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Effect of Yellow and Stop Drosophila Normal Anti-insect Photoselective Nets on Vegetative, Generative and Bioactive Traits of Peach (cv. Suncrest)

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ABSTRACT

The effect of anti-insect photoselective yellow (mesh size of 2.4x4.8 mm) and Stop Drosophila Normal (mesh size of 0.90x1 mm) nets on the generative and vegetative traits of peach (cv. Suncrest) was studied at an orchard near the city of Čakovec, Croatia. Netting significantly affected some vegetative parameters (leaf surface, leaf length and leaf shape index) but there was no significant effect on productivity parameters (yield, yield efficiency, fruit mass and share of decayed fruit). Regarding fruit colouration application of nets significantly affected b* and C* background and L*, b*, C* and h° additional colour parameters. Majority of inner fruit quality parameters (fruit firmness, titratable acidity and total soluble solids / titratable acidity ratio) as well as of bioactive compounds (total polyphenolic content, antioxidant activity, anthocyanin content and share of alkali-soluble pectin) was also under significant effect of netting. Since yellow net only slightly reduced peach fruit quality (compared to control) it can be recommended for application as an anti-insect net. However, Stop Drosophila Normal net more notably reduced quality parameters (especially additional fruit colour) and hence should be used only when other control strategies show to be ineffective.

Keywords: Netting, Light modification, Fruit quality, Bioactive compounds, Anti-insect nets, Sustainability

1. Introduction

Nowadays, modern agriculture is unimaginable without netting. Nets are applied due to numerous reasons, mainly for the protection against various hazards (hail and wind, excessive sun radiation, birds) (Bosco et al. 2015; Giaccone et al. 2012; Middleton & McWaters 2002) and subsequently against insects (Pajač Živković et al. 2018; Tasin et al. 2008). Anti-insect nets are similar to anti-hail nets but they differ from anti-hail nets in mesh size and application method (Pajač Živković et al. 2018). Anti-insect nets overlay the fruit tree canopy and edges of the orchard creating a barrier that disrupts pest propagation by preventing their flight (Tasin et al. 2008). Pajač Živković et al. (2018) successfully applied anti-insect nets with mesh size of 2.4x4.8 mm against oriental fruit moth (*Grapholita molesta* (Busck 1916)) and peach twig borer (*Anarsia lineatella* (Zeller 1839)), two economic pests of stone fruits in the Republic of Croatia. However, relatively recently *Drosophila suzukii* (Matsumura), a highly polyphagous invasive pest to whom peach is one of the host plant species, invaded western countries and became a challenge in the fruit production process (Cini et al. 2012).

Due to the pest's small size, dense nets with smaller mesh sizes are required for successful protection. Smaller mesh in anti-insect nets creates more shade than traditional anti-hail nets of the same colour, which can have a potentially negative effect on vegetative and generative traits of plants cultivated underneath them. However, over a decade ago a relatively new technology emerged – photoselective netting as a tool for light quality and quantity manipulation under practical field conditions (Rajapakse & Shahak 2007). Traditional black nets can influence only light quantity because of their opacity, while translucent nets additionally have the light scattering ability, but neither one can influence the quality properties of light (Ilić & Fallik 2017; Oren-Shamir et al. 2001; Shahak 2008; Shahak et al. 2004b). According to Basile et al. (2012), photoselective nets are made of partially translucent threads that selectively screen-out certain light spectra, in UV and/or visible light spectra, which pass through them and at the same time transform direct light to diffuse. Coloured nets can increase the content of scattered light for two or more times (Oren-Shamir et al. 2001). Scattered light has a better penetration possibility in dense canopies (or in the inner part of canopies), thus increasing the efficiency of light-dependent processes (Lakso & Musselman 1976; Shahak 2014; Shahak et al.

2004a). Moreover, due to light filtration ability (light quality modification) which is defined by pigments incorporated in plastic material (Shahak et al. 2016), they also promote specific plant physiological responses (Shahak 2014). Up to date, the available literature is notably insufficient regarding the effect of photoselective nets on vegetative and generative traits of peaches. Therefore, it is hard to appoint which type of photoselective net should be applied for pest protection so that multiple benefits can be achieved. Moreover, there are no studies that deal with the effect of photoselective nets on the content of bioactive compounds in peach fruit. Hence, this study aims to test the effect of two different photoselective anti-insect nets on peaches 'Suncrest' with emphasis on their effect on the synthesis of the bioactive compounds in fruits.

2. Material and Methods

2.1. Plant material and treatments

The experiment was established at a peach orchard in Vratišinec, near the city of Čakovec, Croatia in April 2015 on the 12 years old peach (*Prunus persica* (L.) Batsch.) cv. 'Suncrest' grafted on vineyard peach (peach seedling). The peaches were trained as an open vase with a spacing of 4x3 m. The experiment consisted of three treatments: uncovered trees that served as control (C), the trees that were covered with yellow photoselective nets (Tenax, Italy) after petal fall (Y) and the trees that were covered with white Stop Drosophila Normal net (Artes Politecnica, Italy) after petal fall (D). Y net had a mesh size of 2.4x4.8 mm while D nets had a mesh size of 0.90x1 mm. Due to smaller mesh sizes both nets have anti-insect properties. In this study yellow net was used because of its promising results obtained in Israel (Shahak et al. 2016). According to the manufacturer D net has protection properties against *D. suzukii* and was therefore selected. The experiment was set up according to a random block schedule in three repetitions for each net and control. Each repetition was physically separated from each other (different net cage) and included three peach trees. Fruits from both treatments and control were harvested on August 6, 2015.

2.2. Vegetative parameters

Vegetative parameters were measured after the end of the vegetative period. Trunk cross-sectional area (TCSA) was measured at a height of 25 cm above soil level and expressed in cm^2. Length of the one-year shoot (cm), internode length (cm), and thickness of one-year shoot (mm) was measured on 10 randomly selected shoots from the middle – the outer part of the tree canopy, per repetition. Thickness (mm) was measured 5 cm from the shoot base by the digital caliper Prowin HMTY0006. Internode length (cm) was measured on three internodes placed at the middle part of the shoot, at 10 randomly selected shoots per repetition. The density of internodes (number of internodes cm^{-1}) was calculated according to the formula:

$$density\ of\ internodes = \frac{number\ of\ internodes\ per\ shoot}{length\ of\ the\ shoot} \tag{1}$$

From each repetition, 10 leaves (30 leaves per treatment) were randomly sampled from the middle part of the shoots located at the middle – the outer part of the canopy. Leaf length (cm) was measured by the digital caliper Prowin HMTY0006 from the top of the leaf to the petiole insertion and leaf width (cm) on the widest part of the leaf. Leaf shape index was calculated as a ratio between leaf length and width. Petiole length (cm) of each leaf was also measured by the caliper Prowin HMTY0006. Leaf surface (cm^2) was calculated by the ImageJ software program (Image Processing and Analysis in Java), frequently used software in scientific community (Schneider et al. 2012), according to a modified method described by Padrón et al. (2016). After setting the length scale in pixels by the determination of the known length in the figure, brightness threshold was modified to highlight the leaf blade and then the leaf area was measured using a Region of Interest manager tool.

2.3. Productivity parameters

Yield, yield efficiency and share of decayed fruit were measured on three fruit trees for each repetition (9 trees per treatment). Yield per tree (kg) was determined in the orchard immediately after harvest by weighing the total yield of fruits for each tree. Yield efficiency (kg cm^{-2}) was calculated according to the formula:

$$yield\ efficiency = \frac{yield\ per\ tree\ (kg)}{TCSA\ (cm^2)} \tag{2}$$

Share of decayed fruit was determined relative to the total yield per tree. Fruit mass was measured on 30 fruit per repetition (90 fruits per treatment) using a digital analytical balance (OHAUS Adventurer AX2202, Ohaus Corporation Parsippany, NJ, USA) with an accuracy of 0.01 g.

2.4. Physico-chemical properties of fruits

Analyses of physico-chemical properties were conducted at the Department of Pomology and Department of Chemistry, Faculty of Agriculture, University of Zagreb, Croatia.

2.4.1. Fruit skin colour parameters

The fruit skin colour parameters were measured according to the CIE L*a*b* and CIE L*C*h° systems, using a colorimeter (ColorTec PCM; ColorTec Associates Inc., USA). In a three-dimensional uniform space L* value is defined as a vertical coordinate which defines lightness, and a* and b* values as a horizontal coordinate which, if negative, indicates intensity of green and blue colour (respectively), or if positive, intensity of red and yellow colour (respectively) (AN 1005.00 2012). According to the most widely accepted international criterion when h° is 0° it assigns to the semiaxis +a* (redness), when is 90° to the semiaxis +b* (yellowness), when is 180° to the semiaxis -a* (greenness) and when is 270° to the semiaxis -b* (blueness) (Carreño et al. 1995). Fruit skin colour were taken separately for the background (yellow) and additional (orange – red) fruit colour on 10 randomly selected fruits from each repetition (30 fruit per treatment).

2.4.2. Fruit firmness, total soluble solids (SSC), titratable acidity (TA) and SSC / TA ratio

All measurements were conducted on 10 randomly selected fruits from each repetition (30 fruits per treatment). The firmness was measured using PCE PTR-200 (PCE Instruments, Jupiter/Palm Beach, USA) fitted with an 8 mm diameter plunger and expressed in kg cm^{-2}. Measurements were taken at four opposite equatorial positions on each fruit at 90° after fruit skin was removed. The SSC was measured with a hand digital refractometer (Atago, PAL-1, Tokyo, Japan) and expressed as °Brix. TA was determined by the titration method with 0.1 mol dm^{-3} NaOH and expressed as % of malic acid. The SSC / TA ratio was calculated from the corresponding values of SSC and TA for each fruit.

2.4.3. Pectin

Pectin fractions (water-soluble pectin, ammonium oxalate-soluble pectin, alkali-soluble pectin) were determined by the method of Robertson (1979) and as well as Fruk (2014). Pectin fractions were isolated from the alcohol-insoluble precipitate of peaches by the distilled water, ammonium oxalate and sodium hydroxide. All absorbance readings were conducted on spectrophotometer Shimadzu UV 1700 (Shimadzu Corporation, Kyoto, Japan). Pectin content of each fraction was calculated according to the standard calibration curve of galacturonic acid. Afterwards obtained pectin content was expressed as mg kg^{-1} of galacturonic acid (mg kg^{-1} GA) and then converted into relative share of fractions according to the formula:

$$UD = \left(\frac{FP}{P}\right) \cdot 100 \tag{3}$$

Where: UD – share of appropriate pectin fraction expressed in %; FP – recorded content of appropriate pectin fraction expressed as mg kg^{-1}; P – total measured amount of pectin in the sample expressed as mg kg^{-1}

2.4.4. Total polyphenolic content and antioxidant potential

2.4.4.1. Preparation of extracts

The peach extraction was carried using a modified method by Komes et al. (2016). Randomly sampled 10 peaches from each repetition were mashed and homogenized with a laboratory mixer (FOSS homogenizer 2094 (Hillerød, Denmark)) until a homogenized fraction was obtained. Then, 10 g of the homogenized fraction was poured with 50 mL of boiled (100 °C) distilled water. Extraction was performed with constant mixing at room temperature (20 °C) for 30 minutes. The suspension was filtered through a metal strainer, cooled, and centrifuged at 900 x g for 5 min. Supernatants were filtered using Whatman No. 4 filter paper in a 50 mL volumetric flask and supplemented with distilled water. Extracts were prepared in duplicates for each sample (and for each respective repetition). The final concentration of obtained extracts was 200 g L^{-1}.

2.4.4.2. Total polyphenolic content

The modified Folin Ciocalteu's method of Singleton et al. (1999) was used for the determination of total polyphenolic content. A mixture of 0.1 mL of peach extract juice with 7.9 mL distilled water and 0.5 mL Folin Ciocalteu's reagent (diluted with distilled water in 1:2 ratio) and 1.5 mL of 20% sodium carbonate was vortexed and left for 2 h to complete the reaction. The absorbance was measured at 765 nm (Ough & Amerine 1988) on spectrophotometer Shimadzu UV 1700 (Shimadzu Corporation, Kyoto, Japan). Total polyphenolic content was determined according to standard calibration curve of galic acid. The data were expressed as gallic acid equivalents, mg GAE 100 g^{-1} of fresh fruit weight (fw).

2.4.4.3. Antioxidant activity

The antioxidant activity was determined using well-known methods with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), according to the procedures of Brand-Williams et al. (1995) and Re et al. (1999), respectively. The obtained data was expressed as μmol Trolox equivalents (μmol TE 100 g^{-1} fw).

2.4.5. Pigments

2.4.5.1. β-carotene determination

The β-carotene content was measured according to the procedure reported by Barros et al. (2008). In a dark test tube, 0.1 g of homogenized sample of randomly selected 10 peach fruit from each repetition was mixed with 10 mL of acetone-hexane (4:6 mL) mixture, vortexed for 1 minute, and filtered through Whatman No. 4 filter paper. The final volume was set to 10 mL. Per each repetition two samples were used and per each sample measurements were conducted in parallel. The absorbance was measured at 453 nm, 505 nm, and 663 nm on spectrophotometer Shimadzu UV 1700. The data was expressed as μg β-carotene g^{-1} fw (μg g^{-1} fw). The following equation was used for the calculation of the β-carotene content:

$$\beta - carotene\ = 0.216\ \cdot A_{663} - \ 0.304\ \cdot\ A_{505} + 0{,}452\ \cdot A_{453} \tag{4}$$

2.4.5.2. Total anthocyanins

The total anthocyanins content were measured in three samples per each repetition, according to the modified method described by Proctor (1974). Per each sample three discs of fresh peaches (fruit exocarp) were obtained (1 mm thick and the surface of one disc was 1.13 cm^2). The disc area was calculated based on the ellipse surface formula:

$$Disc\ surface = \pi\ \cdot a\ \cdot b \tag{5}$$

Where: a – value of the first radius; b – value of the second radius

In order to involve 10 fruits per each repetition in this measurement, discs of last two peaches were halved. The discs were then inserted into a test tube and immersed in 3 ml of acidified methanol solution (1% conc. HCl (v/v)) and vortexed. The tubes were left for three hours at room temperature (20 °C) in the dark chamber. The absorbance was measured at 532 and 653 nm on spectrophotometer Shimadzu UV 1700, and the obtained values of the optical density of anthocyanins were calculated according to the formula described by Wells (1995):

$$Total\ anthocyanins = A_{532} - (0.25\ \cdot (A_{653})) \tag{6}$$

Where: A532 – measured absorbance values at 532 nm; A653 – measured absorbance values at 653 nm

The optical density values were then divided by the molecular extinction coefficient of cyanidin (2.45x10^4) and by the area of the disks (area of three disks - 3.39 cm^2) to convert the values into the concentration of micromoles of anthocyanins per cm^2 (μmol cm^{-2}) (Siegelman & Hendricks 1957).

2.5. Statistical analysis

Data were statistically analyzed using SAS statistical software ver. 9.4 (SAS Institute, NC) by ANOVA and Tukey's HSD test ($P \leq 0.05$).

3. Results and Discussion

3.1. Vegetative parameters

According to ANOVA, treatments did not achieve a significant effect on vegetative parameters of peach shoots (Table 1). However, according to ANOVA, treatments revealed a significant effect on the leaf surface ($P<0.01$), leaf length ($P<0.001$) and leaf shape index ($P<0.05$) (Table 2). Peaches grown under the Y net had significantly higher leaf surface and leaf length values than those grown under the D net or C. Although according to ANOVA leaf shape index was significantly affected by treatments ($P = 0.049$), according to Tukey's HSD test no significant difference occurred between peaches grown under the Y and D net and from C (Table 2). Hence, a strong non-significant trend is evident where leaves of peaches grown under the Y net tend to have higher leaf shape ratio (more elongated leaves) than leaves of peaches grown under the D net and from C (Table 2).

Table 1- Shoot length, shoot diameter, length of internodes, the density of internodes and trunk cross-sectional area (TCSA) of peaches cultivated under anti-insect photoselective nets

Treatment	*Shoot length (cm)*	*Shoot diameter (mm)*	*Length of internodes (cm)*	*Density of internodes (internodes cm^{-1})*	*TCSA (cm^2)*
C	58.53±11.61	6.26 ±1.04	1.92±0.23	0.52±0.07	110.62±27.34
Y	57.56 ±12.27	6.13±1.13	2.02±0.33	0.51±0.09	114.28±25.41
D	53.9 ±12.41	6.06±0.90	2.08±0.25	0.49±0.07	120.33±24.56
	ANOVA				
Treatment	0.34$^{n.s.}$	0.74$^{n.s.}$	0.08$^{n.s.}$	0.19$^{n.s.}$	0.74$^{n.s.}$

[1] Results are expressed as mean ± SD; [2] $^{n.s.}$, nonsignificant

Table 2- Leaf petiole length, leaf surface, leaf length, leaf width and leaf shape index of peaches cultivated under anti-insect photoselective nets

Treatment	*Petiole length (cm)*	*Leaf surface (cm^2)*	*Leaf length (cm)*	*Leaf width (cm)*	*Leaf shape index*
C	0.91±0.11	40.21±5.07 b	15.51±1.05 b	3.59±0.33	4.34±0.33 a
Y	0.97±0.21	45.00±6.79 a	17.28±1.79 a	3.81±0.43	4.54±0.31 a
D	0.89±0.12	40.63±5.81 b	16.15±1.46 b	3.70±0.43	4.38±0.33 a
	ANOVA				
Treatment	0.15$^{n.s.}$	0.008**	0.0001***	0.14$^{n.s.}$	0.049*

[1] Results are expressed as mean ± SD; [2] Means followed by different letters within columns and are significantly different (Tukey's HSD test; $P \leq 0.05$); [3] $^{n.s.}$, *, **, ***, nonsignificant, or significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively

In majority of other studies netting, or application of nets with higher shading properties (when there was no control), enhanced or (less frequently) did not significantly affected leaf vegetative parameters (Amarante et al. 2011; Basile et al. 2014; Brar et al. 2020; Giaccone et al. 2012; Retamales et al. 2008; Vuković et al. 2016). Results obtained in this research are following the majority of the above cited literature. Generally, application of Y net enhanced some leaf vegetative parameters and according to non-significant trend application of Y and D net enhanced internode length. The main reason for such findings is probably due to the shade avoidance mechanism, as proposed in other studies (Basile et al. 2014; Bastias 2011). Reduced red to far-red ratio, which occurs under the white and yellow net (Shahak et al. 2004a, 2004b), is according to Casal (2012) one of the main signals responsible for the shade avoidance mechanism. Shade avoidance mechanism symptoms include higher internode length and leaf elongation (Smith & Whitelam 1997), which was in this study recorded on peaches grown under the Y net. According to Oren-Shamir et al. (2001), the shade avoidance mechanism is not responsible for changes in vegetative growth under all nets. Baraldi et al. (1994) reported that at the bottom part of the peach canopy, where red to far-red ratio and fitocrome equilibrium was reduced (which are signals for triggering shade avoidance mechanism), internode length was enhanced, but leaf surface was reduced in comparison to middle and top part of the peach canopy. All this presents probable explanations why leaves of peaches grown under the D nets did not have enhanced growth as those under the Y nets.

3.2. Productivity parameters

According to ANOVA, treatments did not achieve a significant effect on any productivity trait (Table 3).

Table 3- Yield, yield efficiency, fruit mass and share of decayed fruit of peaches cultivated under anti-insect photoselective nets

Treatment	*Yield (kg / tree)*	*Yield efficiency (kg cm^{-2})*	*Fruit mass (g)*	*Share of decayed fruit (%)*
C	25.97±9.58	0.24±0.09	147.10±39,41	0.83±1.68
Y	31.50±13.69	0.27±0.08	149.50±22.94	1.16±1.60
D	30.76±8.19	0.27±0.10	146.36±26.89	1.24±2.05
	ANOVA			
Treatment	0.45$^{n.s.}$	0.59$^{n.s.}$	0.79$^{n.s.}$	0.87$^{n.s.}$

[1] Results are expressed as mean ± SD; [2] $^{n.s.}$, nonsignificant

Similarly in some other studies netting, or application of nets with higher shading properties (when there was no control), did not achieve significant effect on peach yield or yield efficiency (Giaccone et al. 2012; Vuković et al. 2016). However, the nonsignificant trend is noticeable where trees grown under nets have higher yield and yield efficiency than those from control, although high standard deviation should be also taken into account. If recalculated it presents yield increase of around 4 t per hectare. It must be highlighted that the application of nets did not cause a significant increase in the share of decayed fruit, meaning that possible increase of relative air humidity and plant wetness duration under nets, which was reported by other authors (De Paula et al. 2012; Shahak et al. 2004b), did not have a significant positive effect on fruit fungi infection under nets.

3.3. Fruit skin colour parameters

According to ANOVA, treatments had a significant effect on b* ($P<0.05$) and C* ($P<0.01$) fruit background colour parameters, while on other background colour parameters no significant difference was obtained (Table 4). Peaches grown under the D net had significantly smaller b* and C* background colour values than those grown under the Y net and from C (Table 4).

Table 4- Background colour parameters of peach fruit cultivated under anti-insect photoselective nets

Treatment	*L**	*a**	*b**	*C**	*h°*
C	63.39±2.77	6.27±3.43	40.91±2.80 a	41.53±2.74 a	81.28±4.84
Y	61.82±3.93	6.52±3.61	40.67±4.38 a	41.35±4.33 a	80.87±5.20
D	61.15±3.75	5.28±4.02	37.96±3.93 b	38.57±3.41 b	81.68±6.79
	ANOVA				
Treatment	$0.044^{n.s.}$	$0.34^{n.s.}$	0.016^{*}	0.008^{**}	$0.82^{n.s.}$

[1] Results are expressed as mean ± SD; [2] Means followed by different letters within columns and are significantly different (Tukey's HSD test; P ≤0.05); [3] $^{n.s.}$, *, **, nonsignificant, or significant at P ≤0.05, P≤0.01, respectively

Background colour is a primary criterion maturity used for commercial peach harvest (Lewallen & Marini. 2003), because it changes along with other important parameters such as soluble solids, flesh firmness and violate compounds (Ramina et al. 2008). In most peach cultivars assessment of fruit maturity by skin background colour changes include transformation from green to yellow colour (Crisosto & Valero 2008). Therefore, peaches grown under the D net probably delayed fruit ripening which is indicated by smallest intensity of yellow colour.

According to ANOVA, treatments had a significant effect on all additional fruit colour parameters with exception of a* colour parameter (Table 5). Peaches from C had significantly smaller values of b* and C* additional colour parameters than those grown under the Y and D net. Peaches grown under the D net had significantly higher L* and h° values than those from C, while in comparison to peaches grown under the Y net no significant difference was recorded (Table 5).

Table 5- Additional colour parameters of peach fruit cultivated under anti-insect photoselective nets

Treatment	*L**	*a**	*b**	*C**	*h°*
C	31.22±4.55 b	10.61±2.74	10.74±4.30 b	15.23±4.66 b	44.19±7.86 b
Y	33.01±5.38 ab	13.07±3.61	14.58±5.64 a	19.70±6.32 a	47.29±6.55 ab
D	35.97±5.61 a	11.85±3.27	15.47±5.64 a	19.63±6.07 a	51.62±7.00 a
	ANOVA				
Treatment	0.008^{**}	$0.09^{n.s.}$	0.015^{*}	0.04^{*}	0.0009^{***}

[1] Results are expressed as mean ± SD; [2] Means followed by different letters within columns and are significantly different (Tukey's HSD test; P≤0.05); [3] $^{n.s.}$, *, **, ***: nonsignificant, or significant at P≤0.05, P≤0.01, P≤0.001, respectively

Similarly in some other studies netting, or application of nets with higher shading properties (when there was no control), generally harmed additional fruit colouration (Amarante et al. 2011; Giaccone et al. 2012; Solomakhin & Blanke 2010). Lack of significant difference of some fruit additional colour parameters between peaches grown under the Y net and from C indicates a milder negative effect of the Y net on peach additional fruit coloration. However, application of D net caused notable reduction of peach additional colour. Since red colour for retailers historically presents one of the main fruit quality components (Crisosto & Costa 2008), this is huge drawback for application of D net. Such fruit colour changes are caused by nets light environment modification since according to Hamadziripi (2012) light intensity that reaches fruit skin has crucial effect on colour development. According to Westwood (1993), exposure of the peach fruit to direct light is necessary for the development of red colour, therefore enhancement of scattered light by photoselective nets (Oren-Shamir et al. 2001; Shahak et al. 2004a, 2004b) cannot alleviate negative effects of shading on additional peach colour development. Hence, fruit additional colour parameters and indexes were highly reduced under the D net due to its high shading factor (indicated by smallest mesh size).

3.4. Fruit firmness, total soluble solids, titratable acidity and SSC / TA ratio

According to ANOVA, treatments revealed a significant effect on fruit firmness, TA and SSC/TA ratio ($P<0.001$) (Table 6). Peaches grown under the Y and D net had significantly higher fruit firmness than those from C (Table 6). Peach fruit firmness (with skin background colour) is one of the main maturity indices used to determine and supervise harvesting operations (Crisosto & Valero 2008). In available literature there is inconsistency regarding effect of netting on this fruit trait (Brkljača et al. 2016; Giaccone et al. 2012; Shahak et al. 2004a; Vuković et al. 2016), which may be contributed to the different agro-ecological conditions and hereditary factors.

Although no significant difference was recorded in SSC of peach fruits, a notable non-significant trend is evident where fruits of peaches from C (10.43±1.08%) tend to have higher SSC than those grown under the Y and D net (9.93±0.79 and 9.79±0.91%, respectively) (Table 6). According to Iglesias & Echeverría (2009; after Clareton 2000) soluble solids below 10% are generally unacceptable to consumers. Therefore, only peaches from C scored satisfactory SSC levels, while peaches grown under the D net will probably cause negative consumer response. In most studies netting, or application of nets with higher shading properties (when there was no control), reduced peach fruit SSC levels (Shahak et al. 2004a ; Amarante et al. 2011; Giaccone et al. 2012; Brkljača et al. 2016) or did not achieve significant effect (Amarante et al. 2011; Corollaro et al. 2015; Vuković et al. 2016). Since SSC is under high influence of light availability (Corelli Grappadelli & Marini 2008) it is clear that the highest average reduction of SSC occurred under the D net due to the highest shading factor (indicated by smallest mesh size).

Peaches from C (0.54±0.09%) had significantly smaller TA than those grown under the Y and D net (0.63±0.06 and 0.65±0.08%, respectively) (Table 6). According to a study conducted by Bassi & Selli (1990) variety 'Suncrest' has high levels of acids and they may contribute to its unsatisfactory taste. In available literature there is inconsistency regarding effect of netting on this fruit trait (Shahak et al. 2004a; Giaccone et al. 2012; Lobos et al. 2013; Vuković et al. 2016, 2020).

Peaches from C had a significantly higher SSC / TA ratio than those grown under the Y and D net (Table 6). SSC / TA is important factor in consumer acceptance (Crisosto & Kader 2000).

Table 6- Firmness, total soluble solids (SSC), titratable acidity (TA) and SSC/TA ratio of peach fruits cultivated under different anti-insect photoselctive nets

Treatment	*Firmness (kg cm^{-2})*	*SSC (°Brix)*	*TA (% as malic)*	*SSC / TA*
C	4.29±0.62 b	10.43±1.08	0.54±0.09 b	19.96±4.19 a
Y	4.87±0.46 a	9.93±0.79	0.63±0.06 a	15.83±2.45 b
D	5.20±0.63 a	9.79±0.91	0.65±0.08 a	15.34±2.05 b
	ANOVA			
Treatment	<0.0001***	0.15$^{n.s.}$	<.0001***	<.0001***

[1] Results are expressed as mean ± SD; [2] Means followed by different letters within columns and are significantly different (Tukey's HSD test; P ≤0.05); [3] $^{n.s.}$, ***, nonsignificant, or significant at P≤0.001, respectively

3.5. Pectin

No significant difference was recorded in a share of water-soluble and ammonium oxalate-soluble pectin in peach fruits (Table 7). However, peaches grown under the Y net had a significantly higher share of alkali-soluble pectin than those grown from C, while between those grown under the D net in comparison to those grown under the Y net and from C no significant difference was recorded. However, a strong non-significant trend must be noted where peaches grown under the D net tend to have higher share of alkali-soluble pectin than those from C (Table 7).

Table 7- The share of water-soluble pectin (SWP), the share of ammonium oxalate-soluble pectin (SAOP), the share of alkali-soluble pectin (SAP) of peach fruits cultivated under different anti-insect photoselective nets

Treatment	*SWP (%)*	*SAOP (%)*	*SAP (%)*
C	41.26 ± 6.77	51.15 ± 9.12	7.60 ± 5.10 b
Y	34.31 ± 6.01	34.69 ± 5.57	31.00 ± 9.71 a
D	41.07 ± 5.65	42.93 ± 9.17	15.99 ± 7.67 ab
	ANOVA		
Treatment	0.3$^{n.s.}$	0.1$^{n.s.}$	0.017*

[1] Results are expressed as mean ± SD; [2] Means followed by different letters within columns and are significantly different (Tukey's HSD test; P≤0.05); [3] $^{n.s.}$, *, nonsignificant, or significant at P≤0.05, respectively

Around 15 to 17 days after the full bloom of peaches, protopectins insoluble to water are hydrolysed into pectin fractions that are soluble in water (Selli & Sansavini 1995). Softening of peach fruits is accompanied by the transformation of pectin insoluble in water to water-soluble pectin that gives the fruit the characteristic texture of ripe fruit (Jia et al. 2006). Since peaches grown under the D and Y net also had significantly higher fruit firmness and TA than those from C as well as reduced b* (significantly) and a* (notable non-significant trend) value of background colour for peaches cultivated under the D net, together with pectin fractions results, it may indicate delayed fruit ripening of peaches grown under the D and Y net in contrast to those from C. Similarly, Ordóñez et al. (2016) reported that at commercial maturity apples 'Golden Delicious' grown under the white and black nets (6-7 and 16% of shading, respectively) significantly differ in fruit firmness, SSC and TA. However, according to the same author if data was compared at the beginning of climacteric rise in fruits then no significant difference was recorded. Possibly, by later harvest date, some of negative effects of nets on inner peach fruit quality parameters can be lessen. Additional peach coloration (which is important parameter for peaches) under D net almost certainly cannot be considerably improved by delayed harvest date because direct light exposure of the peach fruit is necessary for the development of red colour (Westwood 1993) and D net has smallest mesh size (hence highest shading factor). Rapid ripening of peaches should also be taken into account.

3.6. Total polyphenolic content, antioxidant potential

According to the ANOVA, treatments achieved a significant effect on the total polyphenol content, ABTS and DPPH antioxidant activity (P<0.001) and total anthocyanin content (Table 8). Total polyphenol content and ABTS antioxidant activity were significantly higher in peaches from the C than in those grown under the Y and D net. DPPH antioxidant activity was significantly smaller in fruits grown under the Y net than in those under the D net and from C (Table 8).

In south Italy, Basile et al. (2012) reported significantly smaller content of total polyphenols and antioxidant activity in the flesh of kiwi 'Hayward' grown under the white net than from C. The scarcity of studies related to these biochemical parameters emphasizes the importance of the results obtained in this study. Light intensity and quality can affect the biosynthesis of antioxidants and phenols (Bakhshi & Arakawa 2006; Jurić et al. 2020), and the concentration of certain polyphenols is being increased when fruits are exposed to UV light because flavonoids can absorb UV radiation and therefore prevent tissue damage (Arakawa et al. 1985). Since it was reported that white and yellow nets absorb UV radiation (Shahak 2008; Shahak et al. 2004b), it can be assumed that the Y and D nets by reduction of UV light transmittance caused reduction in total polyphenol content and ABTS antioxidant activity of peach fruits. Interestingly, fruits of peaches grown under the D net did not significantly differ in DPPH antioxidant activity with peaches from C. Moreover, it must be highlighted that peaches grown under the D net had a significantly smaller amount of total polyphenols and ABTS antioxidant activity than those from C. The main reason for this occurrence is probably because ABTS radical can react with both hydrophilic and lipophilic antioxidants (Prior et al. 2005), while DPPH only with lipophilic antioxidants (Jatoi et al. 2017). Since peach fruit contains a significant amount of carotenoids (Oliveira et al. 2016) (which are lipophilic antioxidants) it is possible that such results were obtained due to a higher amount of carotenoids of peaches grown under the D net (indicated by a non-significant trend).

3.7. Pigments

Regarding the β-carotene levels, in peach fruits, no significant difference was recorded between treatments (Table 8). However, according to a non-significant trend, peaches grown under the D net tend to have a somewhat higher amount of β-carotene. Peaches grown under the D net had significantly smaller total anthocyanin content than those from C, while between peaches grown under the Y net and other treatments no significant difference was recorded (Table 8).

Table 8- Total polyphenol content (TPC), ABTS antioxidant potential (AOP – ABTS), DPPH antioxidant potential (AOP – DPPH), β Carotene content and total anthocyanin content (TAC) of peach fruits cultivated under different anti-insect photoselective nets

Treatment	*TPC (mg GAE 100 g^{-1} fw)*	*AOP –ABTS (µmol TE 100 g^{-1} fw)*	*AOP - DPPH (µmol TE 100 g^{-1} fw)*	*β Carotene (µg g^{-1} fw)*	*TAC (µmol cm^{-2})*
C	21.45±3.06 a	67.38±4.56 a	40.68±3.16 a	5.02±1.30	1.75±1.01 a
Y	15.64±2.26 b	45.08±6.76 b	35.00±4.22 b	5.65±1.81	1.21±0.60 ab
D	18.14±2.18 b	44.90±6.84 b	40.02±1.75 a	7.35±2.44	0.71±0.32 b
	ANOVA				
Treatment	0.0018^{**}	0.0011^{**}	0.007^{**}	$0.1^{n.s.}$	0.047^{*}

[1] Results are expressed as mean ± SD; [2] Means followed by different letters within columns and are significantly different (Tukey's HSD test; P≤0.05); [3] $^{n.s.}$, *, **, nonsignificant, or significant at P≤0.05, P≤0.01, respectively

According to Iglesias et al. (1999) anthocyanin content is directly related to a / b fruit colour ratio and inverse with h and L colour values. It was also the case in this study (Tables 5 and 8) meaning that reduction of anthocyanin content in peaches grown under D net was the main reason for poor additional colour development. Similar results were obtained in Germany by Solomakhin & Blanke (2010) on sun-exposed part of the apple 'Pinova' protected by different types of photoselective nets. In peaches, exposure of fruit to direct light is necessary for the development of red colour (Westwood 1993) and hence for the synthesis of anthocyanins. Therefore, it is evident that D net, due to its high shading factor (indicated by smallest mesh size), achieved the highest average reduction of total anthocyanin content in peach fruits. Changes in light quality can also influence fruit anthocyanin content. Shorter wavelengths, in a range from blue to UV light, show the most prominent influence on the accumulation of flavonoids (anthocyanins) in fruit (Zoratti et al. 2014). White and yellow nets absorb UV light (Shahak 2008; Shahak et al. 2004b) and it may also contribute to the reduction of total anthocyanin content in fruit skin. Since D net has relatively small mesh size, it might had caused the highest reduction of UV light transmittance.

4. Conclusions

Application of nets significantly affected a notable part of studied vegetative and generative peach traits. Application of Y net significantly enhanced some leaf vegetative parameters which were not the case for the D net, while shoot vegetative parameters were not significantly affected by the net application. A significant effect on productivity parameters was not achieved by the application of nets. Application of Y net generally achieved a smaller reduction of peach fruit quality traits than D net, and in some cases did not significantly differ with results obtained by peaches from C. Bioactive compounds and antioxidant activity of fruits were generally reduced as a consequence of net application. It can be concluded that application of Y net only slightly reduced peach fruit quality compared to C (trees grown without net), and therefore can be recommended for application as an anti-insect net. Moreover, due to its additional properties (anti-hail, anti-insect, etc.), its application will overall have a positive effect. However, D net more notably reduced peach fruit quality parameters (and especially additional fruit colour which is important parameter for peaches). Therefore, it should be used only when other control strategies show to be ineffective.

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Abbreviations

C	Control
Y	Yellow photoselective net
D	Stop Drosophila Normal net
SSC	Total soluble solids
TA	Titratable acidity

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