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Occurrence of *Ilarviruses* in sweet and sour cherry in Bulgaria

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Abstract

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Sweet cherry is an important fruit crop in Bulgaria. Field surveys were conducted in 43 commercial and three collection sweet and sour cherry orchards in ten locations of six regions of Bulgaria. Single home-grown sweet and sour cherry trees were included, too. Leaf samples were collected from 2090 trees with and without virus-like symptoms. These samples were tested by DAS-ELISA for three *Ilarviruses* including *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV) and *Apple mosaic virus* (ApMV). While ApMV was not found, 269 and 111 samples tested positive for PDV and PNRSV, respectively and 22 samples for both viruses, showing that 19.2% of the trees were infected with at least one of the two viruses. PDV was the most abundant virus in sweet cherry detected in 87.1% of infected trees. PNRSV was prevalent in sour cherry. Visual observations were made in order to connect the expressed symptoms with the respective virus infection. In 56.2% of infected with PDV trees symptoms as chlorotic spots and rings or chlorosis in the base of central and secondary veins were observed, and 43.8% of infected trees were asymptomatic. PNRSV infection was associated both with and without symptoms. Symptomatology only is not enough for determination of PNRSV or PDV infections due to similar symptoms, thus imposing the need of serological or molecular detection. The almost equal rate of PDV and PNRSV infections found in differently aged orchards suggests their introduction by infected propagating materials.

*Keywords***:** ELISA; cherry; *Ilarviruses*; detection; symptoms; RT-PCR

Introduction

Stone fruits are susceptible to many virus associated diseases (Nemeth et al., 1986). *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV) and *Apple mosaic virus* (ApMV) are one of the most important viruses of sweet (*Prunus avium*) and sour (*P. cerasus*) cherry. These three viruses belong to the genus *Ilarvirus,* family *Bromoviridae* (Roossinck et al., 2005). They occur worldwide in single or in mixed infections within the trees and are serious pathogens of many species of *Prunus*, including sweet and sour cherry (Myrta & Savino, 2008; Sucha & Svobodova, 2010).

Crop losses up to 30-57% caused by PNRSV in fruit-bearing sweet cherry orchards are reported (Posnette et al., 1968). PNRSV is a serious threat also in the nurseries, where the percentage of successful unions may be reduced by 60% (Jacob, 1972). PDV elicit a range of different diseases in sweet and sour cherry and in mixed infections with PNRSV may lead to decline and death of the trees (Cropley, 1968). ApMV has a cosmopolitan distribution (Diekmann & Putter, 1996). Besides sweet and sour cherry ApMV infects many other *Prunus* species as hopes, rose, hazelnut and apple (Fulton, 1983; Nemeth, 1986). All three viruses spread through infected propagating material (Mink, 1992). PNRSV and PDV are also seed-and pollen-born and can be spread by

pollinating insects (Cole et al., 1982; Hamilton et al., 1984; Kelley & Cameron, 1986). Seed transmission of ApMV in hazelnuts is known (Cameron & Thomson, 1985).

Sweet cherry is one of the most important stone fruit crop grown in Bulgaria and presently its production ranks second after the plum. The areas occupied by cherries increase due to the established new plantations in the south region of the country. One of the most positive control measures against plant viruses is the fast and reliable diagnosis.

To gather a better insight of the sanitary status of sweet and sour cherry, a survey was carried out for the presence of PNRSV, PDV and ApMV. Commercial and collection orchards, in several locations of six regions of the country were visually surveyed and collected samples were serologically tested for mentioned above *Ilarviruses*. Infection rates of PNRSV and PDV in symptomatic and symptomless trees and in differently aged commercial orchards, as well in single home-growing trees were determined. The presence of both viruses in limited number samples infected with PDV and PNRSV was molecularly confirmed by RT-PCR analyses.

Materials and Methods

Field survey and visual evaluation of leaf symptoms

Field inspections were conducted and sample collected in spring of 2018 in forty-three commercial and three collection orchards [one of sour cherry at Institute of Agriculture (IA), Kyustendil and two (one sweet and one sour cherry) at Research Institute of Mountain Stockbreeding and Agriculture (RIMSA), Troyan] (Table 1). Single home-growing sweet and sour cherry trees from three locations were also included. Each tree in the field and each leaf sample in the laboratory were evaluated for symptoms. In total 2090 samples of fully developed leaves were collected from trees showing and not showing virus-like symptoms. Samples were taken random from several different branches around the trees, placed in an ice bag and kept at 4°C prior to testing. All positive samples were divided in portions and preserved at -70°C and dried under $CaCl_2$. Dried samples were stored at 4°C.

Serological assays

Collected leaf samples were tested by double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977) for PNRSV, PDV and ApMV following the manufacturer's protocols (Loewe Phytodiagnistica Gmbh). Both, specific and conjugated antibodies were used in quantity of 100 µl and incubated at 37°C for 4h. 150 µl of diluted 1:20 extracts (w/vol) were loaded in duplicate wells of polystyrene microtiter plate and incubated overnight at 4°C.Between each step the plate was washed 3 times with PBS-T (phosphate-buffered saline-Tween). 100 µl freshly prepared p-nitrophenylphosphate in substrate buffer (1mg/ml) were loaded to each well. The plate was incubate at room temperature and photometric measurement was done at 405 nm after 2 h. Samples were considered as positive if their absorbance values were more than three times higher of negative control.

RT-PCR analyses

Serologically detected PNRSV and PDV of limited number sweet and sour cherry samples were analyzed by RT-PCR method. RNA was isolated from fresh leaves by the use of a commercially available RN easy Plant Mini extraction kit (Qiagen, Germany) and used as template to generate single stranded cDNA. Total nucleic extracts were dissolved in 50 µl of RNase free water and stored at -70°C until use.

The coat proteins (CP) of PNRSV and PDV were amplified by two-step RT-PCR method. First, cDNA was synthesized by the use of smART Reverse Transcriptase kit (EURx, LtD.). To a nuclease free micro centrifuge tube 2 µl of template RNA, 1µl (10 mM) of the respective specific antisense primer (listed below) and 2.5 µl (2.5 mM) dNTPs (containing 2.5 mM each dATP, dGTP, dCTP, and dTTP) were added and completed to 12.5 µl with sterile distilled water. The mixture was heated to 65°C for 5 min and quickly chilled on ice. Than 4 µl of 5X first strand buffer, 2 µl of 0.1 M DDT (both, supplied with the kit), 0.5 µl of RNase OUT (40 units/µl) and 1µl smART(200 U/µl) were added, mixed gently and incubate at 50°C for 30 min. The reaction was terminated by heating at 85°C for 5 min.

Secondly, PCR amplifications were performed as 1 µl of cDNA was mixed with 24 µl of the amplification mixture containing 2.5 µl of 10X Buffer (200 mM Tris-HCL pH8.4, 500 mM KCL), 1.5 µl of $MgCl_2$ (25 mM), 2.5 µl dNTPs (2.5 mM), 0.5 µl of each primer (10 mM), 0.25 µl *Taq* DNA polymerase (5 U/µl; EURx, LtD.) and 16.25µl RNase free sterile water.

Oligonucleotide primer sequences (Spiegel et al., 1999) were used to detect PNRSV. Sense primer: 5'-GAGCTCTG-GTCCCACTCAGG-3' and antisense primer: 5'-TCACTCT-AGATCTCAAAGCAG-3' amplify fragment in length of 616 bp, corresponding to the region between 1178 to 1794 nucleotides. Termocycling was carried as follows: 94°C for 3 min, than 40 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 1 min, followed by 72°C for 5 min.

The sense and antisense PDV primers: 5'-GTGTA-GAAAGAAGAGAAGTCCGACAAG-3' and 5'-ATCT-AGAAGCAGCATTTCCAACTACGA-3', respectively

(Vaskova et al., 2000) were used for RT-PCR testing. The expected amplified product was 874 bp long, representing the whole CP open reading frame (ORF). PCR reaction was performed following next thermal cycling program: 94°C for 2 min, than 40 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 90 s, followed by 72°C for 10 min.

Amplified products (5 µl each) were electrophoresed in 1.2% agarose gels in 40 mM Tris-acetate and 1 mM EDTA, pH 8.0 (TAE) and stained with GelRed dye (stock solution 10 000X) (Biotium) at 80–140 V for 1–1.5 h to confirm the expected size of PCR products.

Results and Discussion

Field surveys and symptoms evaluation

In an effort to assess the health status of sweet and sour cherry in respect to PNRSV, PDV and ApMV infections in Bulgaria, a survey was carried in differently aged orchards in ten locations of six regions of the country. A total of 2090 trees were visually observed during the spring of 2018 (from April to June) and collected leaf samples were serologically tested. A slight prevalence of symptomatic infection both for PNRSV and PDV was found. Around 56.2% of collected samples with serologically proved infection of PDV showed symptoms compared to the samples (43.8%) without symptoms in agreement with (Yardimci & Gulac-Kulic, 2011) who observed PDV infection to be commonly associated with symptomatic cherry trees, while (Perez-Sanchez et al., 2015) reported for 65–100% asymptomatic reaction to the virus.

The symptoms caused by PDV (Figure 1) were as follows: A/ yellow rings, lines, spots and wrinkling of the leaves B/ yellowish ringspots and deformation of the leaves; C/ reddish necrosis, spots and rupture; D/ yellow bands; E/ light chlorotic rings. Such symptoms induced by PDV have been already described (Massart et al., 2008; Perez-Sanchez et al., 2015; Smith et al., 1988).

Virus symptoms were seen in serologically proved PNRSV infected trees but many of them were symptomless. The most frequently observed symptoms were necrosis and chlorosis around central and secondary veins, deformation and wrinkling, necrosis of leaf marginal, intervene chlorosis and severe necrosis with shot holes (Figure 2B, C and D). Single infected trees showed “tattered” leaves (Figure 2 A). Many of sour cherry trees infected with PNRSV, however, did not show any symptoms or they were very faint, difficult for detection, consisting essentially of slight mottling between the veins and small necrotic spots with perforation of the tissue. Depending on the isolate the reaction of cherry trees infected with PNRSV range from symptomless to a rugose mosaic

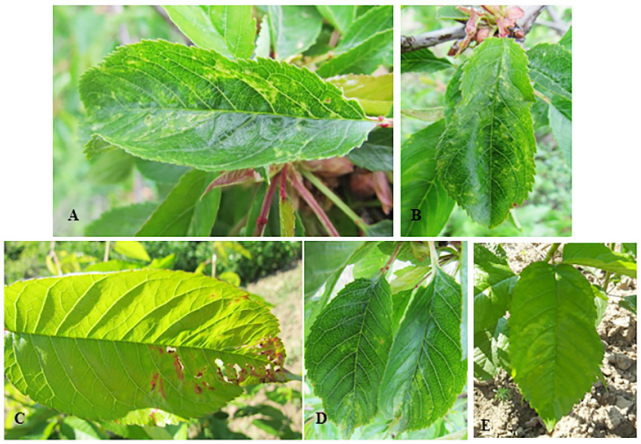

Fig. 1. Symptoms on the leaves of sweet cherry with serologically proved PDV infection. A/ yellow rings, lines, spots and wrinkling of the leaves B/ yellowish ringspots and deformation of the leaves; C/ reddish necrosis, spots and rupture; D/ yellow bands; E/ light chlorotic rings(- original A. Borisova and I. Kamenova)

Fig. 2. Symptoms on the leaves of sweet cherry with serologically proved PNRSV infection. *A*) “tattered” leaf; *B*) necrosis and chlorosis around central and secondary veins, deformation and wrinkling; *C*) interveinal chlorosis; *D*) severe necrosis with shot holes (original A. Borisova and I. Kamenova)

disease (Howell & Mink, 1988). Usually symptoms of PNRSV appear in the first year after infection, during so called acute or shock stage, and later the infection becomes latent (symptomless). There are also strains of PNRSV that cause symptoms annually (Nyland et al., 1976; Wells &Kirkpatrick, 1986). Depending on the symptoms three serologically similar strains of the virus have been distinguished, namely: virus A-does not causes enations (leaf-like growths) on the leaves, and with no apparent recovery of the trees; virus E-with leaf enations and with apparent recovery, and virus G-without enations and apparent recovery (Fulton, 1958; 1968). According Topchiiska (1996) Bulgarian isolates of PNRSV are related to strain G (latent ring spot). The symptoms observed by us in infected trees were relatively mild, without formation of the described above enations. However, on several infected trees in one 5 years old orchard (Kyustendil), more severe symptoms as gumming and drying of skeletal branches was observed.

In general severity of the symptoms caused by PNRSV and PDV vary widely, depending on virus isolate, host specie and temperature (Diekmann & Putter, 1996). In this study many of infected trees exhibited similar symptoms which make it difficult to be connected to each of the viruses, thus imposing the need of their serological or molecular detection. It should also be noted that the described above symptoms were clearer at the beginning of the vegetation period (April, May and early June) and with the progression of the season some of them were masked.

Serological evaluation

In total 2090 samples from sweet and sour cherry were serologically analyzed for PNRSV, PDV and ApMV and the results are presented in Table 1. None of the samples were infected with ApMV and these results are in agreement with the reported absence of the virus in the region of Kyustendil (Borisova & Borovinova, 2014). In a study for sap transmissible viruses in cherry, however, Milusheva & Zivondov (2009) reported infection of 12% with ApMV in the region of Plovdiv. Similarly to our results ApMV was not detected in cherry trees in neighboring countries as Serbia (Mandic et al., 2007) and Greece (Maliogka et al., 2010). In Europe ApMV is more commonly distributed on *Prunus* spp., than on *Malus* spp. (Paunovic et al., 2010). ApMV is often found in mixed infections with PNRSV and PDV, but the frequency

Table 1. Level of infections with PNRSV and PDV in sweet and sour cherry in the surveyed regions of Bulgaria

Region/Location	Number of orchards	Number of tested samples/Number of infected samples (% infection)	Positive for		
			PNRSV	PDV	PNRSV+ PDV
Sweet cherry					
South Western/Petrich	10[a]	430/100(23.3%)	4 (4.0%)	95(95.0%)	1(1.0%)
Western/Kyustendil	9[a]	393/129 (32.8%)	23 (17.8%)	101 (78.3%)	5 (3.9%)
South WesternSimitli	1[a]	41/7 (17.1%)	0	7 (100%)	0
South Western/Sliven	10[a]	544/43(7.9%)	6 (14.0%)	37 (86.0%)	0
South Central/Plovdiv	10[a]	284/17 (6.0%)	0	17 (100%)	0
North Central/Troyan	1[b]	11/0	0	0	0
North Eastern/Kranevo	2[a]	47/11 (23.4%)	0	11(100%)	0
South Central/Strelcha	s.t.[c]	5/0	0	0	0
North Central/Elena	s.t.[c]	22/1 (4.5%)	0	1(100%)	0
Western/Sofia	s.t.[c]	6/1 (16.7%)	1	0	0
Total:		1783/309 (17.3%)	34 (11.0%)	269 (87.1%)	6 (1.9%)
Sour cherry					
South Western/Simitli	1[a]	84/1(1.2%)	1(100%)	0	0
Western/Kyustendil	1[b]	102/79(77.5%)	63(79.7%)	0	16(20.3%)
North Central/Troyan	1[b]	26/2(7.7%)	2 (100%)	0	0
South Central/Strelcha	s.t.[c]	65/10 (15.4%)	10 (100%)	0	0
North Central/Elena	s.t.[c]	11/0	0	0	0
Western/Sofia	s.t.[c]	19/1(5.3%)	1(100%)	0	0
Total:		307/93(30.3%)	77 (82.8%)	0	16(17.2%)
Total sweet and sour cherry		2090/402(19.2%)	111(27.6%)	269(66.9%)	22(5.5%)

Note: [a]commercial orchards; [b]collection orchards; [c]s.t single trees

of its infection seems to be much lower of that with PNRSV and/or PDV, especially in plum, apricot, peach and cherry (Myrta et al., 2003).

DAS-ELISA revealed that 309 (17.3%) of 1783 samples from sweet cherry were infected, of which 269 with PDV, 34 with PNRSV and in 6 sample mix from the two viruses was detected. The highest and lowest infection rates (32.8% and 6.0%) were determined in the commercial orchards of Kyustendil (Western) and Plovdiv (South Central) locations, respectively. No infection was found in the only one surveyed collection orchard (Troyan, North Central). Low infection rate was determined in single home-grown sweet cherry trees since PNRSV and PDV were found each in one sample.

Unlike sweet cherry, sour cherry was infected mainly by PNRSV with 82.8% rate of infection, confirming the higher susceptibility of sour cherry to PNRSV (Paprstein et al., 1995). Our results as those of Rouag et al. (2008) showed a higher occurrence of PNRSV in sour cherry (82.8%), than in sweet cherry trees (11.0%). Low incidence of PNRSV infection in sweet cherry was detected also by Sucha & Svobodova (2010). In 17.2% of infected trees PNRSV was in a mix with PDV.

According to obtained data the overall average infection level of PNRSV and PDV in sweet and sour cherry was 19.2%. Almost a similar infection level (17.7%) of PNRSV and PDV on sweet and sour cherry was reported for Czech Republic (Sucha & Svobodova, 2010). Extended field studies in several countries of the Mediterranean region have shown a high incidence of *Ilarvirus* infection (23.5%), with a prevalence of PDV in cherry (35.4%) (Pallas et al., 2012). PDV, PNRSV and ACLSV are reported as the most commonly spread viruses on cherry (Jordovic, 1955; 1958; Rankovic, 1976).

Comparing the levels of infection with PNRSV and PDV in sweet and sour cherry a higher rate was determined in sour cherry (30.3%), than in sweet cherry (17.3%). That, however, due to the fact that the most of sour cherry trees (63 infected of 102 tested) at the collection orchard of IA, Kyustendil were infected by only PNRSV(79.7%), or by PNRSV in a mix with PDV (20.3%). Hence these results do not reflect the real picture of PNRSV and PDV occurrence; all the more only one commercial sour cherry orchard was included in the study. From the two viruses the most frequently detected in sweet cherry was PDV (87.1%), while in 82.8% of infected sour cherry trees PNRSV was determined. Mixed infections of both viruses were detected in higher number (16 from 307 tested) sour cherry, than in sweet cherry trees. High PDV infection rates in cherry trees have been reported from a number of countries as Algeria, Italy, Serbia, Canada, Turkey, Czech Republic and Jordan (Al Rwahnih et al., 2001; Di Terlizi, 1998; Mandic et al., 2007; Michelutti et al., 2005; Sipahioglu et al., 1999; Sucha & Svobodova, 2010; Rouag et al., 2008). Studding the incidence of PNRSV and PDV in stone fruit species in Bulgaria the highest rate of PDV was found in cherry (15.8%), followed by apricot, plum, peach and almond (Milusheva & Borisova 2005). Mixed infections of two or more viruses are frequently detected on stone fruit species including cherry in Japan (Isogai et al., 2004), California (Sabanadzovic et al., 2005), Turkey (Yardimci & Gulal-Kulic, 2011) and Mediterranean countries (Myrta et al., 2003).

Number of infected with PDV commercial orchards (29) was more than two times higher of the number of orchards without PDV infection (13 orchards), while PNRSV was found in only 6 out of 42 commercial orchards with highest infection (17.8% and 14.0%) in Western (Kyustendil) and South Eastern (Sliven) regions, respectively. Fourteen of the sixteen trees infected with both PNRSV and PDV were detected in a three years old orchard in Kyustendil (Western region).

In order to assess the level of PDV infection in the differently aged sweet cherry orchards, they were conditionally divided as "*young*" (from 1 till 5years old), "*middle*" aged (from 6 till 10 years old) and "*old*" aged (from more than 10 till 15 years old). No significant difference among the orchards was detected, since the levels of infection were 20.7%, 21.1% and 24.2% in the "*young*", "*middle*" and "*old*" aged sweet cherry orchards, respectively. Almost the same situation was observed with PNRSV infection in sweet cherry orchards, as high number of infected trees were found in two orchards, one five years old (Kyustendil, 18 of 120 tested) and one two years old (Sliven, 6 of 15 tested). In the collection sour cherry orchard at IA, Kyustendil planted in 2016, 77.5% of the trees were infected with PNRSV, or with both PNRSV and PDV. These results indicate most likely the introduction of PDV in the orchards by infected plant propagating materials. This applies also for PNRSV infection in the collection sour cherry orchard at IA, Kyustendil, as for the period of two years after its planting the level of infection was 79.7%.

RT-PCR detection

A total RNA was extracted from fourteen leaf samples infected with PDV and from another fourteen samples infected with PNRSV (from sweet and sour cherry). Samples for RT-PCR analyses of the two viruses were selected on the base of their host (sweet or sour cherry in the case of PNRSV) and geographical origin (from four regions).

Amplicons of 874 bp was obtained with PDV primers targeting the coat protein gene of the virus (Figure 3A). A

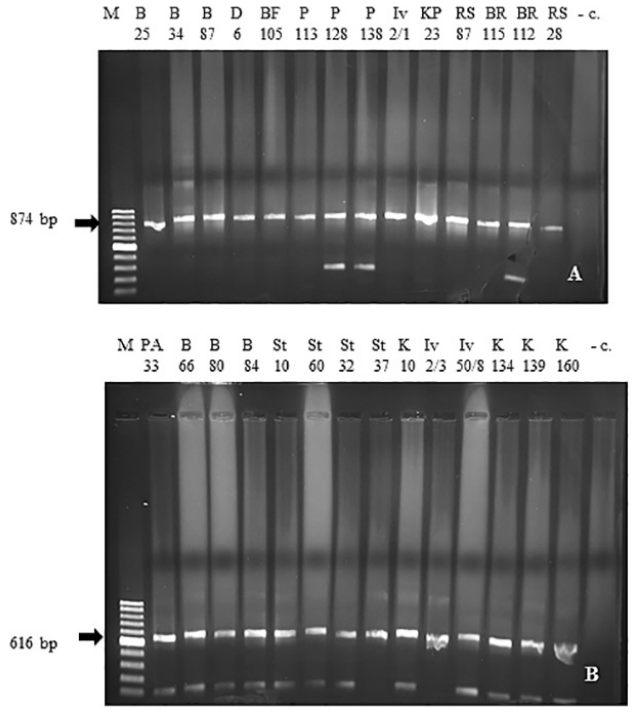

Fig. 3. PCR analyses of A/PDV isolates; B/PNRSV isolates. The amplification of 874 bp and 616 bp fragments of CP gene of PDV and PNRSV, respectively from different locations is shown. Letters and numbers at the top indicate the names of isolates

616 bp fragment was amplified from all analyzed PNRSV isolates (Figure 3B). The amplified fragments were purified and are in process of sequencing of the CP genes of the two viruses for future evaluation of molecular variability of Bulgarian PDV and PNRSV isolates.

Conclusions

DAS-ELISA tests of sweet and sour cherry trees from regions with intensive cherry growing of the country detected infections with only PNRSV and PDV and not ApMV. The established 19.2% rate of infection showed a moderate infestation of the two viruses. PNRSV and PDV are pollen-transmissible and an increase of the infection in the surveyed orchards could be expected. The most abundant in sweet cherry was PDV found in the majority of orchards in all surveyed regions. PNRSV was the main virus found in sour cherry trees. The established rates of infections both, with PDV and PNRSV in "*young*" years old orchards, as well their latent infestations shows the importance of the use of healthy planting material. In this study PDV and PNRSV were detected for the first time at molecular level in Bulgaria and that is a step towards their further molecular characterization.

Disclosure statement

The authors declare that they have no conflict of interest.

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References

Al Rwahnih, M., Myrta, A., Abou-Ghanem, N., Di Terlizzi, B. & Savino, V. (2001). Viruses and viroid's of stone fruits in Jordan. *OEPP/EPPO Bulletin, 13*: 95-98.

Borisova, A. & Borovinova, **M.** (2014). Evaluation of viral status and susceptibility of sweet cherry cultivars to cherry leaf spot. *Blumeriellaja apii* (Rehm). *Rastenievadni nauki*, LI (4-5) (Bg).

Cameron, H. D. & Thomson, M. (1985). Seed transmission of apple mosaic virus in hazelnut. *Acta Horticulturae*, *193*: 131.

Clark, M. F. & Adams, A. N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent as-say for the detection of plant viruses. *Journal of General Virology, 34*: 475-483.

Cole, A., Mink, G. I. & Regev, S. (1982). Location of *Prunus necrotic ringspot* virus on pollen grains from infected almond and cherry trees. *Phytopathology*, *72:* 1542-1545.

Cropley, R. (1968). Synergism and interference between *Necrotic ringspot virus* and *Prune dwarf virus* in fruit trees. *Tagungsbericbte*, *97*:139-142.

Di Terlizzi, B. (1998). Stone fruit viruses and certification in the Mediterranean: problems and prospects. *Options Méditerranéennes*, série B/No*19*: 53–56.

Diekmann, N. & Putter, C. A. J. (1996). Stone Fruits. FAO/IPGRITechnical Guidelines for the Safe Moments of Germplasm № 16.

Fulton, R. W. (1958). Identity of and relationships among certain sour cherry viruses mechanically transmitted to *Prunus* species. *Virology*, *6*: 499-511.

Fulton, R. W. (1968). Relationships among the ringspot viruses of *Prunus*. In: *Tagungsberichte, 97:* From:*VII Enr. Symp.* über *Viruskr der Obstb. 10-16 July*, 1967: 123-138.

Fulton, R. W. (1983). *Ilarvirus* group. CMI/AAB Descriptions of Plant Viruses, 275.

Hamilton, R. I., Nichols, C. & Valentina, B. (1984). Survey of *Prunus necrotic ringspot virus* and other viruses contaminating the exine of pollen collected by bees. *Canadian Journal of Plant Pathology*, *6***:** 196-199.

Howell, W. E. & Mink, G. I. (1988). Natural spread of cherry rugose mosaic disease and two *Prunus necrotic ringspot virus* biotypes in a Central Washington sweet cherry orchard. *Plant Disease*, *72*: 636-640

Isogai, M., Aoyagi, J., Nakagawa, M., Kubodera, Y., Satoh, K., Katoh, T., Inamori, M., Yamashita, K. & Yoshikawa, N. (2004).Molecular detection of five cherry viruses from sweet cherry trees in Japan. *Journal of General Plant Pathology, 70*: 288-291.

Jacob, H. (1972). The frequency of occurrence of ring-spot viruses on stone fruit trees and their influence on the growth of the grafted plants. *Fruit growing*, *14*: 52-54.

Jordovic,´ M. (1955). Plum dwarfing is a viral disease in our country. *Zastita Bilja, 30*: 61-62.

Jordovic,´ M. (1958). About some little-known fruit viruses in our country . *Zastita Bilja*, *47:* 109-111.

Kelley, R. D. & Cameron, H. R. (1986). Location of *Prune dwarf* and *Prunus necrotic ringspot* viruses associated with sweet cherry pollen and seed. *Phytopathology, 76***:** 317-322.

Maliogka, V. I., Charou, A., Efthimiou, K., Katsiani, A. T., Chatzivassiliou, E. K. & Katis,N. I. (2010). Identification of *Ilarviruses* in almond and cherry fruit trees using nested PCR assay. In: 21st International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops. *Julius-Kuhn-Archive*, 427: 281- 283.

Mandic, B., Matic, S., Al Rwahnih, M., Jelkmann, W. & Myrta, **A.** (2007). Viruses of sweet and sour cherry in Serbia. *Journal of Plant Pathology, 89*(1): 103-108.

Massart, S., Brostaux, Y., Barbarossa, L., César, V., Cieslinska, M., Dutrecq, O., Fonseca, F., Guillem, R., Laviña, A., Olmos, A., Steyer, S., Wetzel, T., Kummert, J. & Jijakli, M. H. (2008). Inter laboratory evaluation of a duplex RT-PCR method using crude extracts for the simultaneous detection of *Prune dwarf virus* and *Prunus necrotic ringspot virus*. *European Journal of Plant Pathology*, *122*: 539-547.

Michelutti, R., Myrta, A. & Pallas, **V.** (2005). A preliminary account on the sanitary status of stone fruits at the Clonal Gene bank in Harrow, Canada. *Phytopathologia Mediterranea*, *44*: 71-74.

Milusheva, S. & Borisova, **A.** (2005). The incidence of *Prunus necrotic ringspot* and *Prune dwarf viruses* in *Prunus species* in South Bulgaria. *Biotechnology and Biotechnological Equipment, 19*: 42-45.

Milusheva, S. & Zhivondov, **A.** (2009). Spread of some sap transmissible viruses in cherry. *Rastenievadni Nauki, 46* (1): 63-66.

Mink, G. I. (1992). *Ilarvirus* vectors. *Advances in Disease Vector Research*, *9*: 261-281.

Myrta, A. & Savino, **V**. (2008). Virus and virus-like diseases of cherry in the Mediterranean region. *Acta Horticulturae*, *795:* 891-896.

Myrta, A., Di-Terlizzi, B., Savino, V. & Martelli, **G. P**. (2003). Virus diseases affecting the Mediterranean stone fruit industry: A decade ofsurveys. *Option Méditerranéennes*, Sér. B/ n45: 15-23.

Nemeth, M., Szalay-Marzsón, L. & Posnette, A. (1986). Virus, mycoplasma and rickettsia diseases of fruit trees, Dordrecht (Netherlands): Academia Kiado.

Nyland, G., Gilmer, R. M., & Moore, **J. D**. (1976). "Prunus ringspot group" In: *Virus diseases and non – infectious disorder of stone fruits in North America.* R.M. Gilmer, J. D. Moore, G. Nyland, M. F. Welsh, No 437, USDA, Washington, DC: 104-132.

Pallas, V., Aparicio, F., Herranz, M. C., Amari, K., Sanchez-Pina, M. A., Myrta, A. & Sanchez-Navarro, J. A. (2012). *Ilarviruses* of *Prunus* spp.: a continued concern for fruit trees. *Phytopathology*, *102:* 1108-1120.

Paprštein, F., Karešová, R. & Blažek, J. J. (1995). Dynamics ofdissemination of *Prunus necrotic ringspot virus* in germplasm collection of cherries. *Acta Horticulturae*, *386,*176-182.

Paunovic, S., G. Pasquini, G. & Barba, M. (2011). *Apple mosaic virus*. Chapter 18, pages 91-95 In: *Virus and Virus-Like Diseases of Pome and Stone Fruits*. A. Hadidi, M. Barba, T. Candresse, and W. Jelkmann (eds.) American Phytopathological Society, St. Paul, MN.

Perez-Sánchez, R., Morales-Corts, R., Garcia-Benavides, P. & Gomez-Sánchez, M. A. (2015). Main viruses in sweet cherry plantations of Central-Western Spain. *Scientia Agricola*, *72*(1): 83-86.

Posnette, A. F., Cropley, R. & Smith, **A. J**. (1968). The incidence of virus diseases in English sweet cherry orchards and their effect on yield. *Annals of Applied Biology*, *61*: 351-360.

Rankovic, M. (1976). The most frequent viruses among various plum cultivars in Yugoslavia. Mitt. Biol. Bundesanst. Land.-Forstwirtsch.Berlin-Dahlem, 170: 51-55.

Roossinck, M. J., Bujarski, J., Ding, S. W., **Hajimarad, R., Hanada, K., Scott, S. & Tousignant, M.** (2005). Bromoviridae. In: Fauquet C.M., Maya M.A., Maniloff J., Desselberger U., Ball L.A. (eds.), Virus Taxonomy. In: *Eight Report of the International Committee on Taxonomies of viruses:* 1049-1058. (Netherlands). Elsevier/Academic Press.

Rouag, N., Guechi, A., Matic, S. & Myrta, A. (2008). Viruses and viroids of stone fruits in Algeria. *Journal of Plant Pathology, 90*: 393-395.

Sabanadzovic, S., Abou Ghanem-Sabanadzovic, N., Rowhani, A., Grant, J. A. & Uyemoto, J. K. (2005). Detection of *Cherry virus* A, *Cherry necrotic rusty mottle virus* and *Little cherry virus* 1 in California orchards. *Journal of Plant Pathology, 87*: 173-177.

Sipahioglu, H. M., Myrta, A., Abou-Ghanem, N., Di Terlizzi, B. & Savino, V. (1999). Sanitary status of stone-fruit trees in

East Anatolia (Turkey) with particular reference to apricot. *OEPP/EPPO Bulletin*, *29:* 439-442.

Smith, I. M., Dunez, J., Phillips, D. H., Lelliott, R. A. & Archer, S. A. (1988). European handbook of plant diseases. Blackwell Scientific, Oxford, UK.

Spiegel, S., Tam, Y., Maslenun, L., Kolber, M., Nemeth, M. & Rosner, A. (1999). Typing *Prunus necrotic ringspot virus* isolates by serology and restriction endonuclease analysis of PCR products. *Annals of Applied Biology*, *135*(1): 395-400.

Sucha, J. & Svobodova, **L.** (2010). Incidence of *Prune dwarf virus* and *Prunus necrotic ringspot* virus in orchards of sweet and sour cherry in the Czech Republic. Short Communication. *Horticultural Science* (Prague), *37*: 118-120.

Topchiiska, M. (1996). Sap transmission viruses of stone and nut species in Bulgaria. Etiology, Diagnostics, Distribution and Control. Research work for qualifying for an academic degree, Fruit Growing Institute, Plovdiv: 147-226.

Vaskova, D., Pertzik, K. & Spak, J. (2000). Molecular variability of the capsid protein of the *Prune dwarf virus*. *European Journal of Plant Pathology*, *106:* 573-580.

Wells, J. M. & Kirkpatrick, **H. C.** (1986). Symptomatology and incidence of *Prunus necrotic ringspot virus* in peach orchards in Georgia. *Plant Disease*, *70*: 444-447.

Yardimci, B. C. N. & Ģulal-Kuliç**, H.** (2011). Detection of viruses infecting stone fruits in Western Mediterranean region of Turkey. *Plant Pathology Journal*, *27*(1): 44-52.

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