Aust. J. Plant Physiol., 2000, **27**, 1187–1190

Research Note:

A two-pinhole technique to determine distribution profiles of relative elemental growth rates in the growth zone of grass leaves

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Abstract. A new modified pricking technique, a two-pinhole method, was designed to determine the spatial distribution of leaf elongation of grasses. This new technique makes it possible to obtain the distribution profiles of relative elemental growth rates in the growth zone, to evaluate the effect of pricking on the distribution profile of leaf elongation in the growth zone and to decrease the reduction in the elongation rate of grass leaves due to pricking.

Keywords: leaf elongation rate, relative elemental growth rate, two-pinhole technique.

Introduction

Leaf elongation in grasses occurs in a region at the base of the blade enclosed by sheaths of older leaves, and relative elemental growth rate (R_E) of grass leaves in the growth zone shows the distribution profiles of leaf elongation within the growth zone in one dimension (Kemp 1980). Furthermore, the calculation of the local net deposition rate of substances in growing leaves of grasses, regarded as a useful means of describing physiological and morphological aspects of the growth process (Silk 1984), requires the distribution profile of R_E.

The distribution profile of R_E can be obtained by two methods: an anatomical method or by marking methods. The anatomical method, which determines the distribution profile of R_E by measuring the length of cells, is based on the assumption that the cell flux is conservative throughout the growth zone. Strictly, this is never the case because a cell typically takes 2–3 d to cross the growth zone, which limits the use of the anatomical method. The anatomical method is laborious and time-consuming, and R_E profiles derived from cell-length data are noisy and affected by cell division (Silk *et al.* 1989; Schnyder *et al.* 1990). Marking methods include making pinholes in a growing leaf with a fine needle and making a mark with ink (Davidson and Milthorpe 1966; Schnyder *et al.* 1987). Because it is easy and practical (Schnyder *et al.* 1987), making pinholes has been mostly used to determine the distribution profile of R_E of grass leaves. However, making the pinholes causes a reduction in the leaf elongation rate (L_R) of grasses by 30–50% (Bernstein *et al.* 1993; Ben-Haj-Salah and Tardieu 1995; Hu 1996; Palmer and Davies 1996; Fricke *et al.* 1997). Schnyder *et al.* (1987) reported a similar reduction in L_R by marking with ink. The large amount of artefact may cause a change in the distribution profile of R_E in the growth zone. For a study of stressed plants, the greater reduction in L_R by pricking than by stresses (e.g. drought, salinity or temperature) may result in difficulties in drawing conclusions on the effects of stresses on the spatial distribution of leaf elongation. Therefore, there is clearly a need for a new technique, which cannot only be used to determine the spatial distribution of R_E and to decrease the reduction in L_R, but also to evaluate the effects of the technique on the R_E profile.

With the classical pricking technique, at least 10–15 pinholes are required in the growth zone of about 30 mm in length of grass leaves. To decrease the effect of pricking on L_R, the number of pinholes needs to be reduced. In this study, we designed a two-pinhole technique, i.e. one pinhole is made within the elongation zone and other is made outside the elongation zone for a given leaf, to obtain profiles of R_E in the growth zone of wheat leaves. Since the two-pinhole technique minimizes the number of pinholes, the reduction in L_R can be decreased. Only one pinhole was made in the growth zone. Therefore, the two-pinhole technique makes it possible to test if the distribution profiles of R_E is affected by pricking because the distribution profile of the reduction in L_R can be obtained.

Theory of the two-pinhole technique

R_E can be determined either by directly measuring the relative elongation rates of segments in the growing zone, or by numerical differentiation of the function fitted to displace-

Abbreviations used: *A*, *C*, distance between the two pinholes at pricking and at harvesting; *D*, an increase in the leaf length between pricking and harvesting; D_V, displacement velocity; L_R, leaf elongation rate; PPFD, photosynthetic photon flux density; R_E, relative elemental growth rate; *X*, *B*, distance from the leaf base at pricking and at harvesting.

10.1071/PP00062 0310-7841/00/121187

ment velocity (D_V) data (Erickson 1976; Silk 1984). For roots, Erickson and Sax (1956) estimated R_E by a derivative of the function fitted to D_V data measured by making two marks with ink. In this study, the two-pinhole technique firstly determines the profiles of D_V in the growing zone of grass leaves, and then R_E is obtained from the derivatives of a function of D_V.

A total of *n* plants, used as a set of measurements of the first pinholes (i.e. the pinhole in the growth zone) at different locations of the leaf growth zone, are required. The interval of the first pinhole between two successive plants should be chosen according to the required resolution and the length of the growth zone. The value of *n* depends on the interval of the first pinhole between two successive plants and the length of the elongation zone. Figure 1 depicts of a growing grass leaf both at the time of pricking and harvest. A_i and C_i ($i = 1, 2, ..., n$) are the distances between the two pinholes on a growing leaf of plant *i* at pricking (t_0) and at harvest (t_1), respectively. X_i and B_i are the distances from the leaf base of plant i at t_0 and at t_1, respectively. D_i is the increase in the leaf length of plant *i* during the period of ($t_1 - t_0$).

The displacement velocity of a growing leaf of plant *i* (D_{Vi}) at X_i ($i = 1, 2, ..., n$) was calculated as follows:

$$D_{Vi} = \frac{D_i\,(C_i - A_i)}{t_1 - t_0}\,. \qquad (1)$$

Since it is difficult to find the exact position of the interface between shoot and roots at the time of pricking, the distance (X_i) between the first pinhole and the leaf base was estimated after harvest according to the following equation:

$$X_i = (B_i + C_i) - (D_i + A_i). \qquad (2)$$

After all values of D_{Vi} ($i = 1, 2, ..., n$) are determined, a function of $D_V(X)$ with the distance (X) can be obtained. We then see that:

$$R_E(X) = D_V{}'(X). \qquad (3)$$

Materials and methods

Growth conditions were as described in a previous study (Hu and Schmidhalter 1998). Briefly, six seeds of spring wheat (*Triticum aestivum* L. cv. Lona) per pot were sown in 1.5-L pots containing silty loam. The soil was initially watered to 0.25 g H_2O g^{-1} dry soil with nutrient solution. Soil moisture content was maintained daily at the initial level by replacing water loss. The experiment was conducted in growth chambers with a 16-h light period d^{-1}. The photosynthetic photon flux density (PPFD) was approximately 550 µmol photon m^{-1} s^{-1}, air temperature was 20°C (day/night), and the relative humidity was maintained at 55–65%.

Leaf 4 of wheat plants was chosen in this study because the previous study showed that the growth of leaf 4 is not dependent on the nutrient status of the seed (Hu and Schmidhalter 1998). During the steady phase of leaf growth, i.e. on d 3 after emergence of leaf 4 on the main stem of wheat plants (Hu *et al.* 2000), two pinholes were made by inserting a small needle (diameter = 0.2 mm) through the enclosed leaf sheath 3 h after the photoperiod was initiated. According to the theory discussed above, we varied the location of the first pinhole in the growing leaves from 0 to 35 mm above the leaf base for different plants, while the second pinhole was at approximately 40 mm above the leaf base for all plants. The interval of the first pinhole between two successive leaves was approximately 5 mm, i.e. 8 plants were used as a set of measurements. There were about 50 sets of measurements in this study. A_i (i = 1, 2, ..., 8) and t_0 for plant i were recorded (Fig. 1). Six h after pricking, the growth zone of leaf 4 was carefully freed from surrounding leaf sheaths and then cut with a razor blade from the plant at the leaf base. B_i, C_i and t_1 were recorded. D_{Vi} and X_i were calculated according to equations (1) and (2), respectively. The length of leaf 4 was also measured with a ruler at the time when the pinholes were made and when the plants were harvested. The leaf elongation rate (L_R) of non-pricked plants was measured by using a ruler to record the change in leaf length of non-pricked plants over a 6-h period. The percentage of the reduction in L_R within the growth zone was calculated from the ratio of the pricked leaf L_R to the average L_R of 50 non-pricked leaves.

Results and discussion

Results of the spatial distribution of D_V are presented in Fig. 2. A function of $D_V(X)$ was obtained by fifth-order polynomial fitted to the D_V data. The R_E profile was obtained according to equation 3 (Fig. 3). The reduction in L_R by the two-pinhole technique was 11%, which was much smaller

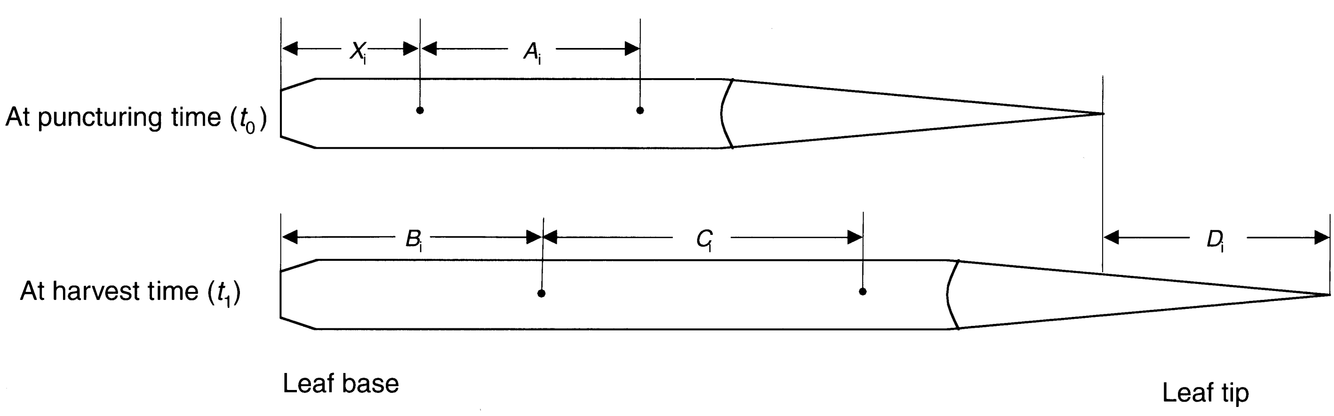

Fig. 1. Diagram of a growing leaf of grasses at pricking time (t_0) and at harvest time (t_1). A_i and C_i are the distances between the two pinholes on leaf at t_0 and at t_1, respectively. X_i and B_i are the distances from the leaf base at t_0 and at t_1, respectively. D_i is an increase in the leaf length during the period of ($t_1 - t_0$).

than the 40–50% reduction by the classical pricking method. The length of the growth zone can be estimated from the distribution profiles of R_E. The length of the elongation zone was defined as the distance from the leaf base to the end of the growth zone (where R_E was less than 0.002 mm mm^{-1} h^{-1}), which was about 3.2 cm.

Results of the distribution profile of the reduction in L_R by two pinholes demonstrates that the reduction in L_R remained almost unchanged with distance (Fig. 4), suggesting that the distribution profiles of R_E were not affected by the two-pinhole technique in this study. However, a change in the reduction in L_R with distance may indicate that pricking affects the distribution profiles of R_E.

Clearly, the two-pinhole technique solves the problems with a classical pricking technique, which causes a greater reduction in L_R and is unable to test how the distribution profile of R_E is affected by pricking. Furthermore, the two-pinhole technique also takes advantages of the easy use of the classical pinhole technique. There are some limits to using the two-pinhole technique. For example, there is no possibility for having the distribution profiles of R_E within the growth zone for one particular leaf, because there is a need to use different leaves to assess the R_E distribution by the two-pinhole technique. When the measured plants are homogenous, the number of plant sets can be reduced. Based on statistics, the number of samples can be estimated (Warrick and Nielsen 1980). For example, the number of sets of plants could be reduced to five, compared to the 50 sets chosen in this study, if one wishes to be within 10% of the correct value at a 0.05 confidence level. In the other cases, the two-pinhole technique may require more replications and a large number of plants and may become time-consuming. By comparing with the R_E profiles obtained by the two-pinhole technique, however, one can be sure whether the classical pinhole technique perturbs the normal growth rate pattern or not.

In order to have the instantaneous R_E (Erickson and Silk 1980), the elongated length of leaves between pricking and sampling should not exceed 20% of the length of the growth zone. Since a longer time was chosen and the elongated length of leaves between pricking and sampling exceeded 20% of the length of the growth zone in this study, R_E was not instantaneous.

In summary, this study demonstrates that the reduction in L_R by the two-pinhole technique is much smaller than that by the former pricking method, and the effect of pricking on the distribution profile of R_E can be evaluated by this two-pinhole technique.

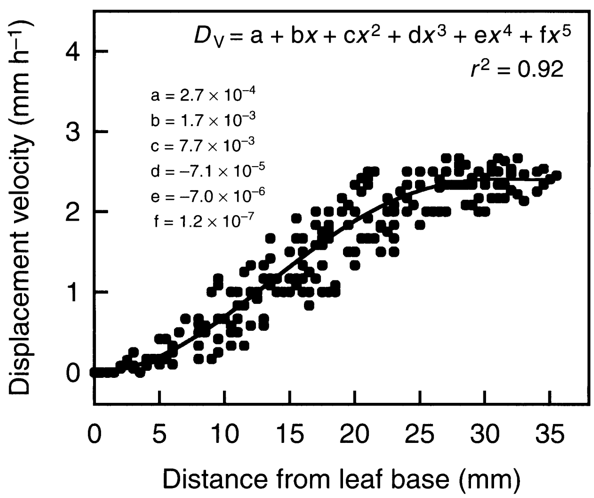

Fig. 2. Spatial distribution of displacement velocity (D_V) of wheat leaf 4 on d 3 after its emergence determined by the two-pinhole method. Fifth-order polynomial was fitted to the D_V data.

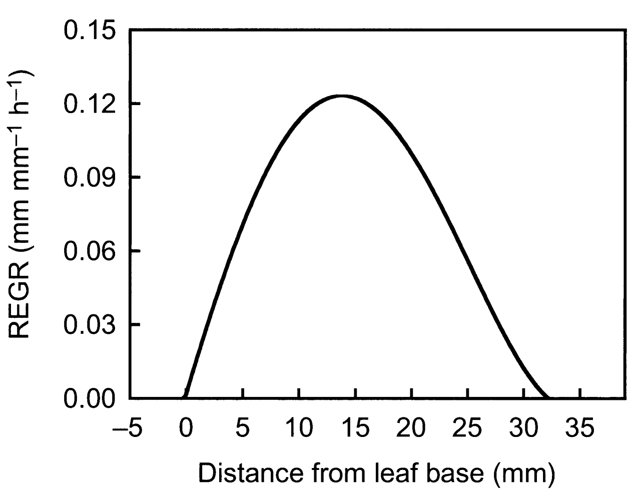

Fig. 3. Spatial distribution of relative elemental growth rate (R_E) of leaf 4 of wheat on d 3 after its emergence.

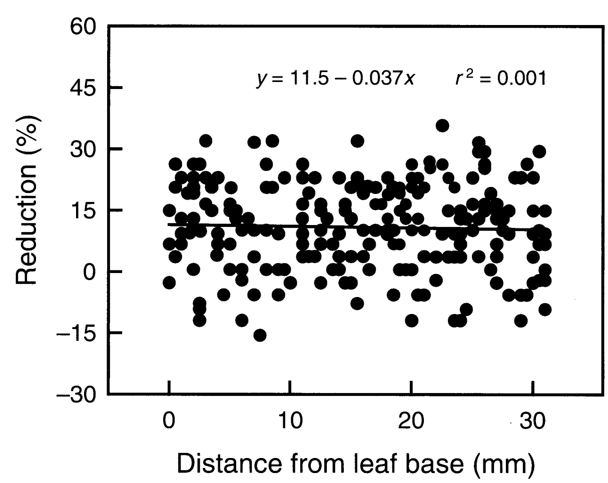

Fig. 4. Spatial distribution of the reduction in the elongation rate of leaf 4 of wheat on d 3 after its emergence by using the two-pinhole technique.

Acknowledgments

We sincerely thank the editor and two reviewers for their critical comments and constructive suggestions on the manuscript.

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Manuscript received 14 April 2000, accepted 14 September 2000