^{-1}
Harvested from non-infected trees	38.2±1.50**	25.5±0.45**
Harvested from infected trees	18.0±1.10	12.0±0.89
Differences	20.2±1.12	13.5±0.70

The asterisks in vertical columns indicate a significant difference between means at $P \leq 0.01$, $n=20$

the present study. The above author also reported that the content of resinous substances in the infected fruits was twice the content in non-infected ones. The fruits of cvs 'Cacanska Rana', 'Cacanska Lepotica', 'Canaski Secer' and 'Stanley' followed a three-stage sigmoid curve growth pattern from fruit setting to maturity, the stages being rapid, slow and very rapid of different duration, depending on the cultivar, the second stage being the shortest, lasting 10–12 days (Milosevic 1997). Under the conditions of the Czech Republic, the average fruit weight in the cultivar concerned was 22.50 g in an orchard with 1,333 tree ha^{-1} in the sixth year after planting (Blažek et al. 2004). Jordović and Janda (1963) reported a decrease in the average weight of infected fruits in 21 plum cultivars examined, but the decrease differed among the cultivars. Namely, the highest decrease in average weight ranged from 34.0% ('Zimmers') to 0.2% ('Green Gage'). The weight of diseased fruits decreased by 20–30% and the fruit yield frequently dropped before harvest (Pribék 2001). The results of the present study are consistent with these cited reports.

Two measurements of fruit drop (20 and 23 August) indicated that there were highly significant differences between infected and non-infected trees (Table 2). Bulatovic-Danilovich et al. (2006) also reported significant fruit drop in susceptible varieties. Since both non-infected and infected trees were grown under identical environmental conditions and using identical cultural and pomological practices, the amount of premature fruit drop from the infected trees could be attributed to the destructive effect of PPV.

The cumulative yield per tree of cv. 'Cacanska Rodna' grafted on 'Wangenheim Prune' seedlings from the third to the sixth year of cultivation in a six year-old high-density plum orchard, was 31.7 kg, and the yield 23.0 t ha^{-1} (Blažek et al. 2004). 'Cacanska Rodna' in Polish orchards produces high yields and excellent fruit quality (Zawadzka et al. 1994). Mika et al. (1998) reported identical findings in the cultivation of the above cultivar in Romania. Staniulis (2006) reported yield losses of 16–48% in PPV-infected plum trees under the environmental conditions of Lithuania. The virus does not kill trees but causes yield losses and reduces the marketability of fruit. For example, PPV has caused considerable losses in Europe, with susceptible cultivars reporting yield losses of 80–100% (Bulatovic-Danilovich et al. 2006). As regards plum susceptibility to PPV, Jordović and Janda (1963) reported the absence of interdependence of the symptom intensity on the leaves and the fruits. This was not the subject matter of the present study. The study, however, focused on the effect of PPV on the vegetative growth and fruit weight, as well as on the yield per tree (kg) and unit area (t ha^{-1}) of cv. 'Cacanska Rodna'. PPV symptoms on the leaves, highly manifested in this cultivar, served only as visual indicators of the virus presence. Bawden (1950) divided plants into five groups according to their response to the virus presence: (1.) infection-localising plants, (2.) plants rapidly dying of systemic diseases, (3.) chronically diseased plants, (4.) plants showing symptoms but suffering relatively low yield losses and (5.) virus-bearing plants showing no symptoms. The cv. 'Cacanska Rodna' could be classified into the fourth group. However, the amount of premature fruit drop from infected trees (Table 2), as well as the difference in the final yield per tree and unit area (Table 3) between the non-infected and infected plants of 'Cacanska Rodna' compromise the cultivation of this cultivar in commercial plum orchards in Serbia and in the growing regions in Europe and worldwide where PPV occurs (Zagrai et al. 2005), although the cultivar is distinguished by its superb qualitative and quantitative agronomic traits - table fruit, processing, drying (Paunovic et al. 1975).

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