ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume LXI 187 Number 6, 2013
http://dx.doi.org/10.11118/actaun201361061693

ASSESSMENT OF ANTIOXIDANT ACTIVITY AND TOTAL POLYPHENOLIC COMPOUNDS OF PEACH VARIETIES INFECTED WITH THE PLUM POX VIRUS

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Received: June 12, 2013

Abstract

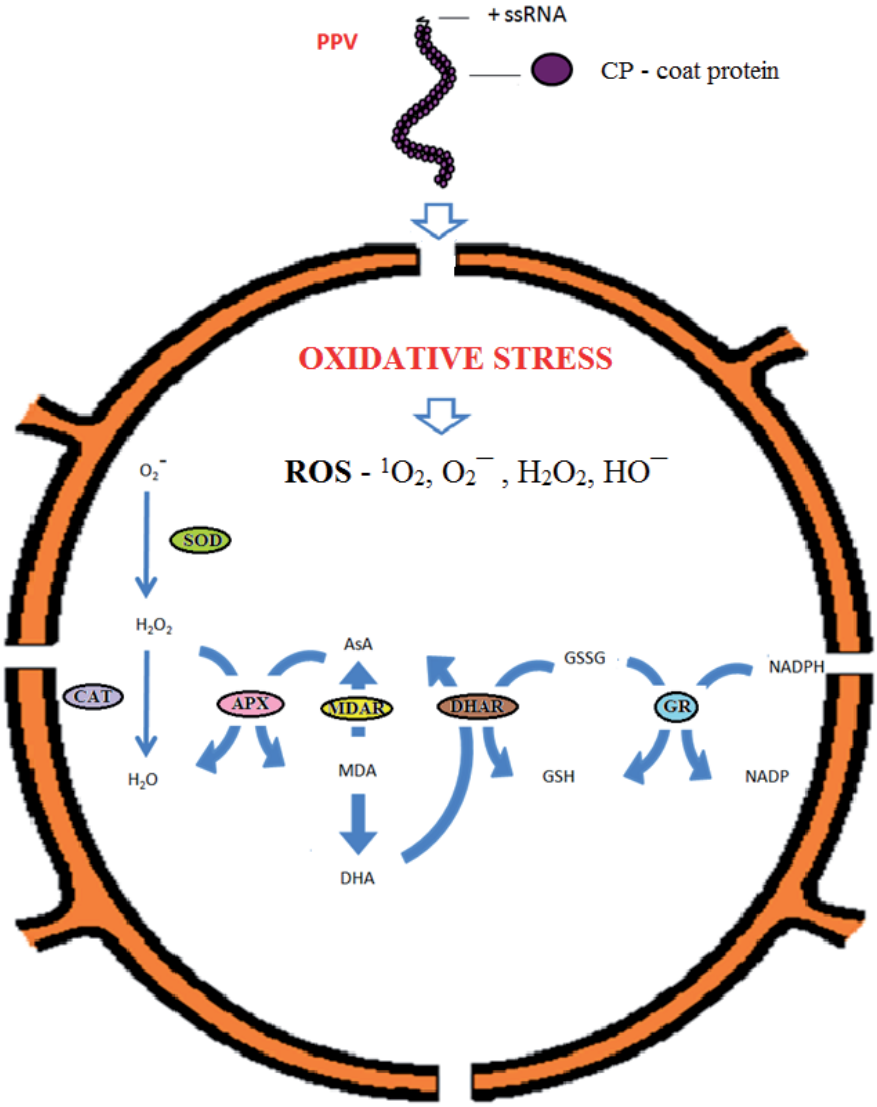
HORSÁKOVÁ JANA, SOCHOR JIŘÍ, KRŠKA BORIS: *Assessment of antioxidant activity and total polyphenolic compounds of peach varieties infected with the Plum pox virus.* Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 2013, LXI, No. 6, pp. 1693–1701

Just like in other stone fruits, also in peach trees, the Plum pox virus is commonly known to be the cause of lower yields, worse quality and smaller size of fruits and it also affects the contained substances. The fruits of peach trees infected with the Plum pox virus (PPV) were subjected to various analyses to determine the content of antioxidant activity and overall polyphenolic compounds. The evaluation took place from 2011 to 2012. To conduct this experiment, two cultivars that had been infected by PPV naturally were selected – 'Royal Glory' and 'Symphony'. Antioxidant activity was established using five principally different methods (DPPH, ABTS, FRAP, DMPD and Free Radicals). The content of total polyphenolic compounds was established using the Folin-Ciocalteu method. The results of these analyses are expressed as the equivalent of gallic acid (GAE) in $mg \cdot kg^{-1}$. Furthermore, in the period from April to October the intensity of PPV symptoms in blossoms, leaves and fruits was also assessed.

It was discovered that as a result of the PPV infection, the content of antioxidant activity as well as of total polyphenols had increased. Average reading of antioxidant activity in the PPV infected fruits had increased by 13.2 % (DPPH), 26.7 % (FRAP), 27.6 % (ABTS), 28.1 % (DMPD), 39.2 % (Free Radicals) and the content of polyphenolic compounds had gone up by about 30.4 % in comparison with the control varieties.

peaches, antioxidant activity, polyphenolic compounds, Plum pox virus

The fruits of peach trees contain, as well as other stone fruit species, a whole range of natural substances which have a positive effect on human body (BYRNE, VIZZOTTO, 2004; ROP *et al.,* 2009). Main antioxidants are thought to be carotenoids, vitamin C and polyphenolic compounds (CHANG *et al.*, 2000; TOMAS-BARBERAN *et al.*, 2001; GIL *et al.*, 2002; BYRNE *et al.*, 2009). Generally, antioxidants can be described as substances which reduce the activity of undesirable free radicals, decrease the probability of their occurrence or transfer them into less reactive or non-reactive conditions. Free radicals represent reactive oxygen species (ROS), these are atoms or molecules which, as a result of one electron missing, show a high reactivity. Under normal circumstances, ROS production in a cell is low, however, oxidative stress such as PPV can result in ROS increase (MITTLER, 2002) which in turn results in balance disruption between ROS production and elimination (ASADA, 1999). However, plants have developed very good defensive mechanisms to neutralise ROS thus protecting cells against oxidative damage. This antioxidant system is created mainly by enzymes but also substances of non-enzymatic nature (VRÁNOVÁ *et al.*, 2002). Main defensive mechanisms are superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (MITTLER, 2002). Ascorbate, ß-Carotene,



1: *Antioxidant defensive mechanisms taking place in a plant cell. Edited by MITTLER, 2002.*
ROS – reactive oxygen species, APX – ascorbate peroxidase, CAT – catalase, DHA – dehydroascorbate, DHAR – dehydroascorbate reductase, GR – glutathione reductase, GSH – glutathione – reduced, GSSG – glutathione – oxidised, MDA – monodehydroascorbate, MDAR – monodehydroascorbate reductase, SOD – superoxide dismutase, AsA – ascorbate, NADP – nicotinamide-adenine dinucleotide phosphate – oxidised, NADPH – nicotinamide-adenine dinucleotide phosphate – reduced

reduced Glutathione and α- Tocopherol are also proven to be very effective antioxidants (SHALATA *et al.*, 2001; CHEN, GALLIE, 2004).

PPV causes oxidative stress which is typical for an increased production of ROS. Protective antioxidant systems avert the initiation of chain oxidation by removing partially reduced oxygen species such as superoxide and hydrogen peroxide (WINSTON, 1990). Superoxide dismutase (SOD) catalyses the transformation of a superoxide radical to hydrogen peroxide, which is subsequently converted by catalase (CAT) or ascorbate peroxidase (APX) into water (MITTLER, 2002). Other processes take place in the so called ascorbate-glutathione cycle when, during the catalase of ascorbate peroxidase, hydrogen peroxide reacts with the ascorbate as two water molecules are formed. At the same time MDHA is formed, which either disproportionates to dehydroascorbate (DHA) and ascorbate or it is reduced to NAD(P)H ascorbate by a dependant monodehydroascorbate reductase (MDAR). Dehydroascorbate is transformed to ascorbate during a reduction with glutathione in a reaction catalised by dehydroascorbate reductase (DHAR). Oxidation of glutathione results in the formation of disulfide (GSSG) amongst the remains of cysteine of two glutathione molecules (HELDT, 2005). Oxidised glutathione is reduced by glutathione reductase which uses NADPH (BUCHANAN *et al.*, 2000).

The relationship of PPV and contained substances in fruit was only examined more closely by BRICIU (2010) who used the PPV infected leaves of the *Prunus domestica* variety to analyse the content of total polyphenols and antioxidant capacity in its fruits. Otherwise, this issue has yet to be described in more detail.

The objective of this study was to determine how PPV affects the content of antioxidant compounds in peach fruits.

MATERIAL AND METHODS

1 Plant material

Starting plant material was provided using the cultivars of peaches 'Symphony' and 'Royal Glory' which had been naturally infected with PPV. The cultivars were part of the trial plantation of fruit trees set up on the premises of the MENDELU Faculty of Horticulture in Lednice in 2004. The rootstock used was 'Julior' and the spacing of trees was 1.5 × 5 m in a shape of slender spindle. Used agrotechnical measures targeted the elimination of diseases and pests (*Taphrina deformans* – Champion, *Sphaerotheca pannosa* – Kumulus, *Monilinia laxa* – Horizon, Pirimor was used to target aphids). Cultivation conditions are those similar to corn cultivation and production. Lednice lies in a temperate climatic belt at an altitude of 176 metres above sea level. The average temperature between the months of April and October 2011 was recorded at 16.3°C with relative humidity of 68% and precipitation of 333.7 mm. Hours of sunshine accounted for 1 566 hours. In the period of April to October 2012, the average temperature was recorded at 16.5 °C. Relative humidity was recorded at 67.7% with precipitation of 329.5 mm and 1 521 hours of sunshine on record. Obtained data was based on the readings from a meteorological station which is located on the premises of MENDELU Horticulture Faculty. The average annual temperature from the years of 1961 to 1990 is 9.2 °C, during the vegetation period (April–October) it was recorded at 14.8 °C with relative humidity of 72.4% and precipitation of 338.6 mm. Average sunshine amount accounted for 1 389.9 hours.

2. Evaluation of PPV occurrence

The tested batch always contained 15 trees of each variety and was subjected to the PPV occurrence analysis test double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA). Uninfected trees of the same cultivars represented the control sample.

2.1 Principle of PPV diagnosis

PPV was detected with the aid of serological method DAS ELISA according to the CLARK, ADAMS, 1977 methodology with the use of commercial kits from the company Bioreba. A detailed methodology procedure describing the immunoenzymatic test was attached. To conduct each reading, at least three leaves (blossoms) were picked from different parts of a tree crown which had been infected with the PPV symptoms. If no PPV symptoms were present, older leaves were picked off middle sections of the shoots.

PPV symptoms were assessed according to the scale for the assessment of symptom intensity in peach trees (POLÁK, SALAVA, 2008) which was modified for the points range of 0–3.

PPV symptoms in leaves

0 – no symptoms
1 – very light, diffused spots or rings in a few leaves (1–5)
2 – medium sized, diffused spots and rings in a larger amount of leaves (50%)
3 – quite severe, diffused spots and rings in the majority of leaves, deformation of leaves.

PPV symptoms in blossoms

0 – no symptoms
1 – very light, diffused spots or rings in blossoms
2 – medium sized, diffused spots and rings in a larger amount of blossoms
3 – quite severe, diffused spots and rings in the majority of blossoms.

PPV symptoms in fruits

0 – no symptoms
1 – very light, diffused spots or rings in a small number of fruits (up to 15%), 1–2 per one fruit
2 – diffused spots and rings of medium intensity in a larger amount of fruits (25–50%)
3 – quite severe, diffused spots and rings in the majority or all of the blossoms, malformation of fruits.

The symptoms in the blossoms were evaluated on 12 April 2011 and 8 April 2012, the symptoms in the leaves were assessed in June (8 June 2011 and 8 June 2012) and the symptoms appearing in fruits were assessed at the time of ripening ('Royal Glory' – 13 July 2011 and 10 July 2012, 'Symphony' – 8 August 2011 and 10 August 2012).

Fruits for analysis were picked at the time of physiological ripeness on 13 July 2011 and 10 July 2012 (Royal Glory) and 8 August 2011 and 10 August 2012 (Symphonie). Subsequently, samples were prepared. At all times, 5 samples were collected and then homogenised with the use of hand mixer (Philips HR 1364, China). For further analysis, 5 g of homogenate was always collected and 10ml of CH_3OH (Penta, Czech Republic) added. In this manner, three samples were collected from each fruit. These samples were later shaken in a horizontal shaker (IKA KS 130 basic, Germany) for 45 minutes and then stored in the temperature of –20 C° until they were required for the actual assessment.

3. Assessment of antioxidant activity

Antioxidant activity was assessed with the aid of five methods – DPPH test, methods ABTS, FRAP, DMPD and Free Radicals. An automated spectrophotometr BS-400 (Mindray, China) was used for the analysis of samples which consisted of a cuvette area (temperature was maintained at 37 ± 0.1 °C), reagent area with a carousel for the reagents and preparation of samples (temperature was maintained at 4 ± 1 °C) and an optic detector. Source of the light is provided by a halogen-tungsten light bulb. Transmition of samples and reagents is provided by a robotic arm with a pipette dispenser. As soon as the reagents or samples of 2–45 µl are added, the content of cuvettes is mixed with an automated agitator. Contamination is minimalized thanks to the pipette dispenser as well as the agitator being flushed with MilliQ water. The readings were assessed at the wave length of λ = 450 nm (Free Radicals), λ = 505 nm (DPPH, DMPD), λ = 605 nm (FRAP), λ = 660 nm (ABTS). The equipment is fully controlled by the BS400 (Mindray, China) software.

Absorbance was calculated according to the calibration curve per the equivalent of gallic acid content (GAE).

DPPH Test

The principle of this test is the ability of the stable free radical 2.2-diphenyl-1-pikrylhydrazyl to react with hydrogen donors. DPPH• presents strong absorbance in UV-VIS spectrum. In this test, after the reduction with an antioxidant (AH) or a radical (R•) the solution is coloured according to this reaction: $DPPH^{\bullet} + AH \rightarrow DPPHH + A^{\bullet}$, $DPPH^{\bullet} + R^{\bullet} \rightarrow$ DPPH-R (PAJERO *et al*., 2000).

ABTS Method

The principle of assessment is based on neutralisation of radical cation created by single electron oxidation of synthetic chromophore ABTS• (2,2'-azinobis (3-ethylbenzothiazolin-6-sulphate) to a radical $ABTS^{\bullet}$ – e- $ABTS^{\bullet+}$ (RE *et al*., 1999).

Frap Method

The principle of this method is the reduction of iron III complexes TPTZ (2,4,6-tripyridyl-S-triazin) with ferric chloride ($FeCl_3$) which are almost colourless, (possibly of light brown colour) and after reduction they create a light blue iron II complex. Limits of this method are those of assessment being carried out at a non-physiologically low pH (3.6) and polyphenolic substances and thiols reacting slowly with the complex are not detected (BENZIE, STRAIN, 1996).

DMPD Method

This method is based on the reaction of DMPD compound (N,N-dimethyl-1,4-diaminobenzene) with iron III chloride in solution whilst a relatively stable and coloured radical form of $DMPD^{\bullet+}$ is created. The compounds with antioxidant activity can diminish the $DMPD^{\bullet+}$ radicals, therefore the solution loses its colour and the absorbance is also decreased (FOGLIANO *et al*., 1999).

Free Radicals Method

The principle of this method is the ability of chlorophyllin (chlorophyllin-sodium copper) to receive and release electrons given the current and stable change of maximum absorbance. This process is dependent on the alkaline environment with the addition of catalysis. Quantification of measured absorbance values is enabled by calibration which reflects the ability of Fe ions in alkaline environment to be transformed from Fe II to Fe III (SOCHOR *et al*., 2010a).

4. Assessment of overall polyphenolic compounds

The content of phenolic compounds was assessed with the aid of the Folin-Ciocalteu method (SINGLETON *et al*., 1999). A 0.5 ml sample was pipetted out into a cuvette and diluted with a 1.5 ml of ACS water. Subsequently, a 0.05 ml of Folin Ciocaltea reagent was added (Sigma Aldrich, USA). Absorbance was evaluated after 30 minutes at a temperature of 22 °C using a double-beam spectrophometer (SPEKOL 210 Carl Zeiss Jena, Germany) at the wave length of λ = 640 nm against the blank. The results were presented as an equivalent of gallic acid in $mg \cdot kg^{-1}$ (SOCHOR *et al*., 2010a).

5. Statistical processing of results

Obtained results were subsequently analysed statistically (a single factor analysis of diffusion, Scheffe's method), using the programme StatSoft (STATISTICA 10).

RESULTS AND DISCUSSION

1. Visual evaluation of PPV symptoms

PPV symptoms were evaluated from April to August (Tab. I). In the time of blossoming – 12 April 2011 and 8 April 2012 PPV symptoms were recorded on the petals of blossoms in the form of diffuse spots of various intensity. First visible signs of infection in leaves were recorded in the month of June and evaluated on 8 June 2011 and 8 June 2012. PPV symptoms in fruits in the time of ripening ('Royal Glory' – 13 July 2011, 10 July 2012, 'Symphony' – 8 August 2011, 10 August 2012).

Symptoms displayed in blossoms are easily visible during a regular viewing of the orchard in the time of blossoming. Therefore, this fact can be used as an early detection of the PPV infection. The intensity of PPV symptoms in the blossoms of infected trees was recorded on the scale from 1–3 points (2011) and 1–2 points (2012). Thus it is obvious that the intensity of PPV symptoms was lower in 2012. When comparing the varieties it was determined that the intensity of PPV symptoms in 'Royal Glory' had

recorded lower readings (ave. 1.66) in comparison with the 'Symphony' (ave. 1.77). The intensity of PPV symptoms detected in leaves was recorded on the scale of 1–3 points (2011) and 1–2 points (2012). The intensity of PPV symptoms was therefore lower in 2012. It was discovered that the 'Royal Glory' showed lower intensity of symptoms (ave. 1.52) than the 'Symphony' (ave. 1.8). The intensity of PPV symptoms recorded in fruits ranged from 0–3 points (2011) to 0–2 points (2012). It is also obvious here that 2012 showed lower intensity of PPV symptoms. As for the comparison of varieties it was found that the intensity of PPV symptoms in 'Royal Glory' was lower (ave. 1.05) than in the 'Symphony' (ave. 1.64).

As the statistical analyses suggest (Fig. 2), major differences of the intensity of PPV symptoms were detected between the control variety and the assessed varieties. The differences on blossoms and leaves were recorded at ($p = 0.000$) whilst in fruits only the 'Symphony' recorded vast ($p = 0.006$).

There were no statistically conclusive differences discovered in the intensity of symptoms on blossoms and leaves ($p = 0.997$; $p = 0.153$), however, conclusive differences of the intensity of symptoms were detected in fruits ($p = 0.050$).

Between the years of 2011 and 2012, statistically inconclusive differences in the intensity of symptoms were discovered in blossoms ($p = 0.871$) and in fruits ($p = 0.313$) of 'Symphony', conclusive differences in this variety were then detected in the intensity of symptoms in leaves ($p = 0.032$). As for the other assessed cultivar, statistically inconclusive differences were detected in the intensity of symptoms in blossoms ($p = 0.999$), conclusive differences in the intensity of symptoms were recorded in leaves ($p = 0.016$) and in fruits ($p = 0.011$).

Generally it is possible to say that from the viewpoint of intensity of PPV symptoms in the leaves and blossoms of the Symphony and Royal Glory cultivars there are no mutual differences. However, these two varieties differ in the intensity of symptoms in fruits.

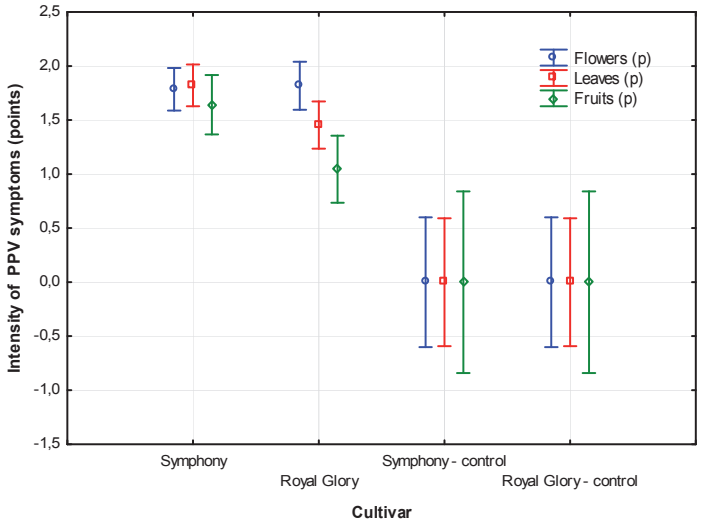
2. Assessment of antioxidant activity and the content of total polyphenols

In the fruits of PPV infected peach trees 5 methods were used to assess the antioxidant activity ((DPPH, ABTS, FRAP, DMPD, Free Radicals). The content of total polyphenols was established using the Folin-Ciocalteu method. Obtained data (Tab. II) have been recorded as the equivalent of Gallic acid in $mg \cdot kg^{-1}$.

Antioxidant activity assessment and the assessment of total polyphenols were conducted in the years of 2011–2012. In 2011, PPV infected 'Royal Glory' fruits recorded higher antioxidant activity as well as the total polyphenols content as supposed to healthy trees. All the available assessment methods, apart from the DPPH method proved this to be the case. In 2012, the 'Royal Glory' fruits of PPV infected trees also presented higher antioxidant activity and total polyphenols as supposed to the fruits of healthy trees. This was also confirmed by all the applied assessment methods except for the FRAP method. Similar results were discovered in 'Symphony' in 2012 where the fruits of PPV infected trees also showed higher antioxidant activity and total polyphenols in comparison with the fruits

I: *Intensity of PPV symptoms*

	Royal Glory						**Symphony**					
	2011			**2012**			**2011**			**2012**		
Tree no.	**Blossoms 12. 4.**	**Leaves 8. 6.**	**Fruits 13. 7.**	**Blossoms 8.4.**	**Leaves 8. 6.**	**Fruits 10. 7.**	**Blossoms 12. 4.**	**Leaves 8. 6.**	**Fruits 8. 8.**	**Blossoms 8. 8.**	**Leaves 8. 6.**	**Fruits 10. 8.**
1	3	1	2	1	1	-	2	2	2	1	1	1
2	2	2	1	1	1	0	3	3	2	2	2	1
3	2	2	1	2	1	1	2	2	3	2	2	1
4	1	2	-	1	1	-	2	2	2	2	1	2
5	2	2	2	1	1	1	1	2	2	2	2	2
6	2	1	2	2	1	0	2	2	1	1	1	1
7	2	1	1	2	1	1	1	2	3	2	2	2
8	1	1	2	2	1	0	1	2	1	1	2	1
9	1	2	-	1	2	-	2	2	1	2	2	2
10	2	2	2	2	1	0	2	2	1	2	1	1
11	2	2	2	2	1	1	2	2	1	2	2	-
12	1	2	1	2	1	1	1	2	1	2	1	1
13	1	2	0	-	-	-	2	2	3	1	1	2
14	1	2	-	2	2	-	2	2	3	1	2	1
15	2	3	2	2	2	0	3	2	2	2	1	-
Ave.	1.67	1.8	1.5	1.64	1.21	0.5	1.87	2.07	1.87	1.67	1.53	1.38
CONT	0	0	0	0	0	0	0	0	0	0	0	0



2: *Statistical evaluation of PPV symptoms intensity in leaves, flowers and fruits*

of healthy trees. This fact was confirmed by all the applied assessment methods.

Antioxidant activity readings of fruits of the PPV infected trees ranged from 246–251 mg·kg^{-1} GAE (DPPH), 411–426 mg·kg^{-1} GAE (ABTS), 830–857 mg·kg^{-1} GAE (FRAP), 499–505 mg·kg^{-1} GAE (DMPD a 1 560–1 578 mg·kg^{-1} GAE (Free Radicals), the fruit of healthy trees recorded the values of 197–242 mg·kg^{-1} GAE (DPPH), 312–344 mg·kg^{-1} GAE (ABTS), 644–688 mg·kg^{-1} GAE (FRAP), 390–394 mg·kg^{-1} GAE (DMPD) a 1 127–1 271 mg·kg^{-1} GAE (Free Radicals). Peaches of the PPV infected trees show higher readings of antioxidant activity and this is the case of all the applied methods. The methods of assessment which were used in this experiment are based on various principles; therefore the results are of varied range.

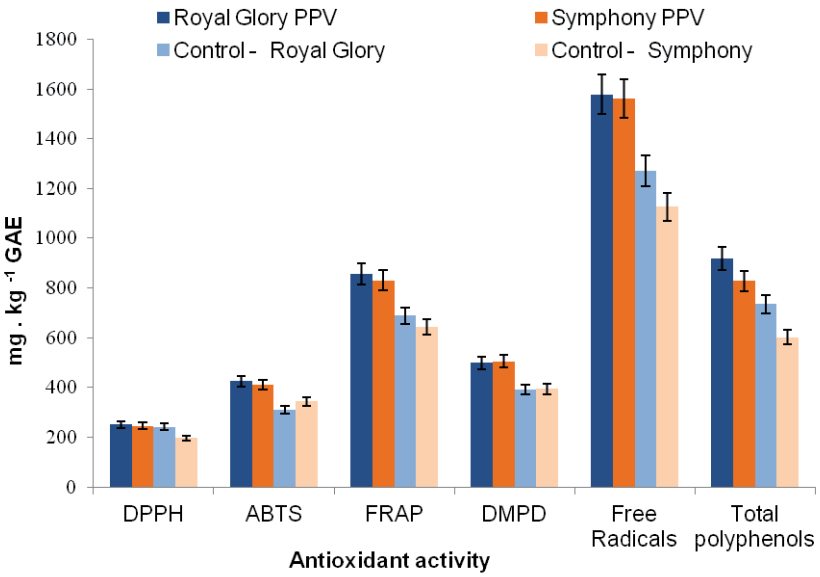
KALOGIROU (2012) states that tomatoes infected with Cucumber mosaic virus (CMV) also recorded higher antioxidant activity when assessed with the DPPH method (2.36 µM·g^{-1} TE) than in tomatoes of non-infected plants (2.03 µM·g^{-1} TE). As a percentage, the difference between infected and non-infected tomatoes recorded at 16.3%, whilst in our case of peach trees and using the same assessment method (DPPH), the difference

II: *Readings of antioxidant activity and total content of polyphenols in fruits*

2011	Antioxidant activity [mg·kg^{-1} GAE]					Total polyphenols [mg·kg^{-1} GAE]
	DPPH	ABTS	FRAP	DMPD	Free Radicals	
Royal Glory	247 ± 9.0	367 ± 2.8	747 ± 4.8	391 ± 4.0	1 223 ± 6.1	823 ± 3.5
Control	300 ± 3.4	271 ± 5.0	679 ± 3.5	340 ± 2.4	1 148 ± 4.6	745 ± 2.3
Symphonie	190 ± 4.0	257 ± 3.6	530 ± 4.2	301 ± 3.3	873 ± 5.1	473 ± 5.2
Control	218 ± 5.1	354 ± 4.5	619 ± 2.9	371 ± 5.1	924 ± 4.2	523 ± 2.7
2012						
Royal Glory	255 ± 2.3	485 ± 4.2	966 ± 2.3	607 ± 2.8	1 933 ± 3.2	1 011 ± 4.8
Control	185 ± 4.5	353 ± 2.5	697 ± 3.5	441 ± 4.6	1 394 ± 5.0	726 ± 3.5
Symphonie	310 ± 3.4	589 ± 3.7	1 177 ± 4.0	740 ± 2.3	2 354 ± 2.6	1 238 ± 4.3
Control	176 ± 3.0	334 ± 3.4	669 ± 2.2	416 ± 3.4	1 330 ± 4.8	682 ± 2.8

III: *Average readings of antioxidant activity and total content of polyphenols in fruits*

Year	Antioxidant activity [mg·kg^{-1} GAE]					Total polyphenols [mg·kg^{-1} GAE]
2011–2012	DPPH	ABTS	FRAP	DMPD	Free Radicals	
Royal Glory	251	426	857	499	1 578	917
Control	242	312	688	390	1 271	736
Symphonie	246	411	830	505	1 560	829
Control	197	344	644	394	1 127	603



3: *Average antioxidant activity and total content of polyphenols*

between the fruits of infected and healthy trees was established at 13.2%. Using the other methods, the values of antioxidant activity were up by 26.7% (FRAP), 27.6% (ABTS), 28.1% (DMPD) and 39.2% (Free Radicals). Average content of total polyphenols in the fruits of infected trees ranged from 829 ('Symphony') – 904 ('Royal Glory') mg·kg^{-1} GAE and in the fruits of healthy trees from 603 ('Symphony') – 736 ('Royal Glory') mg·kg^{-1} GAE. When compared with the control variety, the content of polyphenolic compounds went up on average by 30.4 % when compared with the control cultivar. CHANG *et al.*, (2000) presents the content of total polyphenols in peaches from 467–801 mg·kg^{-1} GAE, BRAT *et al.*, (2006), yellow flesh peach cultivar recorded on average at 593 mg·kg^{-1} GAE whilst our readings of total polyphenols content of the fruit of infected trees are higher (828.59–903.79 mg·kg^{-1} GAE).

As the statistical analyses show, there were no statistically conclusive differences between the assessed varieties ($p > 0.05$). Antioxidant activity positively correlates with the content of total polyphenols (Tab. IV). Furthermore, positive correlation between antioxidant activity and the content of total polyphenols is also confirmed by CEVALLOS *et al.*, (2006), GIL *et al.*, (2002), SOCHOR *et al.*, (2010b), VIZZOTTO (2005, 2007). ROP et al., 2012 also states a positive correlation between antioxidant activity and the total content of phenolic compounds and between vitamin C and flavanoids.

The signs of infection caused by the plum pox virus are not only visual and typical of the particular species but changes in the actual cells are also recorded (HERNANDEZ *et al.*, 2007). The Plum pox virus is one of many stress factors causing oxidative stress in cells. As a result, the production of reactive oxygen forms (free radicals) is increased. MITTLER (2002) confirms that the ROS accumulation can result in peroxidation of membrane lipids, oxidation of proteins, inhibition of enzymes and destruction of nucleic acids. LAMB, DIXON (1997) states that it is the defensive plant reactions that contribute to an increased production of ROS, RNS and also to an increased expression of many defensive genes. Considering the fact that oxidative stress contributes to an increased ROS production, the production of antioxidants also has to be increased as a result of antioxidant defensive systems taking place. This statement is verified by the results of this study where the fruits from the PPV infected trees showed higher content of antioxidant activity.

IV: *Readings of correlation coefficients between the individual assessment methods*

	ABTS	DMPD	FRAP	Free Radicals	Total polyphenols
DPPH	0.893	0.848	0.903	0.829	0.872
ABTS	-	0.937	0.955	0.913	0.918
DMPD	-	-	0.986	0.990	0.963
FRAP	-	-	-	0.980	0.982
Free Radicals	-	-	-	-	0.974

SUMMARY

The aim of this study was to establish how the values of antioxidant activity and total polyphenolic compounds of peach varieties are affected by the Plum pox virus infection.

To assess antioxidant activity and the content of total polyphenolic compounds, two cultivars ('Symphony' and 'Royal Glory') infected with PPV naturally, were chosen. Antioxidant activity was established using five principally different methods (DPPH, ABTS, FRAP, DMPD and Free Radicals). The content of total polyphenolic compounds was established using the Folin-Ciocalteu method. The results of these analyses are expressed as the equivalent of gallic acid (GAE) in $mg \cdot kg^{-1}$. Used methods of assessment are based on various principles, therefore the readings range from 197 $mg \cdot kg^{-1}$ GAE (DPPH) to 1 578 $mg \cdot kg^{-1}$ GAE (Free Radicals). From April to October, the intensity of PPV symptoms in blossoms, leaves and fruits was also evaluated.

It was discovered that on average, and as a result of the Plum pox virus infection, antioxidant activity in peach fruits and the content of total polyphenolic compounds had increased. This fact was confirmed by all the applied assessment methods. Average values of antioxidant activity in the fruits of PPV infected trees have increased by 13.2 % (DPPH), 26.7% (FRAP), 27.6% (ABTS), 28.1% (DMPD), 39.2% (Free Radicals). The content of polyphenolic compounds has, on average, increased by 30.4%.

It was proven that in the context of individual assessment methods, evaluated cultivars had not shown any conclusive differences ($p > 0.05$). It was also discovered that antioxidant activity correlated positively with the total content of polyphenolic compounds. The intensity of PPV symptoms in blossoms, leaves and fruits ranged from 0 to 3 points. In the 'Royal Glory', the intensity of PPV symptoms was recorded as very light and in some cases, the symptoms were not even recorded at all. Statistical analyses show that there were no conclusive differences in blossoms and leaves ($p = 0.997$; $p = 0.153$), however, conclusive differences were recorded in the intensity of PPV in fruits ($p = 0.050$).

Increased content of antioxidant activity 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.

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