КРАТКИЕ СООБЩЕНИЯ И ОБМЕН ОПЫТОМ

УДК 630*161+581.45
DOI: 10.37482/0536-1036-2021-5-185-191

APPLICATION OF THE ADOBE PHOTOSHOP SOFTWARE PACKAGE IN LEAF BLADE AREA MEASUREMENT OF WOODY PLANTS

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Original article */ Received on March 3, 2020 / Accepted on May 14, 2020*

Abstract. There are a number of applied methods for determining morphometric parameters, including the area of leaf blades of woody plants: millimeter graph paper method, method of direct measurements, punching method, and planimetric method. The analysis and practical application of the methods in the test mode revealed their shortcomings related to inaccuracy of measurements, experiment duration, and equipment inaccessibility. Many researchers have successfully acquired digitized images. However, capturing the parameters of the considered structure from the monitor screen is often impossible due to the low availability of morphometric programs and their high cost. The purpose of the work is to evaluate the effectiveness of existing methods for determining the area of leaf blades on the example of the birch genus (*Betula* L.) trees and to accumulate the algorithm for determining morphometric parameters of birch leaf blades in the Adobe Photoshop software package. We have developed a method for determining morphometric parameters of leaf blades of woody plants using the Adobe Photoshop software package. The research subject is the leaf blades of birch trees. This method requires: blank A4 sheets; a ruler with an angle of 90°; transparent adhesive tape (scotch tape); and a scanner. We draw a reference square of random (but fixed) dimensions in a random corner of each sheet, step by 10 mm from its borders, on a blank A4 sheet using a ruler with an angle of 90°. The square is placed strictly parallel to the sheet borders. Then, leaf blades of the desired wood species are attached to the sheet with scotch tape. Using the scanner, we convert the workpiece into electronic form and upload the image to Adobe Photoshop. In order to receive morphometric parameters, you can use the following program tools: “ruler”, “magic wand”, “magnetic lasso tool”. At the same time, it is possible to automatically select the borders of the studied object, which helps to avoid subjective errors. The resulting measurements are displayed in pixels and then converted to the required units. The obtained data is verified using the reference square. Verification showed that the method accuracy is more than 99.9 %. The proposed method allows to perform fast and accurate measurements of morphometric parameters of leaf blades, without requiring the purchase of expensive equipment, which makes it available to any researcher who faces with the task of measuring the surface of leaf blades of woody plants.
For citation: Babaev R.N. Application of the Adobe Photoshop Software Package in Leaf Blade Area Measurement of Woody Plants. *Lesnoy Zhurnal* [Russian Forestry Journal], 2021, no. 5, pp. 185–191. DOI: 10.37482/0536-1036-2021-5-185-191

Keywords: Adobe Photoshop, silver birch, morphometric parameters, leaf blade area.

ПРИМЕНЕНИЕ ПРОГРАММНОГО ПАКЕТА ADOBE PHOTOSHOP ПРИ ИЗМЕРЕНИИ ПЛОЩАДИ ЛИСТОВЫХ ПЛАСТИН ДРЕВЕСНЫХ РАСТЕНИЙ

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Оригинальная статья */ Поступила в редакцию 03.03.20 / Принята к печати 14.05.20*

Аннотация. Существует ряд методик определения морфометрических показателей, в т. ч. площади листовых пластин древесных растений: метод нанесения контуров листьев на миллиметровую бумагу, метод промеров, метод высечек, планиметрический метод. Методы проанализированы и применены в тестовом режиме. Выявлены их недостатки, связанные с неточностью измерений, продолжительностью опыта, недоступностью оборудования. Проблема получения оцифрованного изображения успешно решается многими исследователями, но снятие параметров рассматриваемого объекта с экрана монитора часто невозможно по причине малой доступности морфометрических программ и их высокой стоимости. Цель работы – оценить эффективность существующих методов определения площади листовых пластин на примере березы (*Betula* L.) и аккумулировать алгоритм определения морфометрических показателей листовых пластин в пакете программ Adobe Photoshop. Нами разработана методика определения морфометрических показателей листовых пластин древесных пород с использованием Adobe Photoshop. Предмет исследования – листовые пластины деревьев рода береза. Для применения данной методики использованы чистые листы формата А4, линейка с углом 90°, прозрачная клейкая лента (скотч), сканер. В произвольном углу каждого листа на расстоянии по 10 мм от его границ и строго параллельно им при помощи линейки был вычерчен поверочный квадрат простых размеров. Скотчем на бумаге закрепляли листовые пластины. При помощи сканера переносили заготовку в электронный вид и загружали изображение в Adobe Photoshop. Для получения морфометрических параметров можно использовать следующие инструменты данной программы: «линейка», «волшебная палочка», «магнитное лассо». При этом есть возможность автоматического выделения границ исследуемого объекта, что позволяет избежать субъективных оценок. Результаты отображены в пикселях, а после их переводят в необходимые единицы измерения. При помощи эталонного квадрата производят проверку полученных данных. Точность составляет более 99,9 %. Предлагаемый метод позволяет быстро и без ошибок определять морфометрические параметры листовых пластин, при этом не нужно дорогостоящего оборудования, что делает метод доступным для любого исследователя, перед которым стоит задача измерения поверхности листовой пластины древесных растений.
Для цитирования: Babaev R.N. Application of the Adobe Photoshop Software Package in Leaf Blade Area Measurement of Woody Plants // Изв. вузов. Лесн. журн. 2021. № 5. С. 185–191. DOI: 10.37482/0536-1036-2021-5-185-191

Ключевые слова: Adobe Photoshop, береза повислая, морфометрические параметры, площадь листовых пластин.

Introduction

Due to a wide range of useful features and properties, representatives of the birch genus (*Betula* L.) are constantly within the field of view of foresters, botanists, and environmentalists [2–6, 9, 11, 13, 19, 20]. When carrying out scientific work associated with the determination of the biological potential of trees belonging to this genus, and when introducing them, a necessary condition for substantiating the prospects for their practical use is the study of morphometric parameters of leaves and the determination of the assimilation apparatus strength. The scientific value of these parameters is related to the determination of photosynthetic potential and net productivity of photosynthesis, which is the basis for productivity assessment [7, 8, 15]. The current methods of finding morphometric parameters of the leaf surface are rather cumbersome, not accurate and in some cases their disadvantage is due to the research duration. However, a large volume of tests requires speed and high accuracy of analysis.

The purpose of the work is to evaluate the effectiveness of existing methods for determining the area of leaf blades using the example of birch trees and to accumulate the algorithm for determining morphometric parameters of birch leaf blades in the Adobe Photoshop software package.

Research objects and methods

The research objects were existing methods for determining the area of leaf blades and the proposed method using the Adobe Photoshop software package. Currently, several methods for determining the surface area of leaf blades of woody plants are most commonly used. They are discussed below in descending order from the most used in practice to the least.

Millimeter graph paper method (hereinafter referred to as Method 1). During its implementation, a birch leaf blade was circled along its edges. Then, the area was counted based on the 1×1 mm cells inside the contour. This method for determining the leaf blade area is fairly objective and quite accurate; however, it is a very laborious and time-consuming process that is not suitable for measuring a large number of leaves.

Method of direct measurements (hereinafter – Method 2) [10, 18]. After determining the area of a number of leaf blades by Method 1, their length (L) and maximum width (W) were measured. Then, the circled contours of the leaf blades were described by a simple geometric shape – a rectangle. The average filling factor of the rectangle was 0.7 for the studied birch species. Leaf area (S) was calculated by the following equation:

$$S = LW \cdot 0.7.$$

This method has a high error due to the scatter of the filling coefficient of a simple geometric shape relative to each leaf blade, and the constant determination of the coefficient in the absence of the performed Method 1 makes Method 2 unreasonably laborious. In our case, the deviation with respect to Method 1 was up to 28 % providing its unsuitability for accurate studies.

Punching method (hereinafter – Method 3) [12, 18]. In order to carry out measurements by this method, we used undamaged leaves, which were previously measured by the first and the second methods; their wet mass (M_l) was determined. It

was important that the leaves did not dry out. Punches of a certain diameter were cut from each leaf with a cork drill, 5–10 pcs per one leaf. They were cut so that the sample included both the leaf laminae proper and its central veins. Then, we determined the mass (M_c) of all punches. The leaf area was determined by the following equation:

$$S = M_1 a \pi D^2 / M_p,$$

where S – leaf blade area, mm^2; M_1 – mass of leaves in a sample, g; a – number of punches, pcs; D – drill diameter, mm; M_p – mass of a punch, g.

The main and critical drawback of this method is that the leaf veins falling into the punches significantly increase the value of M_p, as a result the deviation from the results received by Method 1 was up to 31 %.

Planimetric method [8, 14]. According to its description, the harvested leaves are weighed and placed on the moving belt of an electronic device – planimeter. For example, PLANIX planimeters allow to quickly and accurately measure line lengths, calculate areas, coordinates, angles, arcs, and circle radii. Measurements can be taken in millimeters, centimeters, meters, kilometers, and hectares. This method is quite accurate, however it requires expensive equipment for implementation.

A method for determining the morphometric parameters of leaf blades of woody plants using the Adobe Photoshop software package (hereinafter – Adobe Photoshop Method). Adobe Photoshop is a multifunctional graphic editor developed and distributed by Adobe Systems. It mainly works with bitmap images; however, it has some vector tools as well [16, 17]. The proposed method is developed taking into account the shortcomings of existing methods for determining the area of a tree leaf blade and accumulates the possibility for determining other morphometric parameters. The research method was based on the principles of a single logical difference, suitability and expediency of experience [1].

In order to implement the method, we used the following equipment, software, and materials: Kyocera ECOSYS M3540DN scanner; Acculab Vicon VIC-300d3 electronic scales with accuracy up to 0.001 g; ruler with angle of 90°; since the scanner we have chosen for the study has a maximum paper size of A4, blank sheets of this size will be required; transparent adhesive tape (scotch tape); Adobe Photoshop (2020 trial version); freshly cut normally developed silver birch (*Betula pendula* Roth.) shoots with typical leafing out and undamaged foliage after achieving the size and shape peculiar to the species.

The course of the work is shown below.

The reference square of random, but strictly fixed sizes (test object) is drawn on a blank A4 sheet by means of a ruler with an angle of 90° in a random corner, step by 10 mm from its borders. The square is drawn strictly parallel to the borders of the sheet. We have chosen dimensions of 25×25 mm. Thus, we know for sure its height, width, and area – 625 mm^2.

Separate the leaf blades with a whole petiole from the shoot. Each leaf blade with a petiole is weighed on electronic scales Acculab Vicon VIC-300d3 and attached to the prepared sheet of paper with the square using transparent thin scotch tape. Next to each attached leaf blade record the measurement values of its mass. In our case, 7 leaf blades with a petiole can be freely placed on one sheet of A4 paper.

Using the Kyocera ECOSYS M3540DN laser multifunctional device (scanner), A4 sheets with attached samples are scanned. Scan quality is set to 200 dpi. Then, open the resulting image in Adobe Photoshop.

After opening the file, select the tab “Image Size”. Check the image settings in the window that appears. Its dimensions should coincide with the size of the A4 size – 297×210 mm. The next step is to convert the resolution of 200 ppi to pixels/cm for simplicity of measurement. The resulting value is 78.74 pixels/cm. To convert that into millimeters divide that by 10 and get 7.874 pixels/mm. Thus, there will be 7.874·7.874 = 62 pixels/mm^2. If the scan resolution is different from that suggested in the method, it is necessary to independently determine the number of pixels per mm and mm^2 in the specified way.

The next step can be done in two ways.

Variant 1. In order to define leaf blade parameters on the toolbar (on the left side of the screen), select the magnetic lasso tool. Clamp the left mouse button and encircle the leaf blade carefully along the contour. After closing the encircling, the program highlights the leaf blade as a separate contour.

Variant 2. Use the Quick Selection tool on the toolbar (on the left side of the screen) to define the leaf blade parameters. Point the tool to the leaf blade and click on it. The program will automatically highlight the desired contour.

Important. In this method, it is necessary to cancel the selection each time the individual leaf blade data is received; otherwise subsequent values are calculated on an ascending total.

After selecting the image using one of the ways, go to the menu: “image – analysis – record measurements”. The resulting measurements are displayed in pixels per inch at the bottom of the screen.

In order to convert values from pixels per inch to pixels per mm and mm^2, use the appropriate algorithm specified in the paragraph “After opening...”.

In order to measure the parameters of a petiole, use the ruler tool from the menu “image – analysis – ruler tool”. Clamp the left mouse button and measure the desired length/width. The resulting values are displayed in pixels at the top of the screen – the “L_1” parameter. Then, convert to mm in accordance with the paragraph “After opening...”.

In order to verify the test accuracy apply this method on the reference square, the metric parameters of which are already known. When checking the obtained dimensions on the reference square, the following data is obtained: length – 24.98 mm; width – 25.00 mm; and area – 624.5 mm^2. Therefore, the error is less than 0.1 %.

Research results and discussion

When determining the area of leaf blades by various methods, the obtained values differed from each other (see the table below).

The research has revealed that with respect to the existing methods for determining the area of leaf blades, Method 1 is the most accurate. However, this method is very labor-intensive, which is unprofitable in determining the area of a large number of objects.

The method for determining morphometric parameters using the Adobe Photoshop software package is quite reasonable, since it allows to determine many parameters of leaf blades (length, width, and area) at once, almost eliminating the factor of human error.

Moreover, the proposed method allows to archive the obtained primary data in the computer memory and process it when most convenient.

Results of determining the leaf blade areas by various methods

No.	Method 1	Method 2		Method 3		Adobe Photoshop Method	
		mm^2	deviation from Method 1, %	mm^2	deviation from Method 1, %	mm^2	deviation from Method 1, %
1	1262	1734	+27	970	–30	1269	+1
2	1332	1704	+22	1799	+26	1323	–1
3	1319	1730	+24	1459	+10	1332	+1
4	1223	1486	+18	974	–26	1219	0
5	1201	1496	+20	1609	+25	1192	–1
6	1264	1559	+19	980	–29	1254	–1
7	1106	1485	+26	1603	+31	1113	+1

Conclusion

The existing methods for determining the leaf blade area have many shortcomings including the purchase of expensive equipment. The Adobe Photoshop software package allows any researcher to quickly and accurately measure the morphometric parameters of leaf blades.

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