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EFFECT OF INFECTION BY ILAR-VIRUSES ON VEGETATIVE GROWTH OF SWEET CHERRY IN NURSERY AND YOUNG TREES

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Abstract

The effect of infection with Prunus necrotic ringspot virus (PNRSV) and Prune dwarf virus (PDV) on vegetative growth of sweet cherry trees was investigated in a nursery and during the first three years in an orchard. The subjects of the research were trees of cultivars 'Van', 'Kozerska', 'Drogans yellow', 'Lambert' and 'Stefania'. In the spring of 2012 virus-free P. mahaleb rootstocks (IK-M9) were budded with virus-free buds of these cultivars. Fourteen days after that, they were artificially infected with isolates of PNRSV, PDV and mixed infection of them, using 'double chip budding' method. Serological analyses were made by DAS-ELISA method. There was a significant negative virus effect on the vegetative growth in most of the single infected with PNRSV or PDV and all mixed infected trees of Van and Lambert in a nursery. As an average for the period of study there were not significant virus effects on the trunk diameter, the height of the trees and the length of the shoots of virus infected trees of the cultivars 'Stefania', 'Kozerska' and 'Van', compared to the healthy young trees.

Key words*: influence, PNRSV, PDV, sweet cherry, vegetative growth.*

INTRODUCTION

The viral infections are one of the main problems in the production of healthy propagated material from stone fruit species and profitable fruit production. The most common and economically important among the viral pathogens infecting the sweet cherry trees are *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV). These viruses, members of the genus *Ilarvirus,* spread not only by infected propagating material, but also by seed and pollen (Amari et al., 2009; Aparicio et al., 1999; Mink, 1993).

Quantitative data on the relationship between virus infection and the effect on the vegetative and reproductive development of sweet cherry trees is limited and most the publications are from the last century.

PNRSV and PDV may cause a significant reduction in taking of buds, varying by percentages dependent on the rootstocks (Nemeth, 1972; Proebsting et al., 1995).

The infection of *Ilarvirus* also has an adverse effect on the development of young trees in the nursery, and retards their growth (Milbrath, 1950; Milbrath, 1957; Nemeth, 1986; Gilmer et al., 1976; Nyland et al., 1976). In the case of combined infections, the damage is always higher than if the viruses infected separately (Cropley, 1968). In the fruit-bearing orchards these viruses induce also growth and crop yield reduction (Parker et al., 1959; Posnette et al., 1968). They can delay maturity in fruit development up to 2 weeks compared to virus-free trees (Howell and Mink, 1984).

The purpose of our study was to investigate the influence of PNRSV and PDV on the some vegetative properties of five sweet cherry cultivars grafted on *Prunus mahaleb* rootstock.

MATERIALS AND METHODS

The investigation was carried out at the Institute of Agriculture-Kyustendil, Bulgaria. In the spring of 2012, virus-free *P. mahaleb* rootstocks (IK-M9) were budded with virus-free buds of cultivars 'Van', 'Kozerska', 'Drogans yellow', 'Lambert' and 'Stefania'. 14 days after that, they were artificially infected with isolates of PNRSV, PDV and mixed infection of them, using 'double chip budding' method (Table 1). Ten trees of each cultivar were infected with PNRSV, PDV and the combination of both. Virus-free trees from each cultivar were used as controls.

Table 1 Virus treatments and source of inoculum from commercial sweet cherry orchards

Virus treatment	Source of virus
PNRSV	naturally infected cherry, cultivar 'Merchant'
PDV	naturally infected cherry, cultivar 'Superstar'
PNRSV+ PDV I	naturally infected cherry, cultivar 'Diana'
PNRSV+ PDV II	naturally infected cherry, cultivar 'Priusadebnaya'

The 2-year-old nursery trees of cultivars 'Van', 'Kozerska' and 'Stefania' artificially infected with PNRSV, PDV and mix combination, as well as - virus free ones were planted in autumn 2013 in randomised complete blocks, at a spacing 4.80 x 4.00 m.

Each variation of each cultivar was represented by three trees.

The performance of these trees in terms of vegetative growth – trunk diameter, height of the tree and length of the shoots on infected and virus free trees was examined for three years. In February 2015, a pruning of healthy and infected trees was carried out.

The viral status of the used rootstocks IK-M9 and sweet cherry cultivars was determined by ELISA testing. All plant materials were subjected to DAS-ELISA using the PNRSV, PDV, PPV, CLRV and RpRSV detection kit of Loewe Biochemica (GmbH, Germany) and Cocktail-ELISA for identification of ACLSV following recommendations of the supplier. The infection of virus free trees by pollen transfer was followed by regular ELISA testing each year.

The data was statistically processed by analysis of variance using F for test significance and LSD for significance of the differences between variant means and control, at level $P<0.05$, 0.01 and 0.001, depending on data dispersion.

The data analysis was performed by computer programs developed by Maneva, 2007 on the base of standard statistical algorithms suitable for small set of data with biological origin (Sokal and Rohle, 1981).

RESULTS AND DISCUSSIONS

In a nursery

The data from the experiment of the influence of PNRSV, PDV and mixed infection with both viruses on the vegetative properties of trees of investigated five cultivars in a nursery are represented in Table 2.

In most of the single and all mixed infected trees of cultivar 'Van' and 'Lambert' was found a significant negative influence of the viruses on vegetative growth of tree.

The development was the least disturbed by PNRSV infection: the rootstock diameter was less by 8.2% in cultivar Lambert and 10.6% in Van, the diameter of scion was less by 17.0% in Van, the height of the young tree was less by 2.3% in Lambert and 12.4% in Van.

The strongest effect on the development was observed in the combined infection of PNRSV and PDV: the diameter of rootstocks was less by 21.4 - 25.3% in Van, the diameter of scion was less by 21.9 -30.4% in Van, the height of the tree was less by 27.8 - 37.0% in Lambert. In the cultivars 'Drogans yellow', 'Stefania' and 'Kozerska' the viruses and the mixed infections of them insignificantly reduced vegetative properties of the trees.

With the exception of the cultivar 'Kozerska', where PNRSV infection induced reduction of the diameter and the height of trees by 32.5%, mixed infection reduced the scion diameter by 24.7%. In cultivar 'Stefania' the combined infection decreased the diameter of rootstocks by 15.5%, which was statistically proven.

In a non – fruit - bearing sweet cherry orchard:

The effect of virus infections on the vegetative properties of the investigated cultivars in the orchard is shown in Table 3. As an average for the period of study, there were not significant virus effects on the trunk diameter, the height of the trees and the length of the shoots of virus infected trees of cultivars 'Stefania', 'Kozerska' and 'Van', compared to the healthy young trees.

Although it was noted that virus infections insignificantly decreased the trunk diameter by 2.2% in trees of cultivar 'Van' infected with PNRSV to 27.8% in trees 'Stefania' with PNRSV+PDV, the height of the young trees by 3.8% in cultivar 'Stefania' infected with PNRSV to 13.9% in 'Stefania' with PDV, the shoot length by 3.2% in trees of 'Stefania' with PNRSV to 28.9% in trees of 'Kozerska' with mix infection PNRSV+PDV.

Table 2. Vegetative properties of virus-free and virus infected trees in a nursery

Cultivar	Virus treatments	Rootstock diameter, cm	Scion diameter, cm	Height of tree, cm
'Drogans yellow'	virus free	1.82	1.40	146.8
	PNRSV	1.66 ns	1.28 ns	132.6 ns
	PDV	1.76 ns	1.11 ns	126.4 ns
	PNRSV+ PDV I	1.82 ns	1.36 ns	134.6 ns
	PNRSV+ PDV II	1.78 ns	1.32 ns	124.6 ns
Sd		0.1146	0.1660	17.5374
F		0.6574	0.9819	0.4995
LSD 0.05		0.243	0.3518	37.17
'Stefania'	virus free	1.94	1.40	160.4
	PNRSV	1.84 ns	1.52 ns	157.6 ns
	PDV	1.80 ns	1.34 ns	152.8 ns
	PNRSV+ PDV I	1.64 *	1.40 ns	149.2 ns
	PNRSV+ PDV II	1.96 ns	1.36 ns	164.4 ns
Sd		0.1129	9.2518	11,1132
F		2.5830	1.1403	0.5873
LSD 0.05		0.2394	0.1961	23.56
'Kozerska'	virus free	1.92	1.78	185.8
	PNRSV	1.90 ns	1.20 **	125.4 *
	PDV	1.76 ns	1.58 ns	182.0 ns
	PNRSV+ PDV I	1.84 ns	1.34*	145.0 ns
	PNRSV+ PDV II	1.86 ns	1.58 ns	180.8 ns
Sd		0.1843	0.1782	22.4815
F		0.2285	3.2544	2.9007
LSD 0.05		0.3906	0.3778	47.66
'Lambert'	virus free	2.18	1.46	163.6
	PNRSV	2.00 ns	1.48 ns	159.8 ns
	PDV	1.84 *	1.40 ns	143.2 ns
	PNRSV+ PDV I	1.80 *	1.18 *	118,0*
	PNRSV+ PDV II	1.72 *	1.10 *	101.8 **
Sd		0.1582	0.1259	16.0780
F		2.6772	3.7680	5.5424
LSD 0.05		0.3354	0.2669	34.08
'Van'	virus free	2.06	1.64	175.5
	PNRSV	1.84 ns	1.36 *	153.8 ns
	PDV	1.68 **	1.36 *	145.8 *
	PNRSV+ PDV I	1.62 **	1.28 *	129.6***
	PNRSV+ PDVII	1.54***	1.14 **	136.0 **
Sd		0.1278	0.1263	11.1178
F		5.2043	4.1705	5.1885
LSD 0.5		0.2709	0.2678	23.56

During the period of the investigation, each spring the virus free trees of the investigated cultivars were retested for the present of viruses - PNRSV and PDV. The results of the serological analyzes confirmed that all control trees were virus free.

Sweet cherry trees in Bulgaria are grown primarily on Mahaleb (*P. mahaleb*) or Mazzard (*Prunus avium*) seedlings. These rootstocks are generally tolerant to infection by the pollen-borne ilarviruses PDV and PNRSV (Lang and Howell, 2001). It should be noted that in our experiment, it was used only *P. mahaleb* rootstock and this may be one of the reasons for the established insignificant influence of these viruses on the vegetative growth of infected young sweet cherry trees in an orchard.

However, recent research revealed that trees on some new dwarfing rootstock like Gisela 6, Damil, Inmil and others exhibited detrimental reactions to infection by PDV and PNRSV (Lang et al., 1998; Andersone et al., 2002; Lankes, 2007).

Table 3. Vegetative properties of virus-free and virus infected trees in orchard

Cultivar	Virus treatments	Trunk diameter, cm	Height of tree, cm	Length of one-year shoots, cm
		Average (2014-2016)		
'Stefania'	virus free	1.53	228.3	41.89
	PNRSV	1.36 ns	219.6 ns	40.52 ns
	PDV	1.20 ns	196.6 ns	35.08 ns
	PNRSV+ PDV	1.11 ns	200.3 ns	33.99 ns
Sd		0.2503	33.2811	7.5832
F		1.0957	0.4180	0.5341
LSD 0.05		0.6133	81.53	18.57
'Kozerska'	virus free	1.82	260.6	56.95
	PNRSV	1.44 ns	241.6 ns	42.67 ns
	PDV	1.726 ns	248.0 ns	48.00 ns
	PNRSV+ PDV	1.473 ns	238.3 ns	40.46 ns
Sd		0.3005	19.6718	6.8374
F		0.7765	0.5017	2.3030
LSD 0.05		0.7362	48.19	16.75
'Van'	virus free	1.52	249.6	49.34
	PNRSV	1.486 ns	239.3 ns	45.47 ns
	PDV	1.386 ns	232.3ns	40.03ns
	PNRSV+ PDV	1.36 ns	223.3 ns	38.21sn
Sd		0.0879	29.7069	5.853
F		1.5340	0.2808	1.5135
LSD 0.05		0.2155	72.78	14.34

CONCLUSIONS

There was a significant negative virus effect on the vegetative growth in most of the single infected with PNRSV or PDV and all mixed infected trees of cultivars 'Van' and 'Lambert' in a nursery.

As an average for the period of study there were not significant virus effects on the trunk diameter, the height of the trees and the length of the shoots of virus infected trees of the cultivars 'Stefania', 'Kozerska' and 'Van'

grafted on *Prunus mahaleb* rootstock, compared to the healthy young trees.

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