

## **Effect of planting scheme on photosynthetic activity and dry matter accumulation in apple leaves**

K. Laužikė\*, N. Uselis and G. Samuolienė

Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Kauno 30, Babtai, LT-54333 Kaunas distr., Lithuania

\*Correspondence: Kristina.Lauzike@lammc.lt

**Abstract.** This study aims to identify changes photosynthetic rate and dry matter accumulation in apple leaves with decreasing plant to plant distance of the trees. Apple tree ‘Auksis’ was grafted on dwarfing P60 rootstock and planted at different in distances: 0.5 m, 0.75 m, 1 m and 1.25 m between plant to plant distance in rows. Photosynthetic indices were measured at 1.00–1.20 m above ground inside the canopy. 20 randomly selected leaves from the whole apple tree canopy were used to determine leaf area, fresh and dry weight. Measurements were made in three different stages in May, June and September. By decreasing the distance between apple trees from 1.5 m to 0.5 m, photosynthetic rate decreases correspondingly, decreasing by 23% in spring, and decreasing by 31% in autumn. Distance between trees has no significant impact on leave mass per area (LMA), however in spring is higher by 33–51% compared to summer and 42–78% compared to autumn. Dry and fresh weight ratio (DW/FW) significantly increased in summer - by 27% and in autumn - by 37% compared to spring, also DW/FW significantly decreased by the decreasing distance from 1.5 m to 0.5 m by 4–6%. In summary, the decreasing distance reduces the photosynthetic rate, the accumulation of dry matter. Also, photosynthetic rate decreases from spring to harvest time, and on the contrary, the accumulation of dry matter increases as autumn approaches. After evaluating the obtained results, the aim is to further delve into the use and transpiration of water and the impact of the planting scheme on fruit quality.

**Key words:** photosinthetic rate, competitive stress, *Malus domestica*, dry matter, Lithuania.

### **INTRODUCTION**

The efficiency of photosynthesis depends on a complex of environmental factors: temperature, water content, CO<sub>2</sub> concentration, light intensity, nutrient content, etc. (Long, 2006; Yamori, 2016; Suvorova et al., 2017). In order to increase the productivity yield per unit area, the distance between seedlings per unit area is reduced, which causes competitive stress of the fruit trees on the main elements of photosynthesis (Fernández-de-Uña et al., 2016; Al-Namazi, 2017). Increasing density of trees reduces leaf illumination, increases shading, and reduces photosynthetic processes (Ishii, 2012; Manoli, 2017). Light becomes one of the most limiting factors and a key tool for regulating photosynthesis productivity in garden plants (Posada, 2009; Pengelly, 2010). The densely planted, tall, and vigorous trees have reduced light penetration in the lower and interior parts of the canopy, thereby reducing fruit yield, quality, photosynthetic rate (Kami, 2010; Bhusal et al., 2017).

Photosynthetic rate decreases with the penetration of light to the canopy and the illumination of the leaves (Cai, 2011; Dong et al., 2012). The efficiency of photosynthesis is strongly correlated with biomass content, decreasing photosynthesis also inhibits the formation of biomass of the most of plants (Boussadiaa, 2009; Hassiotou, 2009; Ghasemzadeh, 2010; Hassiotou, 2010; Gibsona, 2011; Gaju et al., 2016; Hüner et al., 2016). Also, there is correlation between seasonality and photosynthetic rate, studies show that photosynthetic activity of plants with higher photosynthetic intensity changes more seasonally (Zhang et al., 2017). The illumination intensity of the leaves strongly influences not only mass of biomass but also the size and structure of the leaves as reflected by leaf mass per area (LMA) (Riva, 2016). According to recent studies, leaves with higher LMA in the warm season showed lower photosynthetic rate for woody species and herbaceous plants (He et al., 2019). Although photosynthesis, dry matter accumulation in light deficiency studies have been conducted worldwide, major studies have been performed on tropical and subtropical plants (Posada, 2009; He, 2012), spruce (Ishii, 2012) and others.

Data on changes in medium-broadleaved deciduous trees, garden plants, apple trees, their photosynthesis and dry matter accumulation through tree density changes are lacking. According to global studies, as the distance between trees decreases, it is likely that the rate of photosynthesis and dry matter accumulation will decrease significantly from a certain distance. Therefore, this study aims to identify changes photosynthetic rate and dry matter accumulation in apple leaves with decreasing distance between fruit trees.

## MATERIALS AND METHODS

### Plant material and growing conditions

A field experiment was carried out in an intensive orchard at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Lithuania. The apple tree (*Malus domestica* Borkh.) cv. Auksis was grafted on super-dwarfing rootstocks P60. All trees were planted in 2001. Trees were planted in distances: 0.5 m, 0.75 m, 1 m and 1.25 m between trees in rows, while space between rows was 3 m. Pest and disease management was carried out according to the integrated plant protection practices, the orchard was not irrigated. Soil conditions of the experimental orchard were as follows: clay loam, pH 7.3, humus 2.8%, P<sub>2</sub>O<sub>5</sub> 255 mg kg<sup>-1</sup>, K<sub>2</sub>O 230 mg kg<sup>-1</sup>. Measurements were performed in 3 replicates, 3 trees in each replicate, 3 times per season: in the end of May (leaves fully expanded BBCH 20-25), in the middle of July (beginning of apple maturity BBCH 73-75) and at the end of August (harvest time BBCH 87-88). A completely sunny day is chosen for the measurements.

### Measurements

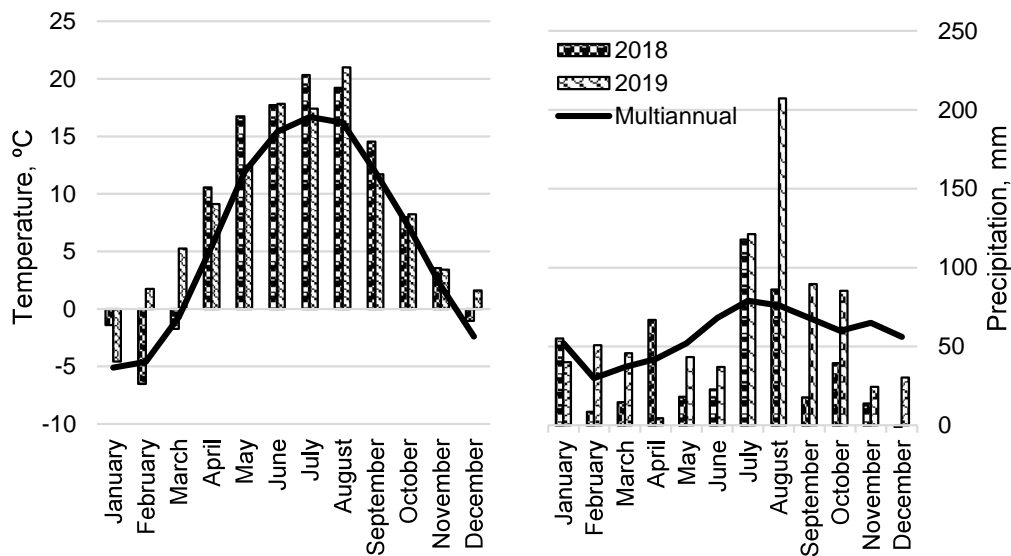
Photosynthetic rate was determined at 9:00–12:00 am by using a LI-6400XT portable open flow gas exchange system (Li-COR Biosciences, Lincoln, USA). Reference air [CO<sub>2</sub>] (400 µmol mol<sup>-1</sup>), light intensity (1,000 µmol m<sup>-2</sup> s<sup>-1</sup>) and the flow rate of gas pump (500 mmol s<sup>-1</sup>) were set. Twenty measurements were made from three leaves (Fully mature leaves are selected) on 1.00–1.20 m above ground.

To determine the leaf area (cm<sup>2</sup>), twenty leaves were randomly sampled from all tree canopy and measured with a leaf area meter (AT Delta - T Device, UK). The dry mass of twenty leaves was determined by drying apple tree leaves in 70 °C for 48 h. (Venti cell 222, Medcenter Einrichtungen, Gräfeling, Germany) to constant weight.

Leaf dry mass per area (LMA) was calculated from twenty leaves dry mass divided from twenty leaves area.

### Meteorological conditions

The meteorological data were collected from 'iMetos' meteorological station at the Institute of Horticulture, LAMMC. Both years temperature was close or higher than multiannual (Fig. 1). 2018 was dry, natural drought was announced in Lithuania in 2018 (Lithuania, lat. 55°N, 2018). However, the trees had sufficient water content. While 2019 had 14% more rainfall than perennial, with particularly heavy rainfall on harvest time in August.



**Figure 1.** Meteorological conditions in two years and the total precipitation in 2018 and 2019 compared to multiannual (average of 100 years) conditions.

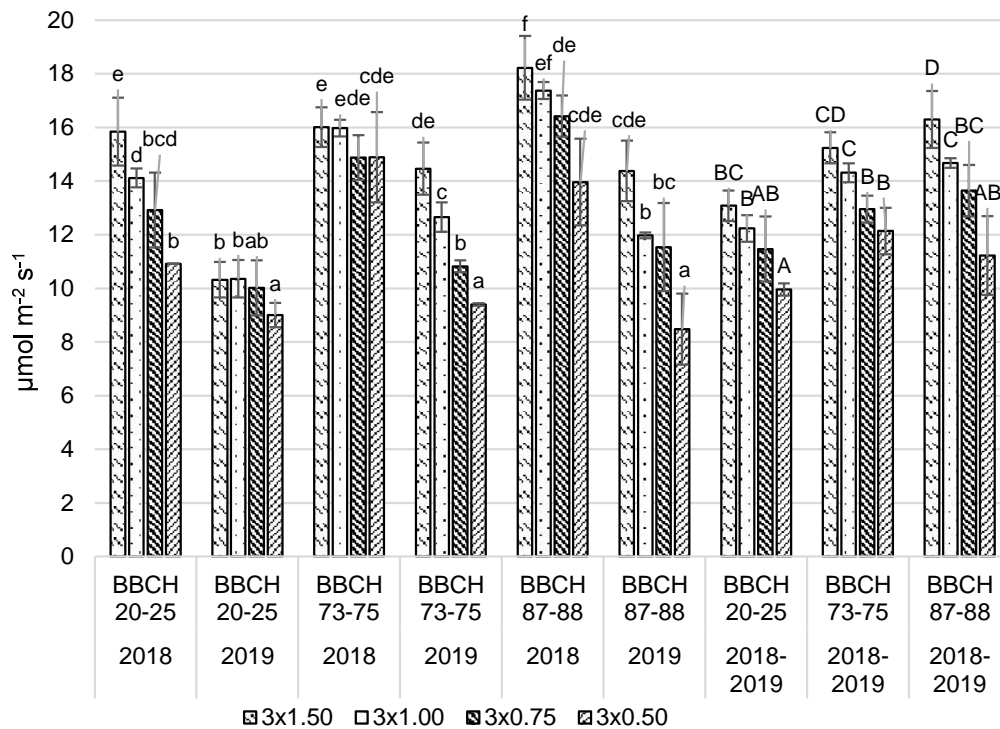
### Statistical analysis

The data was processed using XLStat software (Addinsoft, 2019), and analyzed using Tukey (*HSD*) test at the confidence level  $p = 0.05$ . Data was processed using MS Excel software (version 7.0), standard deviation represents the mean of three replications.

## RESULTS

The distance between trees had a significant effect on the photosynthetic rate. Although the season has influenced Photosynthetic rate ( $P_n$ ) in individual years, no general trend has been observed, nevertheless, in spring the fall in photosynthetic rate

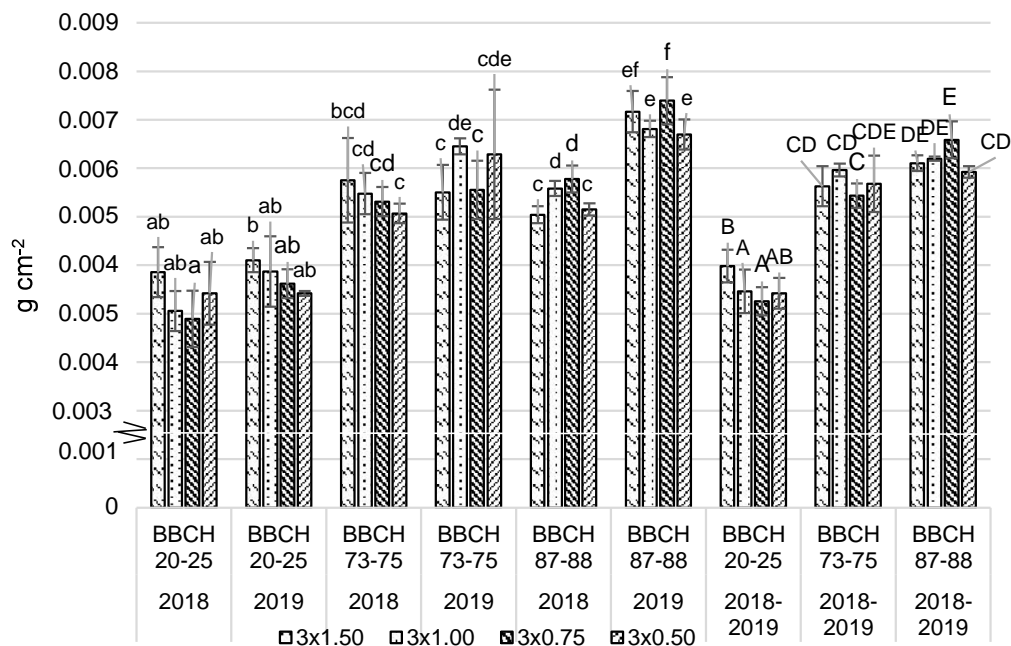
with distance reduction is significantly less than in autumn. By decreasing the distance between apple trees from 1.5 m to 0.5 m, the rate of photosynthesis decreases correspondingly, decreasing by 23% in spring, and decreasing by 31% in autumn (Fig. 2).



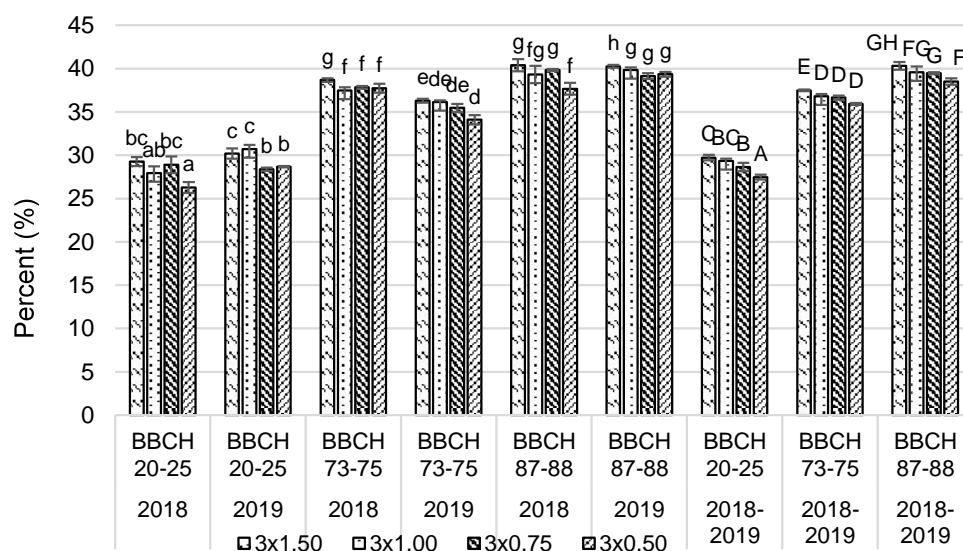
**Figure 2.** Changes in photosynthetic rate during the growing season on ‘Auksis’ apple trees leaves. Means followed by the same letter do not differ significantly at  $P = 0.05$  according to Tukey multiple range; lower-case letters indicate significant differences in 2018 and 2019 and capital letters the significant differences in mean of years. Error bars shows standard deviation.

The increasing distance between apple trees had no significant effect on leaf mass per area (LMA), while the effect of seasonality was significant. The lowest LMA was found during the spring (BBCH 20-25), significantly higher in summer (BBCH 73-75) and autumn (BBCH 87-88), respectively 33–51% and 42–78% compared to spring (Fig. 3).

Dry and fresh weight ratio significantly different not only seasonally, but also by the increasing density of apple trees. DW / FW ratio increased by 27% (BBCH 73-75) and 37% (BBCH 87-88) compared to BBCH 20-25. As the distance between apple trees decreased, the DW / FW ratio decreased, with a significant decrease in the distance from 1.5 m to 0.5 m, which decreased by 4–6% in different seasons.

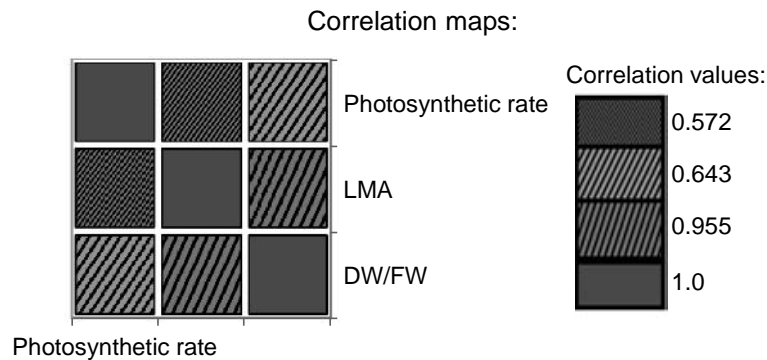


**Figure 3.** Leaf dry mass per area (LMA) in apple trees ‘Auksis’ during 2018 and 2019 (and average of two years) in three seasons of vegetation. Means followed by the same letter do not differ significantly at  $P = 0.05$  according to Tukey multiple range; lower-case letters indicate significant differences in 2018 and 2019 and capital letters the significant differences in mean of years. Error bars shows standard deviation.



**Figure 4.** Dry and fresh weight ratio of ‘Auksis’ leaves during 2018 and 2019 (and average of two years) in three seasons of vegetation. Means followed by the same letter do not differ significantly at  $P = 0.05$  according to Tukey multiple range; lower-case letters indicate significant differences in 2018 and 2019 and capital letters the significant differences in mean of years. Error bars shows standard deviation.

Positive strong and moderate correlations were found between the studied indicators (Fig. 5)



**Figure 5.** Correlation maps (by Pearson correlation matrix) between photosynthetic rate (Pn), leaf mass per area (LMA) and Dry and fresh weight ratio (DW/FW).

## DISCUSSION

The efficiency of photosynthesis is strongly correlated with biomass content, decreasing photosynthesis also inhibits the formation of biomass of the plant (Boussadiaa, 2009; Hassiotou, 2009; Ghasemzadeh, 2010; Hassiotou, 2010; Gibsona, 2011; Gaju et al., 2016; Hüner et al., 2016). However, results from our study show that dry matter accumulation per unit area varied slightly between apple trees planted at different distances (Fig. 2). High LMA (thick) leaves show low water loss due to thick cuticle layers and leaf thickness to be an important feature determining the efficiency of resource acquisition, water retention, and CO<sub>2</sub> assimilation and leaves with higher LMA in the warm season (Riva, 2016; Zhang et al., 2017; He et al., 2019) as well as when plant exposed to the scarcity of water (Bhusal et al., 2020). Although the leaf mass per area did not show a clear trend, the dry to green mass ratio was strongly correlated with the rate of photosynthesis (Fig. 4). Increasing tree density resulted in lower photosynthesis rate, more water retention and less dry matter accumulation in leaves (Fig. 2 and Fig. 4).

Decreasing light: under 30% sunlight, midday leaf net photosynthesis was reduced by 32%–44%, (Guenni et al., 2018). Excessive light can inhibit photosynthesis and biomass growth, shading could improve plant growth in *Torreya grandis* seedling, the optimum shade for these seedlings is 75% (Tang et al., 2015). Recent studies have shown that 20% shading increases the efficiency of apple photosynthesis, research also showed that shading substantially increased the size and weight of apples (Aoun & Manja, 2020). Our studies showed that at 3×1.5 m, apple trees, although shaded by one another, were active in photosynthesis, and with increasing competition and shading, the rate of photosynthesis tended to decrease (Fig. 2).

World-wide studies have found different photosynthetic responses to temperature. Some scientists have reported results that warmed plants have higher photosynthesis rates and higher optimum temperatures that maximize photosynthesis rates (Zhou et al., 2007; Niu et al., 2008; Gunderson et al., 2010; Crous, 2013). Others, meanwhile, say

that warming of the temperature does not significantly affect the rate of photosynthesis (Bronson & Gower, 2010; Chi et al., 2013). However, changes in the length of the day go hand in hand with changes in temperature. Way & Montgomery (2015) in their research showed the importance of day length on tree growth. Some studies showed that photosynthetic capacity decreases with shortening day length. In addition, day length plays an important role in controlling the seasonal variation in gross ecosystem productivity (Busch et al., 2007; Bauerle et al., 2012; Stoy et al., 2014). Still, in Lithuanian conditions, when the day begins to shorten before the harvest, the photosynthesis rates increased, so the length of the day for apple trees of this variety had no negative effect on the intensity of photosynthesis (Fig. 2). Mean temperatures, however, remained similar, although the length of the day fell. From the obtained data we can state that photosynthesis rate of 'Auksis' apple trees was seasonally more influenced by temperature than day length (Fig. 1).

## CONCLUSION

- By decreasing the distance between apple trees from 1.5 m to 0.5 m, photosynthetic rate decreases correspondingly.
- Distance between trees has no significant impact on LMA, however in spring is higher up to 50% compared to summer and up to 78% compared to autumn.
- DW/FW significantly increased in summer and autumn compared to spring, also DW/FW significantly decreased by the decreasing distance from 1.5 m to 0.5 m by 4–6%.
- In summary, the decreasing distance reduces the photosynthetic rate, and the accumulation of dry matter. Also, photosynthetic rate decreases from spring to harvest time, and the accumulation of dry matter inversely increases as autumn approaches.

ACKNOWLEDGMENTS. This work was carried out within the framework of long-term research program 'Horticulture: agro biological basics and technologies' implemented by Lithuanian Research Centre for Agriculture and Forestry.

## REFERENCES

- Al-Namazi, A.A., El-Bana, M.I. & Bonser, S.P. 2017. Competition and facilitation structure plant communities under nurse tree canopies in extremely stressful environments. *Ecology and evolution* **7**(8), 2747–2755.
- Aoun, M. & Manja, K. 2020. Effects of a photoselective netting system on Fuji and Jonagold apples in a Mediterranean orchard. *Scientia Horticulturae* **263**, 109104.
- Bauerle, W.L., Oren, R., Way, D.A., Qian, S.S., Stoy, P.C., Thornton, P.E., ... & Reynolds, R.F. 2012. Photoperiodic regulation of the seasonal pattern of photosynthetic capacity and the implications for carbon cycling. *Proceedings of the National Academy of Sciences* **109**(22), 8612–8617.
- Bhusal, N., Han, S.G. & Yoon, T.M. 2017. Summer pruning and reflective film enhance fruit quality in excessively tall spindle apple trees. *Horticulture, Environment, and Biotechnology* **58**(6), 560–567.
- Bhusal, N., Lee, M., Han, A.R., Han, A. & Kim, H.S. 2020. Responses to drought stress in *Prunus sargentii* and *Larix kaempferi* seedlings using morphological and physiological parameters. *Forest Ecology and Management* **465**, 118099.

- Boussadia, O., Steppe, K., Zgallai, H., El Hadj, S.B., Braham, M., Lemeur, R. & Van Labeke, M. C. 2010. Effects of nitrogen deficiency on leaf photosynthesis, carbohydrate status and biomass production in two olive cultivars ‘Meski’ and ‘Koroneiki’. *Scientia Horticulturae* **123**(3), 336–342.
- Bronson, D.R. & Gower, S.T. 2010. Ecosystem warming does not affect photosynthesis or aboveground autotrophic respiration for boreal black spruce. *Tree Physiology* **30**(4), 441–449.
- Busch, F., Hüner, N.P. & Ensminger, I. 2007. Increased air temperature during simulated autumn conditions does not increase photosynthetic carbon gain but affects the dissipation of excess energy in seedlings of the evergreen conifer Jack pine. *Plant physiology* **143**(3), 1242–1251.
- Cai, Z.Q. 2011. Shade delayed flowering and decreased photosynthesis, growth and yield of Sacha Inchi (*Plukenetia volubilis*) plants. *Industrial Crops and Products* **34**(1), 1235–1237.
- Chi, Y., Xu, M., Shen, R., Yang, Q., Huang, B. & Wan, S. 2013. Acclimation of foliar respiration and photosynthesis in response to experimental warming in a temperate steppe in northern China. *PLoS One* **8**(2), e56482.
- de la Riva, E.G., Olmo, M., Poorter, H., Ubers, J.L. & Villar, R. 2016. Leaf mass per area (LMA) and its relationship with leaf structure and anatomy in 34 Mediterranean woody species along a water availability gradient. *PloS one* **11**(2), e0148788.
- Dong, H., Li, W., Eneji, A.E. & Zhang, D. 2012. Nitrogen rate and plant density effects on yield and late-season leaf senescence of cotton raised on a saline field. *Field Crops Research* **126**, 137–144.
- Fernández-de-Uña, L., McDowell, N. G., Cañellas, I. & Gea-Izquierdo, G. 2016. Disentangling the effect of competition, CO<sub>2</sub> and climate on intrinsic water-use efficiency and tree growth. *Journal of Ecology* **104**(3), 678–690.
- Gaju, O., DeSilva, J., Carvalho, P., Hawkesford, M.J., Griffiths, S., Greenland, A. & Foulkes, M.J. 2016. Leaf photosynthesis and associations with grain yield, biomass and nitrogen-use efficiency in landraces, synthetic-derived lines and cultivars in wheat. *Field Crops Research* **193**, 1–15.
- Ghasemzadeh, A., Jaafar, H.Z. & Rahmat, A. 2010. Synthesis of phenolics and flavonoids in ginger (*Zingiber officinale* Roscoe) and their effects on photosynthesis rate. *International Journal of Molecular Sciences* **11**(11), 4539–4555.
- Gibson, K., Park, J.S., Nagai, Y., Hwang, S.K., Cho, Y.C., Roh, K.H., ... & Edwards, G.E. 2011. Exploiting leaf starch synthesis as a transient sink to elevate photosynthesis, plant productivity and yields. *Plant science* **181**(3), 275–281.
- Guenni, O., Romero, E., Guédez, Y., Bravo de Guenni, L. & Pittermann, J. 2018. Influence of low light intensity on growth and biomass allocation, leaf photosynthesis and canopy radiation interception and use in two forage species of *Centrosema* (DC.) Benth. *Grass and Forage Science* **73**(4), 967–978.
- Gunderson, C.A., O'hara, K.H., Campion, C.M., Walker, A.V. & Edwards, N.T. 2010. Thermal plasticity of photosynthesis: the role of acclimation in forest responses to a warming climate. *Global change biology* **16**(8), 2272–2286.
- Hassiotou, F., Ludwig, M., Renton, M., Veneklaas, E.J. & Evans, J.R. 2009. Influence of leaf dry mass per area, CO<sub>2</sub>, and irradiance on mesophyll conductance in sclerophylls. *Journal of Experimental Botany* **60**(8), 2303–2314.
- Hassiotou, F., Renton, M., Ludwig, M., Evans, J.R. & Veneklaas, E.J. 2010. Photosynthesis at an extreme end of the leaf trait spectrum: how does it relate to high leaf dry mass per area and associated structural parameters?. *Journal of Experimental Botany* **61**(11), 3015–3028.
- He, P., Wright, I.J., Zhu, S., Onoda, Y., Liu, H., Li, R., ... & Ye, Q. 2019. Leaf mechanical strength and photosynthetic capacity vary independently across 57 subtropical forest species with contrasting light requirements. *New Phytologist* **223**(2), 607–618.



- Hüner, N.P., Dahal, K., Bode, R., Kurepin, L.V. & Ivanov, A.G. 2016. Photosynthetic acclimation, vernalization, crop productivity and ‘the grand design of photosynthesis’. *Journal of plant physiology* **203**, 29–43.
- Ishii, H., Hamada, Y. & Utsugi, H. 2012. Variation in light-intercepting area and photosynthetic rate of sun and shade shoots of two *Picea* species in relation to the angle of incoming light. *Tree physiology* **32**(10), 1227–1236.
- Kami, C., Lorrain, S., Hornitschek, P. & Fankhauser, C. 2010. Light-regulated plant growth and development. *Curr. Top Dev. Biol.* **91**, 29–66.
- Long, S.P., Zhu, X.G., Naidu, S.L. & Ort, D.R. 2006. Can improvement in photosynthesis increase crop yields? *Plant Cell Environ.* **29**, 315–330.
- Manoli, G., Huang, C.W., Bonetti, S., Domec, J.C., Marani, M. & Katul, G. 2017. Competition for light and water in a coupled soil-plant system. *Advances in Water Resources* **108**, 216–230.
- Niu, S., Li, Z., Xia, J., Han, Y., Wu, M. & Wan, S. 2008. Climatic warming changes plant photosynthesis and its temperature dependence in a temperate steppe of northern China. *Environmental and Experimental Botany* **63**(1–3), 91–101.
- Pengelly, J.J., Sirault, X.R., Tazoe, Y., Evans, J.R., Furbank, R.T. & von Caemmerer, S. 2010. Growth of the C<sub>4</sub> dicot *Flaveria bidentis*: photosynthetic acclimation to low light through shifts in leaf anatomy and biochemistry. *Journal of experimental botany* **61**(14), 4109–4122.
- Posada, J.M., Lechowicz, M.J. & Kitajima, K. 2009. Optimal photosynthetic use of light by tropical tree crowns achieved by adjustment of individual leaf angles and nitrogen content. *Annals of Botany* **103**(5), 795–805.
- Stoy, P.C., Trowbridge, A.M. & Bauerle, W.L. 2014. Controls on seasonal patterns of maximum ecosystem carbon uptake and canopy-scale photosynthetic light response: contributions from both temperature and photoperiod. *Photosynthesis research* **119**(1–2), 49–64.
- Suvorova, G., Korzukhin, M. & Ivanova, M. 2017. Influence of environmental factors on photosynthesis of three coniferous species. *Annual Research & Review in Biology* **12**(3), 1–14.
- Tang, H., Hu, Y.Y., Yu, W.W., Song, L.L. & Wu, J.S. 2015. Growth, photosynthetic and physiological responses of *Torreya grandis* seedlings to varied light environments. *Trees* **29**(4), 1011–1022.
- Way, D.A. & Montgomery, R.A. 2015. Photoperiod constraints on tree phenology, performance and migration in a warming world. *Plant, Cell & Environment* **38**(9), 1725–1736.
- Yamori, W. 2016. Photosynthetic response to fluctuating environments and photoprotective strategies under abiotic stress. *J. Plant Res.* **129**, 379–395.
- Zhang, Y.J., Sack, L., Cao, K.F., Wei, X.M. & Li, N. 2017. Speed versus endurance tradeoff in plants: Leaves with higher photosynthetic rates show stronger seasonal declines. *Scientific reports* **7**, 42085.
- Zhou, X., Liu, X., Wallace, L.L. & Luo, Y. 2007. Photosynthetic and respiratory acclimation to experimental warming for four species in a tallgrass prairie ecosystem. *Journal of Integrative Plant Biology* **49**(3), 270–281.