Cover letter

Pretoria 28/05/2019

Dear Prof. Dr. Sisir Kumar Mitra

Please find enclosed our manuscript entitled, "**Improving yield and biochemical composition of Avocado by reducing environmental stress after fruit set**" for consideration of publication in Journal Fruits."

Shade netting have been used to protect fruits again environmental factors such as sunlight, hail and wind and have been shown to improve yield and quality of the crop. Shade net also alter the microclimate (light and temperature), thus it is use to manipulate morphological and physiological process in plants. Recent studies have suggested that shade netting set as a permanent structure may disrupt pollinators and thus negatively affect crop yield. To our knowledge, no previous study has evaluated the effect of drape net on avocado productivity.

This study have shown that introducing drape white shade netting after fruit set improved avocado fruit total yield, while black drape net have negative impact on the productivity. C-7 sugars content was not influence by the environment. The drape shade nets negatively affected oleic acid accumulation. Drape shade net had the highest level of ferulic acid and p-coumaric acid

The findings of the study validate that introducing of shade net after pollination may positively influence yield. The modified microclimate and light quality can influence the production of secondary metabolites the phytochemicals.

Thank you for considering our manuscript for review. We look forward to your response

Sincerely,

Helene Fotouo-M (PhD)
Department of crop Sciences
Tshwane University of Technology
Faculty of Natural and Agricultural Sciences
Pretoria
0002
fotouoh@yahoo.fr, fotouomakouateh@tut.ac.za

Original Article

Improving yield and biochemical composition of Avocado by reducing environmental stress after fruit set

Helene Fotouo-M, Owen C. Rathumbo, Peter P Tinyane, Dharini Sivakumar

Phytochemical Food Network to Improve Nutritional Quality for Consumers Group,

Department of Crop Sciences, Tshwane University of Technology, Pretoria, Gauteng, South Africa

Abstract

Protecting fruits from environmental factors such as hails, storm, sunburn using shade net has become a popular practice. However, drape net has an additional advantage since it can be erected after pollination, so that the yield is not compromised. In this study the influence of two drape nets (black and white) on total and marketable yield, accumulation of phenolic compounds, oleic acid and the number taken to ripe in avocado cultivar 'Hass' were compared to the open field and permanent white nets. Fruits were harvested at the commercial maturity stage and classified according to export market standards. Thereafter, fruits were stored at 5.5 °C and at 85% RH for 28 days, followed by 5 to 7 days at 15 °C to simulate the market shelf condition for ripening and subjected to the analysis of phytonutritional compounds. Fruit temperature and photosynthetic radiation (PAR) was found to be highest under open field and the black drape net had the lowest values. The white drape net significantly improved the total and marketable yield while the black drape net had a lower yield than the open field. The C-7 sugar, D- mannoheptulose decreased as the fruit ripened but was not influenced by the growing environment. Oleic acid was lowest in both drape nets. The total phenol decreased with ripening and was highest in open field followed by drape nets. Ferulic acid, p-coumaric acid and syringic acid content increased with ripening. Overall, the white drape net improved yield and maintained fruit biochemical components.

Keywords: Drape shade net, D- mannoheptulose, Oleic acid, Permanent shade net, Total phenol, Total yield

Significance of this study

What is already known on this subject?

- Shade netting have been used to protect fruits again environmental factors such as sunlight, hail and wind and have been shown to improve yield and quality of the crop
- Shade net also alter the microclimate (light and temperature), thus it is use to manipulate morphological and physiological process in plants

What are the new findings?

- Introducing white shade netting after fruit set improved fruit total yield, while black drape net have negative impact on the productivity
- C-7 sugars content was not influence by the environment. The drape shade nets negatively affected oleic acid accumulation
- Drape shade net had the highest level of ferulic acid and p-coumaric acid

What is the expected impact on horticulture?

Introduction of shade net after pollination may positively influence yield. The modified microclimate and light quality can influence the production of secondary metabolites the phytochemicals

1 Introduction

Avocado (*Persea americana* Mill.) is a tropical fruit, belonging to the Lauraceae family. It is widely consumed because of its nutritional value; it is a good source of lipophilic phytochemicals such as phenolics, monounsaturated fatty acids (∼71 %) and vitamins (Hernández et al., 2016; Duarte et al., 2016). However, avocado production is not without challenges; environmental conditions such as wind, hail and excessive solar radiation impact negatively on yield and quality of avocado fruits. Shade net has been successfully used as an alternative to protect crops from adverse environmental conditions (Ilić and Fallik, 2017). Shade netting also have of the ability to diffuse of spectrally modified light to the inner plant canopy and modified the microclimate in the orchards (Engle and Liu, 2016; Ilić and Fallik, 2017). The light receptors phytochromes, cryptochromes, and phototropins become photochemically active only when expose to the specific wavelengths of light, and thus the change in spectra quality can promote desirable physiological responses regulated by light (Sivakumar et al., 2018).

Approximately 45% of the total avocado production volume is exported in 2017 (DAFF, 2017). Improving Class 1 percentage above the current average of 60% (Blakey et al., 2016) and the quality including the nutritional value (Tinyane et al., 2018) is of commercial advantage for growers. Previously, Tinyane et al. (2018) demonstrated that growing avocados under different permanent net colours (Blue, white and red) improved the marketable yield but not the total yield mainly due lack of pollinators activities under nets. The same authors also showed that D-mannoheptulose and phenolic acids were significantly lower in the white net in comparison to other nets colour and open field, and oleic acid significantly higher in white net than other investigated environment. Drape net has similar advantages as the permanent net providing the physical protection, moreover, drape nets are introduced after fruits set and thus pollinators activities are not disrupted (Stander and Cronje, 2016). We hypothesized in this study that drape shade net could improve the total yield and class 1 fruits and maintain its biochemical composition. The aim of this study is to investigate the influence of two drape nets (black and white), permanent white and open field on i) fruit total and marketable yield, and commercial classes; ii) number of day to ripen and biochemical composition of 'Hass' avocado fruit.

2 Materials and method

The trial was conducted at Lombard Avocado farm in Agatha, Tzaneen, Limpopo Province (23.7° South latitude, 30.13° East longitude, 986 m above the sea level). A complete randomised block design was used. The treatment consisted of the following: a permanent white net, 20% shading (Knittex Ltd, Johannesburg, South Africa), two drape nets, white and black net, 20% shading (Drape net, Grabouw South Africa) and open field (control). The trial was conducted as previously described by Tinyane et al. (2018).

2.2 Harvest, packhouse grading and storage trial

Avocado fruit 'Hass' were harvested at commercial maturity with the dry matter content being in the range of 32–36%. After harvesting, fruits from all the treatment replicates were taken to the packinghouse for sorting and grading according to standards commercial classes and the marketable yield was determined. Fruits with damages and defects were considered as waste. Per treatment, ten replicates boxes of class 1 fruits (~16 fruits/boxes) were transported to the laboratory for further evaluation. The boxes were laid out in a completely randomised design at 5.5 °C and 85% RH for 28 days (cold temperature storage), after cold storage, the fruits were held at 15 °C to simulate the market shelf temperature.

2.3 Postharvest evaluation

2.3.1 Number of days to ripen

A set of 33 fruits per replicates per treatment were monitored for ripening on the daily base for seven days. Ripening was assessed by checking the fruit firmness by gentle hand-squeezing daily. The number of days to ripen was expressed as a mean of 100 fruits.

2.3.2 Biochemical analysis

Fruits were assessed at harvest, 28 days after storage and ripe (ready-to-eat) stage. The analyses included quantification of D-mannoheptulose, oleic acid, total phenolic content, and phenolic acids. D-mannoheptulose and fatty acids composition was determined using GC-MS as previously described by Glowacz et al., 2017. Total phenolic content was determined by spectrometry using the modified Folin-Ciocalteau method (Singleton *et al*., 1999). The phenolic acids (ferulic acid, p-coumaric acid and syringic acid) were identified and quantified. Sample extraction was done according to Hertog *et al.* (1992) with some modifications. Freeze dried samples in powder form (0.2 g) was homogenized for 30 S in 1.5 mL of extraction solution containing methanol/water/hydrochloric acid (80:19.5:0.5, v/v/v). Identification and quantification of phenolic compounds were done using HPLC as previously described (Glowacz et al., 2017).

3 Statistical analysis

Yield and classes data were analysed by one-way analysis of variance, and the biochemical compounds analyzed by two-factor analysis of variance using environment and ripening stage as main factors. Significant differences among the treatments were determined using Fisher's protected LSD (least significant differences) test at the level of significance of 5%. All tests were conducted using procedures of the Genstat 18.2 software package (VSN International Ltd., 2016).

4 Results

4.1 Conditions under different environments

The fruit surface temperature and the canopy temperatures were lower under the permanent and drape nets, most particularly at night compared to open field, while the relative humidity was increased. The photosynthetic radiation (PAR) under was 13%, 22%, and 40% lower under the permanent white, white drape, and black drape net respectively (Table 1).

4.2 Total Yield, marketable yield and commercial classes

The total yield in drape white net was similar to that of the open field (t. ha^{-1}) and significantly higher than that permanent white net and black drape net (~2.74 t. ha^{-1}) (Figure 1). Permanent white net and the white drape had the significant highest marketable yield (~2.74 t. ha^{-1}) in comparison to open field and black drape net (~1.5 t. ha^{-1}) (Figure 1). In terms of standard commercial classes, the permanent white net had the significant highest percentage of Class 1 fruit and the significant lowest percentage of Class 1 and Class 2 fruits. The classes did not differ between other environments, except that black net had the significant lowest percentage of Class 3 (Figure 2).

4.3 Number of days to ripen

Fruits from the open field had the significantly highest number of days to ripen and the reach 'ready to ripe stage' for marketing compared to permanent white and black drape nets. The number of days to ripen in white drape net was intermediate and not significantly different from other treatments (Figure 3).

4.4 Biochemical analysis

The effect of growth environment, ripening stage and the environment x ripening stage is presented in Table 2. D-mannoheptulose was not significantly affected by growth environment and environment x ripening stage interaction but was significantly decreased from harvest to ready-to-eat stage (Figure 4). The growth environment and the ripening stage significantly influenced oleic acid as individual factors but not by their interaction was not significant. The content in oleic acid significantly increased with fruit ripening (Figure 5A). Fruit from permanent white net had the significantly highest content of oleic acid compared to those grown under the white and black drape nets. The level of oleic acid in open field was similar to that of the permanent and drape white nets (Figure 5B).

The total phenol content and the investigated phenolic acids (p-coumaric acid, ferulic acid and syringic acid) was influenced by net x ripening stage interaction (Table 2). The overall total phenol content decreased with ripening, while p-coumaric acid, ferulic acid and syringic acid significantly increased irrespective of the environment. The total phenol was found to be significantly highest in open field followed by drape nets, and the permanent white net had the significant lowest value. P-coumaric acid and ferulic acid were significantly highest in white and black drape nets, while the permanent white net had the lowest content in both phenolic acids. Fruit from open field had the significant highest level of Syringic acid followed by permanent and white drape net, whereas the black drape net had the significant lowest level.

5 Discussion

It is evident that the in drape white net in comparison to the white permanent net confirm that improved the total yield at harvest (Fig) Introducing shade netting before flowering and fruit set affect the yield as a result of negative effect on pollination (Stander and Cronje, 2016; Tinyane et al., 2018). The highest marketable yield in drape and permanent white compared to open field is in agreement with previous studies (Blakey et al., 2015; Tinyane et al., 2018) and showed that white colour net effectively protect the fruit from excessive solar radiation winds and hails, and that the PAR and transmittance under the net are favorable to fruit production. The negative impact of black colour netting on crop productivities have been linked to the lower light scattering under the net (Stamps, 2009; Ilić et al., 2017). The significant highest Class 1 fruits found in the permanent white net can be justified by the fact that fruits were protected throughout growth and thus had fewer physical defects, while some damages may have occurred in fruits under white drape net before the introduction of the net. The highest number of days to ripen reported in fruit from open field compared to shaded fruit is in accordance with previous studies (Tinyane et al., 2018). Ripening is usually associated with D-Mannoheptulose content in avocado (Liu et al, 2002). In the present study, although there was no significant difference between environments, fruits from open field had the slightly higher content D-Mannoheptulose (data not shown) in comparison to shaded fruits. The decrease in sugar content and the increase in oleic acid content during ripening was expected. Sugars are suggested to be the precursors of lipid synthesis in avocado (Kilaru et al., 2015). The increase in oleic acid is also associated with an increase in dry matter content in the fruit (Carvalho et al., 2014). The negative effect of direct sunlight on the oleic acid accumulation has been reported (Tinyane et al., 2018), and its accumulation has been shown to improve at low temperature (≤ 19°C) (Ferreyra et al., 2016; Tinyane et al., 2018). Although temperature and PAR are often correlated, Echarte et al. (2010) found that temperature and PAR may influence fatty acid composition independently or through interaction. The fatty acid composition may not directly depend on PAR but on the availability of photo-assimilates in the fruit, and oleic acid was reported to increase with PAR up until a maximum level (Echarte et al., 2013; Aguirrezábaln et al., 2015). It appears that the lower PAR under the white and black drape net may have negatively affected the content in oleic acid in avocado fruits (Figure 5B). Higher content in total phenol content in open field can be related to its role in protecting plant against UV radiation (Zoratii et al., 2014). The slightly higher fruit surface temperature in open field may have also played a role in stimulating the accumulation of total phenol (Yang et al., 2017). Ferulic acid and p-coumaric acid have been suggested as possible ripening markers in avocado because their content gradually increases with ripening (Hurtado-Fernández *et al.,* 2013), which was also the case with syringic acid in this study. The PAR and the light spectra quality have been shown to regulate the accumulation of diverse phenolic compounds (Lekala et al., 2018; Mashabela et al., 2015). However, to explain the mechanism details studies at transcription level is needed.

Our hypothesis was accepted as white drape net improved the yield and maintained the D-mannoheptulose content and total phenol content, whereas the black drape net did not have positive impact fruit.

6 Acknowledgements

The financial support of the National Research Foundation South Africa and the DST-NRF SARChI research chair grant [98352] is greatly acknowledged. Authors wish to thank Drape net S.A and the Lombard's Avocado farm, South Africa for supporting in the installation of the nets and packhouse assessments.

7 References

Aguirrezábal, L., Martre, P. Pereyra-Irujo, G., Echarte, M.M., and Izquierdo, N. (2015). Improving grain quality: ecophysiological and modeling tools to develop management and breeding strategies. In Crop Physiology: Applications for Genetic Improvement and Agronomy, (Sandras V.O., and Calderini, D.F ed.) (Elsevier: Amsterdam) pp. 423–465.

Blakey, R.J., van Rooyen, Z., Köhne, J.S., Malapana,K.C., Mazhawu, E. Tesfay S.Z., and Savage, M.J. (2015). Growing avocados under shade netting – Year 3. South African Avocado Growers' Association Yearbook 39, 201

Carvalho, C.P., Velásquez, M.A., and Van Rooyen, Z. (2014). Determination of the minimum dry matter index for the optimum harvest of 'Hass' avocado fruits in Colombia. Agron. Colomb. 32, 399-406.

Department of agriculture of forestry and fisheries (DAFF) , 2017. A profile of the South African avocado market value chain. www.nda.agric.za

Duarte, P.F., Chaves, M.A., Bores, C.D., and Mendoca, C.R.B. (2016).Avocado: characteristics, health benefits and uses. Cienc Rural. 46, 747-754.

Echarte, M.M., Angeloni, p., Jaimes, F., et al. (2010). Night temperature and intercepted solar radiation additively contribute to oleic acid percentage in sunflower oil. Field crops res. 119, 27–35.

Echarte, M.M., Puntel, L., and Aguirrezábal, L.A.N. (2013). Assessment of the critical period for the effect of intercepted solar radiation on sunflower oil fatty acid composition. Field crops res. 149, 213–222.

Engle, K. J., and Liu, H. (2016). Study on Physical and Mechanical Properties of Agricultural Netting Products . International Textile and Apparel Association (ITAA) Annual Conference Proceedings. 110. Vancouver, British Columbia

Ferreyra, R., Sellés, G., Saavedra, J., Ortiz, J., Zúñiga, C., Troncoso, C., Rivera, S.A., González-Agüero, M. and Defilippi, B.G. (2016). Identification of pre-harvest factors that affect fatty acid profiles of avocado fruit (*Persea americana Mill*) cv.'Hass' at harvest. South Afric. J. Bot. *104*, 15-20.

Glowacz, M., Bill, M., Tinyane, P.P., and Sivakumar, D. 2017. Maintaining post-harvest quality of cold stored 'Hass' avocados by altering the fatty acids content and composition with the use of natural volatile compounds–methyl jasmonate and methyl salicylate. J Sci. Food Agric. 97, 5186-5193.

Hernández, I., Fuentealba, C., Olaeta, J.A., Lurie, S., Defilippi, B.G., Campos-Vargas, R., and Pedreschi, R. (2016). Factors associated with postharvest ripening heterogeneity of 'Hass' avocados (*Persea americana Mill*). Fruits 71, 259-268.

Hertog, M.G.I., Hollman, P.C.H., and Katan, M.B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. J Agric. Food Chem, 40, 2379-2383

Hurtado-Fernández, E., González-Fernández, J.J., Hormaza, J.I., Bajoub, A., Fernández-Gutiérrez, A., and Carrasco-Pancorbo, A. (2016). Targeted LC-MS approach to study the evolution over the harvesting season of six important metabolites in fruits from different avocado cultivars. Food Analyt. Methods *9*, 3479-3491.

Ilić, S.Z, and Fallik, E. 2017: Light quality manipulation improve vegetables quality at harvest and postharvest: A review. Environ. Exp Bot. 139, 79-90.

Ilić, S.Z., Milenković, L., Dimitrijević, A., Stanojević, L., Cvetković, D., Mastilović, J., and Kevrešan, Ž. (2017). Effect of coloured shade-nets on yield and quality of lettuce (*Lactuca sativa* L.) during summer production. Sci. Hort. 226, 389-397.

Kilaru, A., Cao, X., Dabbs, P. B., Sung, H. J., Rahman, M. M., Thrower, N., … Ohlrogge, J. B. (2015). Oil biosynthesis in a basal angiosperm: transcriptome analysis of Persea Americana mesocarp. BMC Plant Biology, 15, 203. https://doi.org/10.1186/s12870-015-0586-2.

Lekala, C.S., Madanic, K.S.H., Phanc, A.D.T., Maboko, M.M., Fotouo, H., Soundy, P., Sultanbawac, Y., and Sivakumar, D. (2018). Cultivar-specific responses in red sweet peppers grown under shade nets and controlled-temperature plastic tunnel environment on antioxidant constituents at harvest.

Liu, X., Sievert, J., Arpaia, M.L., Madore, M., 2002. Postulated physiological roles of the seven carbon sugars, mannoheptulose and perseitol in avocado. J. Am. Soc. Hortic. Sci. 127, 108–114.

Mashabela, M.N., Selahle, K.M.; Soundy, P., Crosby, K.M., & Sivakumar, D. (2015). Bioactive compounds and fruit quality of green sweet pepper grown under different colored shade netting during postharvest storage. *J. Food Sci*. 80, 2612–2618

Singleton, V.L., Orthofer, R., and Lamuela-Raventos, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. Methods in Enzymology 299,152-178.

Sivakumar, D., Jifon, J., and Soundy, P. (2018). Spectral quality of photo-selective shade nettings improves antioxidants and overall quality in selected fresh produce after postharvest storage. Food Rev. Intern. *34*, 290-307.

Stamps, R.H. (2009). Use of Colored Shade Netting in Horticulture. Hortscience 44, 239-241.

Stander, J., and Cronje, P. 2016. Important consideration for citrus production under shade nets. Technology *CRI*, 64-67.

Tinyane, P.P, Soundy, P., and Sivakumar, D. (2018). Growing 'Hass' avocado fruit under different coloured shade netting improves the marketable yield and affects fruit ripening. Sci. Hort. 230, 43–49.

Yang, J., Zhang, X., Jin, Q., Gu, L., Liu, X., Li J., and Ni, Y. (2017). Effect of meteorological parameters and regions on accumulation pattern of phenolic compounds in different mulberry cultivars grown in China. Natural Prod. Res. 31, 1091-1096.

Zoratti, L., Karppinen, K., Escobar, A.L., Haggman, H., Jaakola, L., 2014. Light-controlled flavonoid biosynthesis in fruits. Front. Plant Sci. 5, 1–16.

Tables and figures

Table 1: Fruit surface temperature, Canopy temperature, relative humidity and photosynthetic radiation (PAR) during the growing season (February- June)

	Fruit temperature (°C)		Canopy temperature(°C)		Average RH (%)	Average PAR (μmol m−2 s−1)
	Night average	Day average	Night average	Day average		
Open field	18.695	22.876	19.294	23.319	63.8	1153.111
Permanent white net	17.139	22.339	17.889	23.083	68.5	1006.053
White drape net	17.255	22.092	17.599	22.942	69.84	909.94
Dark drape net	16.480	21.868	17.024	22.218	69.35	696.47

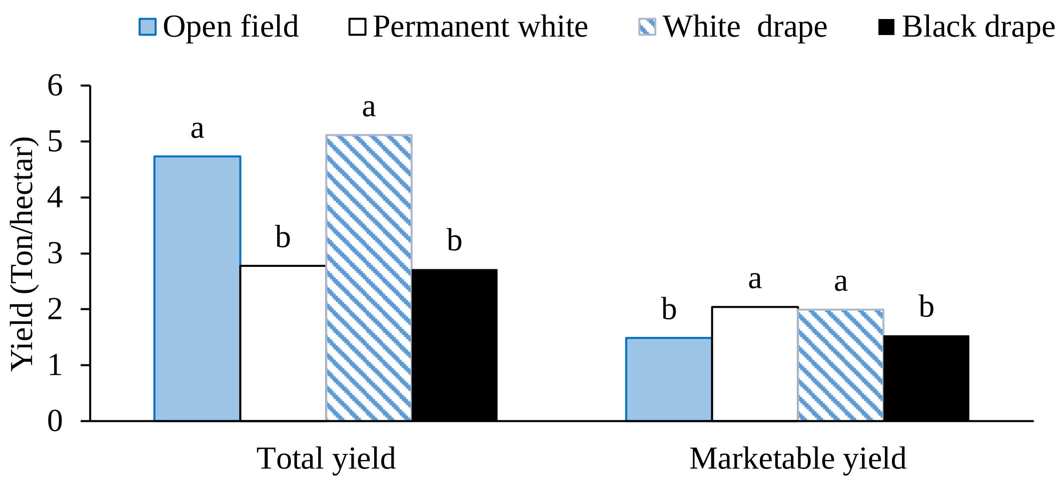


Figure 1. Influence of different drape and permanent shade netting on total and marketable yield of ‘Hass’ avocado. Means in each bar followed by the same letter are not significantly different at $P<0.05$.

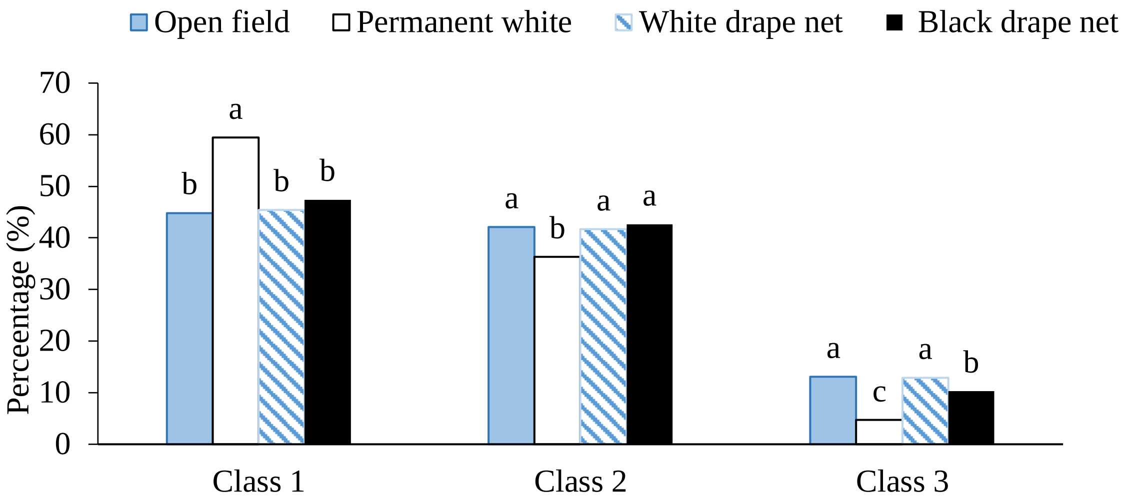


Figure 2. Influence of different drape and permanent shade netting of 'Hass' avocado standard commercial classes. Means in each bar followed by the same letter are not significantly different at $P<0.05$.

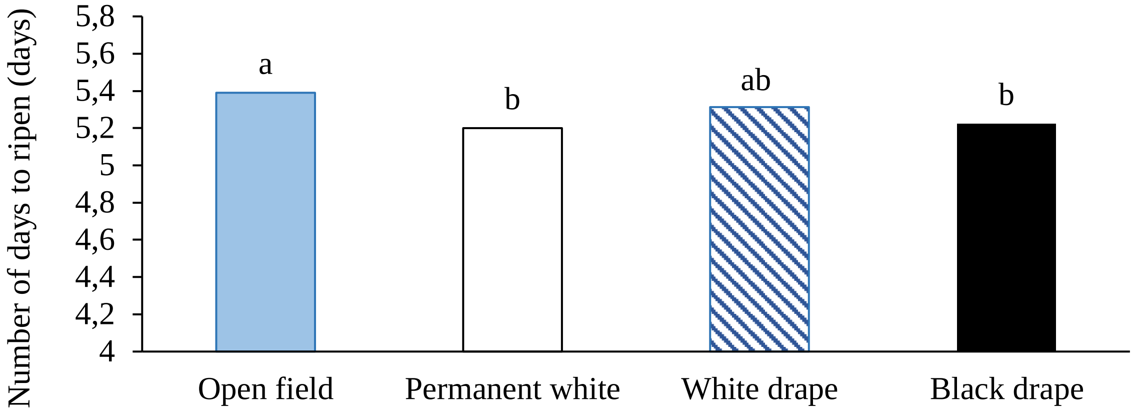


Figure 3. Influence of different drape and permanent shade netting of 'Hass' avocado number of days to ripen. Means in each bar followed by the same letter are not significantly different at P<0.05.

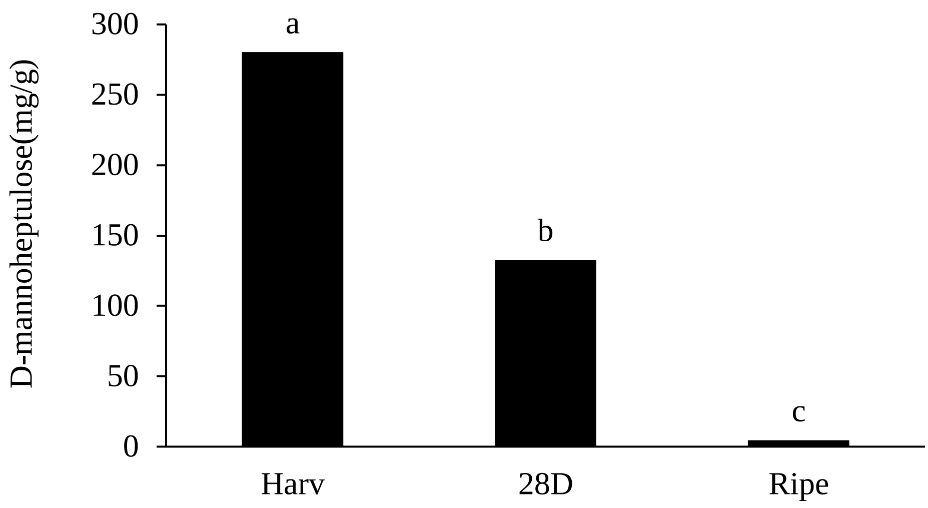
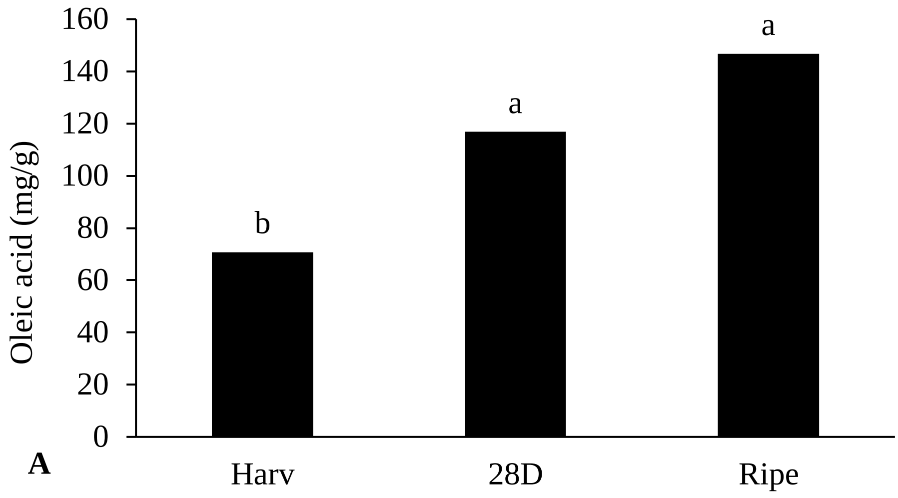


Figure 4. D-mannoheptulose content in ‘Hass’ avocado fruits as affected by ripening stages. Means in each bar followed by the same letter are not significantly different at $P<0.05$.



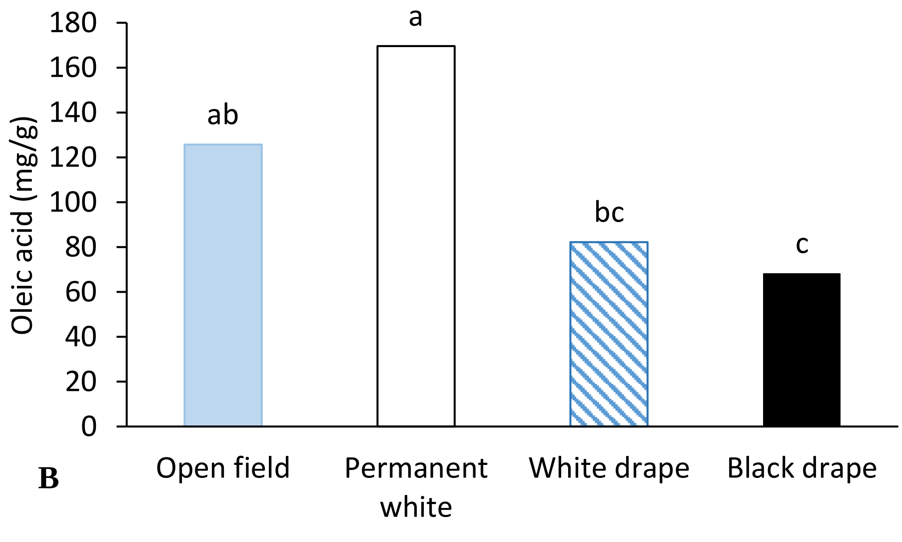


Figure 5: Oleic acid content as affected ripening stage (A) and by permanent white and drape (black and white) net (B). Means in each bar followed by the same letter are not significantly different at P<0.05.

Table 2. Total phenol content and phenolic acids   as affected by growth environment and ripening stage. Means followed by the same letter are not significantly different at P<0.05.

Treatment	Total phenol	P-coumaric	Ferulic acid	Syringic acid
Harv x open field	597.81a	0.00f	8.85hi	7.52f
Harv x permanent white	453.93c	4.99f	5.68i	6.69ef
Harv x white drape	563.39ab	0.00f	12.28g	14.65def
Harv x black drape	568.34ab	0.00f	11.52gh	12.03def
28D x open field	518.12b	41.52d	24.75e	19.14de
28D x permanent white	541.35ab	39.45	20.02f	9.55def
28D x white drape	515.12b	0.00f	12.14g	15.66def
28D x black drape	587.34a	0.00f	13.12g	20.12d
Ripe x open field	347.82d	88.13b	51.31c	246.69a
Ripe x permanent white	217.37e	70.27c	39.88d	206.38b
Ripe x white drape	326.91d	111.57a	97.01a	194.88b
Ripe x black drape	339.95d	114.43a	86.66b	174.35c
$P \leq 0.05$	<.001	<.001	<.001	<.001
LSD	121.7	2.930	3.263	12.49