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ORIGINAL ARTICLE

Does microclimate under grey hail protection net affect biological and nutritional properties of 'Duke' highbush blueberry (*Vaccinium corymbosum* L.)?

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Abstract – Introduction. We tested whether the microclimate under grey hail protection nets (HPN) had an influence on the phenology, vegetative and reproductive potential, and fruit quality traits of 'Duke' highbush blueberry (*Vaccinium corymbosum* L.) in two consecutive years (2013, 2014). **Materials and methods.** Light intensity, air temperature and relative humidity were evaluated together with biological properties (flowering and ripening time, bush height and volume, number of flowers and fruits per bush, fruit set percentage, and yield per bush), and fruit quality including biometrical (fruit weight, index of fruit shape and number of seeds per fruit) and nutritional (soluble solid content – SSC, titratable acidity – TA, total anthocyanins content – TACY, total phenolic content – TPC and total antioxidant capacity – TAC) parameters. **Results and discussion.** In both seasons, light was reduced by 5–20% under HPN, whereas daily maximum temperature was 2.4 °C higher in the open field (OF). This caused two days earlier ripening time in OF compared to the HPN. The increased yield per bush under HPN was the result of larger fruit weight. SSC were unaffected by the net in 2013 while in 2014 significantly higher value of SSC was registered under HPN compared to OF. Although apparent differences in TACY and TPC were not observed between the treatments, TAC significantly increased under HPN in 2014. **Conclusion.** The use of grey HPN could be considered as a suitable alternative for the hail protection of highbush blueberry ensuring an increased fruit production without negative effects on their quality.

Keywords: Serbia / blueberry / *Vaccinium corymbosum* / protective netting / orchard microclimate / flowering / vegetative growth / fruit quality / phenolics

Résumé – Le microclimat sous filet anti-grêle gris affecte-t-il les propriétés biologiques et nutritionnelles du bleuet 'Duke' (*Vaccinium corymbosum* L.) ? Introduction. Cette étude a vérifié si le microclimat sous filet de protection anti-grêle (HPN) coloré influençait la phénologie, les potentiels de végétation et de reproduction, et les critères de qualité des fruits du bleuet en corymbe 'Duke' (*Vaccinium corymbosum*) durant deux années consécutives (2013, 2014). **Matériel et méthodes.** L'intensité lumineuse, la température de l'air et l'humidité relative ont été mesurées conjointement avec les caractéristiques biologiques (durées de floraison et de maturation, hauteur et volume de végétation, nombre de fleurs et de fruits par pied, pourcentage de nouaison et rendement par pied), les critères de qualité biométrique (poids moyen d'un fruit, indice de forme et nombre de graines par fruit) et nutritionnelle des fruits (teneur en matières solubles – SSC, acidité – TA, teneur en anthocyanes totales – TACY, contenu phénolique – PTC et la capacité antioxydante totale – paramètres TAC). **Résultats et discussion.** Sur les deux saisons, l'intensité lumineuse a été réduite de 5 à 20% sous filet HPN, alors que la température maximale quotidienne a été supérieure de 2,4 °C à celle du plein champ (OF). Les filets HPN ont hâté la maturité des fruits de deux jours. Le rendement par pied sous HPN par rapport à OF s'est accru grâce à un poids moyen des fruits supérieur. La teneur en matières solubles des fruits n'a pas été affectée par le filet en 2013 alors qu'en 2014 elle a été nettement plus élevée sous HPN rapport à OF. Bien qu'aucunes différences notables n'ont été observées en TACY et PTC entre les traitements, la capacité anti-oxydante totale (TAC) a augmenté de façon significative sous HPN en 2014. **Conclusion.** L'utilisation de filets gris HPN peut être considérée comme une solution appropriée en protection anti-grêle des bleuets tout en assurant une production accrue de fruits et sans effets négatifs sur leur qualité.

Mots clés : Serbie / bleuet / *Vaccinium corymbosum* / filet de protection / microclimat en verger / floraison / croissance / qualité des fruits / composés phénoliques

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1 Introduction

Highbush blueberries (*Vaccinium corymbosum* L.) are considered commercially important blueberry types and recently have become a popular commercial crop in Europe [1]. The high concentrations of antioxidants and other beneficial compounds in blueberries [2] indicate that the demand for this crop among health-conscious consumers may increase in the near future.

Growing conditions, especially light intensity and temperature, have a strong impact on the quality of fruits [3]. In recent years, some technological innovations were developed to achieve an optimal orchard environment due to global climate changes, especially a more often occurrence of hailstorms, resulting in hail net installation over fruit orchards [4–6]. In general, hailstorms are common weather phenomena in central and southern European countries including Serbia causing a serious threat to the economy and agriculture. The mean annual number of days with hail in the studied region is 1.4 days per year [7], whereby the most frequent occurrence of hailstorms is in May and June. The hail protection net (HPN), besides its primary purpose, also modifies environmental conditions significantly affecting the biological characteristics of plants as well as the biosynthesis of some chemical compounds [8, 9]. Taking into account the total investment of blueberry orchard establishment, yield level and fruit quality, the predicted net profit for orchard exploitation period of 20 years under HPN is almost 190.000 € higher compared to blueberry growing in the open field.

In European fruit orchards, most of the hail nets used are black, some of them are white, and since 2007 different colored hail protection nets have also become available [4]. Black hail nets greatly reduce incident solar light, and they may have a negative impact on vegetative development and the fruit quality in apple [6, 10]. Blanke [11] also reported that black hail nets are often an ineffective colour, which led to the development of grey hail nets, which combine the longevity of the black with the light transmission of the white hail net. They modify the orchard microclimate by decreasing the wind speed (up to 50% reduction) and temperature (by 1−3 °C), as well as by increasing the relative humidity (by 2−6%) and reducing incident light [12].

Light intensity has a large influence on phenological and biological properties such as floral initiation and number and length of shoots [13, 14]. Factors defining fruit inner quality, such as the soluble solid content, organic acids, and phenolics, are also indirectly affected by temperature and light intensity, since the metabolism of fruit is mainly dependent on the photosynthates imported from the leaves [3, 15, 16]. Conditions under HPN were particularly considered as the decisive factors in the analysis. Therefore, the aim of our study was to determine the influence of environmental conditions modified by the grey hail net on the biological plant and nutritional properties of 'Duke' highbush blueberry over the period of increasing productivity. The harvest lasted approximately a month because of uneven ripening. For this reason the variation in the yield per bush, physical and chemical fruit properties across the various harvesting dates was the secondary aim of this study.

2 Materials and methods

2.1 Plant material and net characteristics

The study was conducted in the 'Duke' highbush blueberry plantation located in Mladenovac (Serbia) over two consecutive years (2013, 2014). The orchard is situated at 44°32′ N latitude, 20°42' E longitude, and an altitude of 145 m. This region has temperate continental climate, with an average annual temperature of 10.7 °C and a precipitation of 649 mm.

The orchard was planted in the spring of 2011 with two-year-old nursery trees at a spacing of 3 m × 1 m (3,330 bushes ha^{-1}). The rows were oriented from NE to SW. HPN was set up slightly before flowering, from mid-April in 2013 and from the end of March in 2014.

The grey polyethylene net (SILVERLUX®, Helios Group, Lurano, Italy), with a Leno wave structure and a mesh size of 2.8 × 8 mm was used. This net was black in weft (a single fiber) and transparent in warp (double fibers) with diameters of 0.32 mm both in warp and weft. Lateral and central reinforcement were black. The net was supported with four-meter high poles, with diameter of 8 × 7 cm, located at 8 m intervals. A non-overlapping system with a slight inclination towards the centre of the interrows was applied.

2.2 Experimental design

The trial was set up in a completely randomized block design with 4 replications and 10 bushes per replication. Each plot consisted of bushes covered with grey HPN and uncovered/control bushes in the open field. The total number of bushes was 80 (4 replication × 10 bushes × 2 treatments).

2.3 Light intensity, air temperature and relative humidity

Light intensity was measured weekly at a height of 1.2 m above ground level at 12.00 both under HPN and outside using a digital lux-meter 'Peak teck' PT-5025 (Germany). Air temperature and relative humidity within the canopy were recorded using climate dataloggers (type DT-171, Shenzhen Flus Technology Co., Ltd, China) installed at 1.2 m above the ground level under HPN and outside. Twelve readings per treatment were taken at 2 h intervals every day in each season (*i.e.* from mid-April to mid-July in 2013; and from the end of March to mid-July in 2014).

2.4 Biological properties

For a period of two years the following biological properties were studied: flowering and ripening time, bush height and volume, number of flowers and fruits per bush, fruit set percentage, and yield per bush. The beginning of flowering was considered as being the day when approximately 10% of the flowers were open. The end of flowering was observed when 90% of the petals had fallen [17]. The date of the first harvest was considered as being the beginning of ripening when approximately 8 to 10% of berries were technologically mature as judged by their fully blue color at the scar. The end of ripening was recorded as being the date of the last harvest. The maximum height of the bush was measured after shoot growth had stopped using the PVC tape. The values were expressed in cm. Bush volume was calculated according to the formula:

$$V = \frac{1}{3}A_B H$$

where the volume (V) of any conic solid is one third of the product of the area of the bush base (A_B) and the bush height (H).

The number of flowers and berries per bush were counted to determine the percentage of fruit set. Commercial bumble bee (*Bombus* spp.) colonies were used for the blueberry pollination since honey bees were less efficient at pollinating blueberry flowers in most locations Berries were hand-harvested four times in the 2013 season, *i.e.* five times in the 2014 season and weighed to determine yield per bush at commercial maturity (kg).

2.5 Fruit quality parameters

Biometrical (fruit weight, index of fruit shape and number of seeds per fruit) and nutritional fruit quality (soluble solid content − SSC, titratable acidity − TA, total anthocyanin content − TACY, total phenolic content − TPC and total antioxidant capacity − TAC) were also determined. Fully ripe fruit samples, judged as being a fully blue color at the scar, were collected in four replications during the 2013 and 2014 seasons. The fruit weight was determined by weighing 50 fruit (± 0.1) per replication (200 per treatment) and expressed in grams. For seed counting, the same fruit samples were used within each replication. An index of fruit shape was determined to be the ratio of the maximum height and width. Each sample was pooled to obtain a composite sample and analysed for SSC using a digital refractometer (Pocket PAL-1, Atago, Japan) and the reading was taken in % of soluble solid content in the fruit (% SSC). TA was measured using a digital burette and 0.1 M NaOH to titrate samples to an endpoint. Titratable acidity was based as a percentage of citric acid equivalent.

For the extraction of phenolics, approximately 100 g of the fruits were homogenized in methanol/water/hydrochloric acid at a ratio of 70:30:5 by volume. The homogenates were centrifuged at 10,000 × g for 15 min. Four replications of supernatants were prepared for each sample analysed.

The TACY was measured with the modified pH differential absorbance method [18]. Absorbance was measured at 510 and 700 nm (Multiscan® Spectrum, Thermo electron corporation, Vantaa, Finland) in 0.025 M potassium chloride buffer at pH 1.0 and 0.4 M sodium acetate buffer at pH 4.5. Results were expressed as mg cyanidin-3-glucoside (ε = 29,600) equivalent 100 g^{-1} fresh weight (FW).

The TPC was determined according to the Folin-Ciocalteu spectrophotometric (2501 PC Shimadzu, Kyoto, Japan) procedure [19] using gallic acid (GA) as a standard. Samples were mixed with 0.25 N Folin-Ciocalteu reagent and after 3 min 0.2 M sodium carbonate solution was added, followed by incubation for 60 min. Results were read at 724 nm and expressed as mg GA equivalent g^{-1} FW.

The determination of TAC was done following the ABTS method of Arnao *et al.* [20]. The reaction mixture contained 2 mM ABTS, 15 μM hydrogen peroxide, and 0.25 μM horse radish peroxidase in a 50 mM phosphate buffer pH 7.5. The reactions were monitored at 730 nm (2501 PC Shimadzu, Kyoto, Japan) at 25 °C. Different concentrations (0.1−0.8 mM) of ascorbic acid were added for a standard curve set-up. Absorbance alterations were read on a standard curve and results were expressed as mg ascorbic acid equivalent (asc) g^{-1} FW.

2.6 Data analysis

Statistical analyses were performed using the software Statistica 8.0 for Windows (StatSoft Inc., Tulsa, OK, USA). The data from a 2-year investigation was analysed by ANOVA for each year separately. Significant differences among the means were determined by LSD test at a level of $P \leqslant 0.05$.

3 Results and discussion

3.1 Effects of hail protection nets on light intensity, air temperature and humidity

In both years of observation, incident light was reduced by 5−20% under HPN, with the greater reduction on clear days and higher light intensities (*figure 1*).

Overall, air temperature within the canopy under HPN was lower compared to that in OF (*figure 1*). The temperature disparity was most pronounced during the warmest part of a clear day and therefore, the greatest difference was displayed in daily maximum temperatures (*figure 1*). On average, daily maximum temperature was 2.4 °C higher in the bushes exposed to the sunlight than in those under HPN. Mean daily temperature under HPN was reduced by 1.5 °C on average during both experimental seasons, while daily minimum temperature differed only slightly (0.6 °C). As a consequence of the lower daily maximum temperatures, daily minimum relative humidity under HPN was, on average, 4% higher than in OF (*figure 1*). The average values of daily maximum and mean relative humidity were 2% lower and 1% higher under HPN than those in OF, respectively.

The microclimate measurements during both experimental seasons showed that HPN intercepted more light radiation on sunny days than on cloudy days. Iglesias and Alegre [8] also reported that light interception by black nets was twice as a great as for a crystal nets on sunny days, whereas the difference was smaller on cloudy days. In their study clouds caused a reduction in total incident radiation of around 50%. Light reductions resulting from netting also affected air temperature and relative humidity. In our study, lower daily maximum temperature recorded under HPN caused 4% higher daily minimum relative air humidity than in an outside environment. According to Solomakhin and Blanke [6] the colored hail nets increased the relative air humidity by 2% on a cloudy and by 5% on a sunny day, compared to outside air in the summer.

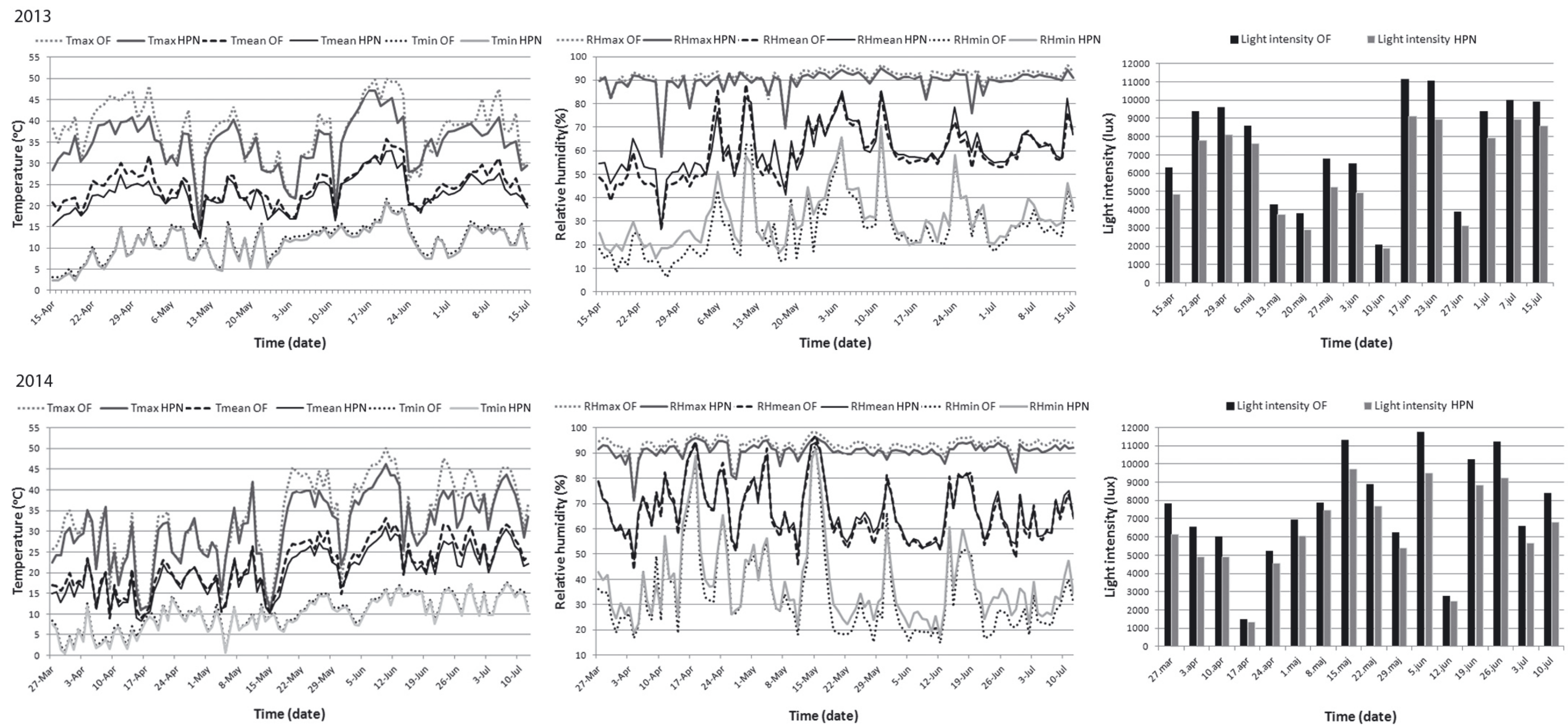

Figure 1. Effects of hail protection nets on air temperature (°C), relative humidity (%) and light intensity (lux) over two consecutive years. OF, open field, HPN, hail protection net.

Table I. Influence of hail protection nets (HPN) on flowering and ripening time of 'Duke' highbush blueberry during the experimental period (2013−2014) (OF: open field).

Year	Treatment	Flowering time			Ripening time		
		Beginning	End	Duration (days)	Beginning	End	Duration (days)
2013	HPN	April 23	May 2	10	June 9	July 9	31
	OF	April 22	May 2	11	June 7	July 9	33
2014	HPN	March 29	May 1	34	June 11	July 14	34
	OF	March 29	May 1	34	June 9	July 11	33

Table II. Influence of hail protection nets (HPN) on biological properties of 'Duke' highbush blueberry in 2013−2014 experimental period (OF: open field). Data are the means of 4 replications ± standard errors (degree of freedom: df = 4).

Treatment	Bush height (cm)	Bush volume (m^3)	Number of flowers per bush	Number of fruit per bush	Percentage of fruit set	Yield (kg per bush)
2013						
HPN	120.0 ± 11.5	0.32 ± 0.07	2,968 ± 88	2,748 ± 115	92.5 ± 1.3	5.2 ± 0.3a
OF	117.0 ± 5.5	0.26 ± 0.03	2,752 ± 87	2,585 ± 98	93.9 ± 0.8	4.1 ± 0.2b
F test	0.069^{ns}	0.632^{ns}	3.096^{ns}	1.160^{ns}	0.830^{ns}	*8.440**
2014						
HPN	154.0 ± 3.0	0.43 ± 0.02a	3,670 ± 108	3,438 ± 171	93.5 ± 1.9	6.0 ± 0.3a
OF	134.0 ± 8.0	0.35 ± 0.01b	3,370 ± 51	3,150 ± 84	93.4 ± 1.2	4.8 ± 0.1*b*
F test	5.480^{ns}	*10.088**	6.408^{ns}	2.293^{ns}	0.002^{ns}	*14.804**

Values within column followed by the same letter are not significantly (ns) different at $P \leqslant 0.05$ (LSD test). *Significant at $P \leqslant 0.05$.

3.2 Biological properties

Environmental conditions in both experimental years were considered favorable for the growing of 'Duke' highbush blueberry. Much earlier flowering was registered in 2014, although the duration of this phenophase was 3-fold longer (34 days) than in 2013, probably as a result of 10.1 °C lower mean air temperatures recorded from the 29th of March – 30th of April, 2014. In 2014, the same dates were recorded regarding the beginning (29th of March) and the end (1st of May) of flowering period between covered and control bushes. Higher daily maximum air temperatures in June and July of 2013 accelerated fruit ripening for two days both under HPN and in OF compared to 2014. The variation at the beginning of the ripening season, can be explained by higher daily maximum air temperature of 2.4 ° C in the bushes directly exposed to the sunlight than in those under HPN. Thus, it is clear that grey nets alter blueberry phenology and could be a valuable tool to displace peak harvest season in blueberries. In both experimental years, grey HPN delayed ripening for 2 days in comparison to OF. The same duration of ripening was observed in the uncovered bushes (33 days) in both years, whereas slightly longer ripening period was recorded in the bushes under HPN in 2014 (34 days) (*table I*).

Most of the parameters of vegetative and reproductive potential, except bush volume in 2014 and yield per bush in both years of investigation, were not affected by HPN (*table II*). 'Duke' has moderate to vigorous bushes with almost 18% higher average height recorded in 2014 since the examined plants were in the period of intensive vegetative development which is characterized by shoot elongation. Bush volume also expressed higher values in the second year, whereby HPN showed a significant influence on this parameter (0.43 m^3). Hancock and Draper [21] have reported that the height of blueberry plants usually does not exceed 250 cm, but in our study the bush height was lower because the plants were examined in the 3rd and 4th year after planting. An increase of bush height and volume in the second experimental season has been expected, considering that bushes were in the period of intensive vegetative development. The number of flowers and fruits per bush did not differ significantly between the treatments, but an increase of these values were observed in the second experimental season contributing to significantly higher yield per bush (6.0 kg – HPN; 4.8 kg – OF). In both experimental years, bushes of cultivar 'Duke' yielded much higher under HPN compared to OF. The yield in the first season was the highest in the 2nd harvest both in OF and under HPN ranging from 2.4 to 3.9 kg per bush respectively. In the next year, the yield per bush was much higher in 4th harvest in both treatments (2.32 kg per bush – HPN; 1.76 kg per bush – OF), 10 times geater than the 1st harvest yield (*figure 2*).

Wach [22] has found that number of canes per bush and berries per cane are significant contributors to yield in six blueberry cultivars. Ehlenfeldt and Martin [23] have investigated whether the small berry size is a consequence of poor pollination resulting in a reduced yield, or whether the small berry size is a result of the plant being overcropped. In their studies, yields across 10 years ranged from 3.5 to 7.4 kg per bush for 'Duke' and a correlation of yield and average berry weight was not significant. Yields per bush obtained in our 2-year study showed similar levels. However, bushes of 'Duke' yielded much higher under HPN compared to OF and differences in

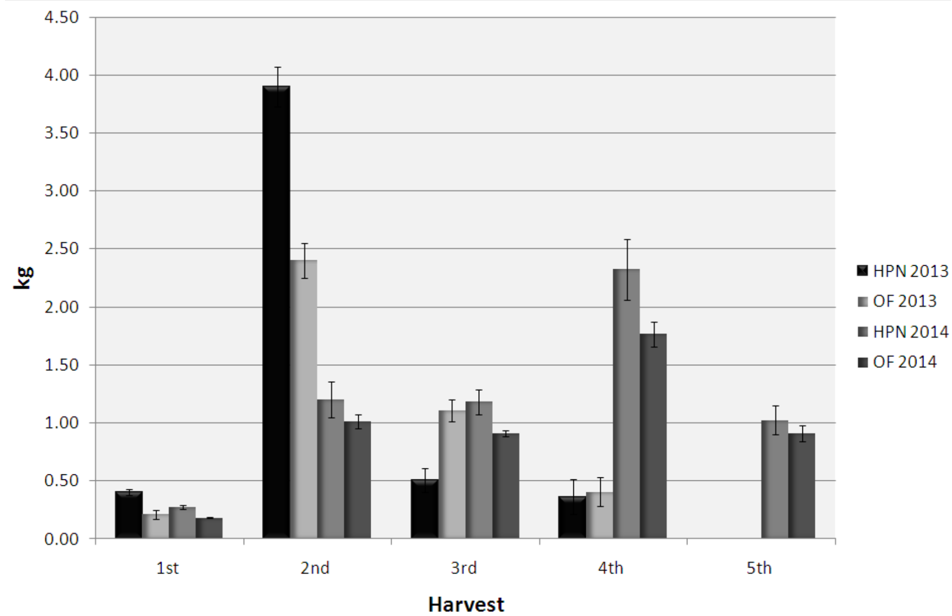

Figure 2. Share of each individual harvest yield in total yield per bush (kg) under hail protection nets (HPN) and in open field (OF) during the first (2013) and the second (2014) experimental seasons. Data are means ± standard errors ($n = 4$). Means marked with different lowercase letters within each harvest time are significantly different at $P \leqslant 0.05$ (LSD test).

yield levels across the harvests were also observed in both experimental seasons. Lobos *et al.* [24] reported that when blueberry plants are subject to the open field conditions, they might be stressed due to excess radiation, high temperature and moisture deficit. In our study light intensities under grey HPN were lower compared with the OF, mean daily air temperatures tended to be lower under the nets (1.5 °C) due to the interception of light radiation, while daily minimum relative air humidity was 4% higher under HPN. These results were in line with those reported by Solomakhin and Blanke [6] and Iglesias and Alegre [8] indicating a reduction of mean daily temperature up to 1.3 °C and a 2–6% increase in humidity associated with the use of nets.

3.3 Fruit weight

'Duke' had a significantly higher average fruit weight under HPN ranging from 1.87 g (in 2013) to 1.75 g (in 2014) in comparison to those in OF. There were also considerable changes in the fruit weight across the harvests (*tables III and IV*). In 2013, the largest berries were from the 2nd harvest (2.01 g) and then average weight was decreased by 16.4% in the 3rd harvest, *i.e.* 28.8% in the 4th harvest. Interaction between treatments had only a significant influence on the fruit weight in 2013. The highest values were achieved in the 1st and the 2nd harvests under HPN, whereas in OF significantly higher fruit weight was recorded in the 1st and the 2nd harvests in comparison to those of the two last harvests. In 2014, the 2nd and the 3rd harvests produced a significantly larger fruits, whereas the lowest value of the fruit weight was found in the last harvest. As opposed to the previous year, interactions between treatments and harvest dates had no significant effect on the fruit weight. The index of fruit shape showed significantly higher value under HPN in 2013 (0.79) corresponding to roundish or flattened forms. In the second year, no significant influence of HPN on this parameter was observed. Conversely, harvest had a significant impact on fruit shape index with somewhat higher values obtained in the last (2013) and the two last harvests (2014). The number of seeds per fruit was found to be considerably higher under HPN ranging from 37.8 in 2013 to 35.0 in 2014. Since the fruit weight decreased between successive harvests, a similar trend was observed in the number of seeds per fruit achieving the highest values in the 1st and the 2nd harvests in 2013, *i.e.* in the 2nd and the 3rd harvests in 2014. Interaction between the treatments had no a significant effect on the number of seeds per fruit in both experimental seasons.

In general, orchards covered by anti-hail nets might have a more efficient water use as a result of lower leaf transpiration rate [25], which might increase fruit weight and yield. Ehlenfeldt and Martin [23] reported that 'Duke' possessed sufficient environmental plasticity to produce fruit with increased berry weight between successive harvests. Significantly higher number of seeds per fruit, accompanied by larger fruit size, was found under HPN demonstrating that favorable environment conditions, in particular a reduction of maximum daily temperatures and decreasing wind speed, both contributing to increased bumble bee activity during the flowering season.

3.4 Fruit quality parameters

The SSC did not differ between the treatments in 2013, whereas a significantly higher value was registered in the fruits harvested under HPN (10.2%) compared to those from OF in 2014 (*tables V and VI*). A variation of SSC was also observed

Table III. Influence of hail protection nets (HPN) and harvesting time on biometrical fruit properties of 'Duke' highbush blueberry in 2013 (OF, open field). Data are the means of 4 replications ± standard errors (df = 16).

	Netting/ Harvesting		Fruit weight (*g*)	Index of fruit shape	Number of seeds per fruit
Netting (N)	HPN		1.87 ± 0.01a	0.78 ± 0.01b	37.8 ± 1.5a
	OF		1.61 ± 0.05b	0.79 ± 0.01a	29.7 ± 1.3b
Harvest (H)	1st		1.84 ± 0.10ab	0.76 ± 0.01c	37.2 ± 2.2a
	2nd		2.01 ± 0.12a	0.74 ± 0.01d	37.1 ± 2.5a
	3rd		1.68 ± 0.04b	0.80 ± 0.01b	32.9 ± 1.8b
	4th		1.43 ± 0.05c	0.83 ± 0.01a	27.8 ± 2.2c
N × H	HPN	1st	2.02 ± 0.12a	0.76 ± 0.01	42.0 ± 1.3
		2nd	2.28 ± 0.03a	0.73 ± 0.00	41.2 ± 2.8
		3rd	1.68 ± 0.07b	0.79 ± 0.00	35.5 ± 3.1
		4th	1.51 ± 0.05b	0.82 ± 0.01	32.5 ± 1.5
	OF	1st	1.65 ± 0.05a	0.76 ± 0.00	32.4 ± 0.1
		2nd	1.75 ± 0.08a	0.76 ± 0.01	33.0 ± 2.3
		3rd	1.68 ± 0.04b	0.82 ± 0.01	30.4 ± 1.0
		4th	1.34 ± 0.05b	0.85 ± 0.00	23.3 ± 1.1
F test	N		*31.601**	*11.50**	*36.172**
	H		*28.002**	*51.50**	*10.925**
	N × H		*5.910**	*1.93*	*0.573*

Values within column followed by the same letter are not significantly (ns) different at $P \leqslant 0.05$ (LSD test). *Significant at $P \leqslant 0.05$.

Table IV. Influence of hail protection nets (HPN) and harvest time on biometrical fruit properties of 'Duke' highbush blueberry in 2014 (OF, open field). Data are the means of 4 replications ± standard errors (df = 16).

	Netting/ Harvesting		Fruit weight (*g*)	Index of fruit shape	Number of seeds per fruit
Netting (N)	HPN		1.75 ± 0.11a	0.79 ± 0.1	35.0 ± 1.6a
	OF		1.49 ± 0.11b	0.79 ± 0.1	31.3 ± 1.9b
Harvest (H)	1st		1.60 ± 0.07b	0.73 ± 0.1c	33.4 ± 1.0b
	2nd		2.10 ± 0.04a	0.77 ± 0.1b	40.3 ± 1.0a
	3rd		1.98 ± 0.10a	0.77 ± 0.1b	39.2 ± 1.0a
	4th		1.42 ± 0.07c	0.84 ± 0.1a	27.8 ± 2.1c
	5th		1.01 ± 0.07d	0.83 ± 0.1a	25.0 ± 1.6c
N × H	HPN	1st	1.75 ± 0.04	0.73 ± 0.01	35.3 ± 0.8
		2nd	2.16 ± 0.07	0.78 ± 0.00	41.9 ± 1.3
		3rd	2.18 ± 0.08	0.76 ± 0.01	40.0 ± 1.5
		4th	1.55 ± 0.04	0.84 ± 0.01	30.8 ± 2.6
		5th	1.11 ± 0.01	0.83 ± 0.02	37.2 ± 1.4
	OF	1st	1.45 ± 0.04	0.74 ± 0.01	31.5 ± 0.9
		2nd	2.04 ± 0.04	0.76 ± 0.01	38.8 ± 1.1
		3rd	1.79 ± 0.06	0.78 ± 0.00	38.5 ± 1.5
		4th	1.28 ± 0.05	0.84 ± 0.01	24.8 ± 2.4
		5th	0.91 ± 0.05	0.83 ± 0.01	22.8 ± 2.5
F test	N		*47.661**	0.010^{ns}	*11.825**
	H		*112.297**	*39.96*	*30.556**
	N × H		1.540^{ns}	0.89^{ns}	0.457^{ns}

Values within column followed by the same letter are not significantly (ns) different at $P \leqslant 0.05$ (LSD test). *Significant at $P \leqslant 0.05$.

Table V. Influence of hail protection nets (HPN) and harvest time on chemical fruit properties of 'Duke' highbush blueberry in 2013 (OF: open field). Data are the means of 4 replications ± standard errors (df = 20) (SSC: soluble solid content; TA: titratable acidity; TACY: total anthocyanin content; TPC: total phenolic content; TAC: total antioxidant capacity).

	Netting/ Harvest		SSC (%)	TA(%)	TACY (mg c-3-g eq 100 g^{-1}FW)	TPC (mg GA g^{-1} FW)	TAC (mg asc g^{-1} FW)
Netting (N)	HPN		11.5 ± 0.4	0.58 ± 0.07b	61.1 ± 4.6	11.0 ± 0.6	1.05 ± 0.04
	OF		11.0 ± 0.2	0.69 ± 0.07a	54.8 ± 3.8	9.9 ± 0.3	1.00 ± 0.03
Harvest (H)	1st		11.0 ± 0.3b	0.98 ± 0.03a	69.8 ± 4.8a	9.7 ± 0.4bc	0.97 ± 0.03
	2nd		10.7 ± 0.2b	0.58 ± 0.06b	67.4 ± 3.6a	11.7 ± 0.8a	1.06 ± 0.04
	3rd		10.9 ± 0.2b	0.58 ± 0.02b	54.5 ± 2.9b	9.3 ± 0.5c	0.99 ± 0.06
	4th		12.6 ± 0.5a	0.40 ± 0.03c	40.4 ± 3.3c	11.0 ± 0.8ab	1.07 ± 0.05
N × H	HPN	1st	10.7 ± 0.3c	0.95 ± 0.05a	74.5 ± 7.9	10.7 ± 0.1	1.03 ± 0.01ab
		2nd	10.8 ± 0.3c	0.46 ± 0.04de	70.0 ± 5.9	13.0 ± 1.1	1.14 ± 0.01a
		3rd	11.3 ± 0.2bc	0.53 ± 0.01cd	58.8 ± 3.4	8.5 ± 0.6	0.88 ± 0.00c
		4th	13.4 ± 0.4a	0.39 ± 0.02e	41.3 ± 6.0	11.6 ± 1.4	1.15 ± 0.07a
	OF	1st	11.3 ± 0.3bc	1.01 ± 0.01a	65.1 ± 5.5	8.7 ± 0.1	0.90 ± 0.01c
		2nd	10.6 ± 0.2c	0.71 ± 0.02b	64.8 ± 4.8	10.3 ± 0.5	0.98 ± 0.06bc
		3rd	10.4 ± 0.1c	0.63 ± 0.02bc	50.1 ± 3.2	10.0 ± 0.3	1.10 ± 0.06ab
		4th	11.8 ± 0.6b	0.41 ± 0.05e	39.4 ± 3.9	10.4 ± 1.0	1.00 ± 0.06bc
F test	N		*4.010*^{ns}	*20.880**	*2.843*^{ns}	*3.877*^{ns}	*2.789*^{ns}
	H		*13.248**	*120.747**	*13.185**	*4.177**	*2.925*^{ns}
	N × H		*3.626**	*4.997**	*0.209*^{ns}	*2.778*^{ns}	*8.459**

Values within column followed by the same letter are not significantly (ns) different at $P \leqslant 0.05$ (LSD test). *Significant at $P \leqslant 0.05$.

Table VI. Influence of hail protection nets (HPN) and harvest time on chemical fruit properties of 'Duke' highbush blueberry in 2014 (OF: open field). Data are the means of 4 replications ± standard errors (df = 20) (SSC: soluble solid content; TA: titratable acidity; TACY: total anthocyanin content; TPC: total phenolic content; TAC: total antioxidant capacity).

	Netting/ Harvest		SSC (%)	TA (%)	TPC (mg GA g^{-1}FW)	TACY (mg c-3-g eq 100 g^{-1} FW)	TAC (mg asc g^{-1} FW)
Netting (N)	HPN		10.1 ± 0.3a	0.37 ± 0.04a	5.10 ± 0.25	83.7 ± 5.5	2.29 ± 1.17a
	OF		9.4 ± 0.1b	0.30 ± 0.03b	4.62 ± 0.21	73.1 ± 7.7	1.82 ± 1.14b
Harvest (H)	1st		9.5 ± 0.2b	0.49 ± 0.03a	4.62 ± 0.47	58.3 ± 7.9b	1.23 ± 0.05c
	2nd		9.2 ± 0.1b	0.41 ± 0.03b	4.60 ± 0.35	67.5 ± 3.5b	1.86 ± 0.26b
	3rd		9.5 ± 0.2b	0.33 ± 0.02c	5.63 ± 0.18	99.2 ± 6.9a	2.51 ± 0.25a
	4th		11.1 ± 0.6a	0.29 ± 0.01c	5.03 ± 0.40	100.8 ± 12.8a	2.25 ± 0.06ab
	5th		9.7 ± 0.2b	0.18 ± 0.01d	4.45 ± 0.34	66.3 ± 6.9b	2.43 ± 0.17a
N × H	HPN	1st	9.6 ± 0.0bc	0.54 ± 0.03	5.20 ± 0.88	73.8 ± 7.7	1.30 ± 0.01
		2nd	9.3 ± 0.1bc	0.45 ± 0.05	4.79 ± 0.23	70.0 ± 3.6	2.30 ± 0.37
		3rd	9.5 ± 0.2bc	0.36 ± 0.02	5.78 ± 0.23	94.6 ± 8.3	2.97 ± 0.17
		4th	12.3 ± 0.7a	0.31 ± 0.01	5.65 ± 0.48	105.3 ± 20.7	2.37 ± 0.05
		5th	10.0 ± 0.4b	0.19 ± 0.03	4.08 ± 0.46	75.1 ± 5.5	2.52 ± 0.29
	OF	1st	9.3 ± 0.2bc	0.43 ± 0.02	4.03 ± 0.08	42.8 ± 3.5	1.16 ± 0.10
		2nd	9.0 ± 0.1c	0.37 ± 0.01	4.40 ± 0.71	65.0 ± 6.4	1.42 ± 0.06
		3rd	9.4 ± 0.4bc	0.30 ± 0.02	5.47 ± 0.30	103.9 ± 12.2	2.06 ± 0.29
		4th	9.8 ± 0.1bc	0.27 ± 0.01	4.40 ± 0.42	96.3 ± 19.3	2.14 ± 0.00
		5th	9.4 ± 0.2bc	0.17 ± 0.01	4.81 ± 0.48	57.5 ± 11.6	2.34 ± 0.24
F test	N		*15.45**	*20.925**	*2.438*^{ns}	*2.170*^{ns}	*13.239**
	H		*11.46**	*52.418**	*1.962*^{ns}	*6.128**	*13.542**
	N × H		*4.78**	*1.359*^{ns}	*1.383*^{ns}	*0.855*^{ns}	*1.891*^{ns}

Values within column followed by the same letter are not significantly (ns) different at $P \leqslant 0.05$ (LSD test). *Significant at $P \leqslant 0.05$.

across the harvests indicating that the cv. Duke yielded significantly higher SSC in the 4th harvest in both experimental years (12.6% and 11.1%, respectively). Zervoudakis *et al.* [26] have stated that some plants may show maximum productivity and photosynthetic activity under moderate shade. Moreover, Iglesias and Alegre [8] noted that SSC decreased when a black net was used, but values appeared to be similar for the crystal net and the control. A variation of SSC across the harvests observed in our study indicates that 'Duke' yielded significantly higher SSC in the 4th harvest of both 2013 and 2014 years In our research, the mean TA in 2013 was higher in OF, whereas in 2014 it was higher under HPN. An opposite pattern can be observed in TA content across the harvests achieving almost 2.5-fold higher values in the fruits of the 1st harvest compared to those of the last harvest in both studied years. There were also differences in the acidity of the fruits between the treatments, and the mean TA in 2013 was 15.3% higher in OF, whereas in 2014 it was 17.9% higher under HPN. Interaction of the applied treatments significantly influenced SSC of the fruits in both experimental years as well as TA content only in the first year. This discrepancy is more a result of the temperature differences between treatments during fruit maturation in each experimental season than the availability of solar radiation. TA content also varied greatly across the harvests achieving higher values in the fruits of the 1st harvest compared to those of the last harvest in both studied years. TA content found by Ochmian *et al.* [27] in organically and conventionally farmed berries of 'Duke' was similar to our results obtained in the first experimental year. Considerably lower TA and SSC content registered in the second year of investigation may be explained by a higher crop load of the plants.

TACY and TPC were not influenced by treatments, whereas a significant effect of harvest on TACY were observed in both experimental years. In contrast to the trend for TACY, harvest only had a significant effect on the TPC levels in 2013.

The cultivar Duke showed 2-fold higher TAC in 2014 (on average 2.06 mg asc eq g^{-1} FW), than in 2013 (on average 1.02 mg asc eq g^{-1} FW; *tables V and VI*). In general, TAC levels were affected by treatments and harvest dates only in 2014. In this year, a significantly higher value was registered under HPN (2.29 mg asc eq g^{-1} FW) and it was found that TAC increased in the three last harvests. Interaction between the applied treatments and harvesting dates had no significant effect on the TACY and TPC in both experimental seasons, while this effect was significant on TAC levels in 2013.

In our research, apparent differences in TACY and TPC were not observed between treatments, whereas significant effect of harvesting on TACY were observed in both experimental years. Kim *et al.* [28] reported 3-fold higher TACY content in berries of 'Duke' grown in Korea and observed differences were probably due to the use of different extraction solvents during sample preparation, diverse cultivation and environmental conditions. Temperature and solar radiation during ripening may influence the accumulation of functional phytochemicals in blueberries [28], but also other factors such as: genotype, site location, altitude, technological measures and year contribute to their synthesis [2]. In contrast to the trend for TACY, harvesting only had a significant effect on the TPC levels in 2013, whereas TAC significantly increased in the three last harvests in 2014 reflecting better light and temperature conditions, especially under HPN. TAC levels detected in our study were 2- to 3-fold higher than those previously reported for 'Bluecrop' and 'Berkeley' grown in Western Serbia [29].

4 Conclusion

A comprehensive investigation of the effect of microclimate under grey HPN on vegetative development, reproductive potential and fruit quality parameters of high bush blueberry was not conducted in European orchards till now. Our research showed that the use of grey HPN caused changes in the blueberry phenology, ensuring increased fruit production without negative effects on its quality. Harvest time also had a significant influence on the fruit weight, number of seeds per fruit, SSC, TA and TACY in both experimental years, while TPC and TAC differed between the harvests either in 2013 or in 2014. An overall conclusion is that grey HPN can be successfully implemented to optimize the production technologies of blueberries.

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