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Research Article

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Physiological basics of sweet cherry productivity depending on rootstocks, interstems and plant density

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Abstract: Optical and physiological parameters in sweet cherry tree canopies at different plant density and rootstock-interstem-scion combinations were studied in order to identify the combinations that would ensure optimal passage of photosynthesis and increase vield and fruit quality. It was shown that cherry cultivars react differently to varying plant density depending on their growth habit and thus require different planting schemes. Leaves in the periphery of tree canopy had higher dry matter content per unit of leaf surface area (LSA) and increased net photosynthetic productivity (NPP) compared to leaves in the center of the canopy. This can be related to higher light interception (LI) levels in peripheral zones of the tree. Trees on interstems had higher yield than trees on own-root clonal rootstock. The complex of photosynthetic and yield indices allowed to select the best scion-rootstock-density combinations: for 'Melitopolska chorna' cultivar - Krymsk 5 and Gisela 5 interstems with 5 x 3 m planting scheme; for 'Krupnoplidna' cultivar – Gisela 5 interstem (5 x 3 m) and Krymsk 5 interstem (5 x 4 m).

Keywords: canopy projection area, leaf surface area, chlorophyll content, net photosynthetic productivity, yield

Abbreviations

CPA = Canopy projected area, m², area of maximum horizontal projection of tree canopy

PSUR = Planting scheme usage rate, %, ratio of canopy projected area to ground area available for the tree

LSA = Leaf surface area, m², combined area of all leaves of tree canopy

LI = Light interception, % of available sunlight intercepted by tree canopy

NPP = Net photosynthetic productivity, g m^2 day¹, amount of dry matter accumulated by 1 m^2 of leaf surface area per day

DM = Dry matter

1 Introduction

Cultivation of modern intensive sweet cherry orchards is impossible without taking photosynthetic activity of the trees into consideration. Solar energy accumulated by plants as a result of photosynthesis is then spent on formation of organs and, ultimately, the yield. Correct passage of photosynthesis is particularly important for trees grafted on vigour-controlling rootstocks, where leaf:fruit ratio is much lower than in vigourous trees.

Sweet and sour cherry have the highest intensity of photosynthesis among all stone fruit crops (Flore and Layne 1999). A large number of indices affect photosynthesis, among which the amount of foliage in tree canopy. Cittadini et al. (2008) indicated that major parameters of sweet cherry fruit quality (weight, pulp density, soluble solids content) increase when leaf:fruit ratio rises. According to Roper and Loescher (2002), an additional 32 cm² of leaf surface area (LSA) are required in order to increase mean fruit weight by 1 g.

In general, sweet cherry trees have more leafy canopies when grafted on clonal rootstocks. According to Kyshchak (2014), trees grafted on Krymsk 5 rootstock formed 1.4 time higher LSA per 1 m² of canopy projected area (CPA) compared to the trees grafted on Mahaleb. Such differences are mainly associated with shorter internodes inherent in dwarfing trees.

Another factor directly affecting photosynthesis is the sunlight penetration into tree canopy. According to studies carried out on other stone fruit species, net photosynthetic productivity (NPP) of leaves that are

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under insufficient light levels is 40 to 80% lower than in leaves on the periphery of the canopy (Alekseeva 1986; Shishkanu and Pitushkan 1970). Lack of light also affects functioning of the leaves: at 30% of light, only 1-3 leaves are formed per cluster of an apple tree, with smaller size and life span of those leaves (Kudryavets and Khromenko 1977).

Finally, photosynthesis is affected by the pigment content of leaf mesophyll. Chlorophyll a, chlorophyll b and carotenoids are the main pigments that absorb photosynthetically active radiation. Photosynthetic productivity decreases with a decrease in the concentration of these pigments in the leaf. Chlorophyll content depends on a large number of factors – light levels (Balan 1978), scion-rootstock combination (Asanica et al. 2015; Moreno et al. 2001), growing conditions (Todaria et al. 1980), stress factors (Küçükyumuk et al. 2015; Viljevac et al. 2013) etc.

In recent years, high density sweet cherry orchards have become increasingly popular. This applies not only to orchards on dwarfing rootstocks, but also to ones where trees are grafted using interstems, as well as rootstocks of medium and high vigour. In many cases, however, high density leads to a decrease in yield and fruit quality. Apparently, this is largely due to the deterioration of light regime in such orchards and, as a consequence, a decrease in the activity of photosynthetic apparatus. Therefore, the aim of this research was to study the optical and physiological parameters of sweet cherry tree canopies in order to identify orchard systems that would ensure optimal passage of photosynthetic processes and increase yield and fruit quality.

2 Materials and Methods

In the field experiment, sweet cherry trees of 'Melitopolska chorna' and 'Krupnoplidna' cultivars, grafted on Krymsk 5 (also known as VSL-2) and Gisela 5 interstems (Mahaleb being the main rootstock) were compared with trees grafted on Krymsk 5 rootstock. Since it is believed that interstems have higher vigour than own-root clonal rootstocks (Senin and Senin 2009; Bielicki and Rozpara 2010), in addition to 5 x 3 m planting scheme (667 trees ha¹) which is considered standard for clonal rootstocks in Ukraine, all variants were planted using 5 x 4 m (500 trees ha¹) scheme. 'Melitopolska chorna' and 'Krupnoplidna' cultivars were chosen for the experiment, as they are the most popular sweet cherry cultivars in commercial orchards of the region.

The orchard was established with one-year-old maiden trees in the spring of 2006 in State Enterprise

"Experimental Farm" Melitopolske" in Fruktove village near the city of Melitopol, in the southeast of Ukraine (46°75'N, 35°19'E). Trees were trained as central leader. The soil of the experimental site is dark chestnut with light-clay texture. Climate is moderately continental, the average daily temperature in January is -3.1°C, in July is +22.8°C; the average annual amount of precipitation is 475 mm. Soil management in the orchard included regular tillage between the rows and herbicide application in the rows. The orchard was not irrigated. Plant protection was carried out in accordance with the recommendations for sweet cherry cultivation in the region.

The experiment was laid using randomized design with three replications of 6 trees. Thus, 18 trees were used in each variant of the experiment. CPA, LSA, yield, mean fruit weight and diameter parameters were determined according to generally accepted methods (Kondratenko and Bublik 1996). Phenological stages of sweet cherry trees are presented according to BBCH scale (Growth stages of mono-and dicotyledonous plants – BBCH monograph 2001).

The content of photosynthetic pigments was determined by spectrophotometric method during the stage of active growth of the fruits (BBCH 76) (Musiyenko et al. 2001). Leaf samples of 150–200 mg were prepared and rubbed with MgCO₃ and 5 ml of acetone. The extract was poured through a glass filter into 25 ml volumetric flask. Bunsen flask was rinsed with small portions of acetone and adjusted to the mark with pure acetone. The obtained acetone extract contains the sum of green pigments. After that, the measurements of optical density (D) at wavelengths of 662 and 644 nm were carried out using UNICO 2800 UV/VIS spectrophotometer. The concentration of pigments (C) was calculated using the formulas:

C (Chl a) =
$$9.784D_{662} - 0.990D_{644}$$
;
C (Chl b) = $21.426D_{644} - 4.650D_{662}$.

The content of pigments (A) in plant material was calculated by the formula:

$$A = 0.01 \text{ C v H}^{-1}$$

where

C – pigment concentration, mg l^{-1} v – volume of the extract, ml (25 ml); H – the sample of plant material, g (0.1–0.2 g).

NPP was determined by Ovsyannikov's method (1985) by circular removal of bark and phloem on individual sweet cherry clusters (spurs), with 3-7 fruits and 15-25 cm² of leaves per 1 fruit on each cluster. This bark and phloem

removal was carried out with the purpose to localize the consumption of accumulated organic matter on fruit growth in the isolated cluster. NPP was calculated using the formula:

NPP =
$$(m_2 - m_1) S^{-1} t^{-1}$$
,

where

 m_1 – dry mass at the beginning of the experiment, g; m_2 – dry mass at the end of the experiment, g; S – leaf area of one cluster, m^2 ; t – duration of the experiment, days. Thus, NPP indicates how much dry matter in grams is provided by 1 m^2 of leaves per 1 day.

In order to measure light interception (LI) levels of the canopy, three model trees were selected in each variant of the experiment. Tree canopy was divided into 6 zones: 4 peripheral zones (northern, southern, eastern

and western) and 2 zones in the central part of the canopy (upper and lower). Peripheral zones extended 1 m inside the canopy. The measurements were carried out 3 times: at 8:00, 12:00 and 16:00, after which the average value for each zone of the canopy was calculated. LI was calculated as a percentage of available sunlight intercepted by the tree.

Analysis of variance of the results was conducted using Minitab 16 software (Minitab Inc., State College, PA). In order to determine the significant difference between the means, Tukey's range test was used with an accuracy of 0.05. In order to determine the relationship between the indices, Pearson's correlation was used.

Ethical approval: The conducted research is not related to either human or animal use.

Table 1: Influence of interstems and plant density on canopy projected area and leaf surface area of sweet cherry trees, average for 2016-2017

Rootstock/	Scheme of planting	CPA, m²	PSUR, %	LSA		
interstem				1 tree, m ²	1 ha, thousand m²	
Melitopolska chorna						
Krymsk 5	5 x 3 m	7.4 a	82 a	49.1 d	32.7 bc	
	5 x 4 m	7.8 a	65 b	57.9 c	29.0 <i>c</i>	
Krymsk 5 interstem	5 x 3 m	7.3 a	81 a	66.0 <i>b</i>	44.0 a	
	5 x 4 m	7.7 a	64 b	78.9 a	39.5 ab	
Gisela 5 interstem	5 x 3 m	7.6 a	84 a	53.2 cd	35.5 b	
	5 x 4 m	7.7 a	64 b	83.0 a	41.5 a	
		Krupnop	olidna			
Krymsk 5	5 x 3 m	8.2 b	91 a	59.0 <i>c</i>	39.4 a	
	5 x 4 m	8.8 a	73 b	52.5 d	26.3 <i>c</i>	
Krymsk 5 interstem	5 x 3 m	8.2 b	91 a	58.7 <i>c</i>	39.2 a	
	5 x 4 m	9.0 a	75 b	66.1 b	33.1 bc	
Gisela 5 interstem	5 x 3 m	8.1 <i>b</i>	90 a	52.3 d	34.9 b	
	5 x 4 m	8.7 a	73 b	78.7 a	39.4 a	
		Average for the sch	neme of planting			
5 x 3 m		7.8 a	87 a	56.4 b	37.6 a	
5 x 4 m		8.3 a	69 b	69.5 a	34.8 a	
		Average for	cultivars			
Melitopolska chorna		7.6 b	74 b	64.7 a	37.0 a	
Krupnoplidna		8.5 a	82 a	61.2 a	35.3 a	

Different letters indicate significant difference between the means according to Tukey's test (p < 0.05)

3 Results and Discussion

The research showed that sweet cherry cultivars in the age of 11th leaf differently adapted to varying plant density. No significant difference between the variants in CPA was found for 'Melitopolska chorna' cultivar that forms narrow pyramidal canopies with sharp crotch angles (Table 1). 'Krupnoplidna' cultivar forms more spreading canopies with wider crotch angles. The trees of this cultivar, when planted less densely (5 x 4 m), had a significantly larger CPA than 5 x 3 m scheme.

When calculating the planting scheme usage rate (PSUR), a technological track for vehicles with a width of 2 m between the rows was taken into account. Thus, area actually available for the trees in case of 5 x 3 m scheme is 9 m^2 , 5 x 4 m scheme – 12 m^2 . It was determined that trees of both cultivars utilized higher density better, with PSUR in range of 81-91%. Value of this parameter for lower density was 64-75%.

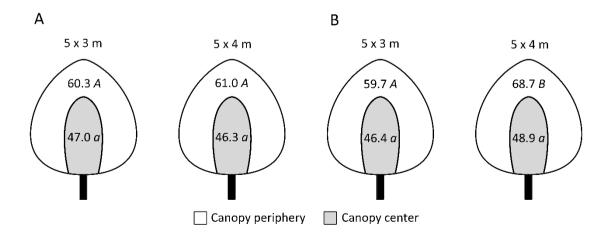
Different letters indicate significant difference between the means for each parameter according to Tukey's test (p < 0.05)

It is generally believed that 30-35 thousand m^2 of leaves (Leaf area index 3-3.5) are required in order to form 20 t ha¹ of sweet cherry yield with high fruit quality (Steiner et al. 2015). Under the conditions of the experiment, most of the variants had sufficient leaf surface area, except for the variant with trees grafted on Krymsk 5 rootstock and use of 5 x 4 m scheme. The highest LSA per hectare was observed in the variant using Krymsk 5 inrestem and 5 x 3 m scheme -41.6 thousand m^2 on average for both cultivars.

The rows in the orchard were placed North to South, as most studies indicate that it makes LI into tree canopy more uniform and promotes an increase in yield up to 20% compared with East to West row direction (Trentacoste et al. 2015; Khemira et al. 1993). When determining LI, it was found that leaves in the central part of the canopy got on average a quarter less sunlight compared to those on the periphery of the canopy. In general, LI levels distributed as follows: the southern side (on average 64.8% of the open area) > eastern side (61.8%) > western side (57.6%) > upper central part (55.5%) = northern side (54.5%) > lower central part (37.0%).

On 'Krupnoplidna' cultivar, LI of the peripheral zones of the canopy was significantly higher in case of lower tree density (Figure 1). For this cultivar a very strong inverse correlation was found between LI level of the canopy periphery and the PSUR (r = -0.976). Thus, it can be concluded that for 'Krupnoplidna' cultivar under the conditions of the experiment, a less dense scheme of 5 x 4 m is more preferable, as it allows sunlight to enter different parts of tree canopy more evenly during the day.

For 'Melitopolska chorna' cultivar there was no significant difference between the LI levels of canopy periphery depending on the plant density, nor the abovementioned correlation was found (r = -0.169). This suggests that a denser planting scheme is better suited for this cultivar, since with lower density, tree canopies did not receive more sunlight. LI levels of the central parts of the canopy were approximately the same in all variants of the experiment.



Different letters indicate significant difference between the means according to Tukey's test (p < 0.05). Capital letters indicate the differences for peripheral zone of the canopy; small letters indicate the differences for central zone of the canopy

Figure 1: Light interception of central and peripheral zones of tree canopies of 'Melitopolska chorna' (A) and 'Krupnoplidna' (B) sweet cherry cultivars, % of the open area, 2017

Chlorophyll is the main pigment absorbing photosynthetically active radiation, so its content in the leaves of the tree is of utmost importance. Total leaf chlorophyll content in the experiment generally matched the data of other researchers both in terms of mg g¹ of DM (Viljevac et al. 2013; Papadakis et al. 2007) and mg m⁻² of LSA (Pilarski et al. 2007).

Given that different parts of tree canopies receive varying amount of sunlight, the photosynthetic processes in their leaves take place in different ways. During this study, it was found that the chlorophyll content of DM of the leaves of the central part of the canopy was on average 1.3 times higher than in the leaves of the canopy periphery (Table 2). This confirms data of Flore and Layne, (1999) and Kudryavets (1987), indicating that a decrease in chlorophyll content in leaves under high levels of sunlight is associated with light saturation and achieving compensation point at which chlorophyll is simultaneously synthesized and decomposed. Increased in terms of DM concentration of green pigments in low lighted leaves is explained by the mechanism of their adaptation to conditions of insufficient LI.

In addition, the same authors indicated that lack of light in the leaves induces the decrease in the thickness of the leaf blade and thus the number of mesophyll layers containing chloroplasts. This point was also proven by the current research: weight of DM of 1 m² of LSA on canopy periphery was on average 33% higher than that of the leaves in the center of the canopy. The fact that such increase is caused by the thickening of the leaf blade, and not by the increase in the proportion of dry matter, is confirmed by the fact that there was no difference in the

water content of the leaves between the variants of the experiment, which was 63-67%.

Thus, when recalculating the concentration of chlorophyll per m² of LSA, it was determined that leaves with a sufficient level of light interception are not only equal, but in a number of variants of the experiment exceed the leaves from the central part of the canopy in chlorophyll content per leaf area. The maximum values of this index for both cultivars were observed on Krymsk 5 rootstock (5 x 3 m scheme), Gisela 5 interstem (both densities), and for 'Krupnoplidna' cultivar - also on Krymsk 5 interstem with a 5 x 4 m planting scheme.

All abovementioned indicators significantly affect the course of photosynthesis in plants. The analysis of NPP showed that its values were significantly lower in shadow leaves compared to higher light level leaves: 2.3 times lower for 'Melitopolska chorna' and 1.6 times for 'Krupnoplidna' cultivars (Figure 2). On 'Krupnoplidna' cultivar, in addition, a strong positive correlation was observed between NPP and LI (r = 0.820), indicating that plants were more active in forming the organic matter due to the greater penetration of photosynthetically active radiation into chloroplasts of the leaf blade.

NPP of 'Melitopolska chorna' trees grafted on Krymsk 5 rootstock was lower than when grafted on interstems, both at the periphery and in the central parts of the canopy. Perhaps, this is because the root system of Krymsk 5 significantly suffered from lack of moisture in the nonirrigated conditions of the experiment. Interstem variants, where Mahaleb is the main rootstock, had a deeper reach of the root system. According to Solovyova (1975), NPP of sweet cherry trees under water stress conditions decreased

Table 2: Chlorophyll content in sweet cherry leaves depending on the zone of the canopy, average for cultivars, 2016-2017

Variant	Scheme of	Specific weight of DM of leaf		Chlorophyll a+b content			
	planting	area,	g m ⁻²	mg g⁻¹	of DM	mg m ⁻²	of LSA
		Periphery	Center	Periphery	Center	Periphery	Center
Krymsk 5	5 x 3 m	75.84 a	57.94 ab	5.46 a	6.51 <i>b</i>	412.9 a	376.4 b
	5 x 4 m	71.76 a	52.63 b	4.93 b	7.18 a	352.7 b	376.9 b
Krymsk 5 interstem	5 x 3 m	73.70 a	52.97 b	4.83 b	6.28 b	355.9 b	330.7 <i>c</i>
	5 x 4 m	77.12 a	61.95 a	4.93 b	6.28 b	380.5 ab	388.7 ab
Gisela 5 interstem	5 x 3 m	76.16 a	57.00 ab	5.34 a	7.13 a	405.2 a	406.2 a
	5 x 4 m	75.99 a	55.88 b	5.21 a	6.30 <i>b</i>	395.2 a	349.7 <i>c</i>
			Average for cano	py areas			
Periphery		75.09 a		5.11 <i>b</i>		383.6 a	
Center		56.3	39 b	6.6	1 <i>a</i>	371	.4 a

Different letters indicate significant difference between the means for each parameter according to Tukey's test (p < 0.05)

by 27%.

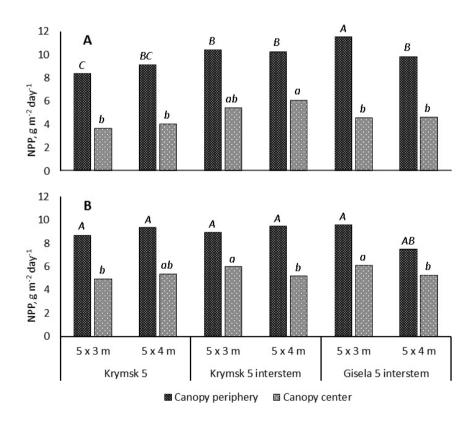
There was no general regularity in the effect of the planting density on NPP. The maximum values of this parameter at the periphery of the canopy on 'Melitopolska chorna' cultivar were observed in the variants with Gisela 5 and Krymsk 5 interstems at 5 x 3 m scheme: 11.52 and 10.38 g m² day¹, respectively. Variants with Gisela 5 (5 x 3 m) and Krymsk 5 (5 x 4 m) interstems stood out on 'Krupnoplidna' cultivar – with NPP of respectively 9.59 and 9.46 g m² day¹.

Organic matter accumulated by plants in the process of photosynthesis, ultimately goes to fruit formation. Frequent frost damage in winter and spring during the years of the research reduced the yield. Therefore, in order to have the best representation of the yield potential of the trees, yield data for the period of 2014-2017 is used.

It should be noted that interstem variants of the experiment had significantly higher cumulative yield compared to trees on Krymsk 5 rootstock: 45% more for Krymsk 5 interstem, and 37% more for Gisela 5 interstem. This confirms the data of other researchers on the subject (Bielicki and Rozpara 2010; Rozpara and Grzyb 2006).

Sweet cherry cultivars had a different reaction to plant density. For 'Melitopolska chorna' the cumulative yield per tree was higher when using 5 x 3 m planting scheme, with the exception of Krymsk 5 variant, where no differences were found between the planting schemes (Table 3). When recalculating the cumulative yield per unit of area, a denser scheme had 1.7 times more yield. The highest productivity for 4 years of research on this cultivar was shown by the variants with 5 x 3 m scheme on Krymsk 5 and Gisela 5 interstems – 30.9 and 25.7 t ha⁻¹, respectively.

For 'Krupnoplidna' cultivar, the yield per tree was higher with 5 x 4 m scheme in variants with Krymsk 5 rootstock and the Krymsk 5 interstem, and was equal regardless of the density when using Gisela 5 interstem. However, when comparing cumulative yield from 1 hectare, the planting schemes evened out, and when using the Gisela 5 interstem, 5 x 3 m scheme was more productive. Thus, it can be concluded that the additional yield per tree on 'Krupnoplidna' cultivar when using lower plant density, which is provided by better penetration of



Different letters indicate significant difference between the means according to Tukey's test (p < 0.05). Capital letters indicate the differences for peripheral zone of the canopy; small letters indicate the differences for central zone of the canopy

Figure 2: Net photosynthetic productivity of 'Melitopolska chorna' (A) and 'Krupnoplidna' (B) sweet cherry trees in different zones of the canopy, average for 2016-2107

Table 3: Influence of interstems and plant density on yield and fruit quality of sweet cherry, average for 2014-2017

Variant	Scheme of planting	Cumulative yield 2014-2017, t ha ⁻¹	Mean fruit weight, g	Mean fruit diameter, mm	
	. , ,	Melitopolska chorr			
Krymsk 5	5 x 3 m	17.3 b	7.2 b	23.3 b	
	5 x 4 m	11.8 <i>c</i>	7.4 <i>b</i>	23.9 a	
Krymsk 5 interstem	5 x 3 m	30.9 a	7.3 <i>b</i>	23.5 ab	
	5 x 4 m	15.7 b	7.9 a	23.9 a	
Gisela 5 interstem	5 x 3 m	25.7 a	7.2 b	23.1 <i>b</i>	
	5 x 4 m	16.6 b	7.8 a	24.0 a	
		Krupnoplidna			
Krymsk 5	5 x 3 m	19.2 b	9.8 c	27.7 c	
	5 x 4 m	20.3 b	10.0 <i>c</i>	27.8 c	
Krymsk 5 interstem	5 x 3 m	25.1 ab	10.3 bc	28.2 bc	
	5 x 4 m	27.9 a	10.8 <i>b</i>	28.6 b	
Gisela 5 interstem	5 x 3 m	30.7 a	10.7 b	28.6 b	
	5 x 4 m	22.5 b	11.8 a	30.4 a	
		Average for rootstocks and	interstems		
Krymsk 5		17.2 b	8.4 b	25.7 b	
Krymsk 5 interstem		24.9 a	8.9 ab	26.0 ab	
Gisela 5 interstem		23.9 a	9.5 a	26.6 a	
		Average for the scheme of	planting		
5 x 3 m		24.8 a	8.7 b	25.8 a	
5 x 4 m		19.1 <i>b</i>	9.2 a	26.4 a	
		Average for cultiva	rs		
Melitopolska chorna		19.7 b	7.4 b	23.6 b	
Krupnoplidna		24.3 a	10.5 a	28.5 a	

Different letters indicate significant difference between the means for each parameter according to Tukey's test (p < 0.05)

light into the canopy, cannot compensate for the decrease of number of trees per area unit. The highest cumulative yield on the cultivar was observed on variants with Gisela 5 (5 x 3 m) and Krymsk 5 (5 x 4 m) interstems - 30.7 and 27.9 t ha-1, respectively.

For both cultivars, the variants that had the highest NPP values also showed the highest cumulative yield per unit area.

The quality parameters of sweet cherry fruit (mean fruit weight and diameter) are very important as larger fruits are more valued by consumers and their price increases (Whiting et al. 2005; Kappel et al. 1996). In the experiment, trees grafted on interstems had higher fruit quality indices than when grafted on Krymsk 5 rootstock. In general, best quality fruit were formed in Gisela 5 interstem variant. When comparing planting density, it was found out that larger fruits are formed when using 5 x 4 m scheme, so on 'Krupnoplidna' cultivar where the yield ha⁻¹ is equal for both densities, this scheme may be more preferable from an economic point of view.

4 Conclusions

In terms of light interception of trees grafted on interstems and vigour controlling rootstocks, 'Melitopolska chorna' cultivar favors 5 x 3 m planting scheme, and 'Krupnoplidna' 5 x 4 m scheme. Despite this, on 'Krupnoplidna' cultivar the yield ha⁻¹ was the same regardless of plant density.

The following scion-rootstock-density combinations showed the best complex of photosynthetic and yield parameters: Krymsk 5 and Gisela 5 interstems with 5 x 3 m planting scheme on 'Melitopolska chorna' cultivar, and Gisela 5 interstem (5 x 3 m) and Krymsk 5 interstem (5 x 4 m) on 'Krupnoplidna' cultivar.

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