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Original scientific paper

High proportion of mixed virus infections in raspberry plantations in Serbia

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SUMMARY

Samples of red raspberry (*Rubus idaeus* L.) from the most important growing areas in Serbia were analyzed for the presence of four most important raspberry viruses: raspberry leaf blotch virus (RLBV), raspberry leaf mottle virus (RLMV), black raspberry necrosis virus (BRNV) and *Rubus* yellow net virus (RYNV), and their incidence in individual and mixed infected samples. Seventy-four samples of eight raspberry cultivars, selected from 32 main cultivation sites were tested, of which 62 leaf samples showed symptoms that could be caused by viruses and 12 samples were asymptomatic. The presence of viruses was tested by appropriate RT-PCR and PCR methods, using virus-specific primers. All four viruses were detected with highly significant differences of incidence in a total of 61 infected symptomatic samples. Infection with one of the four tested viruses was detected in 59.0% of all infected samples. The most prevalent was RLBV, which was associated with raspberry leaf blotch disorder in coexistence with its vector raspberry leaf and bud mite *P. gracillis,* and it was detected in 70.5% of the infected samples. It was followed by RYNV with an incidence of 42.6%, BRNV with 36.1% and RLMV with 9.8% incidence, considering all infected samples. The obtained results for the first time in Serbia showed a high proportion (41.0%) of mixed infections involving between two and four viruses. The most common were infections with two viruses, 68.0% of all mixed-infected samples, and among them coinfections with RLBV and RYNV (44.0%). Viruses of the raspberry mosaic complex, BRNV, RYNV and RLMV, including coinfections of 2-3 listed viruses along with RLBV, were found in a high joined proportion of 14.7% of all infected samples, i.e. 36.0% of mixed infected samples.

Keywords: raspberry, viruses, mixed infections, raspberry leaf blotch virus, Serbia

INTRODUCTION

Raspberry production in Serbia has a very long tradition and special economic importance. Serbia has been one of the leading European countries for decades regarding total annual raspberry production and export of more than 95% of fresh and processed fruits (Petrović et al, 2017). The cultivar 'Willamette' predominates, accounting for 95% of plantations, although other cultivars have also spread in recent years, such as 'Meeker' (3-4%) and 'Tulameen', 'Glen Ample', 'Heritage', 'Polka' and 'Polana' (1-2%).

Raspberry and other *Rubus* spp. are potential hosts of more than 30 viruses. Data obtained after the year 2000 using advanced molecular diagnosis of *Rubus*

viruses showed that viral complexes were the major cause of several raspberry and blackberry diseases in Europe and North America (Martin et al., 2013). Complex infections may affect the growth, yields and fruit quality of red raspberry cultivars which are insensitive or less susceptible to individual viruses of the complex (Martin et al., 2013; Quito-Avila & Martin, 2012).

At least three aphid-transmitted viruses, black raspberry necrosis virus (BRNV), *Rubus* yellow net virus (RYNV) and raspberry leaf mottle virus (RLMV), have been reported as components of the Raspberry mosaic disease (RMD) complex, both in North America and Europe (Martin et al., 2013). These viruses, in mixed infections, are able to decrease plant growth and yield of susceptible varieties. All three viruses were named and described on the bases of their biological properties decades ago, but have been characterized molecularly only recently, enabling their faster and more reliable detection by nucleic acid-based methods, such as RT-PCR or PCR (Jones et al., 2002; Halgren et al., 2007; Tzanetakis et al , 2007).

Symptoms of yellow leaf blotches, twisting of leaves and distortion of leaf margins, usually referred to as Raspberry leaf blotch disorder-RLBD, have been observed for decades in main growing regions of Europe. These symptoms had been attributed to infestation with the raspberry leaf and bud mite (*Phyllocoptes gracillis* Nal.) until McGavin et al. (2012) reported the co-presence of a new emaravirus, the raspberry leaf blotch virus (RLBV) in symptomatic plants in Great Britain and Serbia.

Two types of mosaic disease, named the raspberry chlorotic net and chlorotic leaf spot, were described by Jordović (1963) in the first extensive research of raspberry viral diseases in Serbia over the period 1957-1961. Some two decades ago, Dulić-Marković & Ranković (1997) described the presence of viruses of the raspberry mosaic complex, BRNV, RLMV and raspberry leaf spot virus (RLSV recently identified as a RLMV isolate, McGavin & MacFarlane, 2010), based on indexing on indicator plants of some old cultivars ('Gradina', 'Malling Exploit', 'Malling Promise', 'Valjevska' and 'Zeva'). Only 'Zeva' was infected with both viruses, while the response of indicator plants in the other cultivars exhibited the presence of only one of the viruses.

Despite the recurring virus-like symptoms that have been observed in Serbian raspberry plantations in recent decades, there are scarce data on individual occurrence and spread of some of those viruses based on PCR detection (Jevremović et al., 2016; Paunović & Jevremović, 2017; Jevremović et al., 2019; Jevremović et al., 2020; Paunović & Jevremović, 2020). In particular, there is no information on the prevalence of mixed infections, except our preliminary data (announced at a scientific conference and published as an abstract, Paunović et al., 2017).

Therefore, the aim of this study was to analyze the incidence of four raspberry viruses, RLBV, RLMV, BRNV and RYNV, in raspberry samples, and to determine the proportion of mixed infections and dominant combinations of viruses in the main raspberry growing regions in Serbia.

MATERIAL AND METHODS

Surveys and sampling

Surveys were carried out during 2014-2018 in the most important raspberry growing sites mainly in western and central regions of Serbia. Selected plantations were visually inspected and samples were collected for multiple studies (Jevremović et al, 2019, 2020). A total number of 74 samples, comprising eight raspberry cultivars ('Willamette', 'Meeker', 'Tulameen', 'Fertödi Zamatos', 'Polana', 'Polka', 'Heritage' and 'Glen Ample') originating from 32 main cultivation sites were selected from all collected samples and analysed for the presence of viruses. Each sample consisted of several selected leaves from one plant. Sixty-two samples had virus-like symptoms, while 12 samples were symptomless. Table 1 lists all sites examined, cultivars tested and symptoms observed in the field.

Total nucleic acids extraction and molecular detection of viruses

The collected leaf samples were tested for the presence of four raspberry viruses: RLBV, RYNV, BRNV and RLMV.

Total nucleic acids (TNA) were extracted from fresh or frozen leaves kept at -20^{0}C by the CTAB method (Li et al. 2008). Complementary DNA synthesis was performed using random hexamere primers and Maxima Reverse Transcriptase (Thermo Scientific, 200u/µl). The presence of RLBV, BRNV and RLMV were tested by RT-PCR and the presence of RYNV by PCR using virus specific primers and cDNA or TNA as template. The following primer sets were

used: RLBV 3R 5'-ATCCAGTAGTGAACTCC - 3'/ RLBV 3F5'-CACCATCAGGAACTTGTAATGTTT -3' (McGavin et al., 2012); BRNV1F 5'-ATGCTGAGCCACTTGTGA-3'/BRNV1R 5'-ATCTGGTGTGTTCCGCAT-3' (Halgren et al., 2007); RLMV-specific set of primers CPF5'-CTAAGGAGATATGGCGGA-3'/ CPR5'-CAGTATGGCAGCCTCTTG-3' (Tzanetakis et al., 2007); and RYNV1F 5'-TCCAAAACCTCCCAGACCTAAAAC-3'/ RYNV1R5'-ATAATCGCAAAAGGCAAGCCAC-3' (Jones et al., 2002). PCRs were carried as recommended by the authors cited.

The amplified PCR products were analyzed by 1.5% agarose gel electrophoresis, stained by ethidium-bromide and visualized under UV-light. The presence of fragments of expected size was considered as a positive reaction. Isolates of RLBV, BRNV, RLMV and RYNV whose identities were verified by sequencing of PCR products were used as positive controls: RS-RLBV-7, RS-BRNV-VS1, RS-PLM-M1 (Jevremović et al., 2019; Jevremović et al., 2020; Paunović & Jevremović, 2020) and RYNV-8 (unpublished data). A tissue sample from healthy raspberry plants was used as a negative control.

Statistical analysis of total incidence of individual viruses was performed by one-way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test ($P \leq 0.05$) for mean separation. Before ANOVA, data presented in the form of percentages (Table 1) were subjected to arcsine transformation. Statistical analysis of total incidence of single- and mixed-infected samples was performed by ANOVA ($P \leq 0.05$, Table 2).

RESULTS

The yellow leaf blotch, leaf yellowing, twisting of leaves, and leaf margin distortion were the most common type of symptoms observed in the greatest number of inspected raspberry plantations (Figure 1). This type of symptoms is almost the same as those caused by the raspberry leaf and bud eriophide mite *Phyllocoptes gracilis* Nalepa. Leaf chlorotic mottle, leaf edge chlorosis and vein chlorosis were also symptoms observed in some sampled sites (Figures 2 and 3).

Figure 1. Symptoms of raspberry leaf blotch virus (RLBV) in infected leaves of red raspberry cv. 'Willamette'

Figure 2. Symptoms of BRNV infection on leaves of red raspberry cv. 'Fertödi Zamatos'

Figure 3. Symptoms of BRNV infection on leaves of red raspberry cv. 'Polana'

The results of PCR tests showed virus infections in 61 out of 74 tested samples. All infected samples were with virus-like symptoms. Infection was not detected in a total of 13 test samples, 12 asymptomatic samples and only one symptomatic plant (Table 1).

The amplified DNA fragments of expected sizes, 567 bp, 350 bp, 417 bp and 514 bp specific for RLBV, RYNV, BRNV and RLMV, respectively, were detected in 43 (70.5%), 26 (42.6%), 22 (36.1%) and 6 (9.8%) positive samples (Table 1). Statistical analysis showed highly significant differences ($P = 0.0011$) between the incidences of RLBV, RYNV and BRNV, and RLMV (marked with a, b, c in Table 1).

Infection with only one of four tested viruses was detected in 36 samples (59.0% of all infected samples), while mixed infections with two to four viruses occurred in 25 samples (41.0%). The difference between the total number of plants infected with one virus and those with mixed infections was statistically significant ($P = 0.039$).

Table 1. The list of tested red raspberry samples and detected viruses, RLBV, RYNV, BRNV and RLMV, in Serbian plantations

No.	Sites	Cultivars	Symptoms	Detected virus(es)			
				RLBV	RYNV	BRNV	RLMV
1	Trešnjevica	Willamette	YLB	+	+	-	-
2	Stupčevići	Willamette	LC	-	+	-	-
3	Stupčevići	Willamette	LC	-	+	-	-
4	Ivanjica	Willamette	YLB	+	-	-	-
5	Kotraža	Willamette	YLB	+	-	-	-
6	Trešnjevica	Willamette	YLB	+	-	-	-
7	Zlodol	Willamette	ns	-	-	-	-
8	Ljubovija	Willamette	LC	-	-	-	-
9	Ljubovija	Willamette	LC	+	+	-	-
10	Seča Reka	Meeker	YLB	+	-	-	(+)
11	Gleđica	Willamette	YLB	+	-	-	-
12	Bratljevo	Meeker	YLB	+	-	-	-
13	Bratljevo	Meeker	ns	-	-	-	-
14	Rudno	Willamette	YLB	+	-	-	-
15	Kosjerić	Meeker	YLB	+	-	-	-
16	Kosjerić	Meeker	ns	-	-	-	-
17	Ivanjica	Willamette	LC	-	+	-	-
18	Kriva reka	Willamette	YLB	+	-	-	-
19	Prizren	Willamette	YLB	+	+	-	-
20	Zaglavak	Willamette	LY	-	+	-	-
21	Zaglavak	Willamette	YLB	+	-	-	-
22	Kadinjača	Willamette	YLB	+	-	-	-
23	Priboj	Willamette	YLB	+	+	-	-
24	Buar	Willamette	YLB	+	+	-	-
25	Hrta	Willamette	YLB	+	+	-	-
26	Hrta	Willamette	YLB	+	+	-	-
27	Hrta	Willamette	YLB	+	-	-	+
28	Hrta	Willamette	YLB	+	+	-	-
29	Hrta	Willamette	YLB	+	-	-	-
30	Kostojevići	Glen Ample	YLB	+	-	-	-
31	Kostojevići	Mekeer	YLB	+	-	-	-
32	Kostojevići	Polka	ns	-	-	-	-
33	Zarožje	Willamette	YLB	+	-	-	-
34	Zarožje	Willamette	YLB	+	-	-	+
35	Zarožje	Polka	ns	-	-	-	-
36	Gliječa	Fertodi Zamatos	YLB	+	-	+	-
37	Gliječa	Fertodi Zamatos	LC	-	-	+	-
38	Sevojno	Willamette	ns	-	-	-	-
39	Sevojno	Willamette	LC	-	+	-	-

Table 1 continued. The list of tested red raspberry samples and detected viruses, RLBV, RYNV, BRNV and RLMV, in Serbian plantations

No.	Sites	Cultivars	Symptoms	Detected virus(es)			
				RLBV	RYNV	BRNV	RLMV
40	Jošanička Banja	Willamette	YLB	+	+	-	-
41	Zapadna Srbija	Willamette	YLB	+	-	-	-
42	Ivanjica	Fertodi Zamatos	LC	-	+	+	-
43	Ivanjica	Fertodi Zamatos	LC	-	-	+	-
44	Ivanjica	Fertodi Zamatos	LC	-	-	+	-
45	Ivanjica	Fertodi Zamatos	LC	-	-	+	-
46	Ivanjica	Fertodi Zamatos	LC	-	-	+	-
47	Ivanjica	Fertodi Zamatos	LC	-	-	+	-
48	Ivanjica	Fertodi Zamatos	LC	-	-	+	-
49	Ivanjica	Fertodi Zamatos	ns	-	-	-	-
50	Ivanjica	Fertodi Zamatos	LC	-	-	+	-
51	Ivanjica	Fertodi Zamatos	ns	-	-	-	-
52	Ivanjica	Fertodi Zamatos	LC	-	-	+	-
53	Užice	Polka	ns	-	-	-	-
54	Zapadna Srbija	Tulameen	YLB	+	+	+	-
55	Ivanjica	Heritage	ns	-	-	-	-
56	B. Dobro Polje	Polana	YLB	+	-	-	-
57	Kriva reka	Willamette	YLB	+	-	-	-
58	Jelakci	Willamette	YLB	+	-	-	-
59	Jelakci	Willamette	YLB	+	+	+	-
60	Brus	Willamette	YLB	+	+	+	-
61	Brus	Willamette	YLB	+	+	+	-
62	Milatovići	Willamette	YLB	+	+	-	-
63	Teočin	Tulameen	LEC	-	-	+	-
64	Kraljevo	Willamette	YLB	+	+	+	(+)
65	Kraljevo	Willamette	YLB	+	+	+	+
66	Kraljevo	Willamette	YLB	+	+	+	-
67	Kraljevo	Willamette	YLB	+	+	+	(+)
68	Vrbas	Polana	VC, CM	-	-	+	-
69	Teočin	Willamette	VC	-	+	-	-
70	Teočin	Tulameen	LC	+	-	-	-
71	Vrbas	Polana	ns	-	-	-	-
72	Vrbas	Polana	ns	-	-	-	-
73	Stapar	Willamette	YLB	+	-	+	-
74	Stapar	Willamette	YLB	+	+	-	-
Total number of infected samples / No of PCR positive samples of each virus				61/43 70.5%[a]	61/26 42.6%[b]	61/22 36.1%[b]	61/6 9.8%[c]

YLB: yellow leaf blotch; LC: leaf chlorosis; LY: leaf yellows; VC: vein chlorosis; CM: chlorotic mottle; LEC: leaf edge chlorosis; ns: no symptoms; +: positive PCR reaction; (+): faint expected band in agarose gel; -: virus not detected. Mean values for total incidence of each of four viruses followed by the same letter were not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$)

The largest number of single-virus samples was infected with RLBV, 19 samples (31.1% of infected samples, i.e. 52.8% of single infected samples), followed by 11 samples infected only with BRNV (18.0% of infected samples and 30.5% of single infected samples) and 6 samples infected with RYNV (9.8% of total number of infected samples or 16.7% of single infection samples) (Table 2).

Two coexistent viruses made the highest proportion of mixed infections (68.0%, 17 out of 25 mixed infected samples), followed by infections with three viruses (20.0%, 5 samples) and four viruses (12.0% of mixed infected samples, 3 samples). All viral combinations in mixed infected samples and their incidence were summarized in Table 2.

Table 2. Presence and incidence of detected viruses in 61 infected raspberry samples

Detected virus(es)	Number of PCR positive samples	Percentage (%) of positive samples in total number of single or mixed infected samples	Percentage (%) of positive samples in total number of infected samples
RLBV	19	52.8	31.1
BRNV	11	30.5	18.0
RYNV	6	16.7	9.8
One virus detected in total	**36**[a]	**100**	**59.0**[a]
RLBV + RYNV	11	44.0	18.0
RLBV+ RLMV	3	12.0	4.9
RLBV+BRNV	2	8.0	3.3
BRNV+RYNV	1	4.0	1.6
Coinfection with two viruses – subtotal	**17**	**68.0**	**27.9**
Coinfection with three viruses: RLBV+RYNV+BRNV	**5**	**20.0**	**8.2**
Coinfection with four viruses: *RLBV+RYNV+BRNV+RLMV*	**3**	**12.0**	**4.9**
Coinfection with 2-4 viruses in total	**25**[b]	**100**	**41.0**[b]

Differences between the total number of samples infected with one virus and total number of samples coinfected with 2-4 viruses were statistically significant according to ANOVA at P = 0.039 (a, b)

DISCUSSION

The results of this study, obtained by nucleic acid-based virus detection methods, RT-PCR and PCR, showed the presence of all four raspberry viruses, RLBV, RYNV, BRNV, and RLMV in Serbia, but with highly significant differences of incidence. The identity of viruses was confirmed by sequencing corresponding PCR products of 10 RLBV isolates (samples No. 1, 5, 9, 12, 22, 31, 34, 36, 58 and 61 in Table 1), 4 BRNV isolates (samples No. 36, 44, 50, and 52), one RMLV isolate, sample No. 34 (Jevremović at al., 2019; Jevremović et al., 2020; Paunović & Jevremović, 2020). The identity of 4 RYNV isolates (samples No. 3, 9, 19 and 23) were also verified by sequencing PCR products, which shared 92.8-94.0% of nt identity with the isolate Acc. KF241951 from Canada (our unpublished data).

The most prevalent was RLBV, represented in 70.5% of all infected samples. It was associated with yellow leaf blotch symptoms, registered in almost all surveyed sites and all tested cultivars. RLBV was detected in one-third of the samples with single infection and was also

the prevalent virus in mixed infections. It was detected in the cvs. 'Willamette', 'Meeker', 'Tulameen', 'Fertödi Zamatos', 'Polana' and 'Glen Ample'.

The RLBV was first identified in Great Britain and Serbia by McGavin et al. (2012) and it has been detected also in a few other European countries: Finland, Bulgaria, Poland, Montenegro and Slovakia (Bi et al., 2012; Mavrič Pleško et al., 2014; Cieslinska & Tartanus, 2014; Zindović et al., 2015; Jevremović et al., 2019). In all those countries, it was associated with raspberry leaf blotch disorder. The common presence of RLBV in Serbian plantations had already been confirmed in our previous research (Jevremović et al., 2016; Jevremović et al., 2019). Significant diversity of the portion of the nucleocapsid gene in 21 RLBV isolates was determined, which may be an indication of the virus's long-term presence in Serbia (Jevremović et al., 2019).

Several factors may have contributed to the widespread prevalence of RLBV. Planting material has been imported for years, which may have created a risk of uncontrolled introduction and long-distance spreading of RLBV. Since the virus has only recently been identified and is not yet listed in the EPPO Certification scheme for *Rubus* (OEPP/EPPO, 2009), its presence is not controlled in the production and marketing of planting material. In addition, some growers use shoots from commercial plantations unaware of the state of virus presence, which has certainly contributed to its local spread. Also, the high abundance of raspberry leaf and bud mite *P. gracillis,* already registered in Serbia (Milenković & Marčić, 2012), may have contributed to the local spread of RLBV (McGavin et al., 2012; Dong et al., 2016). 'Willamette', the most preferred raspberry cultivar, is very sensitive to *P. gracillis,* and significant damage caused by this pest has been observed in Serbia (Milenković & Marčić, 2012). Symptoms of YLB had often been attributed to that pest before the virus was identified (McGavin et al., 2012). Further studies are needed to determine the extent to which RLBV and *P. gracillis* contribute to the development of YLB in co-presence.

Our results showed a significantly lower incidence of RYNV (42.6%) and BRNV (36.1%), the viruses that, along with RLMV, have been reported as components of the RMD disorder (Martin et al., 2013). More than two-thirds of RYNV-positive samples (76.9%) were co-infected with other viruses, predominantly 'Willamette' samples, and also 'Ferodi Zamatos' and 'Tulameen' samples. BRNV was detected in mixed infections in 50.0% of the samples of the same cultivars positive for that virus. All 11 tested samples of 'Fertödi Zamatos' were BRNV positive, eight samples were single-infected, while the remaining three were co-infected with one of the RLBV, RVCV and RYNV viruses. Also, BRNV was detected in single infections in the cvs. 'Tulameen' and 'Polana'.

All six raspberry samples which were PCR-positive only for RYNV exhibited symptoms similar to those caused by viral infections, which might suggest that the virus was exogenous, while the endogenous virus integrated in the host genome causes no symptoms (Diaz-Lara, 2016). This assumption will be tested in further research by indexing on *Rubus* indicator plants and testing by RT-PCR using extracted and DNAse digested nucleic acids as template.

The incidence of RLMV (9.8%) was considerably lower than that of RLBV, BRNV and RYNV. It was detected only sporadically and only in mixed infections in cvs. 'Willamette' and 'Meeker'. Nucleotide sequence of the major coat protein gene fragment of one Serbian RLMV isolate was determined and it showed 96.0-99.0% identity with corresponding fragments of four isolates currently available in GenBank (Paunović & Jevremović, 2020).

The choice of raspberry cultivars has changed significantly in recent decades, the old ones are not grown anymore and new ones have taken over, primarily 'Willamette' and 'Meeker', and other cultivars, such as 'Tulameen', 'Polana', 'Polka', 'Fertödi Zamatos' and 'Glen Ample' at a much smaller percentage. Newly grown cultivars were tested several times for the single presence of BRNV, RYNV and RLMV by PCR during 2016-2020. Thus, Jevremović et al. (2016, 2020) detected the presence of BRNV mainly in the cultivar 'Fertödi Zamatos' (83.0-87.5%), and in 'Tulameen', 'Polana' and 'Willamette' at much lower percentages. BRNV was detected both in asymptomatic plants and those with symptoms of venial chlorotic mottle. Sequence analysis of PCR products of the RNA-dependent RNA polymerase fragment (417 bp-long) for five Serbian isolates showed significant divergence both at the country and international levels (Jevremović et al., 2020).

The obtained results for the first time showed a high proportion of mixed infections in raspberry samples in Serbia. Every possible combination of the studied viruses was detected. The most frequent were coinfections with two viruses (68.0% of mixed infected samples) and the coinfection with RLBV and RYNV was prevalent (44.0%).

Viruses of the raspberry mosaic complex, BRNV, RYNV and RLMV, including coinfections with 2-3 listed viruses with RLBV, were found in a high joined proportion of 14.7% of all infected samples, i.e. 36.0% of mixed infected samples.

The significant presence, wide distribution and possibly combined action of RLBV and *P. gracillis* in the most important raspberry growing regions in Serbia have made raspberry leaf blotch disorder the most significant complex in the preferred cultivars 'Willamette' and 'Meeker'. RLBD was also present in other cultivars, grown at much lower percentage, except in 'Fertödi Zamatos', 'Polka' and 'Heritage'. The next most frequent was the joint prevalence of different combinations of viruses of the RMD complex with an addition of RLBV, which was detected in 92.3% of all mixed infected samples.

Raspberry as a perennial plant may be grown in commercial plantings for many years. Such a long growing period provides an extended exposure to viruses and their vectors, so the overall high proportion of mixed infections in Serbia was no surprise.

The high incidence of some raspberry viruses in single and mixed infections imposes a need to take appropriate control measures during the production stage of planting material and in commercial plantations.

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Visoka zastupljenost mešanih virusnih infekcija u zasadima maline u Srbiji

REZIME

Uzorci crvene maline (*Rubus idaeus L.*) iz najvažnijih područja gajenja u Srbiji su analizirani na prisustvo četiri najvažnija virusa maline, virusa mrljavosti lista maline (RLBV), virusa nekroze crne maline (BRNV), virusa žute mrežavosti *Rubusa* (RYNV) i virusa šarenila lista maline (RLMV), njihovu pojedinačnu, kao i zastupljenost u mešanim infekcijama. Testirana su 74 uzorka osam sorti maline, odabrana sa 32 najvažnija lokaliteta gajenja, od kojih su 62 uzorka listova bili sa simptomima koji bi mogli biti izazvani virusima, a 12 uzoraka bez simptoma. Prisustvo virusa je testirano primenom odgovarajuće metode, RT-PCR i PCR, korišćenjem virus specifičnih prajmera. Detektovano je prisustvo sva četiri virusa u zasadima u Srbiji sa visoko značajnim razlikama u zastupljenosti u ukupno šezdeset jednom zaraženom uzorku sa simptomima. Zaraze sa jednim od četiri ispitivana virusa su detektovane u 59,0% svih zaraženih uzoraka. Najzastupljeni je bio RLBV koji je povezan sa sindromom lisne mrljavosti maline u združenom prisustvu sa svojim vektorom, grinjom lista i pupoljka maline *P. gracillis*, detektovan u 70,5% zaraženih uzoraka. Slede RYNV sa učestalošću od 42,6%, BRNV sa 36,1% i RLMV sa zastupljenošću od 9,8% svih zaraženih uzoraka. Dobijeni rezultati su po prvi put u Srbiji pokazali značajnu zastupljenost (41,0%) mešanih infekcija sa dva do četiri virusa. Najzastupljenije su infekcije sa dva virusa, 68,0% svih mešano zaraženih uzoraka, a među njima mešane infekcijie RLBV i RYNV (44,0%). Virusi iz kompleksa mozaika maline, BRNV, RYNV i RLMV, uključujući i mešane-infekcije 2-3 navedena virusa sa RLBV, su utvrđeni u visokoj združenoj zastupljenosti od 14,7% svih zaraženih uzoraka, odnosno 36,0% uzoraka sa mešanim infekcijama.

Ključne reči: malina, virusi, mešane infekcije, virus mrljavosti lista maline, Srbija