Exploring Transpiration Regularity of Single Loquat Tree under Indoor Environmental Conditions

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Abstract: Transpiration has an important impact not only on the growth of plants, but also on the hydrological cycle.Determining quantificationally the plant transpiration rate helps understanding the hydrological cycle more accurately. The transpiration rate of a single loquat tree at different relative humidity, temperature and illumination intensity was measured in a phytotron chamber with a sap flow system (Dynamax, USA).The effects of the three factors on the transpiration rate of single loquat tree was analyzed by Response Surface Methodology (RSM). The results indicated that the effect of relative humidity, temperature and illumination intensity at given conditions followed the importance order of temperature > illumination intensity > relative humidity. The transpiration rate of single loquat tree is increasing with the accretion of relative humidity and reduction of temperature, but decreasing at the initial stage and subsequently kept constant with the illumination intensity increasing. Through response surface analysis, a model of three factors *vs.* transpiration rate was acquired and can provide a reference for the calculation of evapotranspiration.

Introduction

As an important part in hydrology research, hydrologic cycle is closely related to the plant evapotranspiration. The total global precipitation is about 113,500 $km^3\ y^{-1}$ (cubic kilometers per year), among which the infiltration amount is about 80,000 $km^3\ y^{-1}$, and the rest 33,500 $km^3\ y^{-1}$ becomes surface runoff. In the infiltration of soil water, every year about 7,500 km^3 groundwater of the infiltration is supplied to rivers and the rest about 72,500 km^3 becomes "green" water back to the atmosphere, among which the productive and non-productive transpiration stream has been roughly estimated as 30,000-35,000 and 35,000-40,000 km^3, respectively. From the above, it can be seen that the productive transpiration almost occupies a half of the total land surface evaporation. Therefore, it is of great significance to strengthen the research on productive transpiration, namely the role of plants in the water cycle research, for an accurate and comprehensive understanding the whole process of hydrologic cycle.

However, it’s difficult to measure the plant transpiration for its complicated physiological process influenced not only by the adjustment and control of its morphology and physiological conditions, but also by various environmental conditions. The existing methods for the testing plant water consumption, such as empirical formula method, water balance method, eddy correlation method, and remote sensing method, can only determine the total amount of evapotranspiration. In the most cases, the evapotranspiration is required to be divided into transpiration and evaporation. Scholars have tried injury flow method, leaf chamber method and covering method for direct determination of transpiration, but the shortages of these methods are also very obvious, such as interfering the plant growing, consuming time and having low practical value.

So far, in the study of water consumption at different dimension, the research field on the water consumption of single plant has been developed with most technical means, such as heat pulse and heat balance [1]. In the 1932, Huber, the famous physiologist, on the basis of the predecessors' work, adopted a new method to measure the water consumption of per plant of plants- heat pulse velocity record method (HPVR). This method had aroused attention of many experts in the botanical field since its first use. In the 1980s, the method of stem heat balance (SHB) has been developed based on HPVR. Compared with HPVR, the SHB does not need to insert the temperature probe into the stem without calibration,, thus the measurement is more convenient and accurate with the lowest trees damage, and the time for fixed-point measurement can be long; moreover, the invention of the parcel probe can make a more accurate determination for the plants stem flow [2].

In the process of research on the stem sap flux rate of plant, many researchers have done many works on the following aspects: the theoretical basis of calculating the plant sap flux with SHB, rule of stem sap flux change and the error analysis [3]. The results showed that the plant stem sap flux could be monitored effectively by SHB, and could be transformed into transpiration rate. However, most of the previous research work was carried out in the outdoor environment without controlling meteorological factors, and that in the greenhouse is relatively less, let alone the relative research with the phytotron under strict control of meteorological conditions. In this paper, plant transpiration rate was researched with a single loquat tree as the pointer plant under different temperature, relative humidity, and illumination, with single factor analysis and the response surface analysis method to analyze the test data. The effects of different environmental conditions on the transpiration rate of single loquat tree were expected to be clarified, providing a reference for the research of plant evapotranspiration in the hydrologic cycle.

Materials and Methods

The loquat transpiration rate at established conditions was obtained by a Dynamix stem flow measurement system in a PQS type phytotron (5 x 3 x 2.5 m) with a TDR300 for measuring soil moisture and a Vernier caliper for surveying the diameter of objects plant.

The test object is a loquat tree seedlings of 3 years with a trunk diameter of 11 mm and a height of 1.2 m. To avoiding the damage to the seedling roots, a wide range of dig transplanting was employed. The seedling was moved into a deep flower pot with a diameter of 1.5 m and depth of 0.5 m and kept for nine days to restore the original state before test. According to GAO Jun [4] and QIU Guangyang [5], the plant transpiration water consumption in short term, is mainly relative to the intensity of solar radiation (Lx), relative humidity of atmosphere (%) and atmospheric temperature (°C). In the phytotron, the plant transpiration rate under different conditions was observed when the above three variables were mainly controlled with other environmental factors fixed.

The single factor test method and orthogonal experiment method were adopted in this work. All of the experiments were divided into nine groups with three sets of test for each factor, of which the test time lasted 3.5 hours in each group. Illumination (16000, 27000 and 38000 Lx), temperature (5, 20 and 35 °C) and relative humidity of atmosphere (60, 75 and 90 %) were set as the main factor in the orthogonal experiment. At the night before test, the potted loquat tree was watered to ensure the adequate water supply in the soil (the average relative humidity is 43%) and moved into the phytotron, then the packaged stem sap flow gauge in the dark and data collection system were opened to calculate the K_{sh} (0.7164). The experiment is to explore the effect of temperature, light intensity and the relative humidity on the loquat transpiration rate when the water supply in the soil is sufficient, so the CO_2 concentration was set as 400 ppm, similar to that in the atmosphere. Naturally, the air is slowly flowing, hence, the fan in the phytotron was turned on to maintain a wind speed of 0.2 m/s. In the process of test, the soil moisture content would gradually decrease with the continuous plant transpiration, therefore, the soil should be watered to ensure adequate water supplying**.** In the test, the straight part without side branches and scars on the stem of potted plant was chosen and pretreated for the installation of SGA10 probe. Set up the parameters with collecting data interval of 30 min.

Results

Selection of factor and levels of response surface analysis

According to the principles of the center combination design proposed by Box-Behnken, the results of single factor test were summarized to elect the three factors of the light, temperature, and relative humidity for the further orthogonal experiments. The three factors and three levels would be employed for the response surface analysis based on single factor experiment. Factors and levels are shown in Table 1.

Table 1 Factors and levels of response surface methodology

Factor	Level		
	-1	0	1
X_1: Illumination / Lx	16000	27000	38000
X_2: relative humidity of atmosphere	0.60	0.75	0.90
X_3: temperature / °C	5	20	35

The design and results of the response surface experiment

The central composite model was employed to carry out the response surface analysis experiments of 17 test points (5 center points) with three factors and three levels. All of the 17 test points can be divided into two types. One group was the points for analysis, the value of independent variables at the various factors that constitute the three-dimensional vertices, which is a sum of 12 factorial points. The other was zero points, as the center point of the region, which was repeated test 5 times to estimate the test error. The transpiration rate of loquat tree is as the response value (index value). Response surface analysis and test results are shown in Table 2.

Table 2 Design and results of Response surface Box–Behnken experimental

Number	actual value of factor			coded value of factor			Y: plant transpiration rate (g/h)	
	X_1	X_2	X_3	x_1	x_2	x_3	actual value	predicted value
1	27000	0.6	35	0	-1	1	13.8	13.7125
2	27000	0.75	20	0	0	0	14.9	14.9
3	38000	0.75	5	1	0	-1	16.5	16.1875
4	16000	0.75	5	-1	0	-1	19.5	19.5125
5	27000	0.75	20	0	0	0	14.9	14.9
6	38000	0.75	35	1	0	1	14.5	14.4875
7	16000	0.75	35	-1	0	1	14.8	15.1125
8	27000	0.9	35	0	1	1	14	13.7875

9	27000	0.6	5	0	-1	-1	15.9	16.1125
10	27000	0.9	5	0	1	-1	17.4	17.4875
11	27000	0.75	20	0	0	0	14.9	14.9
12	16000	0.9	20	-1	1	0	17.7	17.6
13	27000	0.75	20	0	0	0	14.9	14.9
14	16000	0.6	20	-1	-1	0	16.4	16.175
15	38000	0.6	20	1	-1	0	14.8	14.9
16	38000	0.9	20	1	1	0	14.7	14.925
17	27000	0.75	20	0	0	0	14.9	14.9

After being coded, the testing data were fitted with the Expert Design 7.0 software, as shown in Table 3. The resulting correlation equation of monomial, quadratic term and their interaction items of factors were achieved. Basing on this, the response surface analysis was made. The quadratic equation model relationship between the transpiration rate of loquat tree and variable factors was acquired by multiple regression fitting analysis

$$Y = 19.5532 - 0.0005X_1 + 12.6995X_2 - 0.1749X_3 - 0.0002X_1X_2 + 0.0101X_1X_3$$
$$-0.1444X_2X_3 + 0.001X_1^2 - 1.111X_2^2 + 0.0018X_3^2$$

The reliability of model and correlation of transpiration and all factors in the model were determined through variance analysis of monomial, quadratic term and their interaction items of model. The analysis results are shown in Table 3.

Table 3 Results of variance analysis

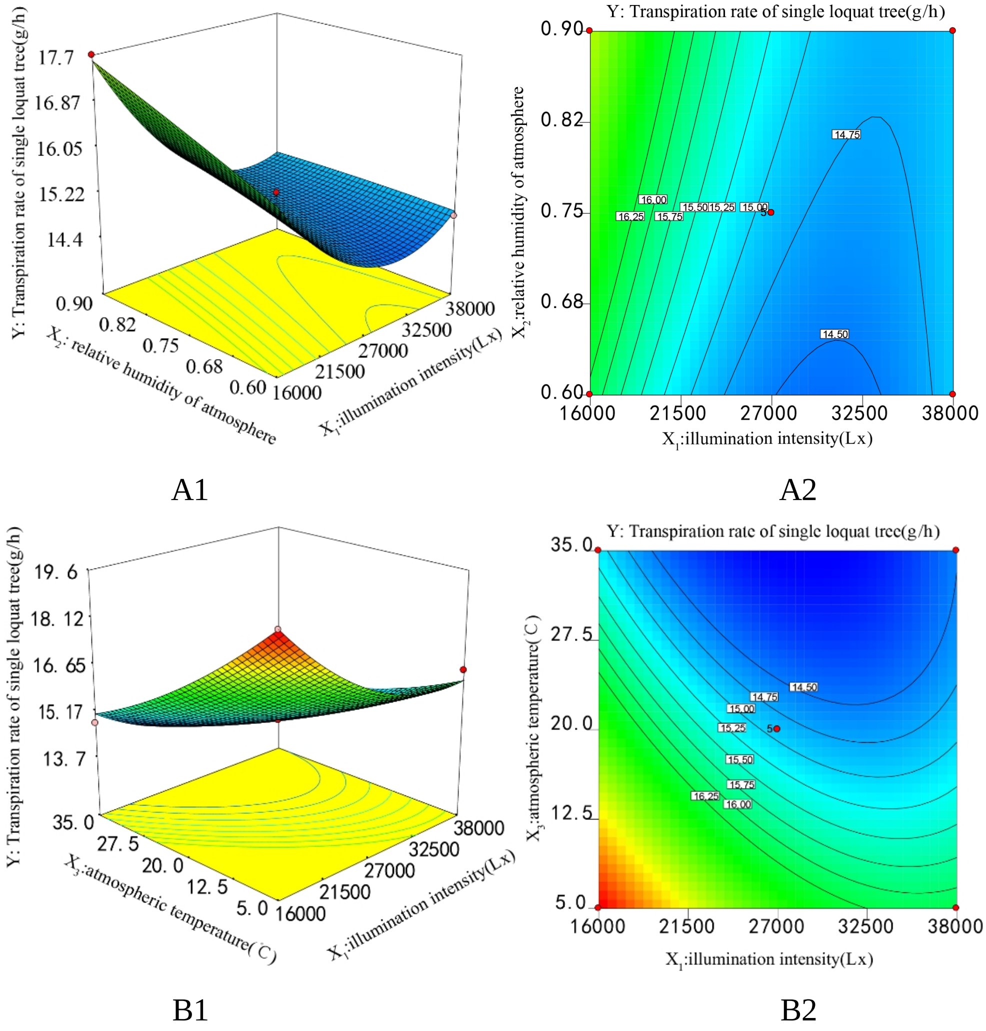
sources of variation	sum of squares	number of degree of freedom	mean of square	F value	P value
Model	35.49868	9	3.944297	65.34931	< 0.0001
X_1	7.80125	1	7.80125	129.2515	< 0.0001
X_2	1.05125	1	1.05125	17.41716	0.0042
X_3	18.605	1	18.605	308.2485	< 0.0001
$X_1 X_2$	0.49	1	0.49	8.118343	0.0247
$X_1 X_3$	1.8225	1	1.8225	30.19527	0.0009
$X_2 X_3$	0.4225	1	0.4225	7	0.0331
X_1^2	4.423684	1	4.423684	73.29181	< 0.0001
X_2^2	0.002632	1	0.002632	0.0436	0.8405
X_3^2	0.673684	1	0.673684	11.16163	0.0124
total residual	0.4225	7	0.060357		
lack of fit	0.4225	3	0.140833		
pure error	0	4	0		

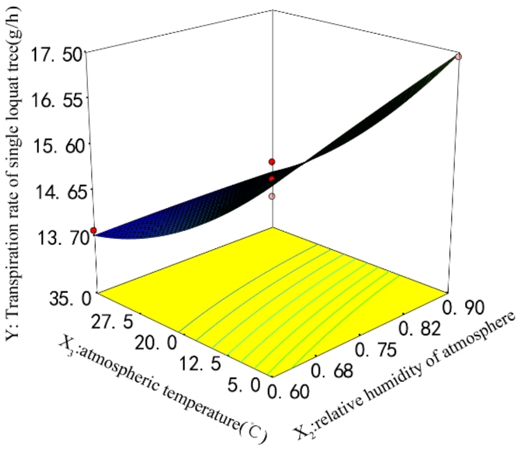
If the above regression equation was used to describe the relationship between the factors and the response value, the influence of independent variables on the dependent variable would be significant, for the significance level of model was less than 0.05, meanwhile, the quadratic regression variance model was very significant. From the results of variance analysis of regression equation, it can also be inferred that the equation could fit testing data well with a slight error, therefore, the regression equation could be employed to analyze and predict the testing results in place of real testing points. The effect turn of the factors for the transpiration rate of loquat trees is $X_3 > X_1 > X_2$ (seen as Table 3).

Response surface analysis

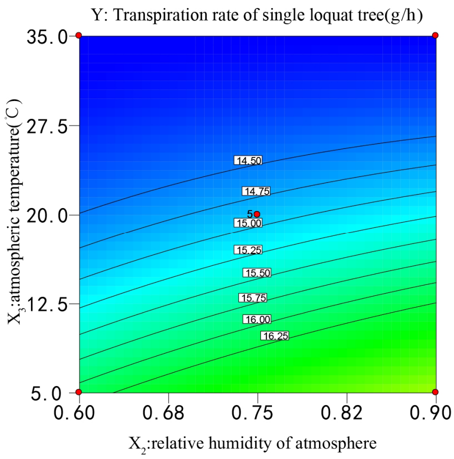
The graphics of the Response Surface Methodology (RSM) method is a three-dimensional space in the two-dimensional plane contour map, which is constituted by a specific response surface (Y) and the corresponding factors X_1, X_2, X_3, each response surface analyzes the two factors, and other two factors fixed at zero level. The effect of every factor on the response values can be directly reflected from the experimental results, and the interaction of these factors can be found in the process of loquat trees transpiration. The regression response surface is shown in Figure 1.

The graph in the RSM is a contour map constituted by a three-dimensional space on a two-dimensional plane. The three-dimensional space that was formed with a specific response surface (Y) and the corresponding factors X_1, X_2, X_3. Two factors were analyzed in each response surface with the other two factors fixed at zero level. The effect of every factor on the response values could be clearly reflected from the RSM graph, and the interaction of these factors could also be found in the process of loquat trees transpiration. The graphs of regression response surface are shown in Figure 1.





C1



C2

Figure.1 Contour map and RSM graph of interactions among illumination intensity, temperature, and relative humidity on transpiration rate at (A) temperature fixed at 20 °C, (B) relative humidity stable at 75 % and (C) illumination intensity immobilized at 27000 Lx, respectively.

The Figure 1 directly reflects the influence of the various factors on the response value. The factor $X_{3,}$ temperature has the most significant effect on plant transpiration, which shows a relatively steep curve. While the factor X_2, relative humidity shows a relatively smooth one, and when it changes, the response value is relatively small.

Discussion

The relationship between plant transpiration rate and relative humidity has not yet been decided whether by model simulation or by experimental study. In our work, the transpiration rate of loquat trees was increased with the addition of relative humidity. The response of plant stomata to relative humidity is the reaction to transpiration rate in nature. Therefore, the increase of relative humidity would not directly induce to the decrease of leaf stomatal conductance, while the stomata conductance directly determines the intensity of transpiration rate. Plants could experience the relative humidity with two possible means, through directly answering the relative humidity (equivalent to a hygrometer) and the transpiration rate. The experiments made by Mott and Parkhurst showed that the plant answered the transpiration rate rather than the relative humidity itself [6]. For loquat trees, the high humidity would not directly lead to the stomatal conductance decreased and stomata closed. It also concluded by CHEN Qin [7] that the stomatal conductance would decrease with the reduction of relative humidity through model simulation.

It was found that the transpiration rate of loquat trees decreased with the increase of temperature, which could be attributed to a self-protection reaction of loquat tree to the temperature changes. Usually, under the stress of low temperature, the water content of plants would decrease to make the solid matter concentrated to increase the concentration of cell sap, which could reduce the possibility of freezing, enhance the ability of cold resistance and anti-freezing, and thus improve the cold resistance [8]. Under the conditions of high temperature stress, the physiological activities of plants would be affected, including the cell membrane damage, inhibition of photosynthesis, and cell aging and death. QU Mingnan [9] has observed that high temperature stress reduced the leaf transpiration and stomatal conductance. When the high temperature put stress on the plant, the stomatal aperture began to decrease, accompany with the transpiration dropping, reducing the plant's transport of mineral ions and the demand for water [10].

According to our testing results and existing theoretical researches and test reports, it can be concluded that transpiration rate of loquat trees would increase with the decrease of temperature at given conditions, which should be attributed to loquat trees increase their transpiration rate because of the pressure of low temperature. One side, increasing transpiration rate could decrease the water content and increase the concentration of solid matters and cell sap; on the other side, it could promote photosynthesis for more sugar to improve the content of soluble sugar in cell, which was in favor of good ability of cold resistance and anti-freezing. If the temperature was high, the stomatal aperture of loquat trees would decrease, resulting in evapotranspiration decreasing and transpiration rate falling.

In our work, the relationship between plant transpiration rate and illumination was studied under manual control of illumination intensity in a phytotron, which was quite different from that under natural state. It was found that the transpiration rate of loquat tree decreased with the illumination intensity at first and then remained stable, which could be attributed to self-adaption of loquat tree to illumination. When illumination saturation state is achieved, the continuous increase of illumination intensity would induce the photoinhibition phenomenon, which means that when the illumination intensity received by the photosynthetic organization exceed the available amounts for photosynthesis, the function of photosynthesis would be weakened, and the continuous increase of illumination intensity would induce the damage of photosynthetic organization. In most cases, when the light intensity decreases, the plant photosynthetic function could be restored gradually. The stomata conductance of plant would be reduced by the strong illumination. In the study of photosynthetic and transpiration rate for different parts of an apple tree, ZHANG Xianchuan found that stomatal conductance in sunny days presented a "concave" type curve, and there was a clear "midday rest" phenomenon, which of the ambient leaves was prominent. Daily variation of stomata for different parts was similar. Within one day, the stomatal conductance for the center leaves was big and that for the ambient leaves was small. It might because of the strong illumination of the ambient leaves, which would induce photoinhibition [11]. The stomatal conductance of leaves with photoinhibition induced by supersaturated illumination intensity would grow smaller, dropping down the transpiration rate.

The research results showed that the transpiration rate of loquat trees decreased with the addition of illumination intensity. Under the test condition, the light saturation point of loquat trees was 16000 Lx. When the illumination intensity grew over 27000 Lx, the photoinhibition arose, and when the illumination intensity arrived at some point, the transpiration rate almost kept stable, which might because that the change of stomatal conductance induced by illumination intensity was not obvious. The illumination intensity of 16000 Lx in this experiment was corresponding to a moderate value. The illumination intensity of summer midday could reach 60000 - 100000 Lx. Under the illumination intensity of 16000 – 27000 Lx, the loquat trees could reach to the light saturation point, indicating high light efficiency, that's to say, the photosynthesis under lower illumination intensity was quite efficient. Moreover, a layer of tomentum was observed on the loquat leaf surface in the experiment and leaf margin slightly curled downwards, which might be the result of the long-term evolution of the environment adaptation, reducing light energy absorption to weaken the damage caused by excessive light.

The transpiration rate of plants was influenced by meteorological factors and biological factors. Under natural conditions, the plant transpiration rate is a comprehensive problem influenced by many ecological environment factors, thus, a lot of experiment research were needed to carry out to understand the relationship between influence factors and transpiration rate. An open environment is inadequate for strict control of the each factors, resulting in the experiment results easily affected by other factors, so it is difficult to clarify the impact of individual factor on plant transpiration rate. Therefore, it is necessary to carry out experiments in a phytotron. Due to the limit of time and experimental conditions, three main factors were chosen from many factors influencing the transpiration rate on the individual loquat, so it was difficult to estimate test error. In the next step, the author will increase the number of test plants, consider more environmental variables, and add the soil water stress and other factors to enhance the accuracy, reliability, and general applicability of the test.

Conclusions

Under the strict control of meteorological conditions in a phytotron, a response surface model was established to find that the most important factor affecting the transpiration rate of a loquat tree was temperature, followed by illumination intensity and relative humidity of atmosphere in turn. The transpiration rate was increased with addition of relative humidity, decreased with the accretion of temperature, decreased at first and then kept stable with the increase of illumination intensity. The P value of the established model is far less than 0.01, indicating that the model is actual available. The model can provide a reference for the region that lacks of parameter to predict the evapotranspiration of per plant only with illumination intensity, temperature, and relative humidity, and for the study of evapotranspiration in the hydrological cycle.

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