PHYSIOLOGICAL AND BIOMETRICAL PARAMETERS OF ORGANICALLY GROWN LETTUCE (*L. SATIVA*)

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ABSTRACT

Global warming is the leading cause of the extreme meteorological events increase in the last three decades. Increase of annual average air temperature and temperature values during winter season and early spring has been recorded in comparison to the (1961-1990) referent period in Plovdiv region. Climate change impacts food security, crop growth, as well as their quality and nutrition value. Therefore, modern scientific research should be focused on growing produce using sustainable and organic methodology that also produces pure and delicious foods. The (type Batavia, variety Maritima) lettuce development was researched in a greenhouse environment in the experimental field of the Agricultural University Plovdiv. During the lettuce growth six different fertilizing variants were used: no fertilizer, one chemical, and four organic fertilizers. The effect of the different fertilizes was studied through specific physiological parameters and productivity. This paper analyzes changes in functional activity of the plant photosynthetic apparatus and productivity of variants with different fertilizers in an unheated greenhouse. The ratio between photosynthetic active radiation (PAR) and quantum yield (qY-Fv/Fm) of PS II was determined to be more optimal in dark-adapted leaves for the organic fertilizer variants, compared to the no fertilizer variant. No significant difference was observed in the values of the minimal fluorescence Fo in reaction centers of PS II after the dark-adaptation of leaves using the different fertilizer variants. Higher values of chlorophyll content index (CCI) were documented for organic and chemical fertilizers compared to the no fertilizer variant.

Keywords: *photosynthetic activity, chlorophyll content index, lettuce, yield, greenhouse*.

INTRODUCTION

In recent years, with the intensification of extreme phenomena of meteorological origin, the advancement of agricultural science and the desire of people to produce qualitative and safe food with minimal risk to the environment, biological farming technologies have aroused scientific interest. Production without mineral fertilizers is important for the environment protection, for the balance and fertility of the soil, as well as for human health. The lettuce is a vegetable, intended only for fresh consumption, which requires good taste and purity of production. Vitamins A, B, C, D, and E can be found in the leaves of the species (Fogg, 1983). The vegetable is one of the main components from the dietary menu and the table in Bulgaria.

The resistance of the species to low temperatures and the duration of the period up to their typical leaf mass reached growth stage (Feller et al., 1995) make it preferred both for autumn-winter production in unheated facilities and for early spring cultivation.

Both the higher temperatures and the changes in the humidification conditions in the country (Marinova et al., 2018; Frantzova, 2014; **Alexandrov et al., 2004**), and the studied area (Georgieva et al., 2017), registered in the recent decades, affect the specific meteorological conditions, the growth and development of the different production **(**Popova et al., 2014**)** and types of lettuces. It is necessary to specify the varieties and the fertilization. Therefore, an experiment in polyethylene greenhouses with a type of lettuce was set (type Batavia, variety 'Maritima') with six different variants of biological fertilization, namely: no fertilization; fertilization by means of one chemical; and fertilization by four organic fertilizers.

There are studies on the interaction of different factors on the physiological status of plants (Shopova and Cholakov, 2014). The physiological condition of plants and effect of various stressful factors thereon have been studied using chlorophyll fluorescence properties by many researchers (Mathur et al, 2014; Kalaji et al.,2016). Chlorophyll fluorescence is a non-invasive measurement of photosystem II (PSII) activity and is a commonly used technique in plant physiology. The sensitivity of PSII activity to abiotic and biotic factors has made this a key technique not only for understanding the photosynthetic mechanisms but also as a broader indicator of how plants respond to environmental change (Murchie and Lawson, 2013). The fluorescence is emitted mainly from chlorophyll *a* of PSII and reflects the primary processes of photosynthesis by light absorption, distribution and transfer of excitation energy and photochemical reactions in PSII. Because of the functional relation of PSII with other components of the photosynthetic apparatus of the chlorophyll fluorescence, it is seen as a proxy for the state of the integral photosynthetic process and the plant organism as a whole (Roháček, 2002). Chlorophyll fluorescence, among others, has been satisfactorily used for monitoring leaf health status in lamb's lettuce (Ferrante and Maggiore, 2007) and storage potential of iceberg lettuce (Schofiled et al., 2005).

The CCM (Chlorophyll Content Meter) 200 plus is useful for improving nitrogen and fertilizer management, and is ideal for crop stress, leaf senescence, plant breeding, health determination, and other studies. Furthermore, the affordability and ease of use make it an exceptional teaching tool for botany and plant science courses (Opti-Sciences 2002; Richardson et al., 2002). The aim of the present study was to monitor the reaction of the leafy lettuce (Batavian variety 'Maritima') to six different fertilization variants by analyzing the temperature conditions and measuring the main parameters of productivity and photosynthetic activity.

MATERIAL AND METHODS

The experiment was conducted on the experimental field of the Agricultural University of Plovdiv in 2019-2020 in unheated greenhouses on alluvial meadow soil (Mollic fluvisol, FAO 2006).. The soil texture is sandy clay loam to clay loam. despite the small amount of total carbonates (2-3%), the soil reaction is slightly alkaline $pH_{(H2O)}$ – 7,7-8,0 (Valcheva et al 2015). The same authors found a high amount of exchange bases (Ca^{2+}+Mg^{2+} - 20-30meq/100g soil) in the composition of the soil sorption complex, and a low content of nitrogen, phosphorus and potassium. The importance of the organic matter of the soil for its fertility is indisputable. However, the nitrogen bound in the organic matter remains hidden in this indicator. The nitrogen in organic form, which is over 95% of total soil nitrogen is the basis of soil fertility. Organic nitrogen is the source that supports the plants throughout the growing season and

ensures an even supply of nitrogen to the plants. The active fraction of soil nitrogen varies with different soil types and depends on a number of factors - degree of cultivation, field history (previous crops in the crop rotation, fertilization system), biotic and abiotic soil characteristics and some environmental factors (mainly temperature and humidity). The lettuce plants (Batavian type, variety 'Maritima') were planted on 8th of November in polyethylene greenhouses in 4 rows according to the scheme 70+30+30+30/30cm with a profile of the soil surface a high level bed (100+60cm.) The experiment was based on the block method with four repetitions, using 28 plants per repetition, and a plot size of 3.36m^2. Organic seeds were provided for seedling production using container technology with 150-hole Styrofoam boards in the following combination – organic seeds - 80%, Perlite - 20%, Lumbricompost for bioproduction of seedlings (Kostadinov & Filipov, 2013). Several variants were tested: 1. NPK (mineral fertilization); 2. Control (non-fertilization); 3. Italpollina; 4. Arkobaleno; 5. LK (Lumbricompost); and 6. Ekoprop NX. The granular fertilizers were introduced as basic fertilization, with soil pre-transplantation at the following norms: N- 12.5kg/da, P_2O_5- 1.25kg/da, + K_2O – 4.75kg/da, Italpollina- 25 kg/da, Arkobaleno - 100 kg/da, and Lumbricompost - 400 l/da. The liquid bio fertilizer Ekoprop NX was applied by double treatment in a dose of 100g/da: before planting - in the 5th leaf seedling phase; and 10 days later on, after the adaptation to the soil. The remaining bio fertilizers are granulated and introduced into the soil before the last tillage and before planting the seedlings. Growing stages were note according BBCH, 2011 and Feller et al., 1995). The biometric measurements were taken three times at one-week intervals in stage-typical leaf mass reached.

Experimental setting: Meteorological observations from Plovdiv AU (Agricultural University) station (42 ° 14′N, 24 ° 75′E and 162 m above sea level) were used for the outdoor temperature analysis. The data were collected according to the recommendations of the WMO (World Meteorological Organization) and are also comparable with the climatic norm for the region. In greenhouse conditions, the air temperature was recorded by means of a weather station M**eteobot® Pro** (https://meteobot.com/). The active temperatures were calculated as the sum Σ^0C) =T-Tb, where T-Tb is taken 0 when T<Tb and T is taken as Tu when T>Tb (WMO, 2011).

Chlorophyll fluorescence imaging: The Chlorophyll fluorescence of the lettuce leaves was measured using a portable device PAR-FluorPen FP 110/D manufactured by Photon Systems Instruments Ltd., Czech Republic. The fluorescence measurement protocol uses short (30 μs) measuring flashes to measure zero level fluorescence (F_0) followed by a strong saturating flash [duration 0.8 s, intensity about 3000 μmol m−2 s−1] to measure the maximum fluorescence (*F*m). Three strong flashes of saturating light probed the effective quantum yield (Qy) of PSII during the actinic light exposure (Maxwell & Johnson, 2000; Nedbal et al., 2000). Light Meter for direct digital readouts of Photosynthetically Active Radiation (PAR) in the range from 400 to 700 nm, the span in which plants use energy during photosynthesis. PAR is measured as Photosynthetic Photon Flux Density (PPFD), which is indicated by units of quanta (photons) per unit time per unit surface area. The chlorophyll fluorescence transients were measured on the same day in the morning. The dates of measurement were 20/03/2020, 28/03/2020 and 04/04/2020 when the plants were in their typical leaf mass reached growth stage. The nine leaves from each variant were dark adapted for about 30 min by detachable leaf-clips prior each measurement. The numeric value of each parameter (Fv/Fm, F_o, PAR) was determined by integrating it over the measured leaf area. ***Physiological estimate of the***

chlorophyll content index (CCI)*:* The Chlorophyll content index of the leaves was measured using a portable apparatus CCM 200 plus Chlorophyll Content Meter manufactured by Opti-sciences, Inc., NH, USA. The physiological assessment was carried out *in vivo* on the field. The measurements were taken on three dates from a sample of leaves at their typical leaf mass reached growth stage. The dates of the measurements were 20/03/2020, 28/03/2020 and 04/04/2020; 20 leaf measurements in the central part of the leaves were taken for each variant (in each of the repetitions). ***Statistical evaluation of the results*****:** To prove differences in the mean values, a one-way ANOVA analysis of variance was performed within the variants of each variety, followed by LSD analysis by means of a Fisher's test. Mathematical processing and statistical analysis were made and visualized using Microsoft® Office products and StatGrafics® statistical software.

RESULTS AND DISCUSSION

Temperature conditions

Table 1. Temperature conditions November 2019-April 2020 and deviation from the reference period 1961-1990

		temperatures ° C				
Months	***Average***	***δ t***	***t max***	***date***	***t min***	***Date***
November	10,8	3,8	23,0	5	2,8	30
Desember	4,4	2,0	17,0	15	-6,5	6
January	3,5	3,2	17,0	28	**-10,5**	8
February	6,4	3,6	21,5	1	-7,0	09
March	8,9	2,1	23,8	13	-4,0	17
April	11,5	-0,7	**27,8**	18	-2,5	8

The observed climatic fluctuations in the study area during the last 3 decades undoubtedly influence the conditions of growth and development of lettuce in the open air and have an economic effect on plants grown in unheated greenhouses. Therefore, the authors believe that temperature, as a key element for the development and productivity of the lettuces is a mandatory element of the study. The average temperature by months and by days for the period of growth and development of the lettuce from planting at the moment of having its 5th true leaf to the stage Typical leaf mass reached - November, December 2019 and January, February, March and April 2020 – has been considered. For November, December, January, February and March the deviation from the climatic norm is positive - by 2.0˚C - December; 2.1˚C in March; 3.2˚C - January and, with the warmest months being November and February (Δ> 3.5 ˚C) degrees above the norm (Table 1). For the period November-April, the lowest reported air temperature was - 10.5˚C, and the highest 27.8˚C, both measured on the 8th day from the first ten days of the month (Table 1). April was cooler than usual (Δ = (- 0.7) ˚C), but the lettuces were harvested at the beginning of the month 04.04 and the low values did not significantly affect their growth and development. In summary, we can say that the period had a warmer winter and a cooler spring than the usual spring, compared to the reference period 1961-1990 and the period 1991-2019 (Fig.1).

As with any plant species, Batavia lettuce defines three cardinal temperature points - minimum, maximum and optimal value. According to some Bulgarian and foreign authors (Kartalov et al. 2007; Lorenz and Maynrad, 1988), the optimal temperature for lettuce development is 16.0-18.0˚C. Morgan (1999) proved that lettuce would produce better quality plants in the cooler conditions of winter and spring. Morgan (1999) also said the optimum temperatures of lettuce were 12 to 21˚C. Well-rooted plants in phenophase 7-9 leaves can withstand temperatures down to -6.0˚C (Cholakov, 1999). The range of development of the species is between 5.0 ˚C and 25.0˚C and below 5.0˚C; the growth slows down and stops (Cholakov, 1999). Other authors (Kristensen et al., 1987; Morgan, 1999) consider the temperature of 4.0˚C to be Tb (base). The temperature conditions have affected the growth and development of the studied plants. The greenhouse is made of polyethylene with a thickness of the cover foil of 0.20 mm. The outside temperature also determines the temperature inside and the excessive values are the reason for the higher values inside in the discussed period (Fig. 1). The lettuces were planted on November 8 in their growth stage: leaf development (Main shoot) - 5th true leaf unfolded with first harvest on March 21 in growth stage typical leaf mass reached. The results show that compared to the conditions outside, the average temperature in the greenhouse is higher by 2.5^{0}C (Fig. 2). From the whole period of 149 days, the days with temperatures below the biological minimum of 5.0˚C (Cholakov, 1999), noted in fig. 1 by a green dotted line, were reduced from 58 outside to 25 inside the greenhouse. The collected active amount for the vegetation period of 124 days was ΣAt^{0}C = 1267^{0}C. The effective temperature amount (ΣEft^{0}C) from the moment of planting to the last harvest of the lettuces in the polyethylene greenhouse was ΣEft^{0}C = 647^{0}C. For the period 21st of March 21- 4th of April 4 there were only two days with temperatures below the biological minimum and the collected active temperatures from the first to the last harvest were 137^{0}C while the value of the effective temperature >Tb was ΣEft^{0}C = 72.0 ^{0}C.

Figure 1. Temperature conditions November 2019-April 2020 and deviation from the period 1991-2019

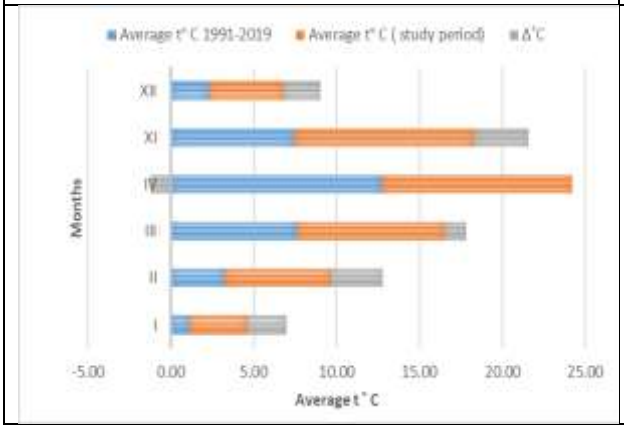
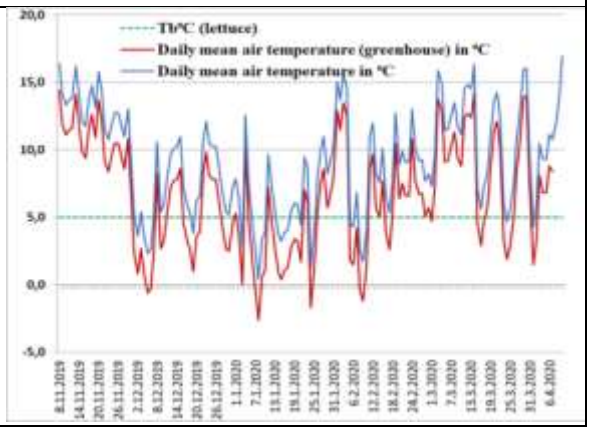


Figure 2. Average daily values of the air temperature outdoors and in an unheated greenhouse during the period of growth and development of the lettuce 2019-2020



It has already been mentioned that in unheated facilities the outside temperature determines the conditions of growth and development of the plants inside. Therefore, a comparison was made between the average temperature values outdoors and indoors (in the greenhouse). A linear relationship ***(y = 0.9421x + 2.8671; r^2=0.83, SS=1, 5; MAE=1.2)*** was obtained (Fig. 3). A model is proposed for calculating the average daily temperature inside the polyethylene greenhouse using the values, obtained from the meteorological site outside. The model was

applicable (Table 2) for the needs of various agrometeorological studies in conventional and organically grown vegetable crops in polyethylene greenhouses.

Figure 3. Plot of fitted model (StatGrafics®)

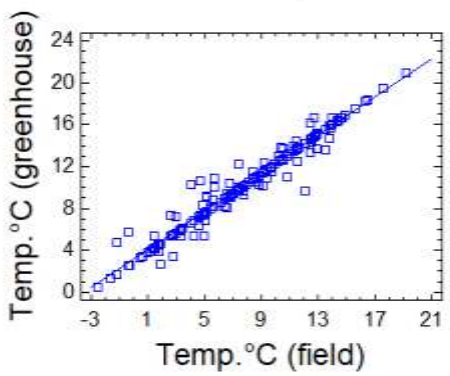


Table 2. Statistical analysis (StatGrafics®)

Dependent variable:

Parameter	Estimate	Standard Error	T Statistic	P-Value
CONSTANT	3.16	0.13	23.37	0.0000
x	0.91	0.015	60.11	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	2945.24	1	2945.24	3614.37	0.0000
Residual	140.972	173	0.81486		
Total (Corr.)	3086.21	174			

Analysis and Statistical evaluation of the fresh mass (g.)

The paper considers the biometric indicator of fresh weight as a key element of productivity. An analysis was made between the control and the tested variants (Fig. 4).

The unfertilized lettuce (the control) has an average weight of 589.18g – starting from 499 when the first measurement was made to 723 during the last one. After mineral fertilization, the weight of the plants varied from 538g to 721g, with an average weight of 629. The average weight of Italpollina was 620.4 with increase from 523.2 to 736.9; of Arkobaleno- 527.4 with the lowest value 472.3 and the highest - 563.9; LK had an average weight of 543.6, starting from 514.3 and reaching 589; Ekoprop - 628.3 with the lowest weight 535.7 and the highest 711.7. It is noteworthy that Italpollina has the best parameters, as in the last measurement it exceeds the results of all the other variants, including for the case of mineral fertilization. The biological variants Arkobaleno and LK have a minimal negative difference compared to the variant with mineral fertilization, while the variants Italpollina and Ekoprop exceed it by 31 g. and 39 g. respectively (Fig. 4).

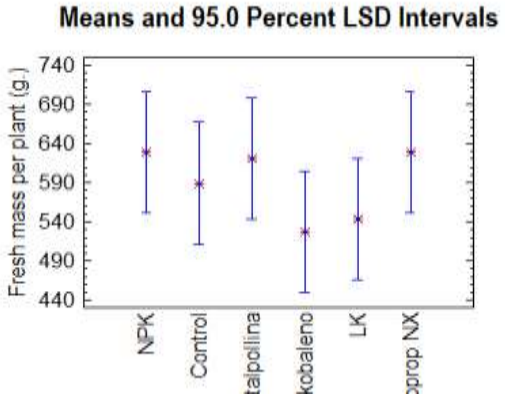


Figure 4. Fresh mass (g.) for the variants of lettuce Batavia variety 'Maritima'

The analysis of the data for this particular year does not show statistically significant differences between the control, the minerally fertilized plants and the ones, fertilized by organic fertilizers (Fig.4 and Tabl. 3). Ekoprop can be considered the best option, and at the last measurement on April 4, Italpollina exceeded all the other fertilization variants. The plants from the variants with organic fertilization have better taste qualities and no deviations from the typical color and shape of the species.

Table 3. ANOVA analysis Fresh mass (g.) of lettuce Batavia variety 'Maritima'

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	29933.9	5	5986.79	0.78596	0.57916	3.10588
Within Groups	91406	12	7617.17			
Total	121340	17				

Physiological parameters

The averaged value of the initial fluorescence (F_0) of the oxidized reaction centers of PSII is highest in case of biological fertilization with LK, and lowest in case of mineral fertilization (Table 4).

Table 4. Chlorophyll fluorescence parameters of the plant leaves for the lettuce Batavia, variety 'Maritima' in an unheated greenhouse

indices / **variants**	***Control***	***NPK***	***Italpollina***	***Arkobaleno***	***LK***	***Ekoprop NX***
Ft=Fo	4413	4668	4468	4761	4130	4103
Ft=Fo	3573	3730	3822	4578	4387	4165
Ft=Fo	4838	4032	4448	3699	4646	4467
mean value	**4275**	**4143** n.s	**4246** n.s.	**4346** n.s.	**4388** n.s.	**4245** n.s.
Qy=Fv/Fm	0,82	0,80	0,80	0,82	0,81	0,81
Qy=Fv/Fm	0,80	0,83	0,81	0,80	0,81	0,83
Qy=Fv/Fm	0,82	0,82	0,84	0,84	0,83	0,83
mean value	**0.814**	**0.816** n.s.	**0.817** n.s.	**0.821***	**0.819** n.s.	**0.823****
PAR	330,7	349,3	480,7	203,7	481,0	200.0
PAR	231,7	290,0	265,7	276,7	285,0	206,3
PAR	83,0	61,0	73,3	97,0	64,7	68,3
mean value	**215.1**	**233.4** n.s.	**273.2****	**192.4** n.s.	**276.9*****	**158.2*****

LSD	F0	Qy	PAR
p=0.05*	191.5	0.0059	33.4
p=0.01**	256.3	0.0079	44.7
p=0.001**	336.5	0.0104	58.7

n.s. no significance difference

With this indicator only no significance of the differences in the average values was established, which proves the weak influence of the fertilization method on it. Also, Zlatev & Kolev, 2012 and Chen et al., 2018 believe that the high temperature leads to an increase in F_0, in which case

the temperature conditions in the greenhouse do not lead to a negative change in the photosynthetic activity of the different variants. The highest value of F_0 for Arkobaleno on the first and second reporting dates can be observed, on average 17.0% higher than the control and significantly the highest value of F_0 of the control for the third reporting date. The average value of the Fv/Fm ratio or the quantum yield Qy of the different variants is close to the normal for healthy leaves - 0.83 (Demmig and Björkman, 1987). The lowest value was registered for the control variant, while the highest value together with a statistically significant difference were measured for the variants with the organic fertilizers Ekoprop and Arkobaleno (Table 4). The comparative characteristic made by dates of measurements shows the largest difference between the unfertilized variant and the variants with organic fertilizers on the third measurement date. With this important indicator of photosynthetic activity, there is a proven positive effect of the organic fertilizers, not only compared to the control variant, but also to the variant with mineral fertilization. On the other hand, Qy values for all variants in this study do not indicate the presence of stress in the plants during the period of reporting their physiological activity. The measured photosynthetically active radiation (PAR) is higher on the first two dates, which is associated with the higher daily temperatures and the increased solar radiation compared to the atmospheric conditions during the third reporting date. The almost double reduction of PAR does not lead to inhibition of the photosynthetic activity. The most optimal ratio between PAR and Qy was reported for Ekoprop and Arkobaleno (Table 4).

In parallel with the readings of some indicators of the chlorophyll fluorescence of the leaves, the chlorophyll index - Chlorophyll Content Index (CCI) was measured (Table 5). With the exception of the variant Arkobaleno, in all the other fertilization variants the CCI has a higher and statistically significant average value compared to the control variant. The leaves of the variants fertilized by organic fertilizers Lumbricompost and Ekoprop have the highest CCI. The lowest and highly variable CCI was reported for Arkobaleno, the low average value being mainly due to the 20.0% lower CCI compared to the control during the third measurement. The values of the chlorophyll index are in a positive correlation with the values of the quantum yield-Qy, which proves the inducing effect of the organic fertilizers on the photosynthetic activity of the plants. The highest average value of fresh mass of the plants, fertilized by Ekoprop, correlates positively with the reported good indicators of the chlorophyll fluorescence and the high chlorophyll content index.

Table 5. Chlorophyll content index (CCI) of the leaves for the lettuce Batavia variety 'Maritima' in an unheated greenhouse

date of estimate / variants	Control	NPK	Italpollina	Arkobaleno	LK	Ekoprop
20.3.2020	8.63	8.46 n.s.	8.84 n.s.	9.06**	8.86 n.s.	8.73 n.s.
28.3.2020	9.12	10.06***	10.35***	9.21n.s.	10.23***	11.12***
04.4.2020	7.35	7.66 n.s.	7.70 n.s.	5.90***	8.56***	7.58 n.s.
mean value	**8.37**	**8.72***	**8.96****	**8.06 n.s.**	**9.22*****	**9.14*****

LSD	**20.03.2020**	**27.03.2020**	**08.04.2020**	**Mean value**
p=0.05*	0.29	0.34	0.40	0.32
p=0.01*	0.39	0.45	0.53	0.47
p=0.001*	0.50	0.58	0.69	0.71

CONCLUSIONS

During the studied period (November 2019 - April 2020) the temperature conditions were determined by a warm winter and cool spring compared to the reference period 1961-1990 and the period 1991-2019. The collected effective temperature sum >Tb (ΣtE^0C) reached up to the typical leaf mass reached for the Batavian lettuce, variety 'Maritima', was 647^0C. The higher by 2.5°C temperature in the greenhouse reduced the days with temperatures below the biological minimum to the number of 25, compared to the number of 58 outside. The evaluation of the fresh mass of the rosette did not show statistically significant differences between unfertilized plants and plants, fertilized by either mineral or biological fertilizers. Ekoprop and Italpollina have the best parameters since Italpollina has the highest productivity of all the other variants for the last reporting date – 4th of April, 2020. Regarding the photosynthetic activity Qy, there is a proven positive effect of the organic fertilizers, not only compared to the control variant, but also compared to the variant with mineral fertilization. The most optimal ratio between PAR and Qy was reported for the variants Ekoprop and Arkobaleno. The leaves of the variants, fertilized with the organic fertilizers Lumbricompost and Ekoprop have the highest CCI.

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REFERENCES

Alexandrov, V. Schneider, M., Koleva, E., Moisselin, J. M (2004). Climate variability and change in Bulgaria during the 20th century. Theoretical and Applied Climatology, 79, 3-4, Springer 133-149.

Chen, J., Burke, J. Xin, Z. (2018) Chlorophyll fluorescence analysis revealed essential roles of FtsH11 protease in regulation of the adaptive responses of photosynthetic systems to high temperature. BMC Plant Biology, 1-13.

Cholakov, D. T., (1999). Vegetable production Plovdiv Academic Publishing House of the Agrarian University, p. 283.

Demmig, B., Björkman O. (1987). Photon yield of O2 evolution and chlorophyll fluorescence Characteristics at 77 K among vascular plants of diverse origins. Planta, 170, 489–504.

Fogg, H. G. W. (1983). Salad crops all year round. UK: Newton Abbot 200 pages.

Frantzova, A. (2014). Remote sensing application in disaster management of natural hazards. 5th International Conference on Cartography and GIS Proceedings, 15-20 June 2014, Riviera, Bulgaria, Vol. 1 and Vol. 2: 715-725.

Ferrante, A., Maggiore, T., 2007. Chlorophyll a fluorescence measurements to evaluate storage time and temperature of Valeriana leafy vegetables. Postharv. Biol. Technol. 45, 73–80. http://dx.doi.org/10.1016/j.postbarvbio.2007.02.003.

Georgieva, V., Kazandjiev V., Stoycheva A. (2017). Assessment of agroclimatic conditions in Plovdiv district. - *In: International conference, 135 Years Agricultural science in Sadovo and 40 Years Institute of Plant Genetic Resources-Sadovo"*: 547-564.

Gide to Agricultural Meteorological Practices (2011). WMO, No.134, Ch. 6-39.

Kristensen, S., Friis, E., Henriksen, K. and Mikkelsen, S. A. (1987). Application of temperature sums in the timing of production of crisp lettuce. Acta Horticulturae, 198, 217-225.

Kartalov, P. I., Doykova, M. P., Boshnakov, P., (1999). Vegetable production with seed production. Sofia: Videnov and son, p. 305.

Kostadinov, K. P., & Filipov, S. V. (2013). Effect of Composition of Mixture on Reproductive Manifestations in Greenhouse Tomato. New Knowledge Journal of Science / Novo Znanie . Dec2013, Vol. 2 Issue 4, 104-110.

Kalaji, H. M., Jajoo, A. Oukarroum, A. Brectic, M. Zivcak, M. Saqmborska, I. Centner, M. Lakazik, I. Goltsev, V. Ladle, R. (2016). Chlorophyll a Fluorescence as a Tool to Monitor Physiological Status of Plants under Abiotic Stress Conditions."*Acta Physiologiae Plantarum*, 38, 102-25.

Lorenz O.A., Maynard DM (2007). Knott's handbook for vegetable growers. John Wiley and Sons, New York

Meier U. (ed.), (Feller et al., 1995 a) (2001). Growth stages of mono-and dicotyledonous plants BBCH Monograph Edition, 118-126

Morgan, L. (1999). Introduction. Hydroponic lettuce production. Australia: Casper Publications Pty Ltd. 112 pages

Marinova, T, Malcheva K., Bocheva L, Trifonova L. (2017). Climate profile of Bulgaria in the period 1988-2016 and brief climatic assessment of 2017. *Bul. J. Meteo & Hydro* 22/3-4 (2017): 2-15.

Murchie, E, Lawson, T. (2013). Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of Experimental Botany*, 64 (13), 3983–3998.

Mathur, S., Agrawal, D. Jajoo, A. (2014) Photosynthesis: Responses to High Temperature Stress. Journal of Photochemistry and Photobiology, 137,116-26.

Maxwell, K, Johnson, G.N. (2000). Chlorophyll fluorescence a practical guide. Journal of Experimental Botany, 51(345), 659-668.

Nedbal, L., Soukupová, J, Kaftan, D, Whitmarsh, J, Trtílek, M. (2000). Kinetic imaging of chlorophyll fluorescence using modulated light. Photosynthesis Research, 66, 3-12.

Opti-Sciences, Inc. (2002). CCM-200 Chlorophyll Content Meter. http://www.optisci.com/ccm.htm.

Popova, Z., Ivanova, M., Martins, D., Pereira, L.S., Doneva, K., Alexandrov, V., Kercheva, M.(2014). Vulnerability of Bulgarian agriculture to drought and climate variability with focus on rainfed maize systems. Natural Hazards, 74, 2, Springer, 865-886.

Richardson, A. D., Duigan, S. Berlyn, G. (2002). An evaluation of noninvasive methods to estimate foliar chlorophyll content.-*New Phytologist*, 153,185-194.

Roháček K, (2002). Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica*, 40, 13-29.

Schofiled, R.A., De Ell, J.R., Murr, D.P., Jenni, S., 2005. Determining the storage potential of iceberg lettuce with chlorophyll fluorescence. Postharv. Biol. Technol. 38, 43–56. http://dx.doi.org/10.1016/j.postharvbio.2005.06.002.

Shopova, N., D. Cholakov (2014). Effect of the age and planting area of tomato (Solanum licopersicum L.) seedlings for late field production on the physiological behavior of plants. Bulgarian Journal of Agricultural Science, 20 (No 1), 173-177.

Valcheva V., K. Trendafilov, M. Almaliev (2015). Nitrogen mineralization potential of alluvial-meadow soil after long-term fertilization, Agricultural science and technology, vol.7, No 4, 476-480.

Zlatev, Z, T. Kolev. (2012) Changes in chlorophyll fluorescence and leaf gas exchange of durum wheat under low positive temperatures. Agricultural science and technology, 4(1), 20-23.