Features of Water Exchange of *Pinus nigra* subsp. *pallasiana* in Conditions of Southern Coast of Crimea

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Abstract—The eco-physiological reaction of *Pinus nigra* subsp. *pallasiana* on the impact of extreme environmental factors was studied; optimum zones and threshold values of soil moisture, temperature and illumination that limits photosynthesis and transpiration of this species in the conditions of the southern coast of Crimea were determined. The optimums of the studied parameters were found: $En = 25\text{-}50$ mg / (m^2s), $I = 450\text{-}1000$ μmol/(m^2s), $Pn = 8,5\text{-}10$ μmol/(m^2s), $Ws = 55\text{-}70\%$ FC. As an indicator of the response (reaction) of species to irrigation, we used the relative water flow rate in the plant shoot and changes in shoot diameter, as well as apical growth of the plant. Analyzing research results allowed us to study the features of water regime of the studied species and reaction to extreme environmental factors. After a severe drought and two irrigations, it took about two days to restore the studied parameters to the initial values. Using the nonlinear regression equations, the dependencies are determined: $Pn = f (I, E)$; $E = f (I, W)$; $E = f (Sf, d)$; $Sf = f (I, W)$. Association between the transpiration rate and the needles temperature under the influence of limiting environmental factors is determined. At a needles temperature above 35°C, the transpiration rate sharply decreases. Regression statistics for the parameters E and Tn: $R = 0,7794$; $R^2 = 0,6076$. When introducing this species into different regions, the results of our studies allow us to compare the hydrothermal characteristics that we obtained with the climatic conditions of a particular region and evaluate the possibilities of its cultivation.

Keywords—*Pinus nigra subsp. pallasiana, water regime, ecological and physiological characteristics, environmental factors.*

I. INTRODUCTION

The climate features of the Southern Coast of Crimea (SCC), which is a region of dry subtropics, allow preserving natural vegetation, planting new ones and reconstructing existing green plantations that are in the microclimate formed by the environment [1].

In the selection of such plant species, it is necessary to study various vital processes in the conditions of their growth.

The objective was to study the dependence of water regime of *Pinus nigra* subsp. *pallasiana* from environmental factors in order to determine the optimal and limiting conditions for their growth [2].

Decrease of transpiration rate and photosynthetic activity during water deficiency under the influence of extreme environmental factors occurs mainly due to stomata closure, which protects this species from dehydration, but disrupts the CO_2 exchange of leaves, which negatively affects photosynthesis.

There are not many papers on these species' reaction to seasonal climate changes in the Crimea and southern Ukraine [3-4] and their tolerance to summer drought, as well as of plants growing in the Mediterranean region of dry subtropics, including various species and subspecies of black pine Pinus nigra subsp.

Black pine *Pinus nigra* subsp. *laricio* inhabit a significant part of the forest area $(3,5*10^6$ ha) in the Mediterranean region – from Morocco to Turkey. In order to study the seasonal functioning and tolerance to summer drought (University of Corsica, France), researches conducted field studies on this species in a natural mountain pine forest [5]. As a response to the summer drought, there is a decrease in gas exchange and xylem flow, which is regulated by stomatal conductance. At the same time, the efficiency of water use is increased and the mesophyll conductance limit is reduced. Such a reaction to summer drought shows the ability of *Pinus nigra* subsp. *laricio* to undergo physiological adaptation to climate change. Amount of precipitation in the Mediterranean basin is expected to decrease by more than 25-30% by the end of the 21st century, probably accompanied by an increase in average annual temperatures by 4-5°C [6].

In addition, these results provide an important basis for further understanding of eco-physiological responses to changing environmental factors.

Features of the water regime of Scots pine (*Pinus sylvestris L.*) in the conditions of the Mediterranean mountain climate were studied at the "Institute of Earth Sciences Jaume Aimera" (Spain, Barcelona). A number of parameters were used to study the response of water regime of this species to soil and atmospheric drought: water potential of leaves, relative xylem flow rate, hydraulic conductivity, leaf gas exchange, stomatal conductance. Research results allowed us to study the response of these parameters to soil and atmospheric drought and to determine the resistance of the species to extreme events during summer drought [7].

Studies on the carbon fluxes from the leaf surface to the landscape level under drought conditions [8] were carried out in the Collserola park (Barcelona, Spain) on various species, including the Aleppo pine (*Pinus halepensis Mill.*). Researchers studied the dependence of seasonal acclimatization of photosynthesis and morphology in sunlit and shaded leaves in natural conditions. Severe drought induced early leaf aging, simultaneously significantly increasing leaf mass per unit area. Shaded leaves (needles) had lower photosynthetic potentials and could not mitigate the negative effects during periods of stress. The studies explain the response of vegetation to abiotic stresses and have

great potential to reduce uncertainty in terrestrial biosphere models, especially in drought conditions.

To develop the scientific basis for growing seedlings and to study the effect of light radiation intensity on the photosynthetic characteristics and water use efficiency of *Pinus tabulaeformis* (native to China), a series of experiments were carried out in a semi-arid region at the experimental station of Beijing Forestry University [9].

The research results showed that the intensity of visible photosynthesis and transpiration increased with the increasing illumination, however, due to lack of water in the soil, stomatal conductance decreases and can create a protective mechanism to prevent more water consumption and leaf gas exchange.

It has been shown that seedlings of *Pinus tabulaeformis* are particularly affected by strong light, which inhibits growth. The obtained research results allowed us to develop scientific basis for growing seedlings for regions with extreme environmental factors.

The objective is to study the ecophysiological reaction of *Pinus nigra* subsp. *Pallasiana* on the environmental impact affecting the characteristics of water metabolism of this species in the autumn growth.

II. METHODS

Crimean pine (*Pinus nigra* subsp. *pallasiana*) is a Mediterranean mountainous, dominant species. The plant range includes Crimea, mainly the southern slope of Yaila, southern Ukraine and the Caucasus [3-4]. The tree is 20-30 (up to 45) m of height. The crown is wide, pyramidal; in older trees it is flat, umbrella-shaped. Branches are horizontal, with shoots bent up. The bark is black or dark brown, deep furrowed, reddish in the upper part of the trunk. Young shoots are yellow-brown, shiny. The buds are large with straight, not bent scales. The needles are dark green, very dense, somewhat curved, 8-12 cm long and 1,6-2,1 mm wide. Crimean pine releases pollen at the beginning of May. Cones are large, with brown and red glossy scales. Seeds are dark gray, speckled, matte, and larger than in ordinary pine. Cones mature in the third year of vegetation in August-September. Trees grow mainly in the area on rocky soils containing lime. They grow well on loamy and clay-limestone. Crimean pine is very photophilous and relatively hygrophilous. It develops well in both the lower, mid-mountain and upper forest zones at altitudes of 500-900 m above sea level. At altitudes above 700 m, in conditions with increased humidity and more frequent precipitation in the form of rain and snow, the Crimean pine tree has the most intensive growth and forms closed forest stands [4].

The studies were conducted in a greenhouse on the territory of the central branch of the Nikitsky Botanical Garden. Plants, seedlings of 3-4 years of age, were grown from cuttings in vegetation vessels in open ground. The conducted studies allowed us to determine these dependences during the period of autumn plant growth (September-October). The time of the experiments is September-October in years 2017-2018.

For continuous automatic recording of gas exchange of intact leaves, plant growth and water balance, the RTM-48A photosynthesis monitor and the PM-11z phytomonitor were used [10]. The PTM-48A monitor system is equipped with four leaf cameras connected to the monitor. The working area

of the standard LC-4B camera is 20 cm² and is applicable to a wide variety of leaves. Temperature sensor is additionally installed in the LC-4B chamber for measuring stomatal conductance of leaves. The measurement of the total photosynthesis rate was determined on young intact leaves in the upper part of the shoot every 15-20 minutes. The natural concentration of CO_2 in the air was approximately at 0,04%. To characterize various processes of plant life, the following parameters were used: values of pure photosynthesis – Pn, μmol/m²s; stomatal conductance – gs, mm/s; transpiration rate – E, mg/m²s in the range of photosynthetic active radiation from 0 to 2000 μmol m²s. Photosynthetically active radiation and other environmental parameters: temperature and air humidity were measured by RTH-48 Meteo-module sensors connected to the digital input of the RTM-48A system. Photosynthetic radiation sensor TIR-4 (I – μmol/m²s), needles temperature sensor LT-1P (Tx °C), soil moisture sensor SMS-5P (%), relative sap flow rate in the shoot by a sap flow sensor SF-5P (Sf, rel.unit), stem diameter growth (d of the shoot) by SD-10z sensor (mm), apical growth sensor axonometer SA-20z (mm).

Statistical data processing was performed using the Statistica 10 software (Statsoft Inc., USA) and Microsoft Excel 2010. The least squares and robust locally weighted regression methods (Statistica 10) were used to model and smooth two-dimensional data. All calculations were carried out at a given significance level of P≤0,05.

III. RESULTS

To study the eco-physiological reaction of *Pinus nigra* subsp. *pallasiana* on the main environmental factors, a series of experiments was conducted in greenhouse conditions, which allowed us to determine the optimal and limiting conditions of the water regime of this species. For various types of plants (drought tolerant or hygrophilous), the optimal value of environmental factors and especially soil moisture can vary over a fairly wide range. In addition, for the same plant species in different phases of its development, this indicator can also vary.

Depending on the growing environment, plants are able to activate or slow down the diffusion of CO_2 molecules and water evaporation by changing stomatal conductance, protecting plants from overheating and creating the necessary conditions for photosynthesis and transpiration, and ultimately a certain homeostasis of the production process [6]. Figure 1a shows a natural change in the parameters E (1) and Pn (2) during soil drought. Regression statistics for the parameters E and Pn: R = 0,7049; R^2 = 0,496. Figure 1b shows the natural change in the parameters E (1) and gs (2). Regression statistics for the parameters E and gs: R = 0,9165; R^2 = 0,8401. An analysis of the results shows that there is a closer correlation between E and gs than between E and Pn.

It should be noted that the opening width of stomata depends on an increase in the level of carbon dioxide in leaves (needles) with increasing air temperature (increased respiration and photorespiration), as well as water deficiency in tissues at high temperatures and low humidity of soil and air [7].

Considering that the relative water flow rate in the shoot of the plant (Sf) and changes in the shoot diameter – d, combine the indicators of climatic factors' impact and available moisture in the soil, and they can be used as an

indicator of the response (reaction) of the species to water supply [5,10]. Figure 1c shows the natural course of the parameters (dp) and soil moisture (Ws) and reaction of the shoot diameter to irrigation. An analysis of the results shows that when the soil moisture changes from 15 vol% to 5 vol% (18-45% FC), the shoot diameter changes by 0,295 mm, which is 2,45% with respect to the initial shoot diameter (12 mm). In Figure 1d, between the xylem flow rate in the shoot (2) and the change in soil moisture (1). With deep intense drought (Fig. 2a), a decrease in shoot diameter in relation to its initial value can reach 3,91%. The reaction to irrigation occurs within a few minutes and up to 1,5-2 hours, and the restoration of the parameters to the initial values depends on the intensity of the drought and can continue for a long time (up to several days).

As can be seen from Figures 1c, 1d and 2a, the parameters used by us are very sensitive to the influence of environmental factors (soil drought). They are determined by the xylem elasticity and, as a result, determine the features of the water regime and drought tolerance of the species [11].
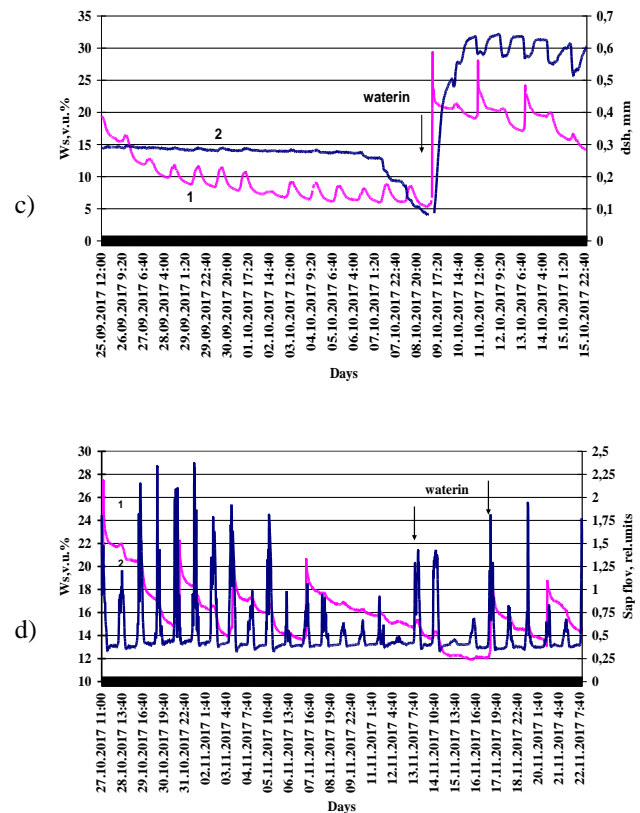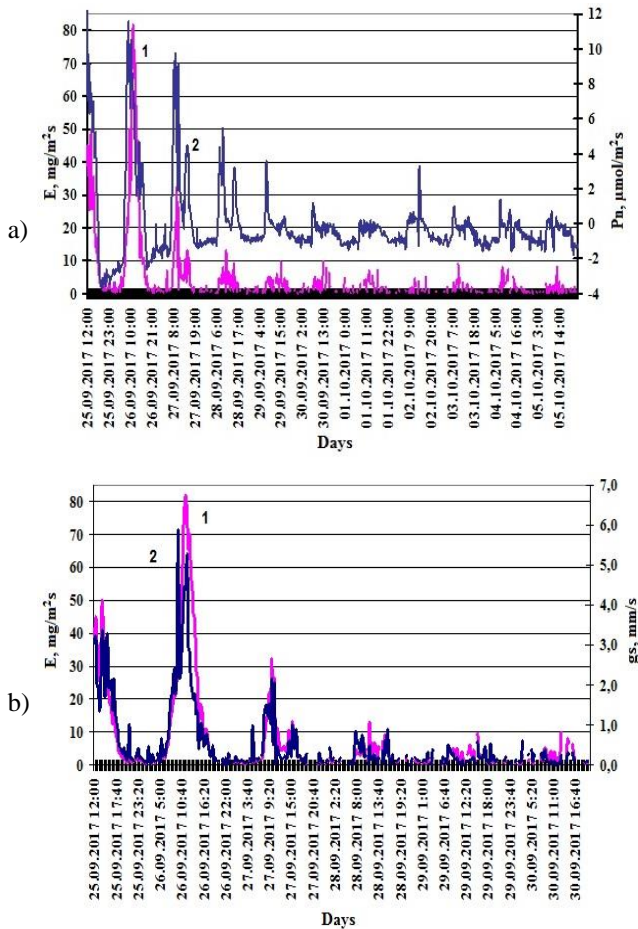

a)

b)

c)

d)

Fig. 1. Natural change in parameters E (1) and Pn (2) during soil drought: a) between transpiration rate – E (1) and stomatal conductivity – gs (2); b) between shoot diameter (2) and soil moisture (1); c) between xylem flow rate in the shoot (2) and soil moisture Ws (1); d) reaction of studied parameters to irrigation

A synchronous decrease in parameters (Sf, dsh) can be used to control the water regime of various plant species (Fig. 2a) and serve as a signal for irrigation [10].

The indicator of the species' response (reaction) to water supply is also apical plant growth. Figure 2b shows the connection between this parameter and soil moisture. At the beginning of the experiment, a decrease in apical growth by 5.8 mm for three days with a decrease in soil moisture from 17 vol% to 7 vol% (42,5-17,5% FC) is observed. Then a plateau is observed eight up to irrigation after eight days. After irrigation, apical growth increase is observed for three days, reaching a plateau on the fourth day. During irrigation, soil moisture increases from 12 to 25 vol% (From 30% to 62,5% FC). Apical growth during this time increases by 6.4 mm in three days. An analysis of the results showed the reaction of this species to soil drought and irrigation – the decrease in apical growth was 1,93 mm. per day during soil drought, and after irrigation this parameter increased by 2,13 mm per day.

Therefore, apical plant growth can be used as an indicator of the water regime and drought tolerance of plants, and its reaction to changes in environmental factors (soil moisture) depends on the intensity and duration of such exposure.

There is a nonlinear regression relationship between the rate of net photosynthesis (Pn), transpiration rate (E) and illumination (I) (Fig. 2c). The zones of ecological and physiological optimum Pn=f (I, E) are determined. For the optimum zone, environmental conditions that ensured the intensity of the studied parameters (gas exchange, water

regime, or other dependent parameter) above 90% of the maximum were taken [12-13]. An analysis of the results and the nonlinear regression equation (Fig. 2c) made it possible to find the optimums of the studied parameters: $En = 25-50$ mg/(m^{2}s); $I = 450-1000$ μmol/(m^{2}s); $Pn = 8,5-10$ μmol/(m^{2}s). There are equations of nonlinear regression of the relationship between these parameters at the top of the graph. The same dependence was found (Fig. 2d) between the transpiration rate (E) by illumination (I) and the change in soil moisture content Ws – $E = f(I, W)$. The optimums of the studied parameters: $En = 25-50$ mg/(m^{2}s); $I = 450-1000$ μmol /(m^{2}s); $Ws = 45-75\%$ FC.

a)

b)

$$Pn= -1,6883+0,4911*x+0,0061*y+0,0032*x*x-0,0007*x*y-3,1076E-6*y*y$$

c)

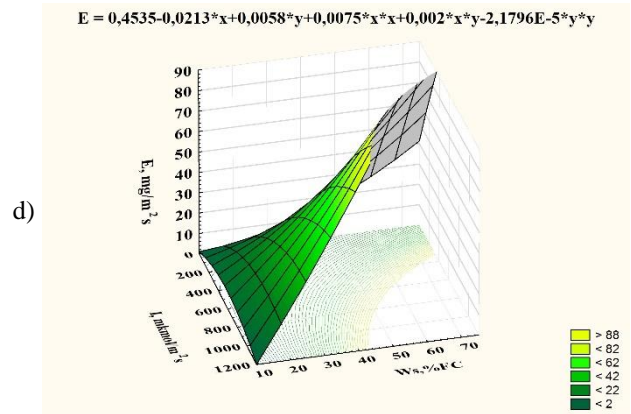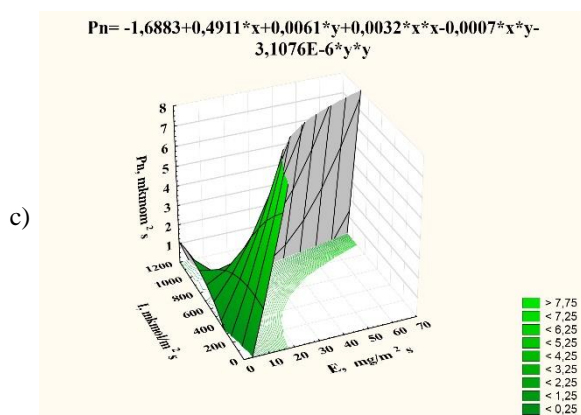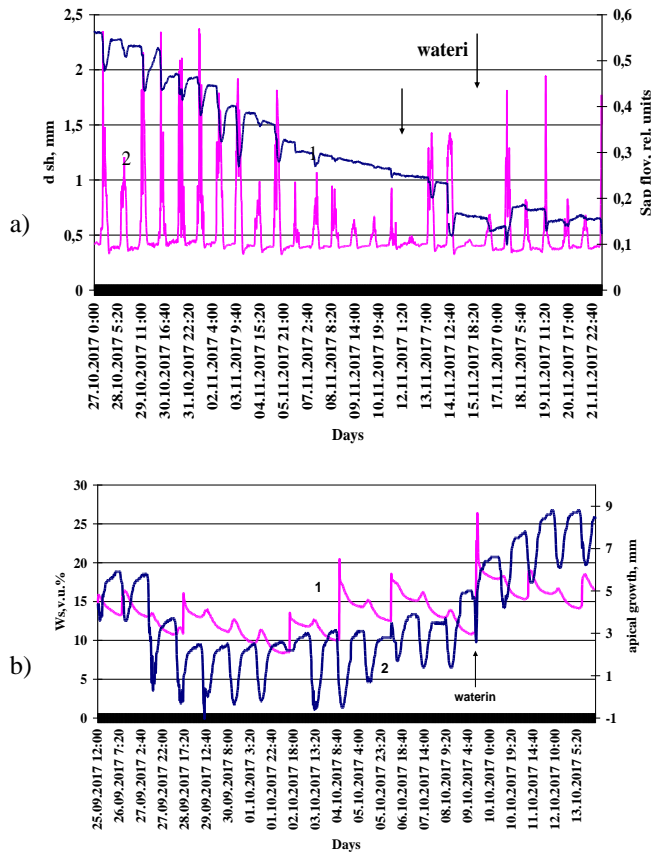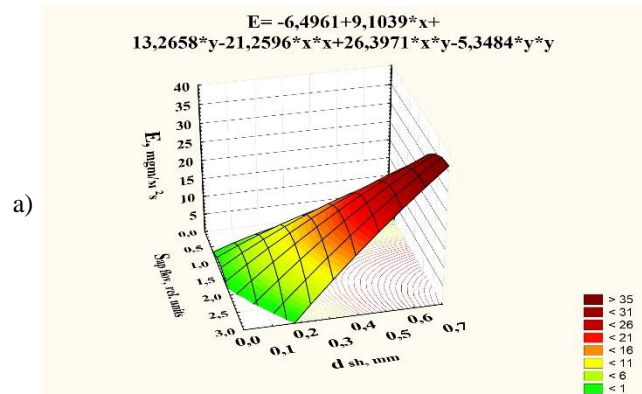$$E = 0,4535-0,0213*x+0,0058*y+0,0075*x*x+0,002*x*y-2,1796E-5*y*y$$

d)

Fig. 2. Dependencies between parameters characterizing various vital processes of *Pinus nigra* subsp. *pallasiana*: a) between xylem flow rate in plant shoot –*Sf* (2) and diameter of this shoot –*d* (1); b) between apical growth rate (2) and soil moisture (1); c) between net photosynthesis rate (Pn), transpiration rate (E) and illumination (I); d) between transpiration intensity (E), illumination (I) and changes in soil moisture – *Ws*

There is a nonlinear dependence between the transpiration rate (E), the relative water flow rate in the plant shoot (Sf) and the change in shoot diameter (Fig. 3a). This dependence is described by the nonlinear regression equation $E = f(Sf, dsh.)$ And it is shown at the top of the graph. Figure 3b shows the dependence of transpiration ratre (E) and the relative rate of the water flow in the plant shoot (Sf) on illumination. It can be seen from the figure that the optima of transpiration rate and relative rate of the water flow are at illumination $I=450-1000$ μmol/(m^{2}s). The dependence of the relative water flow rate (Sf) on illumination (I) and soil moisture (Ws) is shown in Fig. 3c. This dependence also has a nonlinear character – the optimum Sf is at $I=450-1000$ μmol/(m^{2}s) and $Ws=17-28$ v.u.,% (42,5-70% FC).

When the needles are overheated under the influence of various environmental factors, the productivity of photosynthesis and the transpiration rate sharply decrease. Figure 3d shows the natural course of transpiration rate and needles temperature under the influence of environmental limiting factors. At a needle temperature above 35 °C, the transpiration rate sharply decreases.

Regression statistics for the parameters En and Tn: $R = 0,7794$; $R^2 = 0,6076$.

$$E= -6,4961+9,1039*x+13,2658*y-21,2596*x*x+26,3971*x*y-5,3484*y*y$$

a)

$$E= -2{,}3442+7{,}5807*x+0{,}0233*y-0{,}0675*x*x+0{,}0024*x*y-2{,}1881E{-}5 *y*y$$

b)

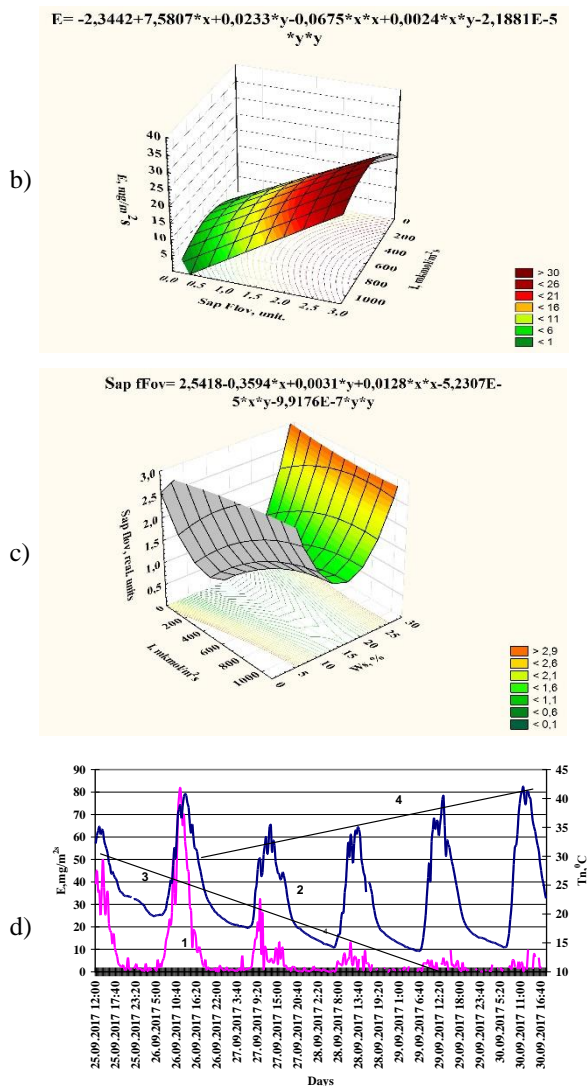$$Sap\ fFov= 2{,}5418-0{,}3594*x+0{,}0031*y+0{,}0128*x*x-5{,}2307E{-}5*x*y-9{,}9176E{-}7*y*y$$

c)

d)

Fig. 3. Quadratic surface of function a) *E=f(Sf, d)*; b) *E=f(I, Sf)*; c) *Sf=f(I, Ws)*; and section contours on the x-y plane; the top of the graph shows the numerical coefficients of the nonlinear regression equations. d) natural course of transpiration rate (1), needles temperature (2), their trend lines (3, 4), respectively

To study the features of the water regime of this species in other periods of vegetation, additional studies are necessary.

IV. Conclusion

We have discovered genotypic features of *Pinus nigra* subsp. *pallasiana* in order to maintain optimal water regime in accordance with environmental conditions. The optimums of the studied parameters are found: $En = 25\text{-}50$ mg/(m²s); $I=450\text{-}1000$ μmol/(m²s); $Pn=8{,}5\text{-}10$ μmol/(m²s); Ws=55-70 % FC.

The eco-physiological response of plants to water deficiency was studied, and information on the effect of intensity and duration of water stress was obtained. After a severe drought and two irrigations, it took about two days to restore the studied parameters to the initial values.

Using the relative water flow rate in the plant shoot and changing the shoot diameter, as well as the apical growth, allowed us to study the features of water regime of this

species and reaction to the influence of extreme environmental factors.

Using the equations of nonlinear regression, the dependencies between the main characteristics of plant life (Pn, E) and environmental factors (I, W) are determined: $Pn = f (I, E)$; $E = f (I, W)$; $E = f (Sf , d)$; $Sf = f (I, W)$.

The transpiration rate sharply decreases at a needle temperature above 35 °C. Regression statistics for the parameters E and Tn are: $R = 0{,}7794$; $R^2 = 0{,}6076$.

When introducing this species into different regions, the results of our studies allow us to compare the obtained eco-physiological characteristics with the climatic conditions of a particular region and evaluate the possibilities of cultivation.

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