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EFFECT OF VIRUS INFECTION ON THE FRUIT QUALITY OF SOUR CHERRY CULTIVAR ŁUTÓWKA

Elżbieta Paduch-Cichal[1], Tomasz Krupa[2], Ewa Mirzwa-Mróz[1], Marek S. Szyndel[1], Karol Staniszewski[1], Wojciech Kukuła[1], Elżbieta Mielniczuk[3], Marcin Wit[1]✉, Wojciech Wakuliński[1]

[1] Warsaw University of Life Sciences, Institute of Horticultural Sciences, Department of Plant Protection, Section of Plant Pathology, 159 Nowoursynowska Street, 02-776 Warsaw, Poland

[2] Warsaw University of Life Sciences, Institute of Horticultural Sciences, Department of Pomology and Horticultural Economics, 159C Nowoursynowska Street, 02-787 Warsaw, Poland

[3] University of Life Sciences in Lublin, Department of Plant Protection, Leszczyńskiego 7, 20-069 Lublin, Poland

ABSTRACT

A survey was carried out on a commercial sour cherry fruit orchard located in Lublin province in Poland to determine the influence of viruses on the fruit quality of sour cherry cv. Łutówka. Leaf samples from trees of sour cherry cv. Łutówka were tested for *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Little cherry virus* 1 (LChV-1), *Little cherry virus* 2 (LChV-2), *Cherry virus A* (CVA), *Cherry green ring mottle virus* (CGRMV), *Cherry necrotic rusty mottle virus* (CNRMV), *Cherry rasp leaf virus* (CRLV) and *Cherry mottle leaf virus* (CMLV) using the RT-PCR technique. The results indicated that PNRSV and PNRSV+CVA infected the samples. PDV, LChV-1, LChV-2, CGRMV, CNRMV, CRLV, and CMLV were not detected in any of the tested sour cherry trees. The effect of virus infection on the chemical composition of sour cherry fruits was investigated. The anthocyanin, total phenolic and vitamin C contents, and antioxidant activity were evaluated. The total phenolic compound, vitamin C contents, and antioxidant activity were significantly higher in PNRSV- and PNRSV+CVA-infected than in virus-free sour cherry fruits. The total anthocyanin content in PNRSV- or PNRSV+CVA-infected fruits was lower than in control trees. To our knowledge, this is the first report in the world about the effect of PNRSV or PNRSV+CVA infection on the anthocyanin compounds, total polyphenolic compounds, vitamin C contents, and the antioxidant activity of sour cherry fruits.

Key words: sour cherry, PNRSV, CVA, anthocyanins, total phenolics, vitamin C, antioxidant activity

INTRODUCTION

Virus diseases occur in all regions of orchard plant cultivation. Usually, viroses have a milder course than bacterioses or mycoses, and they are less likely to lead to the death of an entire plant. However, it should be pointed out that viroses may reduce the number and quality of fruits, lower a plant's resistance to frost, and also increase its susceptibility to diseases caused by pathogenic bacteria or fungi [Németh 1986].

Prunus cerasus L. and *P. avium* L. (sour cherry and sweet cherry, respectively) are crops of economic

✉ marcin_wit@sggw.edu.pl

importance in many world regions. Among over 30 viruses that infect sour and sweet cherry, viruses of the genus Ilarvirus (the family *Bromoviridae*) are prevalent, with *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), and *Apple mosaic virus* (ApMV) being the most common. Trees infected by these Ilarviruses either develop symptoms on leaves, including chlorotic ringspots, yellow line patterns, and rugose mosaics, or are symptomless. Ilarvirus infection may result in a significant yield loss and a delay in fruit maturity [Pallás et al. 2012, Rubio et al. 2017]. Additionally, other viruses are also detected in sour and sweet cherry: *Little cherry virus 1* (LChV-1), *Little cherry virus 2* (LChV-2) and *Little cherry virus 3*, (LChV-3) [Welsh and Cheney 1976, Isogai et al. 2004, Bajet et al. 2008, Ludvíková and Suchá 2011, Rao et al. 2011, Lu et al. 2015, Zong et al. 2015, Ruiz-García et al. 2016, Šafářová et al. 2022], *Cherry virus A* (CVA) [Jelkmann 1995, Isogai et al. 2004, Rao et al. 2009, Zong et al. 2015, Kinoti et al. 2016, Wang et al. 2018, Simkovich et al. 2021], *Cherry green ring mottle virus* (CGRMV) and *Cherry necrotic rusty mottle virus* (CNRMV) [Wadley and Nyland 1976, Desvignes et al. 1999, Zhang et al. 2000, Gentit et al. 2002, Isogai et al. 2004, Sabanadzovic et al. 2005, Mandic et al. 2007, Fiore and Zamorano 2013, Zhou et al. 2013, Cho et al. 2014, Zong et al. 2015], *Cherry mottle leaf virus* (CMLV) [James and Mukerji 1993], and *Cherry leaf roll virus* (CLRV) [Cropley 1961].

Poland's most prevalent sour and sweet cherry tree viruses include PNRSV and PDV [Kryczyński et al. 1994]. Less common examples found in Poland are, among others, CVA and LChV-1 (detected in sour cherry by Komorowska and Cieślińska [2004]), LChV-2 (detected in sweet cherry by Komorowska and Cieślińska [2008]), LChV-1 (detected in sweet and sour cherry by Cieślińska and Morgaś [2010]), and CGRMV (detected in sour and sweet cherry by Komorowska and Cieślińska [2005]).

The influence of viruses on trees is affected by the pathogen and its strain, fruit species, cultivars, and nutrient supply. During the ripening of fruits, some physiological, biochemical, and structural changes occur, which depend on the species, cultivar, ripening stage, and part of the fruit [Usenik et al. 2014, 2017]. However, no report has been published about the effect of PNRSV, PDV, or the other less common viruses mentioned above on the phytochemicals in sour cherry fruits.

To the best of our knowledge, our paper constitutes the first report on the effect of virus infection on the composition of anthocyanin compounds, the total polyphenolic compounds, the vitamin C content, and the antioxidant activity of virus-infected sour cherry fruits.

MATERIAL AND METHODS

Plant material. The research material consisted of 10 leaves collected from each of 20 randomly selected trees of the sour cherry cv. Łutówka (tree age: 23 years), growing in a production orchard (Bialski district). RT-PCR was used to test the material for the presence of *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Little cherry virus 1* (LChV-1), *Little cherry virus 2* (LChV-2), *Cherry virus A* (CVA), *Cherry green ring mottle virus* (CGRMV), *Cherry necrotic rusty mottle virus* (CNRMV), *Cherry rasp leaf virus* (CRLV) and *Cherry mottle leaf virus* (CMLV).

During the experimental period, the tested sour cherry trees were observed in 3 periods: 1st period – before blooming, 2nd period – blooming, 3rd period – fruit picking. All disease symptoms observed in leaf blades and the fruit development were noted.

Molecular detection of cherry viruses in sour cherry trees. Total nucleic acids were isolated from the leaf tissue of each of the 20 studied trees of the sour cherry cv. Łutówka using the silica capture (SC) method described originally by Boom et al. [1990] and adapted to the diagnosis of plant viruses by Malinowski [1997]. For RT-PCR, Titan One Tube RT-PCR System (Roche, Germany) was used to amplify PNRSV, PDV, CVA, CGRMV, LChV-2, LChV-1, CRLV, CMLV, and CNRMV genome fragments with respective primer pairs as described by Zong et al. [2015].

After analyzing the results of the RT-PCR reaction, 3 PNRSV-infected trees of the sour cherry cv. Łutówka cultivar, 3 PNRSV+CVA-infected trees of the sour cherry cv. Łutówka were selected. The control trees were 3 PNRSV-, PDV-, CVA-, CGRMV-, LChV-1-, LChV-2, CRLV-, CMLV- or CNRMV-free trees of the cv. Łutówka. From each selected tree, 1.5 kg of

fruits were collected, and five samples, each of 200 g, were prepared separately for the control and the diseased trees. There were five replicates for each sample. The fruit pulp was separated from the stone, placed in ziplock bags, and stored at –80°C until analysis.

The evaluation of bioactive compounds and antioxidant activity. All reagents used to evaluate bioactive compounds and antioxidant activity were of analytical purity gradient or HPLC grade. All of them were obtained from Sigma-Aldrich (Poznan, Poland) or Merck (Warsaw, Poland).

Separation and anthocyanin content analysis was performed using a Perkin-Elmer 200 series HPLC kit with a Diode Array Detector (DAD), according to the modified method of Szpadzik et al. [2022]. Anthocyanins were identified at 520 nm, with the content of each compound being expressed in mg of cyanidin-3-glucoside equivalent in mg 100 g–1 of FW (fresh weight).

Total phenolic content. The analysis was carried out by the method of Singleton et al. [1999] with Folin-Ciocalteau reagent using a Marcel s330 PRO spectrophotometer (Marcel S.A., Warsaw, Poland). The polyphenol content was calculated based on the standard curve. The total polyphenol content is expressed in mg of gallic acid 100 g^{-1} of FW.

Antioxidant activity was determined by the method of Saint-Cricq De Gaulejac et al. [1999], which is based on the reduction of free radicals derived from DPPH+ (1,1-diphenyl-2-picrylhydrazine, Sigma-Aldrich, Poznan, Poland). The results were expressed in mMol as Trolox equivalent per 1 g FW.

Ascorbic acid (vitamin C) content was analyzed by HPLC with modifications by Krupa et al. [2022]. AAC was determined from the standard and expressed in mg 100 g^{-1} of FW.

Statistical analysis. A one-way analysis of variance (ANOVA) was performed to determine the influence of tree infection by PNRSV or PNRSV+CVA on the composition of anthocyanin compounds, the total polyphenolic compounds, the vitamin C content, and the antioxidant activity of sour cherry fruits. To compare the average values, the Newman-Keuls test was used at the significance level of $p = 0.05$, with all calculations having been made using Statgraphics Plus 4.1 for Windows (Statistical Graphics Corporation, Warrenton, VA, USA).

RESULTS

Subsection comparison of the field symptoms in the case study on multiplex infection with five viruses. The field symptoms of the trees sampled in this survey are presented in Table 1 – observations of the 20 trees of the sour cherry cv. Łutówka selected for testing resulted in either no detected macroscopically visible lesions indicative of the presence of a virus(es) or detected symptoms of varying severity visible on the leaves. The leaves appeared to be rugose with thin and irregular shapes. Weak leaf chlorosis or severe leaf chlorosis or severe leaf chlorosis and necrosis with shoot wholes distributed between the interveinal areas of the upper leaf surface. Additionally, there were enations on the lower leaf surface. A comparison to healthy trees (disease-free, not containing any of the viruses assayed for during the blooming of the infected trees) was performed about 5 or 7 days later, and the flower stalks were shortened. The infected trees bore smaller and more poorly colored fruits compared to the fruits of healthy trees. Growth reduction (stunting) was observed in all the infected trees.

Molecular detection of cherry viruses in sour cherry trees. Electrophoretic analysis of the RT-PCR products revealed the presence of PNRSV and CVA, with the RT-PCR products having the expected sizes. In contrast, no fragment with an expected size was amplified using the primer pairs for PDV, CGRMV, LChV-1, LChV-2, CRLV, CMLV, or CNRMV. As summarized in Table 2, in 8 of 20 samples (40%), only PNRSV was detected. Further analysis indicated that PNRSV and CVA infected the sour cherry trees in a mixed infection. PNRSV infected 45% of the samples simultaneously by two virus species: PNRSV+CVA. PDV, CVA, LChV-1, LChV-2, CGRMV, CNRMV, CRLV, or CMLV were not detected in any of the tested sour cherry trees.

The anthocyanin content in fruits of the sour cherry cv. Łutówka infected with viruses. No statistically significant differences were found in the cyanidin-3-O-glucoside content in the cherry fruits from the PNRSV- or PNRSV+CVA-infected trees compared to the values obtained for the trees of the control trees (Fig. 1).

There were no statistically significant differences in the cyanidin-3-O-rutinoside content between the

Table 1. RT-PCR detection of viruses in sour cherry trees with blight disease symptoms or no symptoms

Symptoms	Tested viruses									
	PNRSV	CVA	PNRSV +CVA	PDV	CLRV	CGRMV	CMLV	CNRV	LChV-1	LChV-2
Leaves										
Severe chlorosis, necrosis, shoot holes, enations	1/20*	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Severe chlorosis, enations	1/20	0/20	4/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Weak chlorosis, enations	6/20	0/20	4/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Flowers										
Delayed blooming, shortened flower stalks	8/20	0/20	9/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Fruits										
Small, poorly colored fruits	8/20	0/20	9/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Trees										
Stunted growth of trees	7/20	0/20	8/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20

* Number of sour cherry trees with virus detected/number of sour cherry trees used in this experiment

Table 2. Detection of viruses in sour cherry trees cv. Łutówka

Virus	PNRSV	CVA	PDV	CLRV	CGRMV	CMLV	CNRV	LChV-1	LChV-2	PNRSV +CVA
Infection (%)	8/20* (40)	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)	9/20 (45)

* Number of sweet cherry trees with virus detected/number of sweet cherry trees used in this experiment

cherry fruits from the PNRSV-infected trees and the control trees, although the anthocyanin content was lower in the fruits from the virus-infected trees. The cyanidin-3-O-rutinoside content in fruits from the PNRSV+CVA-infected trees was significantly lower than the values for the PNRSV-infected trees or the fruits of the control trees (Fig. 2).

A significantly lower cyanidn-3-O-glucosylrutinoside content was detected in fruits from the PNRSV-infected trees compared to the anthocyanin content in fruits from the PNRSV+CVA-infected trees and the fruits of the control trees. There were no statistically significant differences in the cyanidin-3-O-glucosylrutinoside content between cherry fruits from the PNRSV+CVA-infected trees and the fruits of the control trees (Fig. 3).

No statistically significant differences existed between the cyanidin-3-O-soforoside content in cher-

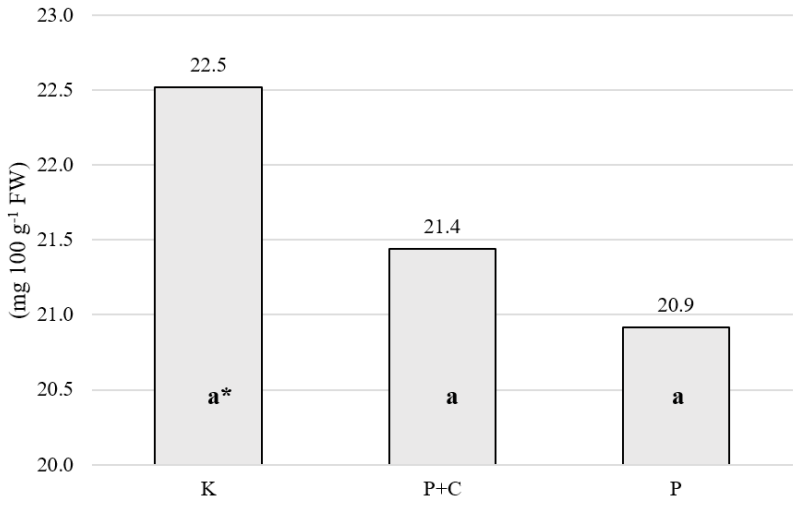

Fig. 1. The average cyanidin-3-O-glucoside content in sour cherry fruits of the cv. Łutówka infected with viruses. K – control trees, P+C – PNRSV+CVA-infected trees, P – PNRSV-infected trees. *a homogenous group according to the Newman–Keuls test. Values marked with the same letter do not differ statistically at the significance level p = 0.05

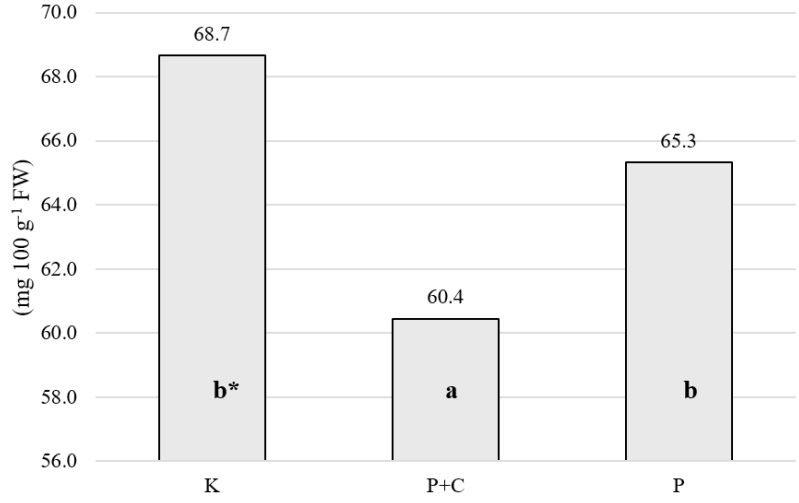

Fig. 2. The average cyanidin-3-O-rutinoside content in sour cherry fruits of the cv. Łutówka infected with viruses. K – control trees, P+C – PNRSV+CVA-infected trees, P – PNRSV-infected trees. *a homogenous group according to the Newman–Keuls test. Values marked with the same letter do not differ statistically at the significance level p = 0.05

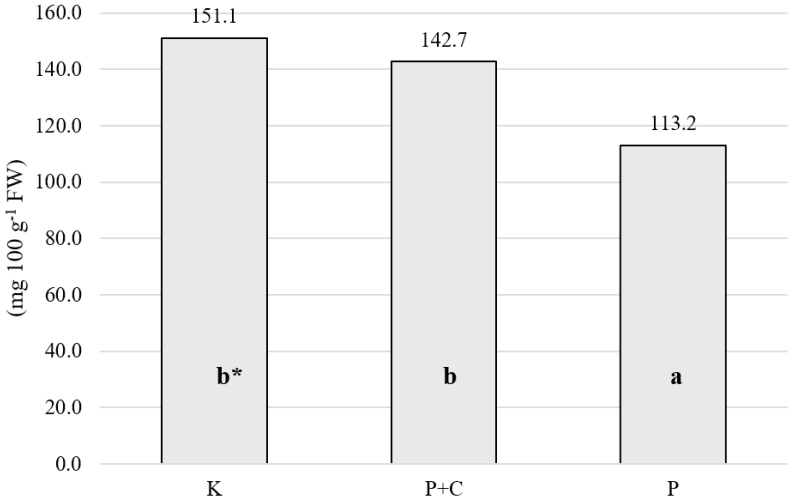

Fig. 3. The average cyanidin-3-O- glucosylrutinoside content in sour cherry fruits of the cv. Łutówka infected with viruses. K – control trees, P+C – PNRSV+CVA-infected trees, P – PNRSV-infected trees. *a homogenous group according to the Newman–Keuls test. Values marked with the same letter do not differ statistically at the significance level p = 0.05

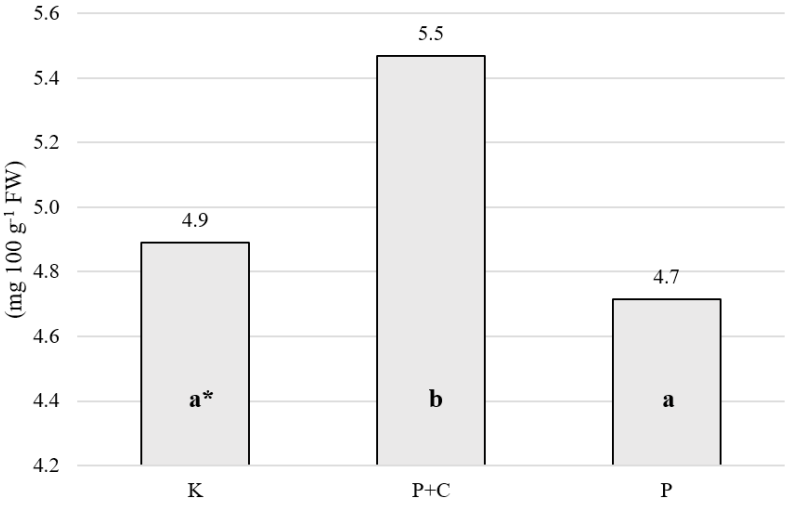

Fig. 4. The average cyanidin-3-O-soforoside content in sour cherry fruits of the cv. Łutówka infected with viruses. K – control trees, P+C – PNRSV+CVA-infected trees, P – PNRSV-infected trees.*a homogenous group according to the Newman–Keuls test. Values marked with the same letter do not differ statistically at the significance level p = 0.05

ry fruits from the PNRSV-infected trees and the anthocyanin content in the control trees. A significantly higher anthocyanin content was obtained in fruits from the PNRSV+CVA-infected trees compared to the values for the PNRSV-infected and control trees (Fig. 4).

The total phenolic content in fruits of the sour cherry cv. Łutówka infected with viruses. Statistically significant differences were found in the total phenolic content in cherry fruits from the PNRSV- or PNRSV+CVA- infected trees compared to the values obtained for the control trees. A significantly higher total phenolic content was obtained when testing fruits from the PNRSV- or PNRSV+CVA-infected trees compared to the values obtained for the control trees (Fig. 5).

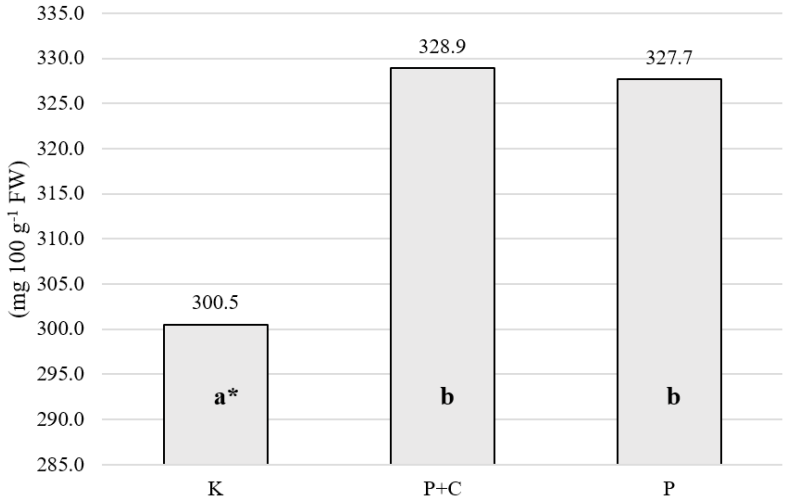

Fig. 5. The average total phenolic content in sour cherry fruits of the cv. Łutówka infected with viruses. K – control trees, P+C – PNRSV+CVA-infected trees, P – PNRSV-infected trees. *a homogenous group according to the Newman–Keuls test. Values marked with the same letter do not differ statistically at the significance level p = 0.05

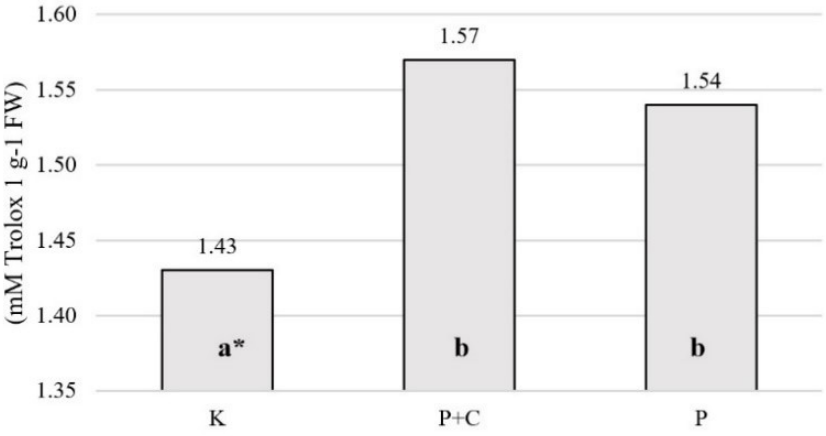

Fig. 6. The average level of antioxidant activity of sour cherry fruits of the cv. Łutówka infected with viruses. K – control trees, P+C – PNRSV+CVA-infected trees, P – PNRSV-infected trees. *a homogenous group according to the Newman–Keuls test. Values marked with the same letter do not differ statistically at the significance level p = 0.05

The antioxidant activity in fruits of the sour cherry cv. Łutówka infected with viruses. Statistically significant differences were found in the antioxidant activity in the cherry fruits from the PNRSV- or PNRSV+CVA-infected trees compared to the values obtained for the trees of the control trees. A significantly higher antioxidant activity was obtained when testing fruits from the PNRSV- or PNRSV+CVA-infected trees than the values obtained for the control trees (Fig. 6).

The ascorbic acid (vitamin C) content in the sour cherry cv. Łutówka fruits infected with viruses.

Statistically significant differences were found in fruits from the PNRSV- or PNRSV+CVA-infected trees compared to values obtained for the control trees. A significantly higher vitamin C content was obtained

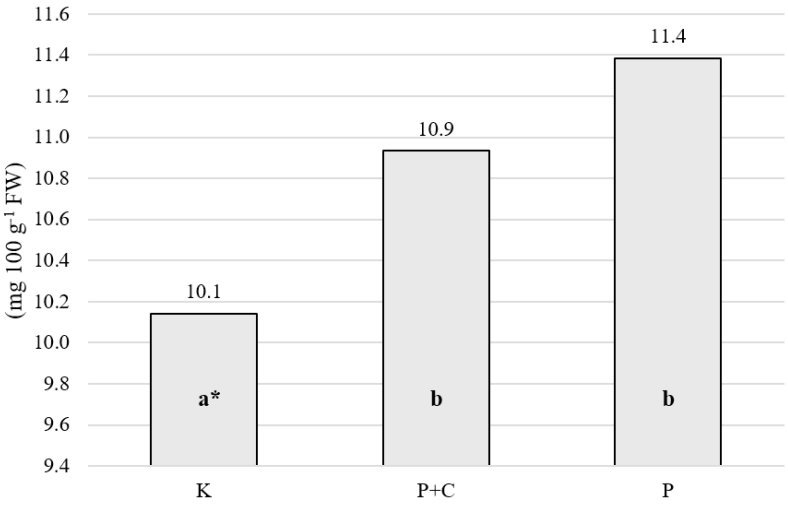

Fig. 7. The average vitamin C content in sour cherry fruits of the cv. Łutówka infected with viruses. K – control trees, P+C – PNRSV+CVA-infected trees, P – PNRSV-infected trees. *a homogenous group according to the Newman–Keuls test. Values marked with the same letter do not differ statistically at the significance level p = 0.05

when testing fruits from the PNRSV- or PNRSV+CVA-infected trees compared to the values obtained for the control trees (Fig. 7).

DISCUSSION

Several papers have reported the incidence of stone fruit viruses in both sweet and sour cherry trees in different regions of Poland. PNRSV and PDV were initially identified via ELISA during the 1960s [Kryczyński et al. 1994]. Subsequently, between 2004 and 2010, numerous viruses, including CVA, LChV-1, LChV-2, and CGRMV, were detected, primarily in sweet and sour cherry trees cultivated in commercial orchards across various parts of Poland or in germplasm collections [Komorowska and Cieślińska 2005, 2008, Cieślińska and Morgaś 2010].

Among the 20 sour cherry trees of the cv. Łutówka selected for study, four trees did not show any macroscopically visible symptoms, while in the remaining 16 trees, symptoms of varied severity were present. The symptoms included weak or severe chlorosis, necrosis, shoot holes and enations on the leaves, delayed blooming, shortened flower stalks, and small, poorly colored fruits. These symptoms are characteristic of trees infected with the *Prunus necrotic ringspot virus*. Such a varied reaction of sour cherry to infection by PNRSV has been reported in the literature [Németh 1986]. The characteristic symptoms of infection by PDV [Németh 1986], LChV-1 and LChV-2 [Welsh and Cheney 1976], CNRMV [Wadley and Nyland 1976], CRLV [Stace-Smith and Hansen 1976], CMLV [James and Mukerji 1993] and CGRMV [Desvignes et al. 1999] were overlooked by the authors of this article. The symptoms associated with infection of sour cherry by CVA have not been described in the literature. However, CVA isolated from an apricot leaf sample exhibited vein-clearing symptoms [Koinuma et al. 2016]. Symptomatic leaf samples exhibiting chlorosis, necrosis, rusty necrosis, mosaic patterns, enations, and shoot holes were systematically collected from sweet cherry plants in the Western Himalayan region of India, as documented by Noorani et al. in 2010. In the present study, sour cherry leaf samples underwent RT-PCR testing to detect the presence of nine distinct virus species. The results revealed the positivity of up to two viruses, PNRSV and CVA, whereas PDV was markedly absent in all the trees tested.

In Poland, according to Kryczyński et al. [1994], PDV is detected in about 70% of the different stone tree species, but it is detected far more frequently in sweet cherry trees, which may explain the absence of the virus in the cv. Łutówka, described in this article. Other viruses (LChV-1, LChV-2, CGRMV, CNRMV,

CRLV, and CMLV) were undetected in any tested tree. These results correspond with those that have been previously obtained in Poland and that show a low rate of infection with lesser-known viruses, such as CVA and LChV-1 [Komorowska and Cieślińska 2004], CGRMV [Komorowska and Cieślińska 2005], LChV-2 [Komorowska and Cieślińska 2008], LChV-1, and CGRMV [Cieślińska and Morgaś 2010]. On the other hand, a high incidence of the lesser known viruses has been reported in other countries: Serbia [Mandic et al. 2007], China [Rao et al. 2009, Zhou et al. 2013, Lu et al. 2015, Wang et al. 2018], Chile [Fiore and Zamorano 2013], Korea [Cho et al. 2014], Japan [Isogai et al. 2004, Zong et al. 2015], the USA (California) [Sabanadzovic et al. 2005], Australia [Kinoti et al. 2016], Canada and the USA [Bajet et al. 2008, Simkovich et al. 2021], and Spain [Ludvíková and Suchá 2011, Ruiz-García et al. 2016].

The authors of this article found that the detection rate for PNRSV (85%) was much higher than for CVA (45%) – the infection of 85% of sour cherry trees of the cv. Łutówka by PNRSV is unsurprising because PNRSV is the most severe sour cherry pathogen worldwide [Pallás et al. 2012]. In Poland, this virus detected sour cherries more frequently than sweet cherries [Kryczyński et al. 1994]. In Japan, Zong et al. [2015] have found that the detection rates for CVA (67.7%) were much higher than for PNRSV (46.8%). A high infection rate with CVA was previously observed by Isogai et al. [2004], with 92% of the Japanese trees tested having been infected with this virus. In Serbia, CVA was found in 84% of the trees tested [Mandic et al. 2007].

Nine of the 20 PNRSV-positive samples examined in this study were concurrently infected with CVA. Such mixed infections have also been documented in other parts of the world, including Japan [Isogai et al. 2004, Zong et al. 2015], the USA (California) [Sabanadzovic et al. 2005], and Serbia [Mandic et al. 2007]. Zong et al. [2015] demonstrated that about 82.3% of the samples carried at least two virus species, with nearly half (29 out of 62 samples) displaying simultaneous infections with four or five virus species. Notably, 21% of the samples were infected by the complex "PNRSV+PDV+CVA+CGRMV+LChV-2", while 14.5% were infected by the complex "PDV+CVA+CGRMV+LChV-2", indicating a considerably higher rate of infection compared to other cases. Furthermore, it was observed that certain viruses occurred more frequently in mixed infections. For instance, of the 29 PNRSV-positive samples, 24 were simultaneously infected with PDV; among the 37 LChV-2-positive samples, 32 also showed infection with CVA. Mandic et al. [2007] reported mixed virus infections involving CGRMV and CNRMV, with both viruses having been detected in 11 samples, while only two single infections with CNRMV and one single infection with CGRMV were identified. Co-infection with multiple virus species often results in synergistic effects and increased symptom severity in host plants. It has been suggested that while CVA infection may not induce significant symptoms individually, it could exacerbate symptom severity when combined with other viruses [James et al. 1999]. Despite initially being identified as the primary pathogen in sweet cherry trees affected by minor cherry disease [Jelkmann 1995], CVA was later found less significant than LChV-2 in causing the disease [Rott and Jelkmann 2001].

In this study, the field symptoms of the trees sampled were primarily represented by three types: i) severe chlorosis, necrosis, shoot holes, enations, ii) weak leaf chlorosis and enations, iii) leaf chlorosis, necrosis, and shoot wholes. Additionally, the blooming days of the infected trees were delayed, the flower stalks were shortened, the fruits were poorly colored, and their growth was reduced on the trees simultaneously infected with PNRSV+CVA and PNRSV. Severe leaf chlorosis, necrosis, and shoot wholes were found on one tree infected with PNRSV. There was no clear relationship between a mixed infection by PNRSV+CVA and the response of the trees.

Sour cherry fruits are a good source of natural antioxidants that strongly influence their quality and contribute to their organoleptic properties and nutritional value. Among the bioactive compounds, phenolic compounds (anthocyanins, flavonoids, phenolic acids) and ascorbic acid impart the health-promoting properties to sour cherry fruits [Wojdyło et al. 2014, Borowy et al. 2018].

Anthocyanins are the primary phenolic compounds in sour cherries, responsible for their vibrant red skin and flesh coloration. The total anthocyanin content varied significantly, ranging from 17.97 mg per

100 g of fresh weight (FW) in the Draden cultivar to 131.28 mg per 100 g FW in the Wieluń 17 cultivar [Sokół-Łętowska et al. 2020], and from 844.77 mg per 100 g FW in the Wisok cultivar to 994.00 mg per 100 g FW in the Sabina cultivar [Wojdyło et al. 2014]. According to data from other sources [Mulabagal et al. 2009, Ferretti et al. 2010, Papp et al. 2010, Khoo et al. 2011, Cao et al. 2015], the anthocyanin content in sour cherries is comprised between 21.0 and 285.0 mg per 100 g FW, 11.3 and 93.5 mg per 100 g FW, 65.1 and 82.4 mg per 100 g FW, 45.0 and 109.0 mg per 100 g FW, and 2.7 and 28.0 mg per 100 g FW, respectively. In the present study, the total anthocyanin content in fruits from control trees of the Łutówka cultivar was measured at 247.2 mg per 100 g FW, being thus below the value of 515.06 mg per 100 g FW reported by Wojdyło et al. [2014], yet above the anthocyanin content of 108.09 mg per 100 g FW as recorded by Borowy et al. [2018] for the same Łutówka cultivar.

In the presented article, cyanidin-3-O-glucosyl-rutinoside was the predominant compound in the sour cherry fruits of the control trees of the Łutówka cv., constituting 61% of the total anthocyanins. The next most abundant anthocyanin was cyanidin-3-O-rutinoside, which accounted for 28% of the total anthocyanins, and finally, cyanidin-3-O-glucoside (9% of the total anthocyanins). The other anthocyanin, cyanidin-3-O-soforiside, had a much lower percentage content (1.9%). Borowy et al. [2018] have observed that among the anthocyanins detected in Łutówka cv. fruits, the most prevalent components were cyanidin-3-O-glucosyl-rutinoside (76.30 mg 100 g^{-1} FW) and cyanidin-3-O-rutinoside (24.34 mg 100 g^{-1} FW). The content of the other individual anthocyanins, cyanidin-3-O-glucoside, and cyanidin-3-O-soforiside, was considerably smaller (0.67 mg 100 g^{-1} FW and 1.98 mg 100 g^{-1} FW, respectively). According to the data provided by Wojdyło et al. [2014], the main anthocyanins in the sour cherry fruits of Łutówka cv. are cyanidin-O-sophoroside (77.91 mg 100 g^{-1} FW) and cyanidin-3-O-rutinoside (70.45 mg 100 g^{-1} FW). Variation in the anthocyanin content in sour cherry fruits of different cultivars has also been reported previously by Simunic et al. [2005], Homoki et al. [2016], and Sokół-Łętowska et al. [2020].

The anthocyanin content and composition in fruits are subject to various environmental factors, post-harvest processing conditions, and the analytical methods employed [Wojdyło et al. 2014]. Factors such as maturity stage, cultivars, cultural practices, geographic origin, and growing season significantly influence the anthocyanin composition and concentration. Within different plant species or even cultivars of the same plant species, the total anthocyanin content can vary considerably, being influenced by genetic factors, light exposure, temperature fluctuations, and agronomic practices [Kim and Padilla-Zakour 2004, Kim et al. 2005, Horbowicz et al. 2008]. For instance, depending on the sour cherry cultivar, the total phenolic compound content ranged from 96.56 mg to 268.98 mg per 100 g of fresh weight (FW) [Sokół-Łętowska et al. 2020], with other studies having reported values in the range of 120.0–407.0 mg of phenolic compounds per 100 g of sour cherry fruits [Kim et al. 2005, Jakobek et al. 2009, Levaj et al. 2010]. In the case of the sour cherry cv. Łutówka, the total phenolic compound content (300 mg per 100 g FW) determined in this study was similar to that reported by Borowy et al. [2018]. The wide variation in the composition and total content of phenolic compounds in sour cherry fruits is attributed to the diversity of cultivars and varieties, with their levels being primarily influenced by factors such as the cultivar characteristics, maturity stage, agronomic practices, and climate conditions [Sokół-Łętowska et al. 2020].

Sour cherry fruits typically boast significant ascorbic acid (vitamin C) levels. However, the ascorbic acid content varies considerably among different sour cherry cultivars, with the concentrations ranging from 2.5 mg 100 g^{-1} FW to 22.11 mg 100 g^{-1} FW [Grzyb and Rozpara 2009, Papp et al. 2010, Wojdyło et al. 2014, Borowy et al. 2018]. In the presented study, the vitamin C content in fruits of the Łutówka cv. was 10.1 mg 100 g^{-1} FW. It was lower than the vitamin C content in the Łutówka cv. fruits described by Borowy et al. [2018] – 20.8 mg 100 g^{-1} FW, and higher than the vitamin C content described by Wojdyło et al. [2014] – 5.91 mg 100 g^{-1} FW. Czynczyk et al. [1988], Głowacka and Rozpara [2010], Jadczuk-Tobiasz and Bednarski [2007], and Milošević and Milošević [2012] believe that the content of vitamin C in fruits of sour cherry cultivars dependents on the cultivar, the rootstock and/or the place and year of study. In the studies carried out by Podsiadło et al. [2009], it has been found that

irrigation tends to cause a decrease in the content of sugars and vitamin C in sour cherry fruits.

Literature data indicate that a vital role in the antioxidant activity is played not only by the total content of polyphenolic compounds but also by their type, i.e., anthocyanins [Usenik et al. 2008, Khoo et al. 2011]. According to these authors, antioxidant activity depends on different chemical properties and is cultivar-specific. In our research, the antioxidant activity was measured by the DPPH method and determined to be 1.43 mM Trolox 1 g^{-1} FW, which corresponds with the literature data. In the previous reports, the antioxidant activity of the sour cherry cultivars measured by the DPPH method was between 0.2 and 2.0 mM Trolox 1 g^{-1} FW [Khoo et al. 2011, Sokół-Łętowska et al. 2020].

In conclusion, the research results obtained by the authors of this paper and the findings of other authors cited in this work confirm that sour cherry is a good source of phytochemicals that contribute to the high antioxidant activity of this fruit. Among the bioactive compounds, phenolics are one of the main groups of phytochemicals present in sour cherry fruits that display a broad spectrum of health-promoting properties. Special attention has been focused on anthocyanins, the polyphenols responsible for the red skin and the flesh color. Fresh sour cherry fruits are good source of sugar, mainly glucose, fructose and saccharose, organic acids, mainly malic acid and vitamin C [Czynczyk et al. 1998, Kim and Padilla-Zakour 2004, Kim et al. 2005, Jadczuk-Tobiasz and Bednarski 2007, Horbowicz et al. 2008, Podsiadło et al. 2009, Głowacka and Rozpara 2010, Milošević and Milošević 2012, Wojdyło et al. 2014, Borowy et al. 2018, Sokół-Łętowska et al. 2020]. Due to their chemical composition, the fresh sour cherry fruits in the daily diet provide many health benefits to humans, especially in preventing and overcoming various diseases [Kunachowicz et al. 2017].

In general, the chemical composition of fruits depends on the specific cultivar, the degree of ripeness, agrotechnical treatments, geographic origin, post-harvest storage conditions, and the virus infection, especially the virus species and the cultivar tolerance to the virus.

The effect of different virus infections on the chemical composition of various host plants has been reported in the literature.

Usenik et al. [2015] have revealed that the content of anthocyanins cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, peonidin-3-O-glucoside, peonidin-3-O-rutinoside in fruits of trees infected with *Plum pox virus* (PPV) was significantly higher than in healthy trees. In their further studies, Usenik et al. [2017] have shown that in plums collected from randomly selected PPV-infected trees, severe symptoms were visible on the leaves and fruits, with the infection having caused a 4.6–6.8 times greater increase in the total anthocyanin content (cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, peonidin-3-O-glucoside and peonidin-3-O-rutinoside) compared to healthy trees. Miletic et al. [2022] have described the influence of PPV-D and PDV-Rec strains of PPV on the content of total anthocyanins [cyanidin-3-O-galactoside, cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside] in fruits of plum of the Čačanska Lepotica cv. Namely, the highest content of total anthocyanins in PPV-Rec strain samples (43.6 mg 100 g^{-1} FW) was followed by healthy samples (33.5 mg 100 g^{-1} FW) and finally by PPV-D-infected samples (24.9 mg 100 g^{-1} FW).

The authors of this paper observed the influence of PNRSV and PNRSV+CVA on the total anthocyanin content in fruits of sour cherry of the Łutówka cv. The total anthocyanin content in PNRSV- or PNRSV+CVA-infected fruits was lower than in fruits from the control trees. Virus infection showed no influence on the cyanidin-3-O-glucoside content in sour cherry, but on the other hand, virus infection showed a statistically significant influence on, i.e., a reduction of the content of cyanidin-3-O-rutinoside, cyanidn-3-O-glucosyl-rutinoside, and cyanidin-3-O-soforoside content. *Grapevine red blotch-associated virus* (GRBaV) infection has reduced the flavonoid and anthocyanin (malvidin-3-O-glucoside, petunidin-3-O-glucoside, delphinidin-3-O-glucoside, pelargodin-3-O-glucoside, and cyanidin-3-O-glucoside) content in grapevine fruits [Blanco-Ulate et al. 2017]. Lee and Martin [2009] have identified five anthocyanins, i.e., glucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin, in berries of the grapevine of the Pinot noir cv. infected with *Grapevine leafroll associated viruses-2* (GLRaV-2) and *Grapevine leafroll associated viruses-3* (GLRaV-3). In the infected samples, the content of all anthocyanins tended to be lower compared to the healthy samples, but only the

content of peonidin glucoside was significantly lower in the infected samples compared to the healthy samples. According to Guidoni et al. [1997], the content of anthocyanins in berries of the grapevine of the Nebbiolo clone 415 infected with *Grapevine leafroll associated virus-3* (GLRaV-3) and *Grapevine virus A* (GVA) has remained consistently higher than in the healthy grapevine berries.

These variations described above could be due to the plant species, plant cultivar, virus species, sampling periods, or seasons. It is unknown how the infection by GLRaV and other viruses, e.g., PNRSV or CVA, influences anthocyanin metabolism and accumulation from fruit ripening to harvest. It would be an interesting subject for future research, as it was not included within the scope of this study.

The authors of the presented paper found that fruits of PNRSV- or PNRSV+CVA-infected trees showed a higher total polyphenol content than fruits of the control trees. According to Horsáková et al. [2013], average total polyphenol content in the fruits of PPV-infected peach trees has ranged from 829 (cv. Symphony) to 904 (Royal Glory cv.) mg 100 g^{-1} FW and, in the fruits of healthy trees, from 603 (cv. Symphony) to 736 (cv. Royal Glory) mg 100 g^{-1}·FW. Jelínek et al. [2012] have detected an increased total phenolic content in hops of the Saaz cv. infected with Hop latent viroids (HLVd). Likewise, Lee and Martin [2009] have observed a higher total phenolic content in fruits from GLRaV-2- and GLRaV-3-infected grapevine of the Pinot noir cv. compared to healthy plants. Chen et al. [2018] have shown that the total phenolic content was significantly higher in the *Telosma mosaic virus* (TeMV)- infected *Passiflora edulis* fruits, with a 19.1% increase compared to the healthy fruits. The total phenolic content has been significantly higher in fruits (33.9%) and leaves (73%) of *Cucurbita moschata* infected with *Tomato leaf curl Palampur virus* compared to the healthy fruits and leaves [Jaiswal et al. 2013].

The authors of this paper found the antioxidant activity assayed with the DPPH method to be significantly higher in sour cherry fruits from infected trees (1.57 mM Trolox 1 g^{-1} FW for P+C, 1.54 mM Trolox 1 g^{-1} FW for P) than in sour cherry fruits from the control trees (1.43 mM Trolox 1 g^{-1} FW). Horsáková et al. [2013] reported that fruits from PPV-infected peach trees of the Royal Glory and Symphony cvs. had a higher antioxidant activity than those from healthy trees. The average value of the antioxidant activity in the PPV-infected fruits assayed with the DPPH method has increased by 13.2% compared to the control cultivars. Likewise, Kalogirou [2012] has stated that tomatoes infected with *Cucumber mosaic virus* (CMV) also possessed a higher antioxidant activity (0.00236 mM Trolox 1 g^{-1} FW) compared to the tomatoes of non-infected plants (0.00203 mM Trolox 1 g^{-1} FW) as assayed with the DPPH method. As a percentage, the difference between infected and non-infected tomatoes was 16.3%.

Horsáková et al. [2013] have discovered that the antioxidant activity of PPV-infected peach cultivars correlated positively with their total content of polyphenolic compounds. This positive correlation has also been confirmed by Vizzotto et al. [2007], Sochor et al. [2010], and Rop et al. [2012], who believe that the increased antioxidant activity and total polyphenolic compound content in peach fruits of PPV-infected trees is probably caused by the function of protective systems which regulate the production of reactive oxygen species and thus protect cells from oxidative damage.

Stress factors, including, among others, plant infection by pathogens, induce oxidative stress by producing increased amounts of ROS. To reduce excessive levels of ROS, plants have developed a specialized defense mechanism that includes enzymatic and non -enzymatic antioxidant systems [Jaiswal et al. 2013, Demidchik 2015, Chen et al. 2018].

The non-enzymatic antioxidant system is also referred to as the ascorbate-glutathione cycle. The AsA−GSH cycle is essential in maintaining antioxidant capacity and signal transduction in plants [Bilska et al. 2019]. Chamika Buddhinie et al. [2017] have observed a 15% increase in ascorbic acid content in the Carica papaya cv. Red Lady fruits. infected with *Papaya ring spot virus* (PRSV). In tomato (*Solanum lycopersicum*) fruits, the total ascorbic acid content increased by about 40% after infection with an attenuated *Cucumber mosaic virus* strain [Tsuda et al. 2005]. In *Brassica rapa* resistant cultivars infected with the *Turnip mosaic virus* (TuMV), about 1.5- to 1.6-fold increases in the total ascorbic acid content were observed in inoculated leaves [Fujiwara et al. 2016]. Likewise, this

paper's authors demonstrated that ascorbic acid content was significantly higher in fruits from virus-infected trees than in control trees. Additionally, ascorbic acid is active not only against viruses but also against other pathogens. For instance, treating rice blast fungus *Magnaporthe oryzae* with ascorbic acid has decreased the percentage of normal appressorium formation [Egan et al. 2007].

However, the mechanisms underlying the accumulation of ascorbic acid in response to pathogen infection remain unestablished. While studies have shown a positive correlation between ascorbic acid content and viral resistance, findings to the contrary also exist. For instance, deficiencies in ascorbic acid have been found to enhance resistance to other pathogens, such as *Pseudomonas syringae* and *P. parasitica*, in vtc mutant lines of *Arabidopsis*. It has been proposed that the elevation in ascorbic acid content following pathogen invasion shifts the ascorbic acid pool towards a more oxidative state [Barth et al. 2004]. Thus, further research is warranted to elucidate the role of ascorbic acid in the ascorbate-glutathione cycle and its interactions with other vital components of the plant's innate antioxidant defense system, particularly concerning redox changes associated with ascorbic acid. For example, in a study investigating antioxidant activity in the fruits of PPV-infected peach trees, Horsáková et al. [2013] speculated that increased oxidative stress leads to heightened ROS production, consequently triggering an upsurge in antioxidant production mediated by the antioxidant defense system. The findings of this study support this notion, as fruits from PNRSV- and PNRSV+CVA-infected Łutówka cv. trees exhibited notable increases in antioxidant activity, as well as in the anthocyanin, total polyphenol, and ascorbic acid content when compared to virus-free control trees.

CONCLUSIONS

Based on the results obtained, it is evident that PNRSV and PNRSV+CVA infections significantly influence the ripening process, particularly fruit coloration, in sour cherry cultivar Łutówka. Compared to virus-free trees, these infections led to notable alterations in anthocyanin, total phenolic, and ascorbic acid levels. Furthermore, PNRSV and PNRSV+CVA infections increased antioxidant activity in the infected trees. This study represents the first documentation of PNRSV and CVA's impact on anthocyanin, total phenolic, ascorbic acid content, and antioxidant activity in infected and virus-free fruits from Łutówka cultivar sour cherry trees sourced from commercial orchards across Poland and Europe. Further research is essential to deepen our understanding of the intricate relationship between biotic stress, such as pathogen attacks, and the aforementioned biochemical properties in infected fruit trees.

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