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Impact of Raspberry Bushy Dwarf Virus on Yield and Fruit Quality of three Red Raspberry Cultivars

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This study aimed to evaluate the yield and assess changes in the chemical composition of fresh red raspberry fruits caused by the presence of Raspberry bushy dwarf virus (RBDV). The study was conducted on fruits collected from RBDV-free and RBDV-infected plants of three raspberry cultivars: Brusviana, Sugana, and Joan J, grown in Kyiv Oblast. Fruits were harvested at the appropriate ripening stages, and their physical parameters (number of fruits, weight, drupelet number) were measured. The biochemical characteristics were analyzed using appropriate methods: soluble solids content (SSC, %) via refractometry; titratable acidity (%) and ascorbic acid (AsA, mg 100 g^{-1}) through acid-base titration; sugar content (%) using colorimetric analysis; and total phenols and anthocyanins (mg 100 g^{-1} fresh weight) by spectrophotometry, applying the Folin-Denis method and the pH differential method, respectively. The results revealed a statistically significant negative impact of viral infection on yield across all three cultivars, with reductions reaching up to 62%. Infected plants produced smaller, deformed, crumbly, and lower-weight berries with fewer drupelets compared to healthy ones. The AsA content in infected fruits was significantly lower in all three cultivars (7.7–9.6%). In contrast, the TA content was 40–50% higher in infected plants of Brusviana and Sugana. The levels of phenolics were notably higher in virus-infected plants of Joan J (up to 28.7%) and Sugana (up to 16%) than in the control, while anthocyanin content significantly increased in Sugana (60.4%) and Brusviana (53.7%). Statistical analysis confirmed the undeniable impact of RBDV infection on the studied traits. Considering all experimental and statistical data, it can be concluded that RBDV affects marketability, fruit quality, and chemical composition in all three examined red raspberry cultivars.

Keywords: RBDV, *Rubus idaeus*, yield, soluble solids, titratable acids

Introduction

The physiological condition of plants is significantly altered under the influence of biotic stress, particularly viral infection (Simeone et al., 2000; Ivascu et al., 2002). The impact of viruses on yield depends on many factors, including the strain and isolate of the virus, the plant cultivar and age, cultivation methods, the presence of vector species, the time and way of infection, as well as climatic conditions (Sastry, 2013).

Red raspberry (*Rubus idaeus* L.) is a key component of berry plantations in Ukraine, ranking third in cultivation area with 4,900 hectares and accounting

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for 28% of berry exports (UKRSTAT, 2022). Among the most popular cultivars, the primocane Brusviana (Ukrainian breeding), the Sugana (Swiss), and Joan J (Great Britain) which are highly valued by growers for their high yield potential, exceptional flavor, and adaptability to various growing conditions.

Raspberries produce juicy fruits rich in vitamins, particularly ascorbic acid, minerals, anthocyanins, and flavonoids, which are beneficial to health (Bobinaitė et al., 2016). However, raspberry productivity can be compromised by viral infections. Over 40 viral and virus-like pathogens are known to infect *Rubus* species, leading to significant yield losses (McGavin and MacFarlane, 2010). One such pathogen is Raspberry bushy dwarf virus (RBDV). It is transmitted via pollen, affecting germination and growth of pollen tubes. Disruption in drupelet formation results in crumbly fruit, with symptom expression varying among raspberry genotypes (Sproge and Strautina, 2020). Crumbly fruit is a typical symptom of RBDV infection. Studies on the Marion blackberry cultivar have shown that RBDV infection reduced drupelet number per fruit from 60 to 10, leading to distorted fruit and a yield loss of about 40% (Martin et al., 2001; Strik and Martin, 2003). Notably, the presence of crumbly fruit can lead to yield reductions of 50 to 60%, even if the total number of drupelets remains unchanged (Martin and Mathews, 2001). Clearly, viral pathogens like RBDV pose significant threats to high plant productivity.

Research on the harmful effects of RBDV is of high importance for Ukraine, as previous studies have shown a high prevalence of this virus. Therefore, this study aimed to investigate the impact of viral infection on the yield, quality, and biochemical composition of raspberry fruits. Assessing the productivity of economically important raspberry cultivars, as well as fruit quality, is essential, as viral infections can reduce consumer appeal and taste characteristics.

Material and Methodology

A visual inspection of raspberry plantations for virus infection symptoms was conducted on primocane raspberry cultivars Brusviana, Sugana, and Joan J, located in the Kyiv region. Visually infected and healthy samples were selected and tested for raspberry ringspot virus (RRSV), strawberry latent ringspot virus (SLRV), tomato black ringspot virus (TBRV), cherry leaf roll virus (CLRV), apple mosaic virus (AMV), cucumber mosaic virus (CMV), arabis mosaic virus (ArMV) using the ELISA method (DAS-ELISA) with specific antisera LOEWE (Germany) and BIOREBA (Switzerland) according to the manufacturer's recommendations. For DAS-ELISA, young raspberry leaves were homogenized 1 : 20 (wt/vol) in extraction buffer (phosphate buffer +0.05% Tween-20 +2% polyvinylpyrrolidone). 100 mL of reagents were added per well at each step of the ELISA process, and absorbance values at 405 nm were recorded with an ImmunoChem-2100 spectrophotometer. Samples with absorbance values more than twice as high as the average of the negative control, evaluated in the same ELISA plate, were considered positive for pathogens.

RT-PCR was done for raspberry vein chlorosis virus (RVCV) and raspberry leaf blotch virus (RLBV). Total RNA extraction was performed using the Genomic DNA Purification Kit (Thermo Scientific, Lithuania), with 75 mg of plant tissue taken for analysis. The quality of the extracted RNA was evaluated using a DeNovix DS-11 spectrophotometer at wavelengths of 260, 260/230, and 260/280 nm. RVCV identification was conducted using RT-PCR with primers 1527-F and 1528-R with an expected fragment size of 499 bp specific to the part of the CP coding region (McGavin et al., 2011). RLBV was identified using RT-PCR with primers 1287-F and 1095-R, which amplify a 570 bp fragment specific to the NP coding region (McGavin et al., 2012).

Physical Traits of Fruits

The study focused on plantations and fruits of three raspberry cultivars: Brusviana, Sugana, and Joan J. Based on the testing results, seven plants free from viral infection were selected for Brusviana and Sugana cultivars, and six for Joan J. Yield was assessed by evaluating the infected RBDV plants of each cultivar alongside an equivalent number of healthy plants. The berries were collected at the optimal ripeness stage, counted, and weighed according to the harvest dates. The average yield per bush was then calculated. The fruit weight was measured on digital scales. Depending on the quality indicators following the requirements of SSTU 7179:2010, the fruits were classified into two commercial grades: first grade (weighing at least 2.5 g) and second grade (weighing at least 2.2 g), as well as fruits for processing. Commercial-grade raspberry fruits also met the following criteria: healthy, fresh, intact, uniform in size, free from excess moisture, and free from foreign smells and tastes.

Biochemical Composition of Fruits

The biochemical composition of red raspberry fruits was analysed at the Post-harvest quality laboratory of fruit and berry products at the Institute of Horticulture (IH, NAAS of Ukraine). Fruits at the consumer ripeness stage were examined following the methodology by Kondratenko et al. (2008). All measurements were conducted in triplicate.

Soluble Solid Content Determination

Soluble solid content (SSC) was determined using a portable refractometer ATAGO PAL-1 (Japan). For analysis, 10 fruits were crushed, and the juice was evaluated with the refractometer, taking temperature deviations into account. The data were recorded as a percentage of fresh weight.

Titratable Acidity Determination

Titratable acidity (TA) was determined by transferring 25 g of the crushed fresh sample into a 250 mL flask, washing with no more than 150 mL of hot distilled water. The flask was heated in a water bath at 80 °C for 30 minutes, cooled, adjusted to volume with distilled water, and filtered. A 20 mL aliquot was titrated with 0.1 N NaOH to a pink color (pH 7.0) using phenolphthalein. The acid content was calculated as a percentage of the raw mass (fresh weight), using the titration data and a conversion factor for citric acid

Total Sugar Content Determination

Total sugars were extracted from 25 g of fresh raspberry fruits using 100 ml of hot distilled water and purified by precipitating proteins and pigments with 2 ml of 10% lead acetate. Sucrose was hydrolyzed with 10% hydrochloric acid (2.5 ml for each sample), then oxidized with 3 ml of Fehling's solution. The test tubes were shaken and placed in a boiling water bath for 10 minutes, then cooled on ice. The supernatant was transferred into centrifuge tubes and centrifuged for 15 minutes at 3,000 rpm. The optical density of the solutions was measured at 640 nm using a ULAB 102UV spectrophotometer. Sugar content was calculated based on a graduated curve constructed using standard glucose solutions, and the data were expressed as a percentage of raw mass (fresh weight).

Measuring pH of Berry Juice

The pH of the berry juice was measured with a pH meter (Mettler Toledo, Switzerland).

Ascorbic Acid Content Determination

Ascorbic acid (AsA) was extracted from 10 g of fresh sample by grinding it in a porcelain mortar with broken glass and a mixture of 2% $C_2H_2O_4$ and 1% HCl (80 : 20, vol/vol). In 10 minutes, the extract was transferred to a 100 mL volumetric flask, diluted to the mark with the acid mixture, and filtered. Titration was performed using a 2,6-dichlorophenolindophenol solution (Tilmans reagent), and the mixture was left for 30 minutes to allow complete phase separation. AsA content was calculated based on the Tilmans paint titer and expressed as mg per 100 g of raw mass (fresh weight).

Total Phenolic Compound Determination

To determine the total phenols, 5 g of the sample fresh was ground with 10 ml of 95% ethanol and filtered. In a test tube, 7.9 mL of distilled water, 0.1 mL of extract, and 1 mL of Folin-Denis reagent were mixed, and after 3 minutes, 1 mL of 1 N Na_2CO_3 was added. The optical density was measured after one hour at 640 nm using a ULAB 102UV spectrophotometer (USA). The control sample was prepared by mixing 8 mL of distilled water with 1 mL of Folin-Denis reagent, and after 3 minutes, adding 1 mL of 1 N Na_2CO_3. The total phenol content was calculated using a graduated graph with chlorogenic acid as mg per 100 g of raw mass (fresh weight).

Anthocyanin Content Determination

The anthocyanin content was determined using the pH differential method, which relies on the reversible color change of anthocyanins between their oxonium form at pH 1.0 and hemiketal form at pH 4.5. Anthocyanins were extracted using acidified ethanol (70% ethanol with 0.1% HCl) and 1 g of plant material with following by filtration. The extracts were diluted in two buffer systems: 0.025 M potassium chloride (KCl) at pH 1.0 and 0.4 M sodium acetate ($CH_3CO_2Na \cdot 3\ H_2O$) at pH 4.5. Absorbance was measured at 520 nm and 700 nm using a ULAB 102UV spectrophotometer with 1 cm path-length cuvettes. The anthocyanin content was calculated and expressed as cyanidin-3-glucoside equivalents (mg per 100 g fresh weight) (Giusti and Wrolstad, 2001).

Statistical Analysis

Statistical analysis of the research data was carried out using Minitab 19 software (Minitab LLC, 2019). The data were subjected to a one-way analysis of variance after the Kolmogorov–Smirnov normality test. To determine significant differences between the means, Tukey's post-hoc test at $p \leq 0.05$ was used.

Results and Discussion

Results

To detect viral infections, 90 raspberry samples (30 from each cultivar) were analyzed using ELISA. RBDV was detected in all samples showing leaf chlorosis symptoms (24 samples) and fruit crumbliness symptoms (28 samples). Additionally, RBDV was found in 18 samples collected from asymptomatic plants. In contrast, 20 visually healthy plants tested negative for all viruses. All samples were free from other tested viruses such as RRSV, SLRV, TBRV, CLRV, AMV, CMV, ArMV, RVCV, and RLBV.

Effect of RBDV on Yield and Commercial Fruit Quality

Infected plants of all three studied cultivars showed a trend of reduced yield. The most significant yield loss was observed in Joan J, reaching up to 62.2%. Cultivars Sugana and Brusviana also exhibited yield reductions of 61.8 and 58.6%, respectively. Notably, the virus identified in this study negatively affected all yield components. Observations revealed fruit deformities, characterized by drupelet irregularity and crumbliness in RBDV-infected plants. However, the number of berries per fruiting lateral did not show statistically significant differences between infected and virus-free plants for any cultivar (Figure 1). Despite this, the most pronounced difference between infected (RBDV+) and healthy (RBDV−) plants was recorded in Joan J, where the number of fruits per plant decreased by 16.3%.

Only a minimal proportion of the berries harvested from RBDV-infected plants were classified as commercial-grade according to SSTU 7179:2010. Specifically, 14% of the berries met the criteria for the grade I category, while 17.6% were designated as grade II. The majority of the collected berries, accounting for 68.4%, did not meet the required quality standards for fresh market consumption and were therefore categorized as material for processing.

Healthy plants of cv. Brusviana produced a high proportion of first-grade commercial berries (82.3%), whereas RBDV-infected plants had almost no fruits that met the criteria for grade I (10.3%). Instead, the majority of these fruits were classified as material for processing (79.7%). In infected plants of the cv. Sugana, the proportion of fruits classified as first-grade commercial was higher than in cv. Brusviana (28.6%), with 14.3% falling into the grade II category, and a significant proportion (57.1%) classified as material for processing. Among the fruits harvested from healthy plants of Joan J, a significant proportion met the criteria for grade I (87.5%). In contrast, infected plants of this cultivar produced no fruits that could be classified as commercial grade.

The results demonstrate a significant reduction in fruit weight across all three raspberry cultivars affected by RBDV, as illustrated in Figure 2. The reduction in fruit weight ranged from 35.7 to 46.1%, with the greatest loss observed in the cv. Joan J.

Additionally, infected plants exhibited a significant reduction in the number of drupelets on the berries,

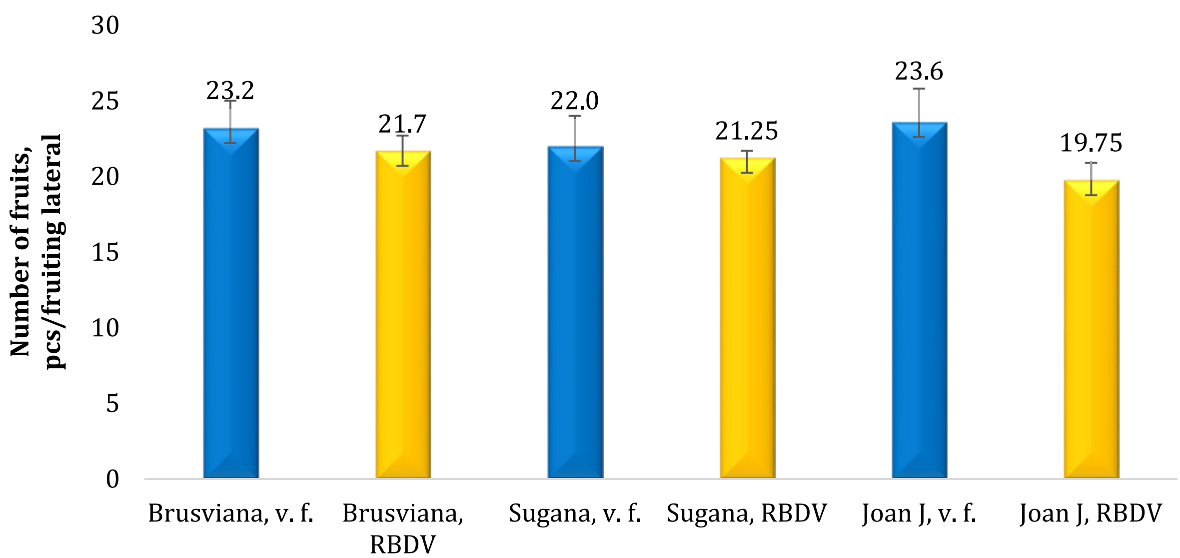

Figure 1 Impact of RBDV on the number of fruits, pcs per fruiting lateral

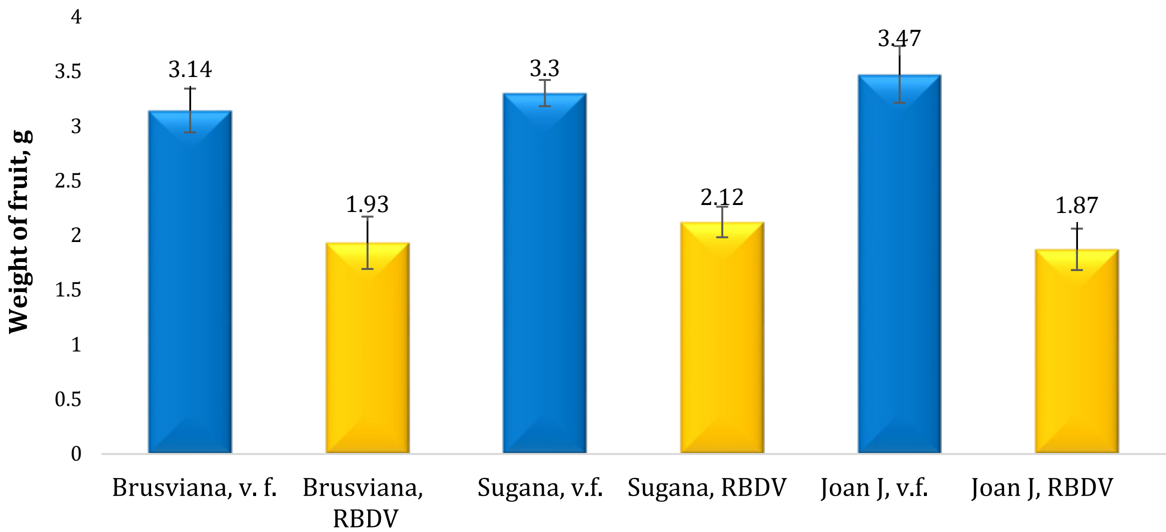

Figure 2 Impact of RBDV on the weight of fresh fruit, g

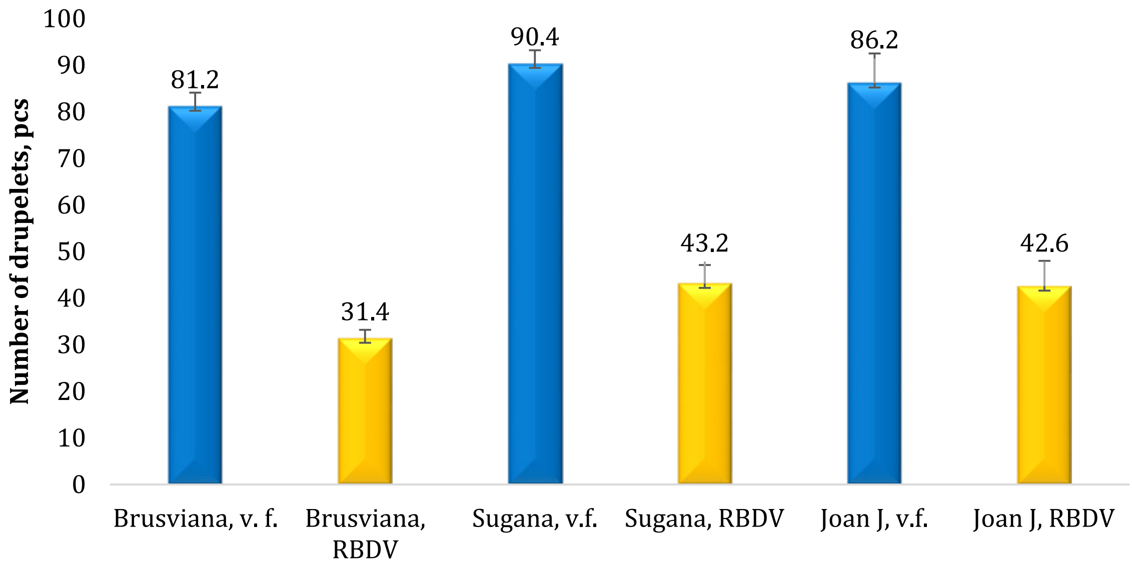

Figure 3 Impact of RBDV on drupelet number, pcs

further confirming the negative impact of RBDV on fruit development (Figure 3). The most considerable decrease in drupelet count was observed in the cv. Brusviana (61.3%), followed by cv. Sugana (52.2%) and cv. Joan J (50.6%). Thus, while the decrease in drupelet number was smallest in cv. Joan J, it experienced the greatest fruit weight loss, which may be a consequence of the disruption of drupelet development.

Effect of RBDV on Biochemical Composition of Raspberry Fruits

The effect of RBDV on total sugar content, SSC, TA, pH, as well as on the levels of phenols, anthocyanins, and AsA in fruits is detailed in Table 1. Analysis of sugar content and pH revealed a tendency for these parameters to decrease under the influence of the virus, with no significant differences between RBDV+ and RBDV– plants. Total sugar content in the fruit decreased by 11.8% only in the cv. Brusviana, while remaining stable in the other cultivars. The pH values of the juice ranged from 2.9 to 3.02 for infected plants, and from 2.93 to 3.08 for healthy plants across the cultivars.

The total SSC content decreased in all raspberry cultivars affected by the virus. However, a statistically significant reduction was observed only in cv. Brusviana, where it dropped by 12.5%. The TA content

Table 1 Content of chemical compounds in RBDV-free and RBDV-infected cultivars of raspberries

Cultivar	Soluble solids content (%)	Titratable acidity (%)	Total sugars (%)	pH	Total phenols (mg 100^{-1} g)	Ascorbic acid (mg 100^{-1} g)	Anthocyanins (mg 100^{-1} g)
Brusviana, v. f.	9.6 ±0.1a	0.52 ±0.02a	5.56 ±0.14a	2.95 ±0.01a	283 ±2.3a	17.75 ±0.18a	31.1 ±0.3a
Brusviana, RBDV	8.4 ±0.2b	0.73 ±0.05b	4.9 ±0.28a	2.9 ±0.02a	294 ±3.6a	16.04 ±0.25b	47.8 ±0.7b
Sugana, v. f.	10.4 ±0.2a	0.52 ±0.06a	5.86 ±0.15a	3.08 ±0.03a	237 ±2.5a	18.8 ±0.25a	32.8 ±0.5a
Sugana, RBDV	10 ±0.3a	0.78 ±0.06b	5.82 ±0.23a	3.02 ±0.07a	275 ±2.2b	17.35 ±0.11b	52.9 ±0.3b
Joan J, v. f.	10.6 ±0.1a	0.52 ±0.07a	6.16 ±0.09a	2.93 ±0.04a	202 ±3.9a	16.05 ±0.19a	15.9 ±0.8a
Joan J, RBDV	9.7 ±0.3a	0.52 ±0.04a	6.11 ±0.17a	2.98 ±0.05a	260 ±2.1b	14.8 ±0.26b	14.8 ±0.4a

Notes: v. f. – virus free; different letters after the values indicate a significant difference at $p < 0.05$

was 40–50% higher in infected plants of cv. Brusviana and cv. Sugana but remained unchanged in cv. Joan J.

A significant increase in polyphenol levels was observed in the berries of RBDV-infected plants of cv. Joan J (28.7%) and cv. Sugana (16%). In cv. Brusviana, polyphenol content also increased; however, the difference between infected and healthy plants was not statistically significant.

Anthocyanin levels were also significantly higher in RBDV-infected plants of cv. Sugana and cv. Brusviana, increasing by 60.4 and 53.7%, respectively. In contrast, in cv. Joan J, anthocyanin content slightly decreased in infected fruits. Moreover, this cultivar had a considerably lower anthocyanin content overall compared to the other two. The AsA content was significantly lower in RBDV-infected plants compared to virus-free ones across all three cultivars, with reductions ranging from 7.7 to 9.6%.

Discussion

The primary consequence of RBDV infection in raspberry plants was a significant reduction in the yield of RBDV+ plants compared to virus-free ones. The average yield loss across all cultivars was 60.8%. The results of this study confirm that RBDV negatively impacts the yield of the three examined cultivars, with the most significant impact observed in the Joan J cultivar. Similar yield losses were reported in blackberries, specifically the cv. Marion, where RBDV infection led to a 40–50% decrease in yield (Strick and Martin, 2003). At the same time, the number of fruits from infected and healthy plants did not differ significantly, which aligns with previous findings (Strick and Martin, 2003).

RBDV also affected fruit weight, which directly influenced the yield of the three raspberry cultivars studied. The number of drupelets per berry and its weight are key quality indicators that determine the classification of fruits for marketability. A 68.4% reduction in the number of first and second-grade fruits negatively impacts the economic viability of growing RBDV-infected raspberries. The fruit quality from virus-positive plants significantly deteriorated, as quality is determined by both fruit mass and integrity. Similar findings were reported for three out of five raspberry cultivars infected with RBDV, where both the number of drupelets and fruit weight were reduced (Moore et al., 2012). Likewise, under the influence of another virus affecting raspberries, RLBV, significant reductions in fruit size and weight (9.15–27.5%) were observed in the Willamette cultivar (Jevremović et al., 2022).

The impact of the virus on the biochemical composition of raspberry fruits was evaluated. No significant differences were observed between healthy and infected plants in terms of total sugar content and juice pH. However, a slight decrease in both parameters was noted across all varieties, with the most pronounced reduction in total sugar content found in the Brusviana variety. A similar trend has been reported in grapes, where GLRaV-2 and GLRaV-3 infections had no substantial effect on juice pH (Lee & Martin, 2009). In contrast, in grape cultivars Cabernet Franc and Merlot infected with tomato black ring virus (TBRV), total sugar content was higher than in healthy plants, but the increase was statistically significant only in cultivar Merlot (Dewasme et al., 2019). Both parameters remained unchanged in raspberries infected with RLBD (Jevremović, 2022). Slight differences were also observed in the SSC of raspberry fruits. Similar findings were reported for the Willamette raspberry infected with RLBV. Although the statistical difference in this study was minor, RBDV infection led to a decrease in SSC, whereas RLBV caused a slight increase (Jevremović et al., 2022). In peaches, SSC levels showed no consistent variation between trees infected with plum pox virus (PPV) and healthy ones (Samara et al., 2017). In contrast, *Physalis peruviana* infected with groundnut ringspot virus (GRSV) produced fruits with a 6.8% lower SSC, while infection with physalis rugose mosaic virus (PhyRMV) resulted in a 7% increase (Kraide et al., 2023). Similarly, in the strawberry cultivar Benihope, SSC decreased by 21.09% following infection with strawberry mottle virus (SMoV) and strawberry vein banding virus (SVBV) (Fan et al., 2022).

The taste formation of fruits is largely determined by the balance between sugars and acidity content. Thus, the RBDV-induced increase in TA in raspberry fruits negatively affected their flavor balance and suitability for processing. Although ACLSV did not have a clear effect on the TA of Golden Delicious apples, slightly higher values were observed in fruits from infected trees, which the authors attributed to lower crop load (Cieślińska and Rutkowski, 2008). In contrast, a significant TA reduction of 36.11% was reported in strawberry fruits infected with SMoV and SVBV (Fan et al., 2022).

AsA plays a crucial role in plants as an antioxidant, protecting cells from oxidative stress caused by pathogens. It also contributes to plant growth, hormone regulation, and defense responses, influencing resistance to infections (Hossain et al., 2017). In this study, a reduction in AsA content was observed in the infected fruits of the raspberry cultivars, with an average decrease of 9.6%. The response of AsA content to various plant-virus interactions varied. A 15% increase in AsA content was recorded in the fruits of *Carica papaya* cv. Red Lady was infected with papaya ring spot virus (PRSV) (Chamika Buddhinie et al., 2017), while tomato yellow leaf curl virus (TYLCV) led to a significant reduction in AsA content in the fruits of two tomato cultivars, with decreases ranging from 35.3 to 51.5% (Tajul et al., 2011). These variations suggest that the effect of viral infections on AsA content is complex and influenced by factors such as plant species, virus strain, and the plant's defense mechanisms.

The total polyphenol content was higher in RBDV+ plants of Sugana and Joan J, which had a significant impact on the potential fruit quality of raspberries. These findings are consistent with previous results, where under the influence of RLBV, total phenolic content varied depending on the year and location of cultivation for the cultivar Willamette (Miletic´ et al., 2024). An increase in phenolic compounds was also observed in Saaz hops infected with hop latent viroid (HLVd) (Jelínek et al., 2012), and in Pinot noir grape fruits infected with grapevine leafroll-associated viruses GLRaV-2 and GLRaV-3 (Lee and Martin, 2009). However, the impact of viral infection on phenolic levels may depend on the cultivar's genotype. For example, the cultivar Čačanska Lepotica showed no change in phenolic content when infected with PPV, suggesting its tolerance to the virus (Miletić et al., 2022). A statistically significant increase in anthocyanin levels (up to 60.4%) was recorded in two out of the three studied raspberry cultivars, which may indicate a protective response of the plant to viral stress.

At the same time, the effect of viral infection on anthocyanin accumulation varies across different crops and pathogens. For example, in Lutowka cherry fruits infected with prunus necrotic ringspot virus (PNRSV) and cherry virus A (CVA), anthocyanin levels were lower compared to the control (Paduch-Cichal et al., 2024). An increase in anthocyanin content was observed in *Vitis vinifera* grapes infected with grapevine fanleaf virus (GFLV) (Rupnik-Cigoj et al., 2018). Meanwhile, infection with RLBV did not affect anthocyanin content in raspberries (Miletic et al., 2024), confirming that pigmentation changes depend on the specific interaction between the virus and the plant.

Conclusions

The results of this study demonstrate that RBDV infection significantly affects the productivity and biochemical composition of raspberry plants. Infected plants exhibited a marked reduction in fruit yield, with the cv. Joan J showing the greatest loss. Furthermore, the virus led to significant changes in fruit quality, with infected berries being classified as lower grade compared to healthy plants. While sugar content and pH were slightly affected, the most notable changes were observed in phenolic and anthocyanin levels, which increased in infected plants of cv. Sugana and cv. Brusviana, but decreased in cv. Joan J. This suggests that while RBDV infection does not substantially alter all biochemical parameters, it has a detrimental impact on fruit marketability, particularly in terms of size, weight, and overall quality. These findings emphasize the importance of effective virus management in raspberry cultivation to maintain both yield and fruit quality for commercial production and processing.

Conflict of Interest

The authors have no conflicts of interest to declare.

Ethical Statement

This article doesn't contain any studies that would require an ethical statement.

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