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Assessment of Spatial Distribution of Growth in the Elongation Zone of Grass Leaf Blades[1]

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ABSTRACT

Knowledge about the spatial distribution of growth is essential for understanding the leaf growth process. In grasses the elongation zone is located at the base of the leaf blade and is enclosed by sheaths of older leaves. Assessment of spatial growth distribution, therefore, necessitates use of a destructive method. We used a fine needle to make holes through bases of tillers at the location of the leaf elongation zone of tall fescue (*Festuca arundinacea* Schreb.), then measured the displacement of the holes after a 6 or 24 h interval. Needle holes caused a 22 to 41% decrease in daily leaf elongation so experiments were conducted to investigate if the spatial distribution of growth in the elongation zone was altered. Leaf elongation rate was reduced similarly when needle holes were made within or above the zone where cell elongation occurs. Distribution of elongation within the zone was the same when estimated by displacement of needle holes or ink marks placed on the epidermis of the elongation zone after surrounding tissue had been removed. Making holes at different locations within the elongation zone did not differentially affect the relative contribution of the damaged or undamaged parts to leaf elongation. These findings demonstrate that needle holes or ink marks in paired leaves can be used to estimate the relative distribution of growth in the elongation zone of undamaged tall fescue leaf blades.

Yield of tall fescue (*Festuca arundinacea* Schreb.) in vegetative growth stages is closely related to LER[3] (5), leading us to focus on the leaf growth process (7, 11, 12) and associated carbohydrate metabolism (13, 14). Information on the spatial distribution of growth will be helpful for investigating anatomical and physiological changes associated with the elongation process.

Leaf elongation in grasses occurs in a region at the base of the blade that is enclosed by sheaths of older leaves (4). As it is concealed, distribution of growth in the elongation zone can be assessed only by use of a destructive technique. When cell division does not occur in the elongation zone, data on the spatial distribution of cell lengths together with data on leaf elongation rates can be used to calculate local elongation rates, as shown in experiments with broad bean roots (8). In tall fescue, however, mitotic figures of epidermal and mesophyll cells are maximal at 3 to 4 mm above the point of leaf blade attachment and extend up to 10 mm in the elongation zone (7).

Tissue surrounding the elongating blade of grasses can be removed, ink marks placed on the elongating tissue, and displacement of marks followed over time (11). Measurements of epidermal cell lengths in control leaves confirmed that the length of the elongation zone of tall fescue leaf blades was accurately determined by ink mark displacement (11). Removal of tissue around the growing leaf can be avoided when marks are made in the elongation zone of leaves by horizontally piercing the base of the tiller with a fine needle (2). We have evaluated this technique by piercing holes through basal parts of vegetative tillers containing elongating leaves. Holes were made at 3-mm distances and displacement of holes relative to their original position was measured after a short growth interval.

In our experiments and those of others, LER was decreased after making holes (2) or placing ink marks (11). Therefore, experiments were performed to determine if displacement of holes in elongation zones can be used to estimate the distribution of growth within elongating regions of undamaged leaf blades. The effect on LER due to holes in the elongating zone was compared with the effect due to holes in the nonelongating part of the growing blade. In a second experiment displacements of holes and ink marks were compared for describing the spatial distribution of elongation within the zone. Finally, we examined if location of holes affected the spatial distribution of growth within the elongation zone. Our data show that holes reduce LER and absolute rates of segmental elongation, but all segments are affected similarly causing the length of the elongation zone and the spatial distribution of elongation to be unaltered.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Plants of a tall fescue (*Festuca arundinacea* Schreb.) genotype selected for high LER were propagated vegetatively and grown as described previously (13). After establishment, plants were transferred to a growth chamber and grown in continuous light (300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD) at constant air temperature (20°C) and relative humidity (70%). Thereby, a constant 21°C was maintained at the base of tillers, and elongation of selected leaves occurred at a constant rate for at least the duration of the measurements (up to 2 d). Plants were watered twice daily, keeping the soil near field capacity.

In another experiment plants were grown in a 15/9 h light/dark cycle with 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD during the photoperiod. A constant temperature of 21°C was maintained at the base of tillers by adjusting air temperature to 19/23°C (light/dark). We have found tiller base temperature is similar to soil surface temperature due to the close proximity. Soil temperatures are warmer than air temperatures during the photoperiod and cooler during the dark period. The relationship was not altered when plants were watered daily to keep the soil near field capacity.

Leaf Elongation Measurements. LER was measured continu-

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[3] Abbreviations: LER, leaf elongation rate; RSE, relative segmental elongation; SER, segmental elongation rate; PPFD, photosynthetic photon flux density.

ously as described previously (12). Briefly, the tip of the elongating blade on a vegetative tiller was taped to nylon fishline, which was wrapped one and one-half times around the 1.5-cm plexiglass wheel of a rotating potentiometer (Helipot model 5101). Potentiometers were fastened to ringstands above the plants. Weight (<10 g) was attached to the free end of the line to overcome friction of the potentiometer and prevent slackness of the line.

Making Holes. Needles were constructed from a 0.2 mm diameter plain steel guitar string. A sharp point was made with a fine file. The whorl of leaf sheaths was pierced directly above the small basal thickening of the tiller which is associated with the location of the terminal meristem of the vegetative tiller. This caused the lowermost hole to be placed close to the origin of the elongating blade, which is near the ligule. Spacing of holes was estimated by holding a ruler parallel to the tiller. Unless indicated otherwise, 10 holes were made with 3-mm distances between holes. This 27-mm interval included the entire elongation zone.

Approximately 6 h (24 h in Fig. 3B) after making holes, the tillers were removed from the plant. Distances between holes were measured with a calibrated ocular, first in the outermost nongrowing sheath and then, after exposure, in the elongating leaf. The former values indicated the initial distance between holes. Leaves were discarded if the lowermost hole was not within 1 mm of the ligule of the elongating blade.

Marking. Sheaths surrounding the elongating leaf were carefully removed from the tiller that remained intact on the plant. Ink marks were placed 3 mm apart along the epidermis of the elongation zone, then the lower part of the leaf was wrapped with moistened paper as previously described (11). Displacement of marks was measured with an ocular after approximately 6 h.

Calculations. RSE_i of a given segment was calculated from displacements of the holes or ink marks:

$$RSE_i = 2(D_{i,t_n} - D_{i,t_o})\cdot(D_{i,t_n} + D_{i,t_o})^{-1}$$

where D_{i,t_o} represents the initial distance between neighboring holes or ink marks, defining segment i at the time of making the hole or mark (t_o), and D_{i,t_n} represents the distance between these same holes or marks after a period ($t_n - t_o$) of growth. For data comparison the RSE_i values were converted to percentage RSE_i values (% RSE_i) as follows:

$$\%\ RSE_i = 100\ RSE_i\cdot(RSE_1 + RSE_2 + \ldots + RSE_n)^{-1}$$

where n is total number of segments in the elongation zone. All data were calculated from at least five separate tillers.

RESULTS AND DISCUSSION

Effect of Holes on LER. After leaves were taped to the fish line and leaf elongation had attained a steady rate, holes were made equidistantly in (0–30 mm from the ligule) and above the elongation zone (30–60 mm from the ligule). Control leaves (handling only in Fig. 1A) were handled identically but without making holes. The LER responded instantaneously to both handling and making holes, showing a rapid increase (not shown) followed by a period of no elongation (Fig. 1A). Clearly, this response was not due to holes since control leaves (handling only) behaved similarly. The subsequent lag in LER, however, was longer when holes were made.

Making holes above or making only two holes within the elongation zone resulted in a shorter lag than when eight holes were made within the elongation zone (Fig. 1A). Thus, duration of the lag appeared to be influenced by the extent of damage done to the elongating leaf. After the lag, LER of leaves that were only handled returned to their initial rate. In contrast, leaves with holes reached a steady LER that was 64 to 75% of the initial rate. The decrease in steady LER due to holes was similar, regardless of the number or location of holes, suggesting that the reduction was largely independent from the site of damage.

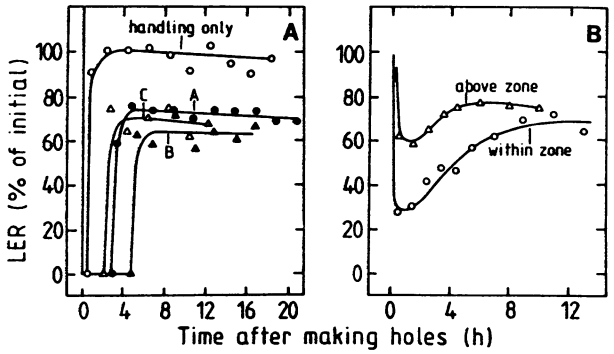

FIG. 1. Response of LER to making holes in or above the elongation zone. A, Control leaves (handling only) were handled identically to other treatments, but without making holes. Equidistant holes were made either within the elongation zone (A, two holes and B, eight holes between 0 and 30 mm from the ligule) or above the elongation zone (C, five holes between 30 and 60 mm from the ligule). B (but not in A), tillers were immobilized prior to LER measurements and making holes (see text). LER was recorded continuously. Data points are averages of three measurements.

Since handling alone affected LER only initially it was suspected that this response was an artifact. Tillers of tall fescue normally emerge at angles and are slightly curved. The tillers had to be straightened slightly in order to make the holes. The sudden increase and subsequent lag in all treatments may occur while tillers slowly regain their original curvature and position after handling. This latter effect could mask leaf elongation occurring during the period of reorientation. A second experiment was conducted where such effects were avoided.

Tillers to be measured were taped to a small glass test tube with duct tape, then tubes were held firmly by clamps mounted on a ring stand. This setup did not influence LER, and allowed us to make holes in tillers during LER measurements without affecting the geometry of the system.

After LER had attained a steady rate eight equidistant holes were made within the elongation zone (0–30 mm from the ligule) or distal to the elongation zone (30–60 mm from the ligule). Alternatively, bases of tillers were pressed with the rear end of the 4-mm-diameter glass rod that held the needle. Similar pressure was applied as would occur when the needle penetrated the tiller and the glass rod contacted the surface. No effect on LER was observed (data not shown), suggesting that the effect of handling *per se* on LER observed in Figure 1A was indeed spurious.

Similar to the earlier experiment (Fig. 1A) LER decreased rapidly after holes were made, with the decrease being greatest when holes were made within the elongation zone (Fig. 1B). Measurements on a shorter time scale and increased resolution confirmed the rapid reduction, but there was not a cessation of growth immediately after making holes (data not shown). The initial rapid decrease was followed by a slow partial recovery. The LER attained a steady rate which was 76 and 69% that of the previous steady rate, approximately 4 and 8 h after making holes within or above the elongation zone, respectively.

The initial response of LER to making holes in the elongating zone was greater than when holes were made in nonelongating tissue. Later the LERs were similar, indicating that the early effect of holes was partially related, but later growth was not related, to the site of damage.

Distribution of Growth Within the Elongation Zone. Holes were made through the elongation zone of 10 tillers. On a matched set of 8 tillers, the outer leaf sheaths were removed and ink marks made on the elongation zone of the exposed blade as

before (11). Distances between holes and marks were measured after 6 h. Previous investigations had shown a 27 and 31% decrease in daily LER after making holes and marks, respectively.

The spatial distribution of growth in the elongation zone of tall fescue blades estimated by holes and marks was the same (Fig. 2). The contribution to leaf elongation was small in the lowermost segment of the blade (% RSE of 9.1 and 7.1), and increased towards 6 to 9 mm from the base (% RSE of 23.6 and 25.6 for holes and marks, respectively). Distal to 9 mm the % RSE decreased and cessation of elongation occurred at 24 to 27 mm from the leaf base in both treatments. The data show that the methods used did not differentially affect the distribution of growth in the elongation zone of tall fescue leaf blades.

Effect of Location of Holes on Spatial Distribution of Growth. Figure 3A shows segmental contributions to leaf elongation obtained when holes were made throughout the elongation zone, and when holes were made only in the basal, or distal part of the elongation zone. Elongation of segments was not influenced by the pattern used. The presence of holes in the distal part of the elongation zone did not cause a significant change in the contribution of distal segments to leaf growth. Similarly, when holes were made only in the basal part of the elongation zone the basal segments elongated at rates similar to control rates.

Similar results were obtained in another study (Fig. 3B). The basal portion of the elongation zone (0–12 mm from the ligule) contributed similarly to total leaf elongation when holes were present (total of 63% RSE for control) or absent (total of 62% RSE for trace B) in the distal part of the elongation zone. Likewise, the distal part of the growth zone (16–28 mm from the ligule) contributed 10% to leaf elongation when holes were absent (A) and 15% when holes were present (control) in the elongation zone. Thus, location of holes did not differentially affect the spatial distribution of growth in the elongation zone.

Estimating the Spatial Distribution of Elongation in Undamaged Leaves. The mechanism for reduction in LER due to making holes or marking has not been explained with our experiments. The response of LER to making holes was very rapid, indicating the primary effect was on cell elongation. Effects on cell division may also have occurred, but cell division alone does not contribute to LER. Thus, a decrease in rate of cell division due to making holes would not be observed until the number of cells in the rapidly expanding tissue starts to decrease. Cells move very slowly, however, taking more than 2 d to move from the zone of cell division towards the zone of rapid growth (H Schnyder, CJ Nelson, unpublished data).

Portions of leaf blades with holes did not show any visual abnormality other than the presence of holes when they were allowed to emerge above the surrounding sheaths, *e.g.* there were no constrictions at the location of holes. Frequently, three holes were observed at one position, indicating that the needle had pierced through the overlapping parts of the rolled blade. Wounding when making holes or when removing outer leaves to mark the elongation zone may have caused ethylene production (6) and associated effects on LER. Likewise, a change in water status due to holes may explain part of the response (1), but water status of the marked leaves should not be adversely affected, especially when outer leaves are removed to reduce transpiration and the elongation zone is covered and kept moist.

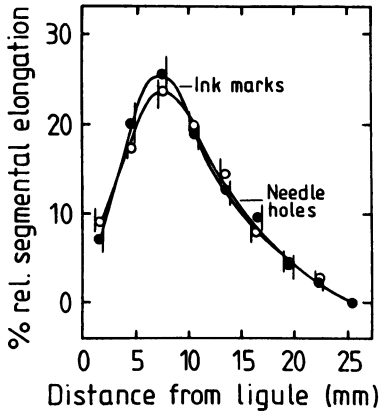

FIG. 2. Distribution of growth within the elongation zone of leaf blades assessed by measuring displacement between needle holes and ink marks. Tillers with elongating leaves were divided into two uniform groups of 10 and 8 tillers for the needle hole and marking treatments, respectively. Holes and marks were made as described in text.

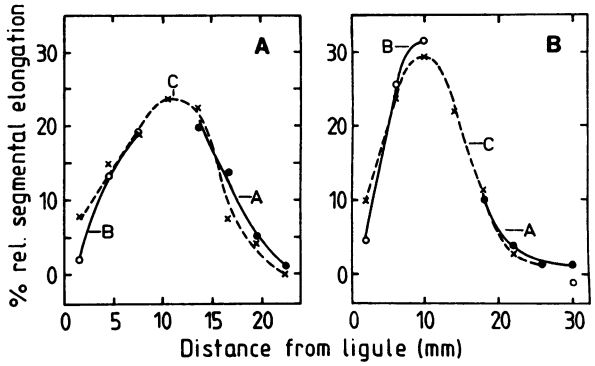

FIG. 3. Distribution of growth within the elongation zone of leaf blades as affected by needle holes through different regions of the zone. A, tillers growing in continuous light were divided into three uniform groups of six tillers each. In the control treatment (C) nine holes were made through tillers at 3-mm distances between 0 and 24 mm from the ligule. In treatments A and B nine holes were made at 3-mm distances between 0 and 33 mm from the ligule, with no holes being made in the basal part of the elongation zone in treatment A (0–12 mm from the ligule) and no holes in the distal part in treatment B (9–21 mm from the ligule). Displacement of holes was measured after approximately 6 h. B, Data from an experiment where eight holes were made at 4-mm distances. Holes in the control (C) were made throughout the elongation zone (0–28 mm), in treatments A and B holes were made between 0 to 40 mm, with no holes being made between 0 to 16 mm in treatment A and no holes being made between 12 and 28 mm in treatment B. Displacement of holes was measured after 24 h. Data points are means of five measurements. Data are not shown for distances above 24 and 32 mm for (A) and (B), respectively, as no elongation occurred.

Regardless of the mechanism involved, wounding affected only the rate and not the spatial distribution of elongation. Data on displacement of holes can thus be used to estimate the spatial distribution of elongation in undamaged leaves by correcting for the reduction in LER caused by holes. For this purpose the LER before making holes or the LER of identical but undamaged leaves must be measured. Then SER ($mm \cdot mm^{-1} \cdot h^{-1}$) in undamaged leaves can be obtained by calculating

$$SER_i = LER \cdot RSE_i \cdot (RSE_1 + RSE_2 + \ldots RSE_n)^{-1} \cdot L^{-1}$$

where LER represents the rate of undisturbed leaf elongation (*i.e.* before making holes) in $mm \cdot h^{-1}$, n the total number of segments in the elongation zone, and $RSE_i \cdot (RSE_1 + RSE_2 + \ldots RSE_n)^{-1}$ the fractional contribution of segment i to LER as obtained from measurements of hole displacement. The last term, L, represents the length of segments (mm). This is used to convert elongation rate per segment to elongation rate per mm leaf length.

Use of Data for Growth Anaylsis. To be accurate, an analysis of growth must be both elemental and instantaneous (3), *i.e.* segments must be short in relation to the length of the elongation zone, and elongate little between the time of making holes and the time of measurement of hole displacement.

Erickson and Silk (3) reported that maize (*Zea mays* L.) roots elongated at 2 $mm \cdot h^{-1}$. In their experiments, SERs, *i.e.* relative elemental growth rates, calculated from a 1-h observation interval, were close to the instantaneous rates obtained from streak photograph data. The roots in their experiment elongated 2 mm during the 1-h observation interval corresponding to 20% of the length of the elongation zone. In our experiments (except in Fig. 3B) leaves elongated a mean of 3.8 mm in the 6 h between times of making holes and measurement. This increase in blade length corresponds to 16% of the length of the 24-mm elongation zone, suggesting that SERs obtained from our data are close to instantaneous rates.

In addition to being instantaneous, growth rates should also be elemental. In our experiments the growth zone was divided into eight 3-mm long subzones. Because of their relative size we termed these subzones segments rather than elements (9) of the growth zone, and accordingly, growth rates are termed segmental rather than elemental.

A higher resolution of the spatial distribution of growth could probably be obtained by decreasing the length of segments, *e.g.* to 1 mm. With 1-mm spacing, however, the average displacement of holes with respect to neighboring holes would be decreased to one-third of the displacement observed with 3-mm spacing. Experimental errors would be amplified since absolute errors in measuring distances between holes would remain the same. Figure 2 shows that the spatial distribution of growth is described with good detail when the growth zone is divided into nine segments.

CONCLUSION

The spatial distribution of growth in the elongation zone of tall fescue leaf blades was the same whether obtained from measurement of short-term displacement of ink marks or holes. Previous work had demonstrated that the length of elongation zones determined by cell lengths was accurately estimated by following the displacement of ink marks in the elongation zone (11). We have also found that the elongation of segments from different locations in the elongation zone was not differentially affected by the presence or absence of holes in any other location. Therefore, the decrease in leaf elongation caused by making holes in the elongation zone was due to proportional decreases of elongation of segments throughout the zone. Thus, the relative contribution of segments to leaf elongation can be used together with data on LER during undisturbed growth to calculate the spatial distribution of growth velocities in intact leaves. We found that making holes was more convenient and less time consuming than making marks.

We are using the spatial distribution of growth velocities and substance concentrations in the elongation zone of leaf blades, along with the continuity equation, to calculate the spatial distribution of substance incorporation rates. The power and use of this system in describing physiological and morphological aspects of the growth process has been demonstrated by Silk *et al.* (10).

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