Expl Agric. (1997), volume 33, pp. 65–72
Printed in Great Britain

A SIMPLE AND LOW-COST METHOD FOR LEAF AREA MEASUREMENT OF DETACHED LEAVES

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(*Accepted* 26 *March* 1996)

SUMMARY

A technique for leaf area measurement utilizing water spray as an inexpensive substitute for electronic equipment was developed and tested with leaves of potato (*Solanum tuberosum* L.). The leaf areas measured by the spray method were highly correlated with those measured by an electronic area meter. Measurements of leaf area obtained by the spray method were significantly more highly correlated with those obtained by the area meter than were the measurements of dry weights. The main advantages of the new method are precision, accuracy and immediate results at a low cost.

INTRODUCTION

Advanced equipment for accurate and fast leaf area measurement is often expensive. Cheaper methods are needed if leaf area measurements are to become a viable tool for researchers with restricted budgets. Before the introduction of modern electronic area meters and image analysis techniques, a variety of techniques were used. Sesták and colleagues (1971) provide an extensive description of the most common methodology available. These include counting squares on millimetre paper, hand-planimetry, the gravimetric method, dot counting, photoelectric planimetry, airflow, linear measurements of the leaves, leaf weighing, detached leaf counting, and the rating method.

Most of the methods available are accurate and precise but slow (for example, the dot counting method, Bleadsdale, 1984), rapid but inaccurate (for example, linear measurements, Tieszen, 1982), or require expensive specialized equipment such as the area meter described above. Composite leaves are especially problematic. Leaf weighing, though simple and inexpensive, requires knowledge of the specific leaf area (SLA), which is the area : mass relationship. The development of the water spray method is an attempt to combine acceptable speed, accuracy and reliability with equipment available and affordable to researchers with restricted budgets. In this paper, the spray method is described, and its reliability is tested with an electronic device and compared to the SLA method.

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MATERIALS AND METHODS

To use the spray method, a top-loading balance ranging from 0 to at least 10 g, and with a precision of ±0.01 g is required. It should be well protected against moisture. We used an Ohaus model E120 (Ohaus Scale Corporation, Florham Park, NJ, USA). A room plant spray bottle or other similar device is necessary to produce a fine, homogeneous spray. Additionally, a light but rigid sheet of any non-porous material, for example, cardboard covered with plastic, and a cloth to wipe the sheet dry between the measurements, is necessary.

The water-proof sheet of known total sheet area (*SA*) was set on top of the balance and tared. The leaves, the total area of which was to be measured, were distributed evenly over the sheet surface, avoiding overlap, and the weight, *a*, was recorded. The sheet and the leaves were sprayed wet so that the water deposition on the sheet was as even as possible. The weight, *b*, was recorded, the leaves were removed, and the weight, *c*, was recorded. Instead of picking the leaves off they could be dropped off by turning the sheet upside down. About 3 g of water were used on a plastic sheet of about 690 cm^2. The amount of water and droplet size were adjusted so that the water was held to the inverted sheet by adhesion.

The calculation of the area was based on the assumption that the proportion of water taken off with the leaves $(b - a - c)$ to the total amount of water $(b - a)$ is equal to the proportion of the leaf area (*LA*) to the total sheet area (*SA*). The leaf area (*LA*) was calculated as follows:

$$LA = \frac{(b - a - c)}{(b - a)} \times SA \qquad (1)$$

where *a* was the weight of the sheet + leaves, *b* was the weight of the sheet + leaves + water spray, and *c* was the weight of the sheet + spray after leaf removal. *SA* was the sheet area.

To test the method, a Licor LI-3000 area meter (Lambda, Lincoln, Nebraska, USA), consisting of a sensor and a readout unit, was used as a control. In one half of the sensor a row of light sources is located every 1 mm, and light detectors are located in the other half. The leaf is passed between the rows of light sources and detectors. With the help of a length encoding cord that is pulled out with the leaf, the readout unit records for every millimetre the number of light beams shaded by the leaf, and an accumulated value is shown in cm^2 (Tieszen, 1982). The principle is identical to that of the dot counting method (Bleadsdale, 1984).

Three field-grown plants of *Solanum tubersum* L. ssp. *andigena* varying in leaflet size were harvested. Leaf areas of 20 randomly selected leaves (petioles included) from each plant were measured by the electronic area meter and by the spray method, and their dry weights were recorded. The area meter reading was repeated three times per leaf using simple acetate envelopes. Dry weights were determined after 24 h at 80°C. Ten of the 20 leaves per plant were weighed individually and, to generate variation, another ten leaves per plant were weighed in pairs. This procedure gave a total of 45 observations from the three plants.

Prior to the experiment, the electronic area meter was calibrated with rectangular paper sheets of known area cut into pieces of different sizes. The spray method was also verified with pieces of plastic of known area, on which occasion spraying patterns were also examined. Evaporation was a possible source of error, especially if there was a difference between that occurring from the leaves and that occurring from the sheet surface. For this season, evaporation was compared from an empty sheet, from one covered with fresh leaves, and from one with withered leaves.

RESULTS

The area meter worked reliably when tested with paper pieces of known area (coefficient of variation = 0.66%), although the instrument was old and needed calibration ($A_{real} = -2.8\ \text{cm}^2 + 1.004 \cdot A_{measured}$). The repeated measurements of the leaves had a greater coefficient of variation of 3.3%, which was probably due to the rearrangement of overlapping leaflets, and the leaflets not being completely flat.

The leaf data consisted of two overlapping, presumably normally distributed populations: the leaves measured individually and those measured in pairs. A logarithmic transformation of both area meter and spray data produced a normal distribution overall, which was acceptable for regression analysis. The variation of the residual (deviation from the regression line) depended proportionally on the area, which supported the use of a logarithmic transformation of the dry weight (Snedecor and Cochran 1967). Although necessary for statistical validity, the transformations did not change the results fundamentally compared to those obtained with untransformed data.

The correlation between the spray method and area meter method was very high (Fig. 1, for transformed data, $r = 0.976$). The correlation between leaf dry weight and leaf area measured with the area meter was significantly lower (Fig. 2, $r = 0.948$, $p < 0.05$). About half of the sheet area could be covered with leaves easily, and this roughly defined the maximum capacity of one spray measurement. The precision of the spray method, measured as a residual standard deviation from the regression line, was better than 12 cm^2, corresponding to 1.7% of the plastic sheet area, or about 3.5% of the sheet capacity. The specific leaf area was 155 $\text{cm}^2\ \text{g}^{-1}$ dry matter. The variation in dry weight from the regression line was linearly dependent on the area, about 17%. Thus, the spray method performed better than the dry weight method when more than 10% of the sheet area was covered with leaves.

The intercept of the regression between untransformed spray area and true area was about 1.5% of the sheet area, not all of which was due to residual variation. This means that the direct result of the Equation (1) would slightly overestimate leaf area (Fig. 1). In the verification of the technique with plastic pieces of known size, the spray pattern was made more uniform (Fig. 3) by moving the sprayer toward and away from the person, as well as from left to right, passing

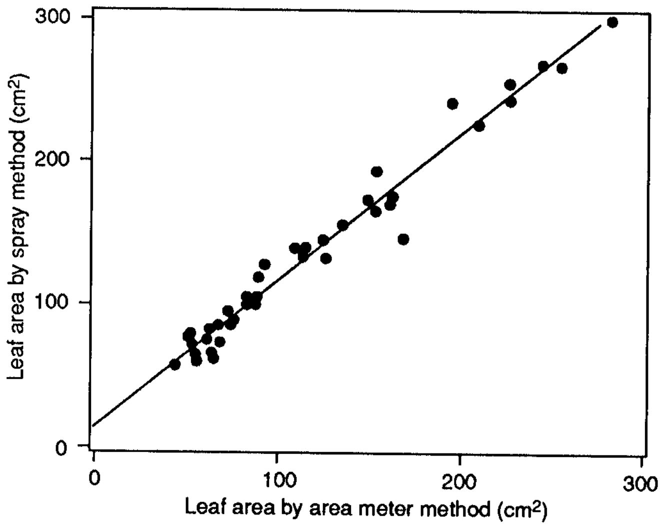

Fig. 1. Leaf area measured by the spray method (y) compared to leaf area measured with the area meter (x). $y = 1.02x + 13.9$, $R^2 = 0.96$.

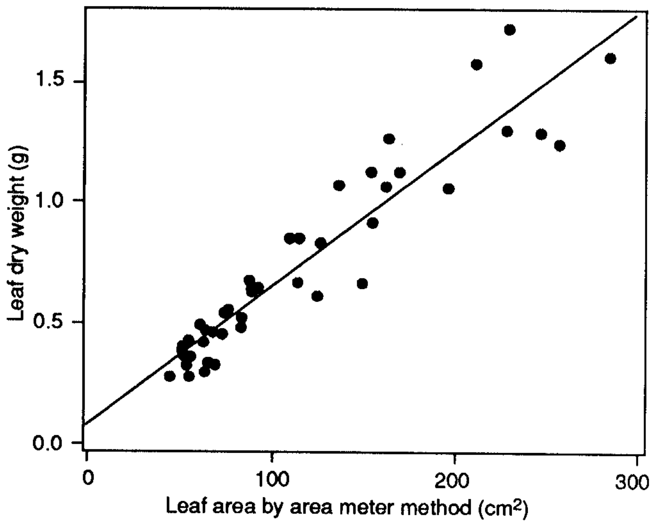

Fig. 2. Dry weight of the leaves (y) compared to the leaf area (x) measured with the area meter. $y = 0.00579x + 0.0750$, $R^2 = 0.88$.

well beyond the edges of the sheet. With a more even water deposition, the intercept disappeared, but the regression coefficient was slightly more than 1 (Fig. 4).

Evaporation occurred at a similar rate from fresh leaves, withered leaves and the sheet surface. Thus, the time between readings b and c was much more important than the time between a and b. The evaporation after spraying 3 g

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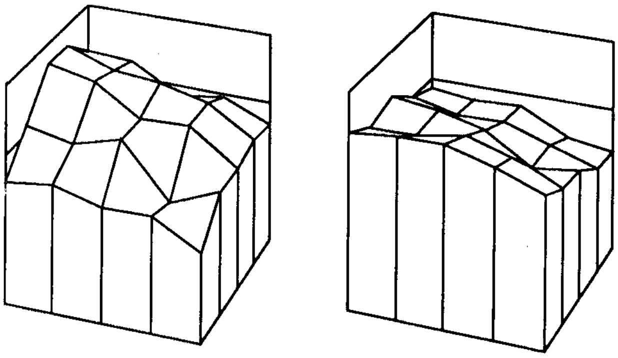

Fig. 3. Examples of water spray patterns, left: without moving the spray; right: trying to move the spray in both directions. The heights of the intersections represent the amounts of water received by rectangular areas in a grid pattern.

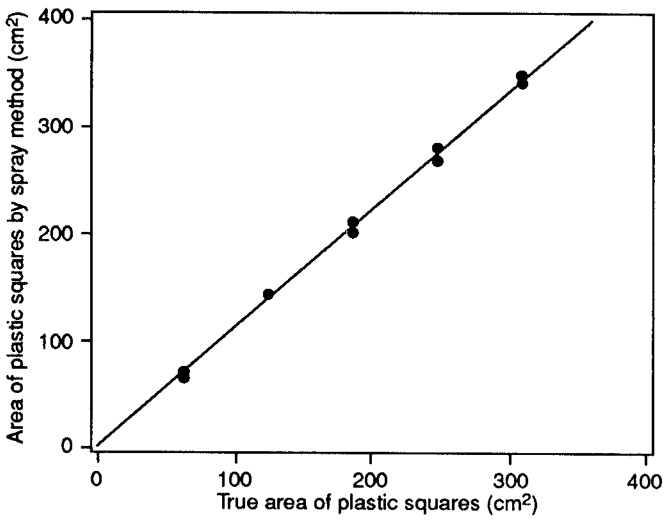

Fig. 4. The area of plastic squares of known area, cut into pieces of about 12 cm^2, measured by the spray method (y) compared with their true areas (x). $y = 1.09x + 1.83$, $R^2 = 0.998$.

water on an area 690 cm^2 was 1.14 mg s^{-1}. Therefore, the extra area measured, caused by evaporation, would have been about 0.21 cm^2 s^{-1}, which is only about 0.035% of the sheet area per second between the readings. If, under the same conditions, half of the plastic sheet was covered with leaves, a delay of about 10 s would cause an overestimation of the leaf area by less than 1%.

DISCUSSION

Although accuracy and precision cannot be separated completely, the absence of systematic bias is taken here as accuracy, and the reproducibility, or low variation

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among individual measurements, as precision. A similar classification of error has been used by O'Brien & van Bruggen (1992).

Some factors affecting the accuracy of the spray method are: (1) lack of randomness of the pattern of the leaves on the plastic sheet; (2) lack of evenness of the water deposition pattern; (3) evaporation of water occurring between recordings of weights; (4) dropping of water from the leaves onto the sheet, or loss of water from the sheet caused by sweeping the leaves while picking them up, or loss of water when inverting the sheet; and (5) leaflet overlapping and other leaf preparation factors, including the problem that leaves are not always flat.

If water was evenly distributed, the pattern of leaves on the sheet would have no effect on the accuracy. It seems to be difficult to produce a fully homogeneous water deposit, and therefore avoiding excessive systematic (centre-oriented) leaf patterns is important. The trial with plastic pieces showed that with some effort, both water distribution and leaf pattern can be maintained adequately, and the accuracy is satisfactory.

Evaporation of water between weight readings could be another potential source of error, but under tropical highland conditions of low ambient temperature (less than 20°C) and high relative humidity it was minimal, perhaps contributing an over-estimation of 1% of the area. Since the delay between readings depends on leaf quantity, the effect would be primarily on the regression coefficient, not the intercept.

Water dropping from the leaf surface to the sheet was not a problem when using about 3 g of water on a sheet of 690 cm^2. That would have led to an underestimation of leaf area, which did not occur in our experiments. The slight deviation from 1 of the regression coefficient between the leaf area measured by spray and that measured by area meter (Fig. 1 and 4) is probably explained by the leaves sweeping the sheet and carrying off some water while being picked up. Usually this can be ignored. Dropping the leaves by inverting the sheet helps, while also minimizing the delay between readings, and thus reducing the overall time needed.

Some factors having effect on the precision of the spray method are: balance precision, reading precision, leaf preparation and overlapping of the leaves.

The precision of the balance and the care taken by the person reading it are important. Let us simulate these effects using Equation (1), 3 g of water and a sheet of 690 cm^2. In the case of a leaf of 160 cm^2, an error of 0.1 g in one of the readings a, b, c, or the tare, would cause an error in the result of 18, 17, 23, or 23 cm^2, respectively. The effect of an error in reading c, or in the tare, remains constant when the leaf size changes, whereas for readings a and b, larger leaf areas are less affected. If the reading precision was 0.1 g, the theoretical overall precision of our example would be 20%, but when the reading precision is improved to 0.01 g and the sheet is filled nearly to its maximum capacity to hold leaves (300 cm^2), the precision becomes 1.0%.

The errors originating from leaves not being perfectly flat, overlapping leaflets and similar factors are common to both the spray and the area meter methods.

This is included in the residual variation in our tests. Other possible factors affecting the precision are lack of homogeneity of the spray (droplet size), or other variations in the procedure. With reasonable care, the precision is satisfactory.

For the best possible accuracy and precision our recommendations are: (1) use a balance as precise as ±0.01 g or better; (2) cover the sheets as fully as possible; (3) protect the working area from direct sunshine and wind; (4) minimize the time between the measurements before and after removing the leaves from the sheet, which is done the fastest by turning the sheet upside down and (5) try the method in the beginning with samples of known area.

If a top-loading balance is available, the spray method is cheaper than most other methods. Plastic spray bottles are available at a very low price. Compared to the electronic equipment, the capital cost of the spray method is much lower, precision is somewhat lower, and the measuring durations are similar.

In our experiments, the spray method was more precise than using leaf dry weight for leaf area measurement. Furthermore, specific leaf area (SLA) is not constant but varies greatly in time and among sites, even within one genotype (Lactin & Holliday 1995). Therefore, it is common practice to calibrate the method for each time and place, that is, to assess the SLA from a sub-sample by some other means, often with an electronic device. The error in assessing SLA depends on the sample size, and adds to the variation of dry weight as compared above. The spray method may not be an appropriate substitute for SLA when measuring large quantities of leaves, but it can substitute for the electronic device needed for its calibration, if no device is available. The spray method can be applied without calibration, and the results are immediate. It can also be used in combination with SLA using fresh weights instead of dry, and so an oven is not required.

The spray method was used successfully to calibrate another, non-destructive leaf area method (Korva and Forbes 1995). A method was required for potato leaves, which varied in the degree of fine structure. The results presented in this paper should apply to any leaves which are nearly flat, independent of their shape.

Acknowledgments. We want to thank all the CIP colleagues who assisted and gave their valuable suggestions during the experiment. We appreciate the useful comments on the manuscript by Dr. Anton Haverkort.

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