Testing four methods to assess leaf area in young olive trees

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Abstract

Assessment of leaf area is of fundamental importance to calculate diverse physiological parameters such as the total rate of transpiration or photosynthesis of a plant or its leaf area index (LAI).

This paper reports the determination of total leaf area in 3 year-old olive trees of two varieties using four different methods and leaf counting as the control method.

Leaf area was determined on 7 plants of each variety, Arbequina and Cobrançosa, by thoroughly counting the total number of leaves on each plant and then multiplying by the average leaf area for each variety

Leaf area of the same plants was then estimated by (1) the analysis, with software ImageJ, of a photo of each plant from a side or from the top, (2) the crown light scattering measured with a ceptometer (AccuPAR Model LP-80) in the ground at 10 and 20 cm from the trunk, (3) the analysis with software Hemisfer of a photo of the plant from the top, and finally (4) by the determination of the transpiration (mmol s^{-1}) of each plant over a 2 hour period by a weighing method and dividing this value by the mean transpiration rate (*E*) of each plant, calculated from the measurement of stomatal conductance (g_s) on 10 leaves with a porometer and mean air RH and leaf and air temperature over the same period.

Both the ceptometer and the Hemisfer software give a value of leaf area index (LAI). In order to obtain the leaf area, LAI was divided by the projected area of the crown obtained on photos from the top.

Average leaf area (n = 7 × 30) was 0,238 × 10^{-3} m^2 and 0,264 × 10^{-3} m^2 for Arbequina and Cobrançosa, respectively. Total leaf area of each plant varied from 3,38 m^2 and 4,85 m^2 for Arbequina and 0,86 m^2 and 3,99 m^2 for Cobrançosa. Ceptometer and analysis of photos from a side gave estimates of leaf area under 50% of the actual leaf area. Analysis of photos from the top gave on average 70% the actual values. The most accurate methods were analysis with software Hemisfer and by the ratio of transpiration by *E* which gave values 5% bellow or 20% above actual values, respectively.

Introduction

Assessment of leaf area (Jonckheere et al. 2004; Weiss et al. 2004) is of fundamental importance to calculate diverse physiological parameters such as the total transpiration or photosynthesis of a plant or its leaf area index (LAI).

This paper reports the determination of total leaf area in 3 year-old olive trees of two varieties, Arbequina and Cobrançosa, using five different methods, leaf counting (as the control method), photographs from a side or from the top, ceptometer, Hemisfer software and finally by measuring plant transpiration and leaf conductance.

Materials and methods

Leaf area was determined on 7 plants of each variety, Arbequina and Cobrançosa, by thoroughly counting the total number of leaves on each plant and then multiplying this figure by the average leaf area for each variety. Average leaf area was determined by the measurement, on photographs, of the exact leaf area of seven samples of 30 leaves each using ImageJ software. This procedure gave a value for each plant's total leaf area with an estimated error of less than 10%.

Leaf area of the same plants was then estimated by four different methods. On the first method, a ceptometer was used (AccuPAR Ceptometer Model LP-80, Decagon Devices, Inc., U.S.A.) to measure the crown light scattering in the ground at 10 and 20 cm from the trunk. The second method consisted simply on the analysis of plant photos from the top with software Hemisfer (ref). The third method to determine leaf area was done by the analysis, with software ImageJ (Rasband, 1997), of photos of each plant from two sides and from the top, against a white background. Finally, leaf area was estimated by the determination of the transpiration (mmol s^{-1}) of each plant over a 2 hour period (10 to 12 am) by a weighing method and dividing this value by the mean leaf transpiration rate (E, mmol m^{-2} s^{-1}) obtained from the measurements of mean leaf conductance (g_s) measured at 11 am on 10 leaves, 7 sun-exposed and 3 shadowed, with a porometer (AP4, Delta-T devices, U.K.), and the mean difference in the molar fraction of water vapor between the leaf and the air obtained from leaf temperature and air humidity and temperature at 11 am.

Both the ceptometer (AccuPAR Model LP-80) and the Hemisfer software give a value of leaf area index (LAI). In order to obtain the leaf area, it was necessary to get a value for the projection of the crown. This value was obtained analysing the photos from the top with ImageJ and looking at the total area (including blank spaces) of the crown (Fig. 1, C).

Results

Actual leaf area of each plant was initially estimated by counting the number of leaves on each plant and multiplying this value by the average leaf area for each variety. Average area of a leaf (n = 7 × 30) was 0,238 × 10^{-3} m^2 and 0,264 × 10^{-3} m^2 for Arbequina and Cobrançosa, respectively. Total leaf area of each plant varied from 3,38 m^2 and 4,85 m^2 for Arbequina and 0,86 m^2 and 3,99 m^2 for Cobrançosa.

After that, leaf area was indirectly assessed by four other methods.

Figure 1 shows an example of the determination of leaf area by these indirect methods.

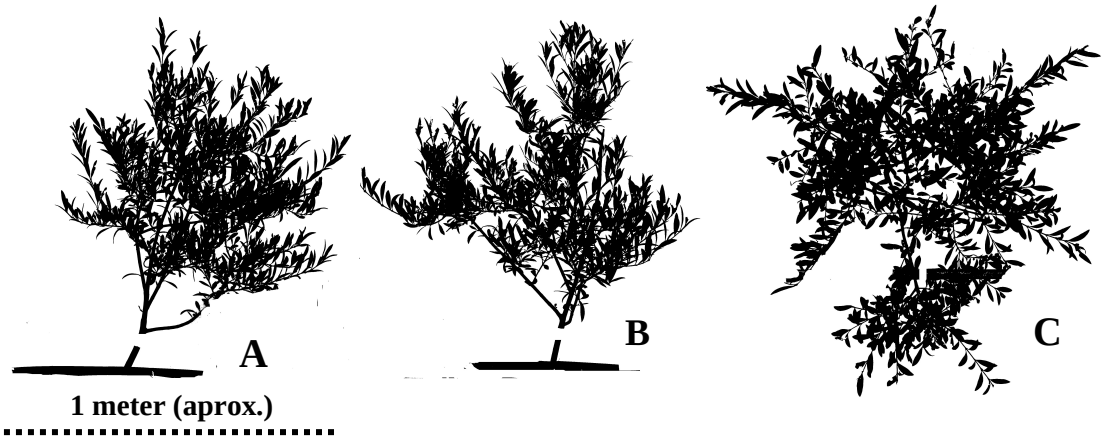

Figure 1. Example of the determination of the leaf area and leaf area index (LAI) of a young olive tree by four different methods. In this example, figure shows adjusted photos from a side (A and B) and from the top (C) of an Arbequina tree (Arb 3 on Table 1.). Leaf area determined by leaf counting on this tree was estimated to be 0,350 m^2. Leaf area determined with ImageJ on photo A was 0,138 m^2, 0,118 m^2 on photo B and 0,229 m^2 on photo C (from the top), i.e., 34%, 39% or 64% of the actual leaf area, respectively. The projected area of the crown was estimated with ImageJ on photo C to be 0,284 m^2. The mean values of LAI measured on this tree with the ceptometer and Hemisfer software were 0,30 and 1,54, respectively. For the measured projected area, this would give a leaf area of 0,085 m^2 with the ceptometer and 0,438 m^2 with Hemisfer, i.e., about 25% and 125% of the actual leaf area, respectively. Leaf area estimated by the ratio between transpiration (0,762 mmol s^{-1}, measured by the decrease in weight of the pot over a 2 hour period) and transpiration rate (2,40 mmol m^{-2} s^{-1}, calculated from the average stomatal conductance measured with a porometer, 155 mmol m^{-2} s^{-1} , and the difference in molar fraction of water vapor, 0,016) was 0,317 m^2, i.e., 9% below actual leaf area.

Table 1 shows the results from these direct and indirect methods to assess leaf area.

Table 1 shows that ceptometer and analysis of photos from a side underestimated leaf area by about 50%. Analysis of photos from the top was, on average, around 70% the actual values. The most accurate methods were analysis with software Hemisfer and the ratio of transpiration by *E* which gave values 5% bellow or 20% above actual values, respectively

Table 1. Leaf area (m^2) of the seven Arbequina (Arb) and seven Cobrançosa (Cob) young olive trees. Actual leaf area was calculated by leaf counting. Leaf area was also estimated with a ceptometer, Hemisfer software, image analysis of photographs from a side and from the top and finally by dividing the transpiration of each plant by its transpiration rate.

Plant	*Actual leaf area*	*Ceptom.*	*Hemisfer*	*Side photos*	*Top photo*	Transp/*E*
Arb 1	0,485	0,299	0,491	0,184	0,285	0,477
Arb 2	0,413	0,168	0,450	0,153	0,259	0,360
Arb 3	0,350	0,085	0,438	0,128	0,229	0,317
Arb 4	0,408	0,159	0,679	0,206	0,297	0,418
Arb 5	0,430	0,203	0,314	0,171	0,331	0,369
Arb 6	0,404	0,071	0,359	0,162	0,251	0,297
Arb 7	0,338	0,168	0,376	0,144	0,228	0,375
Cob 1	0,221	0,069	0,127	0,121	0,154	0,329
Cob 2	0,399	0,129	0,384	0,144	0,240	0,471
Cob 3	0,260	0,032	0,182	0,095	0,163	0,231
Cob 4	0,095	0,013	0,075	0,064	0,100	0,157
Cob 5	0,224	0,057	0,274	0,117	0,178	0,308
Cob 6	0,064	0,004	0,031	0,034	0,037	0,123
Cob 7	0,086	0,057	0,060	0,037	0,066	0,143

Nevertheless, analyses of photos from a side or from the top were reasonably consistent, i.e., the ratio of measured values *vs* actual values showed little variation. Therefore, using a proper correction factor, this method should also give a good estimate of leaf area.

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