

## FIRST REPORT ON IDENTIFICATION OF RASPBERRY BUSHY DWARF VIRUS IN RED RASPBERRY (*RUBUS IDEAEUS* L.) IN BULGARIA

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### Abstract

*Raspberry bushy dwarf virus* (RBDV), classified to genus *Idaeovirus*, is a seed- and pollen-borne virus that is commonly found in red raspberry (*Rubus idaeus* L.). The virus seems to occur worldwide, including the neighbouring to Bulgaria countries – Romania and Serbia. In Bulgaria, however, RBDV has not been reported so far and, therefore, this is the first report for RBDV occurrence in our country.

The investigations were carried out during four successive vegetative cycles in two raspberry plantations located in Plovdiv district and included two cultivars: ‘Lyulin’ grown in an experimental plot at the Fruit Growing Institute in Plovdiv, and ‘Heritage’ grown in a commercial plantation. The RBDV identification and corroboration was done using visual observations, serological analyses by enzyme-linked immunosorbent assay (ELISA), and biological tests by mechanical inoculations onto herbaceous indicators.

In some bushes, visual observations revealed decreasing of cane vigour (dwarfing), leaf yellows and sometimes crumbly fruits. ELISA data showed that RBDV was identified in single infection in 12 % of the tested samples from ‘Lyulin’ (experimental plantation) and in 8.1 % of the samples from ‘Heritage’ (commercial plantation). The virus was detected also in mixed infection with other raspberry viruses as the infection levels in ‘Lyulin’ and ‘Heritage’ being 32 % and 16 % respectively.

Biotests were carried out by mechanical inoculation onto *Celosia argentea*, *Chenopodium amaranticolor*, *C. foetidum* и *C. quinoa*. RBDV infected *C. amaranticolor* and *C. quinoa*. Both species reacted with local and systemic symptoms.

The presence of RBDV in Bulgaria was confirmed by both methods: ELISA and mechanical inoculation onto herbaceous indicators.

**Keywords:** raspberry, bushy dwarf, identification, serological assay, mechanical inoculation

### Introduction

*Raspberry bushy dwarf virus* (RBDV), genus *Idaeovirus*, is a seed- and pollen-borne virus (Murant et al., 1974) that is commonly found in red and black raspberry. It seems to occur worldwide. Natural hosts of the virus belong to the genus *Rubus*, red raspberry (*Rubus idaeus*), black raspberry (*R. occidentalis*), loganberry (*R. ursinus* x *R. idaeus*) and boysenberry [(*R. ursinus* x *R. idaeus*) x (*R. baileyanus* x *R. argutus*)] (Barnett and Murant, 1970; Converse, 1973). Recently, RBDV was detected in grapevine (*Vitis vinifera* L.) (Mavric et al., 2003; Jeremovic and Paunovic, 2008). Experimentally RBDV could be transmitted to more than 50 herbaceous host plants.

In sensitive red raspberry cultivars (*R. idaeus*), the virus can cause leaf curling, necrosis, premature defoliation, decreased vigour, and drupelets abortion leading to crumbly fruits. Although the virus causes mild symptoms in most raspberry cultivars, it may affect fruit quality, especially in mixed infections. Because of its debilitating effects on growth and fruit yield, RBDV is regarded as a serious disease of red raspberries (Wood & Todd 1976; Murant 1987).

In Bulgaria, RBDV has not been reported so far and, therefore, this is the first report for RBDV occurrence in our country.

### Materials and Methods

Investigations were carried out during four successive vegetative cycles in two raspberry plantations located in Plovdiv district and included two cultivars: ‘Lyulin’ grown in an experimental plot at the Fruit Growing Institute in Plovdiv, and ‘Heritage’ grown in a commercial plantation. The RBDV identification and proving was done by visual observations, serological analyses by enzyme-linked immunosorbent assay (ELISA), and biological tests by mechanical inoculations onto herbaceous indicators.

Two approaches were applied for ELISA sampling - randomized and individual samples. On the first stage of the study, samples were composed of 8-10 leaves, taken randomly from three bushes. On the second stage the individual leaf and fruit samples were collected from both marked canes, showing disorders in growth and yield and healthy ones. Besides RBDV, the samples were also analysed for *Arabis mosaic virus* (ArMV) and *Raspberry ring spot virus* (RpRSV).

ELISA was performed in variant double antibody sandwich (DAS) according to Clark and Adams (1977) using commercial antisera by Bioreba AG for detection of RBDV and Loewe GmbH for identification of ArMV and RpRSV.

Biological tests were carried out by mechanical inoculation of the following herbaceous indicators: *Celosia argentea*, *Chenopodium amaranticolor*, *C. foetidum* и *C. quinoa*. The leaf tissues, source of inoculum, were grinded in phosphate buffer with 1% nicotine.

## Results and Discussion

### Symptoms observed

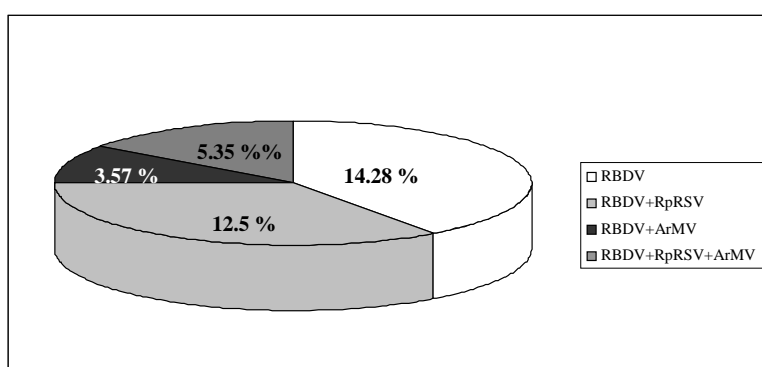
In some bushes, visual observations revealed decreasing of cane vigour (dwarfing), leaf yellows and sometimes crumbly fruits.

### DAS ELISA results

In the course of study 56 randomized and 81 individual samples from `Lyulin` and 57 individual samples from `Heritage` were tested by DAS ELISA.

In the first (preliminary) serological test of `Lyulin` plants, eight (14.28 %) of total number of 56 randomized samples, reacted positively only with antisera against RBDV. The virus was detected in mixed infection with RpRSV in seven samples (12.5 %) and with ArMV in two samples (3.57 %). Moreover, in three (5.35 %) of the analyzed samples, triple virus infection was detected (fig. 1).

Figure 1. Results from DAS ELISA of randomized samples. Single and mixed infection with RBDV.



DAS ELISA of the individual samples identified RBDV in single infection in ten (12 %) of 81 tested samples from `Lyulin` (experimental plantation) and in seven (8.1 %) of 57 analyzed samples from `Heritage` (commercial plantation). The virus was detected also in mixed infection with other raspberry viruses, the infection levels in `Lyulin` and `Heritage` being 32 % and 15.78 % respectively (tab. 1). Mixed infection with RpRSV was identified in 16 samples of `Lyulin` and in four samples of `Heritage`. Four samples from `Lyulin` and three samples from `Heritage` reacted at the same time with antisera against RBDV and ArMV. Triple virus infection was found in six samples of `Lyulin` and two samples of `Heritage`.

Table 1. Infection levels in `Lyulin` and `Heritage` in single and mixed infection, detected in individual samples.

Cultivar	N	n-1	IL-1 (%)	n-2	IL-2 (%)
`Lyulin`	81	10	12.30	26	32.00
`Heritage`	57	7	8.10	9	15.78

N – a total number of tested samples per cultivar

n-1 – number of DAS ELISA positive samples in single infection

n-2 - number of DAS ELISA positive samples in mixed infection

Infection level-1 (%)

### Biological indexing

Biological tests were carried out by mechanical inoculation onto *Celosia argentea*, *Chenopodium amaranticolor*, *C. foetidum* и *C. quinoa*. RBDV infected *C. amaranticolor* and *C. quinoa*. Initially, both species

reacted with local spots and patterns on the inoculated leaves and later on - with systemic symptoms on the non-inoculated leaves. Biological tests resulted in determination of three RBDV isolates.

RBDV is seed-transmitted, but in established plants spread occurs through pollen, and both the maternal parent and seeds may become infected. No vector is known (Murant, 1976). Therefore, the virus is distributed on long distances mainly by planting material.

As it was mentioned above, RBDV is widespread among *Rubus* species and cultivars. The virus was reported in the neighbouring to Bulgaria countries – in Romania (Isac et al., 2004; Isac et al., 2008), as approximately 31% the tested germplasm of *Rubus* was infected by RBDV and in Serbia (Dulic-Markovic and Rankovic, 1992; Jeremovic et al., 2004), where the virus was detected in two cultivars `Willamette` and `Meeker`. However, the virus has not been reported so far. On the base of the data obtain of the current study, the presence of RBDV in Bulgaria was proven by both methods: ELISA and mechanical inoculation onto herbaceous indicators.

### Conclusions

This is the first time when RBDV was detected in red raspberry cultivars `Lyulin` and `Heritage`, grown in Plovdiv district of Bulgaria.

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