Maize Leaf Elongation: Continuous Measurements and Close Dependence on Plant Water Status

Abstract. *A simple method was developed for measuring extensive growth of intact leaves of monocots on a minute-by-minute basis. Growth was markedly reduced by a slight reduction in leaf water potential. When plants mildly deficient in water were irrigated, growth resumed virtually instantly. The transitional rapid growth after watering suggests that water deficit increased cell extensibility.*

It is well known that cell turgor, hence favorable water status of plants, is important to growth by cell expansion. Green (*1*) demonstrated that the expansion of internodal cells of *Nitella* was closely dependent on turgor pressure and that the gross extensibility of the cell increased when extension was checked by a lack of turgor. Studies of expansive growth in higher plants have been conducted mainly on excised *Avena* coleoptiles. In a detailed study of short-term growth of green leaves, Boyer showed, largely with excised tissue, that growth is reduced by a slight reduction in leaf water potential (*2*). Such studies of green leaves have been hampered because of lack of an accurate method for measuring short-term growth. We have developed such a method capable of monitoring the growth of leaves of intact monocots on a minute-by-minute basis and have observed an extreme dependence of growth on plant water status (*3*).

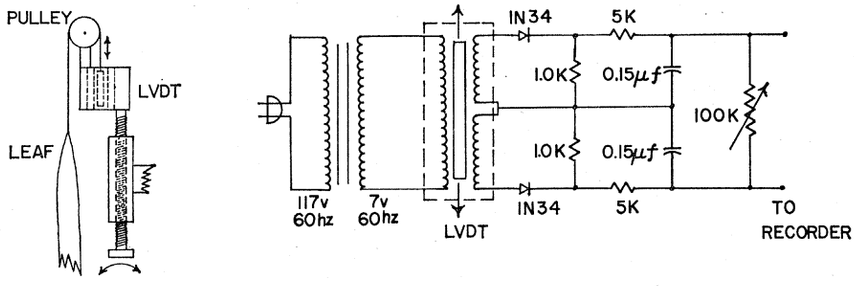

Fig. 1. Apparatus for recording leaf elongation. The attachment of the leaf to the linear variable differential transformer (model 586DT-050, Sanborn, Waltham, Mass.) is shown on the left and the circuitry on the right.

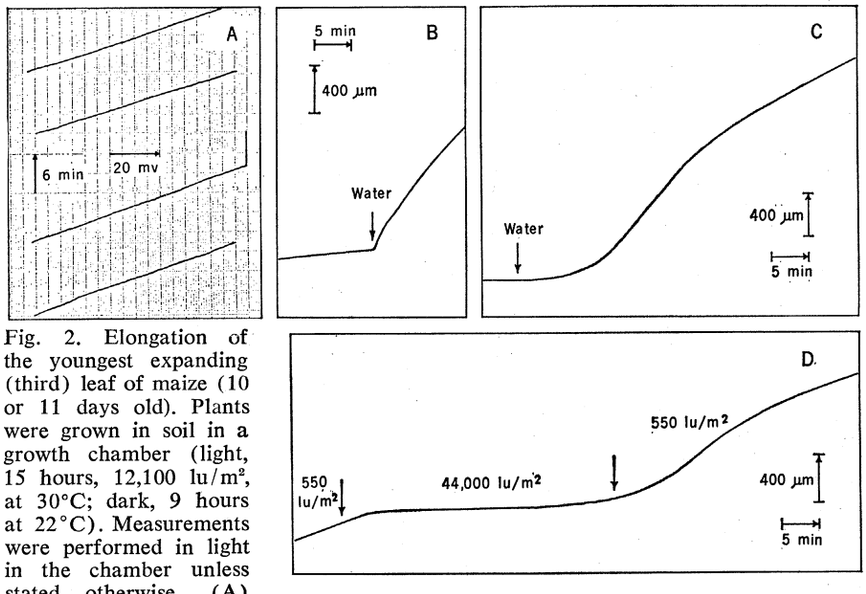

Fig. 2. Elongation of the youngest expanding (third) leaf of maize (10 or 11 days old). Plants were grown in soil in a growth chamber (light, 15 hours, 12,100 lu/m², at 30°C; dark, 9 hours at 22°C). Measurements were performed in light in the chamber unless stated otherwise. (A) Recorder tracing for a well-watered plant. The soil water potential was −0.15 bar. The sensitivity of the LVDT was 7.5 μm per millivolt. Recorder pen started at bottom left. After the pen spanned the chart width, the position of the LVDT core was adjusted to bring the pen to about 10 mv. The series of lines thus represent essentially a continuous record of elongation. (B) Effect of watering a plant mildly deficient in water. Soil water potential was about −1.9 bars at watering. Water was added to the soil at the time indicated by arrow. Note that the water deficit prior to watering still permitted slight growth (4 μm per minute). (C) Effect of watering a plant moderately deficient in water. Soil water potential was −2.8 bars at watering. Note that the water deficit prior to watering was severe enough to stop elongation. (D) Effect of light intensity on a well-watered plant (about −0.2 bar of soil water potential). Elongation was measured in the laboratory. At the start of the experiment the plant had been in laboratory light (550 lu/m²) for several hours, and growth rate was steady. Arrows indicate the time when the strong light (Sylvania iodine-quartz lamps, filtered through 7 cm of water) was turned on or off.

A linear variable differential transformer (LVDT) was used to transduce changes in the position of the leaf apex brought about by elongation to a voltage output which was continuously recorded (Fig. 1). One end of a sewing thread (1 to 2 dm long) was taped (Scotch Magic Mending) to the apex above the elongating region of a leaf of maize (*Zea mays* L., var. WF9 × M14), and the other end was tied to the iron core (weighing 4 g) of the LVDT. By looping the thread over a small pulley (1.2 cm in diameter, and low in friction) and by positioning the plant directly under the pulley, the leaf, anchored at the base by the roots in the soil, was kept taut and erect by the slight weight of the LVDT core. The primary coil of the LVDT was connected to a 7-volt a–c source. The output of the secondary coils was rectified, and displayed on a millivolt recorder. To convert the output to increase in leaf length, the apparatus was calibrated with a micrometer.

When a growing young leaf was attached to the apparatus, there was a remarkably constant increase in voltage output with time (Fig. 2A) after an initial period of adjustment. Fully grown leaves or growing leaves of plants under water stress gave little or no increase in voltage. The increase in output apparently represented leaf growth by elongation. The method was then tested for potential sources of error.

When first attached to the apparatus, the leaf showed some mechanical stretching from the weight of the LVDT core, as indicated by a transitory rapid rate of elongation lasting less than 10 minutes. Therefore, all measurements were made after this period. Doubling the core weight did not increase the measured growth rate. Another test was conducted in this connection. Cells are extended irreversibly when the extensive force exceeds the

minimum yield stress (*4*). In the present case the extensive force may be considered as the sum of forces due to turgor and the weight of the core. The turgor force was eliminated by cutting off the root system, and hence, the water supply. Elongation accelerated slightly upon cutting, probably from the release of xylem tension and water. After 10 minutes, elongation stopped and the leaf started to shrink, although no obvious wilting was observed even after 30 minutes. Thus, the core weight alone was not sufficient to sustain any prolonged elongation, which indicated that the force due to the core weight did not exceed the minimum yield stress.

Another potential source of error was temperature fluctuation, which may cause differential thermal expansion. Attaching the core to a fully grown leaf and varying the temperature of the environment revealed that the measured length increased 45 μm for each degree of increase in temperature. However, since the rate of elongation of a growing leaf was around 60 μm per minute, error due to temperature fluctuation is insignificant when growth is measured for many minutes. For very brief growth measurements, it would be important to control the temperature.

Slight water deficits greatly reduced elongation. Drying of the soil, reducing leaf water potential from -3 bars (well-watered plants) to -4 bars, reduced elongation about 10 percent under the conditions used. Elongation stopped completely when leaf water potential was reduced to -6.5 bars, even though wilting was still not obvious. These results generally agree with those of Boyer (*2*).

Growth recovery was virtually instant (*5*) when plants were watered if the prior water deficits were not severe (Fig. 2B). It is improbable that the added water moved so rapidly to the expanding zone in the leaf. The extremely rapid response was more likely the result of the water continuum in the xylem acting as a cohesive and incompressible liquid transmitting the change in pressure and the associated small change in volume from roots to leaves.

There was a transitory period of fast elongation upon watering, which changed to a slower steady rate several minutes later. If we assume that growth rate is the product of gross extensibility and turgor pressure (*1*), the fast rate in the transitory period suggests that the gross extensibility was increased by water deficits. Turgor pressure during this period should be lower than that associated with steady-state growth. Therefore, extensibility must be higher to account for the faster rate.

The rapidity of the response in elongation points to the essential role of water in providing the driving force for cell enlargement. The transitory fast elongation following watering suggests that the main factor in reducing growth during mild water deficits is the reduced driving force, not alterations in metabolism. Apparently, accumulation of metabolites necessary for growth during the period of water deficiency permitted the transitory fast elongation when turgor pressure suddenly began to increase.

With more severe water deficits, however, plant response was different (Fig. 2C). There was a lag after watering before elongation gradually resumed. It is expected that with greater water deficits in the cells more time would be needed for the turgor pressure to increase to the minimum yield stress. After the initial lag, the transitory rapid elongation was again observed (Fig. 2C).

The detail in elongation that this method is capable of resolving is demonstrated in another experiment that determined the effect of varying light intensity (Fig. 2D). Elongation was greatly reduced within a few minutes when light was increased from about 550 to 44,000 lu/m^2, conditions that are expected to increase leaf temperature, stomatal aperture, and transpiration. Upon return to the low light, elongation not only resumed in a few minutes but also showed a transitory fast period similar to the response to watering. Although the results can be best explained in terms of changes in transpiration and resultant changes in leaf water balance and turgor, the possibility is not ruled out that growth was affected through other effects of light.

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References and Notes

1. P. B. Green, *Plant Physiol.* **43**, 1169 (1968).
2. J. S. Boyer, *ibid.*, p. 1056.
3. After completion of this study, we were told that G. Meijer [*Acta Bot. Neer.* **17**, 9 (1968)] monitored the inhibition of elongation of dark-grown gherkin hypocotyl by blue light, also with an LVDT.
4. Minimum yield stress is the threshold force above which the plant tissue begins to deform irreversibly. J. A. Lockhart, in *Plant Biochemistry*, J. Bonner and J. E. Varner, Eds. (Academic Press, New York, 1965), p. 826.
5. The lag of several or more seconds, not obvious in Fig. 2B because of the scale, is accounted for, at least in part, by the time required for water to permeate the soil to the root zone.
6. Supported in part by grant B-029-CAL from the Office of Water Resources Research, United States Department of the Interior, by a grant from the Water Resources Center, University of California, and by NSF grant GB 5658.

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Vitamin B_{12} Binders of Chicken Serum and Chicken Proventriculus Are Immunologically Similar

Abstract. *Two substances that bind vitamin B_{12} are found in chickens, one in the serum and another in the proventriculus. Their molecular weights, as estimated from gel filtration on Sephadex, are approximately 113,000 and 96,000, respectively. Antibody elicited in rats against the proventriculus binder reacts against both binders.*

Two immunologically different substances that bind vitamin B_{12} are found in rats and mice; these are intrinsic factor (IF) and transcobalamin II (TC-II). They are found in the gastric mucosa and serum and are synthesized in the gastric mucosa and liver, respectively (*1*). Another binding substance of higher molecular weight (110,000 as compared to 55,000 for IF and 38,000 for TC-II) is found in human serum, saliva, and gastric mucosa. This has been termed R binder or transcobalamin I (*1*). These three immunologically distinct B_{12} binders probably arose from a single ancestral B_{12} binder during evolution, but little is known about the types of B_{12} binders found in nonmammalian vertebrates.

Chicken serum has a high concentration of substances that bind B_{12} (*2*). Our experiments show that this binder is similar in size to R binder, immunologically similar to the binder found in chicken proventriculus, and immunologically distinct from the R binder.

The extent of B_{12} binding in serum