Biological and nutritional properties of black currant berries (*Ribes nigrum* L.) under conditions of shading nets

Boban Djordjevic[1,*], Katarina Šavikin[2], Dejan Djurovic[1], Robert Veberic[3], Maja Mikulič-Petkovšek[3], Gordana Zdunić[2], Todor Vulic[1]

[1]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia

[2]Institute for Medicinal Plants Research "Dr Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia

[3]University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

[*]corresponding author: +38162227383; fax +381112199805

e-mail address: b.djordjevic@agrif.bg.ac.rs

postal address: Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia

Abstract

BACKGROUND:

Changes of environmental factors, created under influence of various shading nets, could significantly affect the biological characteristics of plants grown in such conditions as well as biosynthesis of primary metabolites and ascorbic acid. Five black currant cultivars 'Ben sarek', 'Ben nevis', 'Ben lomond', 'Ometa', and 'Čačanska crna' were cultivated in the shade of two green polyethylene nets and exposed to direct sunlight during two experimental seasons.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jsfa.6962

RESULTS:

In the control treatment, all cultivars contained the highest amounts of soluble solid content and number of flower buds per shoots in both years of cultivation. The bushes exposed to direct sunlight had the highest sunburn damage of the berries and leaves, and loss of yield. The greatest yield loss caused by berries damage in present study during experimental period had cultivar 'Ben sarek', in 2010 year 9.0% and 15.4% in 2011. Growing in shade of light green net contributed the highest content of ascorbic acid. Control bushes and bushes in the shade of light green net had significant higher radical scavenging activity with values between 1.15 to 1.22 mg/ml.

CONCLUSION:

Plants in shade of nets in both years of cultivation had lower damage of leaves and berries and percentage of loss yield, and the usage of the net economically advantageous for the growers. Fruit of black currant cultivars grown in shading conditions still represent a good source of valuable nutritive and biologically active compounds.

Keywords: Black currants; shading nets; biological traits; yield, ascorbic acid, DPPH

Introduction

Berries are an important constituent in a human nutrition with a special focus on health-promoting compounds.[1-3] They are grown all over the world in the cooler and humid climates zones with most of the commercial currant production occurring in Northern Europe.[4-5]

Currants (*Ribes* spp.) are perennial shrubs best known for their tart-tasting fruit.[6] Black currants are an important source of biologically active compounds, especially phenolics and ascorbic acid.[7-9] Due to these compounds present in high quantities black currant cultivars show very strong biological activity, such as antioxidant, anti-inflammatory, antimicrobial and anti-cancer activity.[10-12]

The quality of black currants is influenced by the amount and composition of primary metabolites, especially different organic acids and sugars. Fructose and glucose are the major sugars that contribute to the sweetness of berries. Berries with a high content of sugars also have pleasant organoleptic characteristics[13] and can be used for fresh consumption. Fresh berries are highly perishable, and their quality and shelf life can be greatly affected by different pre- and postharvest factors. The major acid, malic acid, causes a sour and bitter taste. These sensory components are affected by the genotype, harvesting time, and origin of black currants.[6]

Environment conditions such as light intensity, day length, temperature, humidity, geographic origin have a strong influence on growth.[14] Temperatures during fruit development and ripening may influence the accumulation of health functional phytochemicals in black currant berries.[15] Fruit colour is also reported to be affected by the mean temperature in the growing season.[16]

Light intensity has a large degree influence on metabolism of secondary metabolites,[17-18] firmness[19] and flavour of the fruit.[20] Also, phenological and biological properties such as floral initiation, number and length of nodes depend on solar radiation.[21]

The effect of light on plant development is complex, involving the combined effects of several photo receptor systems.[22] Solar radiation is known to stimulate the enzymes phenylalanine ammonium lyase (PAL) and chalcone synthase (CHS) and other branch-point enzymes of the phenyl propanoid pathway. PAL catalyses the transformation of phenylalanine into *trans*-cinnamic acid, which leads to the formation of complex phenolic compounds such as flavonoids, tannins and lignin.[23]

However, high temperatures and intensive solar radiation in summer cause a lot of problems in fruit growing. Sunburns on fruit result in browning and necrosis as a consequence of increased surface temperatures more than 14° C compared with air temperature.[24] Utilization of shade nets or anti-hail nets decreases the fruit damage and has a positive effects on fruit yield.[25-27]

Shading is a cultural practice which is often used to alleviate the excessive sunlight exposure of overhead plants.[28] Shading can also decrease water evaporation from plants and soil, and decrease the environmental temperature around the plants, especially in summer. However, fruit growing under the shade of nets affects the synthesis of bioactive compounds,[29-31] mainly causing a decrease of their content.

Due to the great commercial, nutritional and medicinal importance of black currants and also the lack of the data about the effects of shading nets in currants cultivation, the aim of our study was to determine the influence of changed environmental conditions caused by the use of two green shading nets on biological properties and biosynthesis of primary metabolites of different black currant cultivars over two successive seasons.

Materials and methods

Experimental conditions

Five black currant cultivars were analyzed: ‘Ben sarek’ (Scotland), ‘Ben nevis’ (Scotland), ‘Ben lomond’ (Scotland), ‘Ometa’ (Switzerland), and ‘Čačanska crna’ (Serbia). Experimental fields were conducted in village Mislodjin (Serbia), situated between 44°30’and 44°45’N latitude and 20°00’ and 20°20’E longitude, altitude between 80 and 90 m. Mislodjin is located almost in the centre of the northern warm temperate belt, with a milder climate than the typical continental. The average annual temperature in this area is ca. 11°, and during the year, the amount of rainfall is 640 l/m^2. The plantation was established on sandy loam soil, with an average aq. pH of 6.3. Planting was done with one-year nursery trees at a distance of 1.8 m between rows, and 0.8 m in the row, what resulted in ca. 6950 plants/ha. Two factorial experiments (cultivar × treatment) were set up in random field and samples were taken from five bushes. Currant bushes were either grown in the shade of two green polyethylene nets (shade treatments, light green color –LGN and dark green color-DGN, mass 36 and 50 g/m^2, respectively) which according to the manufacturer's specifications (Sigma Promet, Bečej, Serbia) retain 30% and 50% of sun light or exposed to direct sunlight (control treatment). Polyethylene network was constructed from UV stabilized materials (300Kly). Network was set up across the shrubs of the shade treatment at a height of 2.5 m.

The shading nets were placed above the bushes from end of April till early of May depending of stage of fruit set and removed about one month after harvest for each cultivar. After the network had been set up above the plants, environmental measurements were performed every day at 8, 12 and 17 h. Measurements included the measurement of air temperature and the surface temperature of berries and leaves, relative humidity and light intensity[31]. The air temperature and relative humidity were measured at a height of 2 m using automatic weather stations Meteos Compact, PESSLINSTRUMENTS GmbH, Austria set in the immediate vicinity of the planting. Surface temperature of leaves and berries were measured with infrared thermometer DT-8188, PCE – GmbH, Germany (measurement range −50 to 550°C, accuracy ±1.5%; resolution between 0.1–50 and +200°C), and light intensity was measured with lux meter PRO-LX 1108, Electricals Electronics Enterprises, India (ranges 0.1–400,000 lx, accuracy ±3%, resolution 0.01–100 lx).

Biological properties

During two years, the following biological properties were studied: harvest time, shoot length, number of flower buds per shoot, mass and length of fruit cluster, number of berries per fruit cluster, berry mass , percentage of damaged berries and leaves, yield per bush and percentage of loss yield. Clusters mass and berry mass was scale-weighed and expressed in g, whereas cluster length was measured with a ruler and expressed in cm. Shoot length was measured by ruler in cm.

Berries were hand-harvested in the fourth and fifth years of vegetation in June and July 2010–2011, depending on the commercial ripening time for each cultivar (90% colored fruit). All cluster observations were made on 50 clusters sampled randomly from all the positions of the shoots. Berries were collected from five bushes (500 g per bush) of each cultivar. All of the samples from each cultivar were consolidated, thus representing average sample for further analysis. After harvesting, the berries were stored at +5°C and analysed within 24 h.

2.3. Determination and Extraction of Soluble Solid, Sugars and Organic Acids

Primary metabolites (soluble solids content, glucose, fructose, sucrose, citric, tartaric and malic acid) were analysed in the whole fruit without seeds. For each cultivar, five repetitions per sampling date were carried out (n=5); each repetition included several fruit. Soluble solids content was determined by refractometer (Atago, pocket PAL-1. Kyoto, Japan). For the extraction of primary metabolites, 10 g of fruit were homogenized in 50 mL of double distilled water using Ultra-Turrax T-25 (Ika-Labortechnik) and left for 30 min at room temperature. After the extraction, the homogenate was centrifuged (Eppendorf Centrifuge 5810 R) at 12000 rpm for 7 min at 10 °C. The supernatant was filtered through a

0.45 μm cellulose ester filter (Macherey-Nagel) and transferred into a vial, and 20 μL of the sample was used for the analysis. The analysis of primary metabolites was carried out using high-performance liquid chromatograph (HPLC) of Thermo Separation Products. The separation of sugars was carried out using a Rezex RCM-monosaccharide column (300 mm × 7.8 mm) from Phenomenex operated at 65 °C. The mobile phase was double distilled water, and the flow rate was 0.6 mL min^{-1}; the total run time was 30 min, and a refractive index (RI) detector was used to monitor the eluted carbohydrates.[32] Organic acids were analyzed on a HPLC using an Aminex HPX- 87H column (300 mm × 7.8 mm) with a UV detector set at 210 nm.[33] The column temperature was set at 65 °C. The elution solvent was 4 mM sulphuric acid in double distilled water at a flow rate of 0.6 mL min^{-1}. The duration of the analysis was 30 min. The concentration of an individual metabolite was calculated according to a calibration curve of corresponding standard solutions. The content of all analysed sugars was summed up and presented as total analyzed sugars. In a similar way, total analysed organic acids were calculated. Both values were used for the determination of total sugar/organic acid ratio. The sweetness index was calculated by multiplying the sweetness coefficient of each individual sugar (glucose = 1, fructose = 2.3, and sucrose = 1.35).[33]

2.4. Extraction and Determination of Vitamin C.

Vitamin C was quantified using the reflectometer set of Merck Co (Merck RQflex).[34] Fruit sample (5 g) and 20 mL oxalic acid (1%) were mixed, homogenized for 1 min, and filtered. PVPP (polyvinylpolypyrrolidone) (500 g) was added to 10 mL of the filtered sample to remove phenols, and 6−7 drops of H_2SO_4 (25%) were added, to reduce the pH level below 1.14 Results were expressed as mg ascorbic acid 100 g^{-1} fresh mass (FW).

2.5. DPPH radical scavenging activity

The free radical scavenging activity (RSA) of berries on the stable1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical was carried out according to the procedure described previously with slight modifications.[30] The antiradical capacity of each extract was evaluated using a dilutions series, in order to obtain a large spectrum of sample concentrations. The extracts (100 µl) were mixed with 1400 µM methanolic solution of DPPH. The antiradical capacity of each sample was evaluated using a dilutions series, in order to obtain a large spectrum of sample concentrations. Absorbance at 517 nm was measured after 20 min. The percentage of inhibition was calculated using equation:

$$\text{Inhibition} = A_0 - A_i / A_0 \times 100,$$

where A_0 is absorbance of the control and A_i is absorbance of the samples. IC_{50} values were estimated using a nonlinear regression algorithm. All test analyses were run in triplicate. Trolox was used as a positive control.

2.6. Statistical analysis.

All data were reported as mean ± standard error of triplicate determinations. Since all observed parameters were analysed by two-way analysis of variance (ANOVA) showed significant interactions between treatments (cultivar × shading), null hypothesis of equal means for each treatment separately has been analysed using one-way ANOVA with *post hoc* Fisher's least significant difference test (LSD) as a method for comparing treatment group means at $P < 0.05$ level. Critical values for pairwise comparisons of the means are declared in tables for each treatment. Radical scavenging activity data were presented in original values in table, but statistical test was performed on its reciprocal data since IC_{50} value is inversely proportional to antiradical activity. Degree of linear dependence between metabolites was calculated by Pearson's correlation (r), and significance of correlation was performed using SPSS 11.5.3. For determination of sequential classification according to the desirable properties among all examined varieties, Ivanovic's distance (I-distance).[35]

Results and discussion

Environmental measurements

Values of environmental parameters are shown in figure 1. The average values of air temperature, leaf and berries, and light intensity were higher in 2011, while only the average value of the relative humidity was higher in 2010. The highest leaf and berry temperature in both experimental years were recorded in the third measurements (at 17^h). At bushes exposed to the direct sunlight a higher light intensity of 35-65% was measured. Berry and leaf temperatures were lower on plants grown in shade of dark green net, for about 3.1 to 7.2°C, respectively, compared to control plants.

Biological properties

Biological properties were significantly depended on cultivars and intensively of solar insolation. The earliest ripening time in both experimental years had cultivar 'Ben sarek', and the latest cultivar 'Ometa'(table1). All cultivars had earlier ripening time in 2011 probably as a result of higher temperatures during the maturation. In 2010 using LGN and DGN delayed ripening time for 5 to 7 days and 6 to 10 days, respectively, compared to non-shade shrubs. In 2011 ripening time was less delayed, for 2 to 6 and 6 to 9 days in LGN and DGN treatments, respectively. Cultivars 'Ben sarek' and 'Čačanska crna' matured in year 2011 in average for 5 days earlier regarding to the year 2010. Our results confirmed these findings reported by Rubinskiene *et al*.[36]

Percentage of damaged leaves due to sunburn depended of cultivars and treatments (figure 2). In 2010 year cultivars 'Ben sarek' and 'Ben nevis' had statistically significant higher percentage of damage leaves. Both cultivars in non-shade bushes had more than 33% damage leaves. In year 2011 all cultivars except 'Ben lomond' had higher percentage damage. Cultivar 'Ben sarek' had statistically significant the highest leaves damage (16.1%) and 'Ben lomond' (5.4%) the smallest. Unlike in the first season, in the second experimental year existed significant difference between treatments. Control bushes of cultivars 'Ben sarek', 'Ben nevis' and 'Ometa' had more than 30% damage leaves.

Like in the case of leaves, significant damage of berries was measurement on bushes directly exposed to sunlight (figure 2). Temperature of leaves and fruits surface higher than 14°C compared with air temperature leads to sunburn occurrence.[24] In 2010 year the highest berry damage had cultivar 'Ben sarek' (9.02%), while the smallest had 'Ben lomond' (2.46%). Berries of all cultivars in shade of dark green net had the lowest percentage of damage, but a significantly difference in level of damage between nets in this experimental season was not recorded. In second year total damage of berries increased for 78%. All cultivars except 'Ben lomond' showed increased level of berry damage. Cultivar 'Ben lomond' have moderate to very vigorous bushes with large leaves which cover berries, and due to affect to low level of berries damage. Also, was recorded significant correlation between length of one-year old shoots and berries damage, in year 2010 $r=0.95$ and $r=0.97$ in 2011. The cultivars which had longer shoots had lower percentage of damage berries. In 2011 year statistically significant differences between all treatments were noted. In both experimental years only cultivar 'Ben lomond' did not have damaged berries in shade of dark green net. The greatest loss of yield caused by berries damage in present study in both years had cultivar ‘Ben sarek’, in year 2010 9.0% and 15.4% in 2011 and the smallest with 2,5% in both years cultivar ‘Ben lomond’(figure 2). All cultivars, except ‘Ben lomond’ had increase of loss of yield in 2011 year. Influence of cultivars and treatments and their interaction were statistically significant to percentage of loss yield. Also, a significant correlation between the degree of susceptibility of berries and leaves to damage were recorded in both years, in year 2010 r=0.78 and r=0.82 in 2011.

In year 2010 cultivar ‘Ben sarek’ had the shortest one-year old shoots while cultivar ‘Ben lomond’ had the longest (table 1). Obtained results in our study confirmed the results by Kampuss and Strautina[37] and Sasnauskas *et al*.[38] All cultivars in non-shade bushes had statistically significant shortest shoots (96.7 cm), while in LGN treatment shoots were the longest (104.7 cm). In this year the cultivars ‘Ben sarek’ and ‘Ben nevis’ did not show significant influence of treatments on the shoots length. In the second year average shoot length in all cultivars was shortest. Cultivars ‘Ben sarek’ (79.8 cm) and ‘Ben nevis’ (86.7 cm) had the shortest shoots length and the longest was measured in the ‘Ben lomond’ cultivar. In this year most of the cultivars had the longest shoots in shade of dark green net.

Number of flower buds per one-old shoot depended on the cultivar and treatment, and their interaction in year 2010 was not significant (table 1). Cultivar ‘Ometa’ had the greatest amount of flower buds (15.5) and cultivar ‘Ben nevis’ (11.6) the lowest. In this year all cultivars in the shade of dark green net had significantly less flower buds. In second year, in all examined cultivars the number of flower buds per shoots increased. Cultivar ‘Ben lomond’ had the highest while cultivar ‘Ben nevis’ still had the lowest number. In this year the control bushes had the highest number of flower buds due to increased differentiation. The increase of floral buds per shoots was affected by higher exposure to sun radiation.[21] Only in shade of dark green net all cultivars had lower number of flower buds.

Cultivar 'Ben sarek' in first year had a significantly higher mass of clusters compared to other cultivars (table 1). In the year 2011, in all cultivars except 'Ben sarek' mass of clusters was higher. In this year cultivar 'Ometa' had the highest mass of clusters and cultivar 'Čačanska crna' still had the lowest. Our results in present study are in agreement with findings by Giongo *et al*.[39] In both experimental years bushes in shade of dark green net had the highest mass of clusters. Also, interaction between treatments and cultivars had a statistically significant influence to cluster mass. In both years cultivars 'Ben lomond' and 'Ometa' had the highest number of berries per clusters. The treatments did not have influence while genetic predisposition of cultivars had significantly impact.

The highest mass of berries in experimental period had cultivars 'Ben nevis' and 'Ben sarek' and the lowest had 'Ben lomond' and 'Čačanska crna'. Also, shading nets had statistically significant effects of berries mass. Generally, all of control bushes in both years had significantly lower mass of berries. The strongest effects on mass of berries had percentage of damage leaves, which confirmed coefficient correlation (r=0.82 in 2010 year and r=0.87 in 2011). Smaller mass of berries was probably the results of sunburn necrosis of damage leaves. According to Racskó *et al*.[40] thermal death of cells caused inactivation normal photosynthetic system.

Cultivar 'Ometa' in the year 2010 had the highest and 'Čačanska crna' the smallest yield per bush. In this year differences between treatments were not significant. In second year despite cultivar 'Ben sarek', all cultivars showed a decrease in yield, values of decrease in yield ranged between 2 to 31%. Also, the highest decrease of yield recorded in treatment with DGN, 18% compared with previous year. Cultivar 'Ben sarek' was the only which had significantly higher yield in the control bushes compared with other treatments, despite increased of berry damage. That was the result of a significant increase of generative buds in those bushes. Moreover, the strongest effects on the yield had number of generative buds, where correlation coefficient in year 2010 was r=0.81 and in 2011 r=0.89.

Soluble Solid, Sugars, Organic and Ascorbic Acids

Chemical composition of berries depended on cultivars, treatments and their interaction (table 2). The relationship between sunlight exposure and temperature of clusters is important to berry composition and metabolism.[41] In both experimental years, the highest soluble solid content (SSC) were detected in berries of cultivar 'Ometa' (16.6 and 17.0°Brix) similarly with findings of Pedersen.[42] Berries of fully exposed bushes in 2010 and 2011 had significantly the highest soluble solids content.[18] High commercial parameters such as organic acids and sugars, and their ratios, play important roles in the character and quality of the flavour of berries.[43]

Cultivar 'Ometa' had the highest content of total sugars, in both years over 100 g/kg fresh fruit in control bushes (table 2). Dark green net contributed a significant reduction in the total sugar content of the berries, in some cultivars more than 2.3-fold. Sugars in black currant fruit are mainly mono- and disaccharides (glucose, fructose, and sucrose), and the relative proportion of these individual sugars is important for the perception of sweetness.[13,44] The highest contents of these sugars in both experimental years had cultivar 'Ometa' and the smallest 'Ben lomond'. In all cultivars fructose was the most abundant sugar, and also is characteristically sweeter than glucose or sucrose.[45] In the year 2011 four out of five cultivars had higher content of fructose in bushes of shade of light green net. In this year only LGN resulted in the increase of fructose content. The fructose to glucose ratio was 1:0.8 in 2010 and 1:0.7 in 2011 year. Contents of glucose were in generally the highest in the control bushes. In all cultivars and treatments in year 2011 a decrease of glucose was recorded. The amount of sucrose was lower and varied among different treatments and cultivars. The fruit sugars concentrations showed a significant difference between control and shading treatments, and the sucrose and glucose concentration was inversely proportional to the level of shading.[46] Zhang *et al*.[13] showed that contents of fructose, glucose, and total sugar were different between the varieties and weather conditions. Differences in the sugar distribution between cultivars during the experimental period influenced the sweetness index showing a similar tendency. In both years cultivar ‘Ometa’ had the greatest sweetness index. Much lower sweetness index was recorded in the second year of investigation due to lower amounts of individual sugars contained in the berries.

The predominant organic acid in both years was citric acid (table 2). In the total content of organic acids content of citric acid was more than 75%. The highest amount of citric acid in the year 2010 had cultivar ‘Ben nevis’. Also, in this year control bushes and bushes in shade of light green net had significant higher values of citric acid. Joscelyne *et al*.[17] determined that grape berries in the shadow had a lower acid concentration compared with fully exposed ones. However, in the following year bushes in shade of dark green net had an increased content. Malic and tartaric acids were present in both years in about 4.1- (malic) to 16.8-fold (tartaric) lower quantities compared to citric acid, which is more than in the research done by Bordonaba and Terry[47] and Milivojević *et al*.[6] The highest amounts of malic acid in 2010 and 2011 year had cultivars ‘Ben nevis’ and ‘Ben lomond’, respectively, and the smallest in both years had cultivar ‘Čačanska crna’. Comparing total analysed organic acids among the cultivars, ‘Ben nevis’ cultivar contained the highest amounts, while cultivar ‘Čačanska crna’ contained the lowest. Among the treatments non-shade bushes contained the highest amounts of total acids in both years. A good measure for the perception of sweet and sour taste is the sugar/organic acid ratio. Because of a high amount of sugars and low amount of organic acids, this ratio was highest in 'Čačanska crna' and ‘Ometa’. Both cultivars were therefore sweeter tasting as compared to others where the ratio was lower.

Extraction and Determination of Vitamin C

The highest content of ascorbic acid in the year 2010 (table 2) was found in cultivar 'Ometa' (2.53 g/kg FW), and the lowest had 'Čačanska crna' (2.16 g/kg FW). Bushes in shade of light green net had significantly higher amounts of ascorbic acid compared to other treatments. Also, interaction between cultivars and treatments influenced significantly to ascorbic acid content. However, cultivar 'Ben lomond' had the highest amounts of ascorbic acid in control treatment and whereas 'Ometa' in shade of dark green net. In the year 2011 in all cultivars and treatments a decrease in ascorbic acid content for over 25% compared to previous year was noticed. In this year amount of ascorbic acid was the highest in the 'Ben nevis' cultivar and the lowest in 'Ben lomond'. In all cultivars the highest vitamin C level was measured in bushes in shade of light green net. The diversity of obtained results might be due to the difference of plant adaptability to shading. When plants are cultivated in such environmental conditions that differ from their native habitat, their ability to grow and develop will mainly depend on their capacity to acclimatize at the level of photosynthesis.[48] Zhang *et al*.[13] emphasized negative correlations of ascorbic acid amounts with the radiation during the last month before harvest and during the last week before harvest in all of the varieties. Vagiri *et al*.[49] found, using a method of principal component analysis, that strongest influence on the content of most of the bioactive compounds had location and year.

Radical scavenging activity

In our study, all tested cultivars expressed high value of radical scavenging activity against DPPH radical and significant differences among cultivars and treatments were noticed (table 2). In the year 2010 the ‘Ometa’ cultivar showed the strongest DPPH radical scavenging activity with an IC_{50} value of 1.13 mg/ml, while the lowest was noticed in variety

‘Ben nevis’ (1.28 mg/ml). Also, control bushes and bushes in the shade of light green net had significant higher radical scavenging activity. Significant correlation ($r=0.57$, $P<0.01$) between contents of ascorbic acid and radical scavenging activity was noticed. In the second experimental year, in all cultivars, decreased level of radical scavenging activity was noticed probably due to a lower amount of total phenols which was presented in our previous paper.[30] The highest DPPH radical scavenging activity showed bushes in shade of light green net. In this year, significant correlation between ascorbic acid and radical scavenging activity was not observed ($r=0.31$, $P<0.01$).

Sequential classification according to Ivanovic’s distance

For sequential classification of cultivars, yield, vitamin C content, soluble solid content, leaf damage and number of flower buds have been chosen as desirable properties, and yield has been taken into account as the most important parameter. Discrimination effects are calculated as the distances from a fictitious cultivar being defined with minimum values of desirable properties. The developed rank list (table 3) indicates the current position of the analysed black currant cultivars in these environment conditions. In both experimental years cultivar ‘Ometa’ had the highest ranking in control treatment and in the plants in shade of light green net.

Conclusion

Successful growing of black currant depends on the influence of a large number of environmental factors, especially light and temperature. A moderate change of environmental factors by using different types of shading nets resulted in the improvement of conditions for the cultivation. In our study, all black currant cultivars in the shaded treatments demonstrated a lower generative potential when compared to the fully exposed ones based on the number of generative buds. All control plants had higher values of primary metabolites, especially soluble solid content and total organic acids. Plants in shade of nets in both years of cultivation had lower damage of leaves and berries and percentage of loss yield, and the usage of the net economically advantageous for the growers. Bushes in LGN treatment had significantly the highest amounts of ascorbic acid in both experimental years. Also, in second year all cultivars in LGN treatment had higher level of radical scavenging activity. Fruit of black currant cultivars grown in shading conditions still represent a good source of valuable nutritive and biologically active compounds. In these environment conditions better biological and biochemical properties compared to other cultivars was recorded for the cultivar 'Ometa'.

Acknowledgment

The authors acknowledge their gratitude to the Ministry of Education, Science and Technological Development of Serbia for financial support, project number 46013.

Reference

1. Gulcin I, Topal, F, Cakmakci, R, Bilsel, M, Goren, AC and Erdogan, U, Pomological Features, Nutritional Quality, Polyphenol Content Analysis, and Antioxidant Properties of Domesticated and 3 Wild Ecotype Forms of Raspberries (*Rubus idaeus* L.). J Food Sci **76**(4): 585-593 (2011).
2. Hudec, J, Bakosÿ, D, Mravec, D, Kobida, L, Burdovaä, M, Turianica, I and Hlušek, J, Content of Phenolic Compounds and Free Polyamines in Black Chokeberry (Aronia

melanocarpa) after Application of Polyamine Biosynthesis Regulators. J Agric Food Chem **54**: 3625-3628 (2006).

3. Mikkonen, TP, Määttä, KR, Hukkanen, AT, Kokko, HI, Torronen, AR, Karenlampi, SO and Karjalainen, RO, Flavonol content varies among black currant cultivars. J Agric Food Chem **49**: 3274–3277 (2001).
4. Godjevac, D, Tesevic, V, Vajs, V, Milosavljevic, S, Zdunic, G, Djordjevic, B Stankovic, M, Chemical Composition of Currant Seed Extracts and Their Protective Effect on Human Lymphocytes DNA. J Food Sci **77**(7): 779-783 (2012).
5. Hummer, KE and Dale, A, Horticulture of Ribes. Forest Pathol **40**: 251−263 (2010).
6. Milivojević, J, Slatnar, A, Mikulić-Petkovšek, M, Stampar, F, Nikolić, M and Veberič, R, The influence of early yield on the accumulation of major taste and health-related compounds in black and red currant cultivars (*Ribes spp*.). J Agric Food Chem **60**: 2682−2691 (2012).
7. Djordjević, B, Šavikin, K, Zdunić, G, Janković, T, Vulić, T, Pljevljakušić, D and Oparnica, Č, Biochemical Properties of the Fresh and Frozen Black Currants and Juices. J Med Food **16**(1): 73-81 (2013).
8. Kondakova, V, Tsvetkov, I, Batchvarova, R, Badjakov, I, Dzhambazova, T and Slavov, S, Phenol compounds—qualitative index in small fruits. Biotechnol Equip **23**(4): 1444–1448 (2009).
9. Maatta-Riihinen, KR, Kamal-Eldin, A, Mattila, PH, Gonzaälez-Paramaä, AM and Torronen, AR, Distribution and contents of phenolic compounds in eighteen scandinavian berry species. J Agric Food Chem **52**: 4477-4486 (2004).
10. Beattie, J, Crozier, A and Duthie, GG, Potential health benefits of berries. Curr Nutr Food Sci **1**: 71-86 (2005).

11. Mazza, G, Anthocyanins and heart health. Ann Ist Super Sanità **43**(4): 369-374 (2007).

12. Tabart, J, Kevers, C, Evers, D and Dommes, J, Ascorbic acid, phenolic acid, flavonoid, and carotenoid profiles of selected extracts from Ribes nigrum. J Agric Food Chem **59**: 4763–4770 (2011).

13. Zhang, J, Yang, B, Tuomasjukka, S, Ou, S and Kallio, H, Effects of latitude and weather conditions on contents of sugars, fruit acids, and ascorbic acid in black currant (Ribes nigrum L.) juice. J Agric Food Chem **57**: 2977−2987 (2009).

14. Uleberg, E, Rohloff, J, Jaakola, L, Trôst, K, Junttila, O, Häggman, H and Martinussen, I, Effects of Temperature and Photoperiod on Yield and Chemical Composition of Northern and Southern Clones of Bilberry (Vaccinium myrtillus L.). J Agric Food Chem **60**: 10406−10414 (2012).

15. Krüger, E, Dietrich, H, Hey, M and Patz, CD, Effects of cultivar, yield, berry mass, temperature and ripening stage on bioactive compounds of black currants. J Appl Bot Food Qual **84**: 40-46 (2011).

16. Wang, SY and Zhang, W, Effect of Plant Growth Temperature on Antioxidant Capacity in Strawberry. J Agric Food Chem **49**(10): 4977-4982 (2001).

17. Joscelyne, VL, Downey, MO, Mazza, M and Bastian, SP, Partial Shading of Cabernet Sauvignon and Shiraz Vines Altered Wine Color and Mouthfeel Attributes, but Increased Exposure Had Little Impact. J Agric Food Chem **55**: 10888–10896 (2007).

18. Ubi, BE, External stimulation of anthocyanin biosynthesis in apple fruit. Food Agric Environ **2**: 65–70 (2004).

19. Ordidge, M, Garcıa-Macias, P, Battey, NH, Gordon, MH, John, P, Lovegrove, JA, Vysini, E, Wagstaffe, A and Hadley, P, Development of colour and firmness in

strawberry crops is UV light sensitive, but colour is not a good predictor of several quality parameters. J Sci Food Agric **92**: 1597–1604 (2012).

20. Schwartz, E, Tzulker, R, Glazer, I, Bar-Yaakov, I, Wiesman, Z, Tripler, E, Bar-Ilan, I, Fromm, H, Borochov-Neori, H, Holland, D and Amir, R, Environmental conditions affect the color, taste, and antioxidant capacity of 11 pomegranate accessions' fruits. J Agric Food Chem **57**: 9197−9209 (2009).
21. Heide, OM and Sønsteby, A, Floral initiation in black currant cultivars (Ribes nigrum L.): Effects of plant size, photoperiod, temperature, and duration of short day exposure. Sci Hortic **138**: 64–72 (2012).
22. Oren-Shamir, M, Gussakovsky, EE, Shpiegel, E, Nissim-Levi, A, Ratner, K, Ovadia, R, Giller, YE and Shahak, Y, Coloured shade nets can improve the yield andquality of green decorative branches of Pittosporum variegatum. J Hortic Sci Biotechnol **76**(3): 353–361 (2001).
23. Dixon, RA and Paiva, NL, Stress-induced phenylpropanoid metabolism. Plant Cell **7**: 1085–1097 (1995).
24. Schrader, LE, Zhang, J, Sun, J, Xu, J, Elfving DC and Kahn C, Postharvest Changes in Internal Fruit Quality in Apples with Sunburn Browning. J Amer Soc Hort Sci **134**(1):148–155 (2009).
25. Iglesias, I and Alegre, S, The effect of anti-hail nets on fruit protection, radiation, temperature, quality and profi tability of 'Mondial Gala' apples. J Appl Horti **8**(2): 91-100 (2006).
26. Lobos, GA, Retamales, JB, Del Pozo, A, Hancock, JF and Flore, JA, Physiological response of Vaccinium corymbosum 'Elliott' to shading nets in Michigan. Acta Hortic **810**: 465–470 (2009).

27. Retamales, JB, Montecino, JM, Lobos, GA and Rojas, LA, Colored shading nets increase yields and profitability of highbush blueberries. Acta Hortic **770**: 193–197 (2008).

28. Liu, C and Liu, Y, Impacts of shading in field on micro-environmental factors around plants and quality of pineapple fruits. J Food Agric Environ **10**(2): 741–745 (2012).

29. Jakopic, J, Stampar, F and Veberic, R, The influence of exposure to light on the phenolic content of 'Fuji' apple. Sci Hortic **123**: 234–239 (2009).

30. Šavikin, K, Mikulič-Petkovšek, M, Djordjević, B, Zdunić, G, Janković, T, Djurović, D and Veberič, R, Influence of shading net on polyphenol profile and radical scavengingactivity in different varieties of black currant berries. Sci Hortic **160**: 20–28 (2013).

31. Spayd, SE, Tarara, JM, Mee, DL and Ferguson, JC, Separation of sunlight and temperature effects on the composition of Vitis vinifera cv. Merlot berries. Am J Enol Vitic **53**: 171–182 (2002).

32. Dolenc-Sturm, K, Stampar, F and Usenik, V, Evaluating of some quality parameters of different apricot cultivars using HPLC method. Acta Aliment **28**: 297−309 (1999).

33. Keutgen, A and Pawelzik, E, Modifications of taste-relevant compounds in strawberry fruit under NaCl salinity. Food Chem **105**: 1487−1494 (2007).

34. Pantelidis, GE, Vasilakakis, M, Manganaris, GA and Diamantidis, G, Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and cornelian cherries. Food Chem **102**: 777−783 (2007).

35. Ivanovic, B, Classification Theory. Institute for Industrial Economics, Belgrade, Yugoslavia (in Serbian), pp. 75-102 (1977).

36. Rubinskiene, M, Viškelis, P, Stanys, V, Šikšnianas, T and Sasnauskas, A, Quality changes in black currant berries during ripening. Sodininkyste ir Daržininkyste **27**(2): 235-243 (2008).

37. Kampuss, K and Strautina, S, Evaluation of blackcurrant genetic resources for sustainable production. J Fruit Ornam Plant Res **12**: 147-158 (2004).

38. Sasnauskas, A, Rugienius, R, Šikšnianas, T, Uselis, N, Raudonis, L, Valiuškate, A, Brazaityte, A, Viškelis, P and Rubinskiene, M, Small berry research according to COST 863 Action. Sodininkyste ir Daržininkyste **27**(2): 389-400 (2008).

39. Giongo, L, Grisenti, M, Eccher, M, Palchetti, A, Vrhovsek, U and Mattivi, F, Horticultural and nutritional qualities of white, red and black currants. Acta Hort **777**: 167-172 (2008).

40. Racskó, J, Szabó, T, Nyéki, J, Soltész, M, Nagy, PT, Miller, DD and Szabó, Z, Characterization of sunburn damage to apple fruits and leaves. Int J Hort Sci **16**(4): 15–20 (2010).

41. Spayd, SE, Tarara, JM, Mee, DL and Ferguson, JC, Separation of sunlight and temperature effects on the composition of Vitis vinifera cv. Merlot Berries. Am J Enol Vitic **53**(3): 171-182 (2002).

42. Pedersen, HL, Juice quality and yield capacity of black currant cultivars in Denmark. Acta Hortic **777**: 510-516 (2008).

43. Battino, M and Mezzetti, B, Update on fruit antioxidant capacity: a key tool for Mediterranean diet. Public Health Nutr **9**: 1099–1103 (2006).

44. Milivojevic, J, Maksimovic, V and Nikolic, M, Sugar and organic acids profile in the fruits of black and red currant cultivars. J Agric Sci **54**: 105−117 (2009).

45. Djordjevic, B, Šavikin, K, Zdunić, G, Janković, T, Vulić, T, Oparnica, Č and Radivojević, D, Biochemical Properties of Red Currant Varieties in Relation to Storage. Plant Foods Hum Nutr **65**(4): 326-332 (2010).

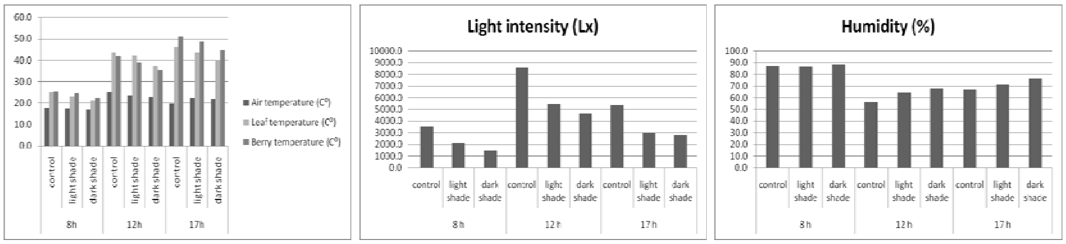
46. Watson, R, Wright, CJ, McBurney, T and Linforth, RS, Influence of harvest date and light integral on the development of strawberry flavour compounds. J Exp Bot **53**: 2121-2129 (2002).

47. Bordonaba, JG and Terry, LA, Biochemical profiling and chemometric analysis of seventeen UK-grown black currant cultivars. J Agric Food Chem **56**: 7422–7430 (2008).

48. Pastenes, C, Santa-María, E, Infante, R and Franck, N, Domestication of the Chilean guava (Ugni molinae Turcz.), a forest under storey shrub, must consider light intensity. Sci Hortic **98**: 71–84 (2003).

49. Vagiri, M, Ekholm, A, Oberg, E, Johansson, E, Andersson, CS and Rumpunen, K, Phenols and Ascorbic Acid in Black Currants (Ribes nigrum L.): Variation Due to Genotype, Location, and Year. J Agric Food Chem **61**: 9298-9306 (2013).

2010



2011

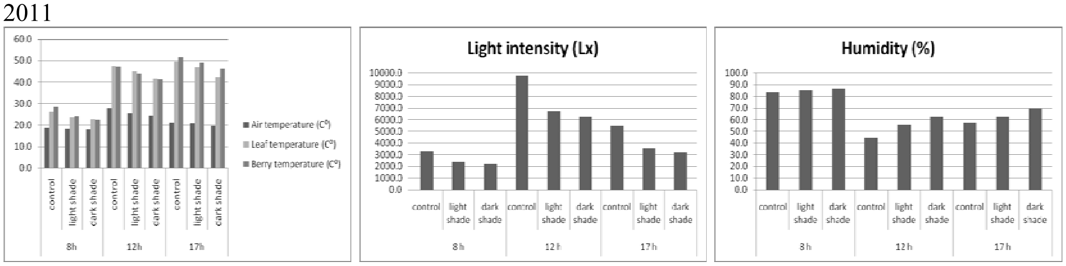
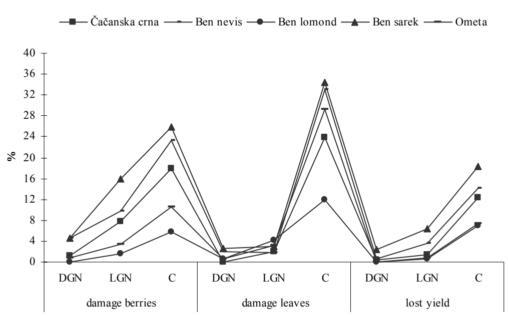


Fig. 1. Temperature of air, leaves and berries surface, relative humidity and light intensity during the first (2010) and the second (2011) experimental season

2010



2011

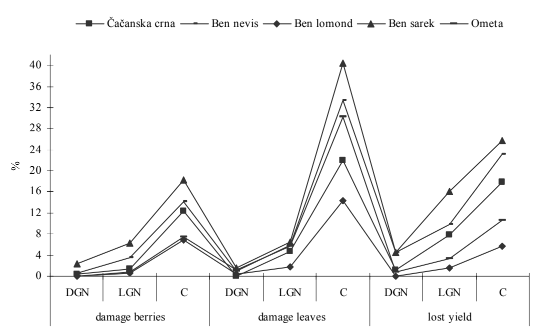


Fig. 2. Percentage of damage berries and leaves and lost yield during the first (2010) and the second (2011) experimental season

Table 1.Biological and generative properties of black currant cultivars in experimental period (2010-2011 year)

	Čačanska crna			Ben nevis			Ben lomond			Ben sarek			Ometa			Critical values		
	DGN	LGN	C	DGN	LGN	C	DGN	LGN	C	DGN	LGN	C	DGN	LGN	C	cultivar	shading	interaction
2010																		
harvest time	01.07	28.06	21.06	25.06	23.06	18.06	05.07	30.06	24.06	24.06	22.06	16.06	15.07	12.07	05.07			
length of shoot (cm)	106.7	109.8	100.2	87.9	89.3	87.6	129.8	127.1	114.2	83.2	81.9	75.4	110.9	115.3	106.2	4.98	3.85	8.62
no. of flower buds	11.8	12.8	11.8	11.1	11.6	12.1	14.2	14.4	15.8	12.1	12.6	14.1	13.9	15.5	17.1	1.91	1.48	3.31
mass of cluster (g)	6.3	5.5	5.1	6.9	6.5	5.5	6.9	5.2	5.3	6.7	7.8	7.3	7.2	7.1	5.2	0.65	0.50	1.12
length of cluster (cm)	8.7	7.8	7.2	5.4	4.6	4.7	9.1	8.1	8.1	6.0	5.5	6.3	6.7	6.8	6.2	0.59	0.46	1.02
number of berries	7.7	6.7	6.5	5.6	5.5	4.9	8.9	8.1	8.4	5.3	5.5	6.1	8.0	7.5	7.6	0.57	0.48	0.98
mass of berry (g)	0.9	1.0	1.1	1.5	1.5	1.3	0.9	0.8	0.7	1.3	1.4	1.2	0.9	1.0	0.9	0.13	0.10	0.22
yield per bush (kg)	0.88	0.83	0.71	0.84	0.83	0.81	0.96	1.02	1.08	1.13	0.86	0.69	1.37	1.35	1.57	0.17	0.13	0.29
2011																		
harvest time	27.06	22.06	16.06	22.06	18.06	14.06	30.06	25.06	21.06	19.06	14.06	12.06	12.07	09.07	01.07			
length of shoot (cm)	106.9	107.4	97.1	86.0	88.1	86.1	124.8	124.7	115.3	83.8	80.5	75.2	117.7	110.4	103.9	6.15	4.77	10.66
no. of flower buds	11.1	12.1	16.2	9.4	12.2	15.6	13.4	16.0	20.1	11.7	14.8	21.5	12.6	15.4	19.0	1.96	1.52	3.40
mass of cluster (g)	7.1	6.5	5.9	7.6	7.2	6.7	6.7	7.1	7.4	8.9	6.3	5.3	9.8	8.7	9.3	0.66	0.51	1.15
length of cluster (cm)	8.9	8.1	8.1	5.1	5.0	4.3	6.7	7.4	7.4	5.0	4.8	4.3	7.6	6.8	7.0	0.36	0.28	0.62
number of berries	8.4	8.5	8.2	5.7	5.3	5.5	8.7	9.0	9.6	5.9	5.8	5.1	9.9	8.5	8.8	0.54	0.42	0.93
mass of berry (g)	0.8	1.0	0.6	1.2	1.3	1.1	0.9	1.0	0.8	1.5	1.2	1.2	1.1	1.1	1.1	0.14	0.11	0.24
yield per bush (kg)	0.74	0.70	0.83	0.65	0.86	0.88	1.11	0.83	1.07	0.88	1.15	1.56	0.91	1.09	0.98	0.15	0.12	0.27

Data are presented as mean (n = 5)
Critical values for pairwise comparisons of the means at $^{*}P < 0.05$ level (LSD 0.05)

Table 2. Soluble solid content (°Brix), individual and total sugars and organic acids (g/kg FW), content of ascorbic acid (g/kg FW), radical scavenging activity (IC_{50} mg/ml), as well as sugar/acid ratio and sweetness index of black currant cultivars in experimental period (2010-2011 year)

	Čačanska crna			Ben nevis			Ben lomond			Ben sarek			Ometa			Critical values		
	DGN	LGN	C	DGN	LGN	C	DGN	LGN	C	DGN	LGN	C	DGN	LGN	C	cultivar	shading	interaction
2010																		
soluble solid	14.2	15.2	16.1	13.9	15.0	16.0	14.2	15.0	16.8	15.8	15.9	17.2	14.0	16.9	18.9	0.24	0.19	0.42
sucrose	7.6	7.7	9.6	18.1	14.9	15.5	9.5	8.3	9.0	9.1	17.9	10.7	10.8	14.0	25.1	1.75	1.35	3.03
glucose	26.6	26.3	33.6	23.2	28.3	30.4	21.9	19.0	22.5	12.7	28.7	15.7	20.4	30.1	52.2	3.32	2.57	5.76
fructose	37.2	37.5	45.9	32.9	37.2	39.4	29.0	25.0	30.1	17.5	38.4	20.4	26.4	39.5	64.2	4.25	3.29	7.36
total sugars	71.4	71.5	89.1	74.1	80.4	85.3	60.4	52.3	61.6	39.4	85.0	46.8	57.6	83.6	141.6	8.87	6.87	15.36
citric acid	20.8	20.5	25.5	29.9	30.3	29.5	24.5	18.7	22.4	18.8	29.8	18.0	17.5	22.2	27.8	2.11	1.64	3.66
malic acid	2.6	2.8	4.9	10.5	9.8	9.3	6.8	5.0	5.9	4.6	9.1	4.3	2.9	4.1	5.5	0.74	0.57	1.28
tartaric acid	1.0	1.1	1.7	1.6	2.0	1.9	1.1	1.0	1.1	0.7	1.5	0.7	1.0	1.5	2.3	0.18	0.14	0.31
total organic acids	24.4	24.3	32.0	41.9	42.1	40.7	32.4	24.7	29.4	24.0	40.3	23.1	21.4	27.9	35.7	2.92	2.26	5.06
ascorbic acid	1.49	2.72	2.27	2.02	2.83	2.13	2.05	2.18	2.29	2.39	2.86	1.76	2.64	2.45	2.50	0.09	0.07	0.15
RSA*	1.21	1.11	1.16	1.48	1.25	1.11	1.34	1.26	1.22	1.31	1.10	1.15	1.11	1.14	1.13	0.05	0.04	0.07
sugar/acid ratio	2.92	2.94	2.78	1.77	1.91	2.10	1.87	2.12	2.09	1.64	2.11	2.03	2.69	3.00	3.97			
sweetness index	122.4	122.9	152.2	123.1	134.0	141.9	101.5	87.6	103.9	65.4	141.1	77.0	95.7	139.7	233.9			
2011																		
soluble solid	14.2	16.3	17.6	13.2	14.2	14.9	12.4	13.9	14.2	11.8	14.0	15.3	14.9	17.7	18.3	0.27	0.21	0.47
sucrose	11.1	3.7	5.7	11.2	13.3	6.3	6.5	14.5	7.7	13.4	11.2	17.0	7.5	21.6	18.0	2.20	1.70	3.81
glucose	33.0	19.4	28.3	15.0	16.1	17.7	12.4	27.9	18.4	14.6	16.7	22.0	18.9	36.1	33.5	2.41	1.87	4.18
fructose	26.6	49.1	39.5	22.3	23.2	23.0	20.2	45.8	29.2	26.5	22.0	34.3	26.3	53.4	48.7	3.89	3.02	6.74
total sugars	70.8	72.3	73.5	48.5	52.6	46.9	39.1	88.2	55.3	54.5	49.9	73.2	52.6	111.2	100.2	8.24	6.38	14.28
citric acid	25.3	9.7	20.3	26.4	21.5	19.1	19.6	26.6	25.4	23.2	19.1	25.9	18.1	23.9	28.4	1.73	1.34	2.99
malic acid	3.8	1.3	2.9	10.9	6.8	5.3	6.6	10.3	9.2	6.0	4.9	8.0	3.9	5.8	7.4	0.76	0.59	1.31
tartaric acid	1.3	0.4	1.0	1.1	0.8	0.8	0.9	1.6	1.4	0.8	0.7	1.3	1.0	1.6	2.3	0.14	0.10	0.23
total organic acids	30.4	11.4	24.2	38.4	29.1	25.2	27.1	38.5	36.0	30.0	24.7	35.2	22.9	31.3	38.1	2.58	1.99	4.46
ascorbic acid	1.73	1.89	1.49	1.84	1.99	1.84	1.55	1.75	1.71	1.72	1.73	1.61	1.69	1.81	1.76	0.07	0.05	0.12
RSA*	1.24	1.14	1.20	1.52	1.15	1.25	1.37	1.22	1.31	1.31	1.10	1.21	1.18	1.12	1.14	0.06	0.04	0.08
sugar/acid ratio	2.33	6.35	3.04	1.26	1.80	1.86	1.44	2.29	1.54	1.82	2.02	2.08	2.30	3.55	2.63			
sweetness index	109.4	137.5	126.9	81.4	87.4	78.9	67.7	152.9	96.0	93.7	82.4	123.7	89.4	188.2	169.8			

Data are presented as mean (n = 5)Critical values for pairwise comparisons of the means at *P < 0.05 level (LSD 0.05); RSA* - radical scavenging activity (Šavikin et al., 2013)

Table 3. Rank list of black currant cultivars according to Ivanovic's distance (2010-2011 year)

	control		light green net		dark green net	
Cultivar	I-distance	rank list	I-distance	rank list	I-distance	rank list
2010						
Čačanska crna	0.966	IV	1.247	IV	0.80	IV
Ben nevis	0.835	V	1.782	III	0.72	V
Ben lomond	1.873	III	3.874	II	4.19	I
Ben sarek	2.226	II	1.020	V	3.58	II
Ometa	3.460	I	3.896	I	3.33	III
2011						
Čačanska crna	1.013	V	1.710	III	2.129	II
Ben nevis	1.805	III	1.710	III	1.180	V
Ben lomond	1.737	IV	0.723	V	1.993	IV
Ben sarek	2.815	II	1.481	II	2.242	II
Ometa	4.999	I	4.326	I	3.837	I