

**Zeitschrift:** Archives des sciences et compte rendu des séances de la Société  
**Herausgeber:** Société de Physique et d'Histoire Naturelle de Genève  
**Band:** 50 (1997)  
**Heft:** 3: Archives des Sciences

**Artikel:** Computer-assisted measurements of plant growth with linear variable differential transformer (LVDT) sensors  
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**DOI:** <https://doi.org/10.5169/seals-740286>

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Communication présentée à la séance du 11 décembre 1997

## COMPUTER-ASSISTED MEASUREMENTS OF PLANT GROWTH WITH LINEAR VARIABLE DIFFERENTIAL TRANSFORMER (LVDT) SENSORS

BY

**Robert DEGLI AGOSTI\*, Laurent JOUVE & Hubert GREPPIN**

### ABSTRACT

**Computer-assisted measurements of plant growth with Linear Variable Differential Transformer (LVDT) sensors** - A system is described which allows to measure plant growth with a high precision ( $\sim 2 \mu\text{m}$ ) and high frequency (1 measurement per sec). It is based on LVDT sensors and driven by a dedicated computer with a software that allows to perform almost immediate out-line data analysis and treatment. The software automatically controls the position of each sensor for optimal measurements over elongation of 15 cm with respect to the plant by triggering motors that lift up the LVDT's. Twelve LVDT sensors channels with 4 environmental sensors (temperature, relative humidity and light intensity) monitor the plant growth and environmental conditions. The floral stem growth of *Arabidopsis thaliana* has been followed in a photoperiod of L:D 12:12 and then followed by continuous light. L: D synchronizes the stem extension rate, with the highest growth rate in the light phase. In continuous conditions, fluctuations of high and low rates persist with a period of  $\sim 24$  h. This property is typical of a system under control of the biological clock. At an other time scale (hours), stem extension rates measurements present oscillations with an ultradian periodicity ( $\sim 30$  min). These examples illustrate well the large dynamics capacity and resolution of the developed installation.

**Key-words:** *Arabidopsis thaliana*, growth, floral stem, LVDT.

**Abbreviations:** LVDT, Linear variable differential transformer or Linear voltage displacement transducer; GDU, Generator demodulator unit.

### INTRODUCTION

Plant growth is an important phenomenon as it drives a significant biomass production on earth. Since a long time plant physiologists have attempted to follow this process and particularly, in higher plants, the stem elongation. For this purpose, rulers have been used since 1727 by TREWS (cited in SACHS, 1887). This method is simple, inexpensive and robust and still in practice. However, it suffers of important drawbacks: it is not precise and does not allow fine continuous growth monitoring. Moreover, touching the plant at intervals trigger stress responses that significantly affect the growth process itself (JAFFE *et al.*, 1980; LECHARNY & JACQUES, 1980).

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A review of different methods used in the past allowing for better precision and/or continuous measurement has been presented by RUGE (1961). Devices range from the early mechanical systems such as the "Zeiger am Bogen" from SACHS (1870), the auxanograph from WIESNER (1876), the auxanometer from BOSE (1928), to the optical interferometric-based system from MEISSNER (1929) and LAIBACH (1932) and kinematography of PFEFFER (1900). More modern techniques involve video image digitizing (JAFFE *et al.*, 1985; MACDONALD *et al.*, 1987) and electromechanical methods. These latter are often designated as LVDT sensors in plant physiology. The LVDT acronym is for Linear Voltage-Displacement Transducer (LECHARNY & WAGNER, 1984), or for Linear Voltage Differential Transformer (RUIZ-FERNANDEZ & WAGNER, 1994; PRAT & PARÉSYS, 1995); although, in the physical and technical sciences the LVDT acronym is for Linear Variable Differential Transformer (DECKER & KOSTKA, 1989). Displacement transducers (LVDTs) have been used since the 70's for plant growth measurements (HSIAO *et al.*, 1970; PENNY *et al.* 1974) and are currently in use (DEPEGE *et al.*, 1997; KERCKHOFFS *et al.*, 1997; KRISTIE & JOLLIFE, 1986). More detailed descriptions of the different applications and history of LVDT technology in plant growth studies can be found in RUIZ-FERNANDEZ & WAGNER (1994) and JAFFE *et al.* (1985). The LVDT method is particularly well suited to monitor the growth with high time and sensitivity resolution. The purpose of this work is to present such a system and its application to the measurement of the floral stem elongation of *Arabidopsis thaliana*.

## MATERIALS AND METHODS

### *Plant material*

*Arabidopsis thaliana* (L.) Heynh. Landsberg *erecta* ecotype seedlings were grown in a potting compost under L:D (8:16) photoperiod for 3 weeks after sowing. Then they were individually transplanted in a new pot and cultivated under L:D (12:12) for 3-4 weeks further waiting for the initiation of inflorescence primordia. Once the floral stem was about 2-5 mm long, they were attached to the measuring sensors (LVDTs) by a cotton thread. During growth light was provided by fluorescent lamps (Sylvania 36W Luxline-Plus) with an intensity of  $75 \mu\text{mol m}^{-2}\text{s}^{-1}$  (PAR). Experiments were done in thermo and hygro-regulated chamber: temperature was  $22.5 \pm 0.5$  °C and relative humidity of  $80 \pm 6$  %. Light during measurements was provided by fluorescent lamps (Sylvania 36W Gro-lux,  $45 \mu\text{mol m}^{-2}\text{s}^{-1}$ , PAR).

### *The LVDT sensor and Generator Demodulator Unit (GDU)*

The Fig. 1 presents the aspect and the structure of a LVDT together with its associated GDU. A LVDT (L20, Chauvin-Arnoux, France) is a cylindrical case with a central bore in which an iron core (20 mm) moves practically freely. A primary coil generates an alternating magnetic field as the current through it is modulated at 2000 Hz by the GDU (GDL 8070-05, Chauvin-Arnoux, France). As a result, voltages are induced in the secondary coils according to the position of the iron core and finally demodulated by the GDU.

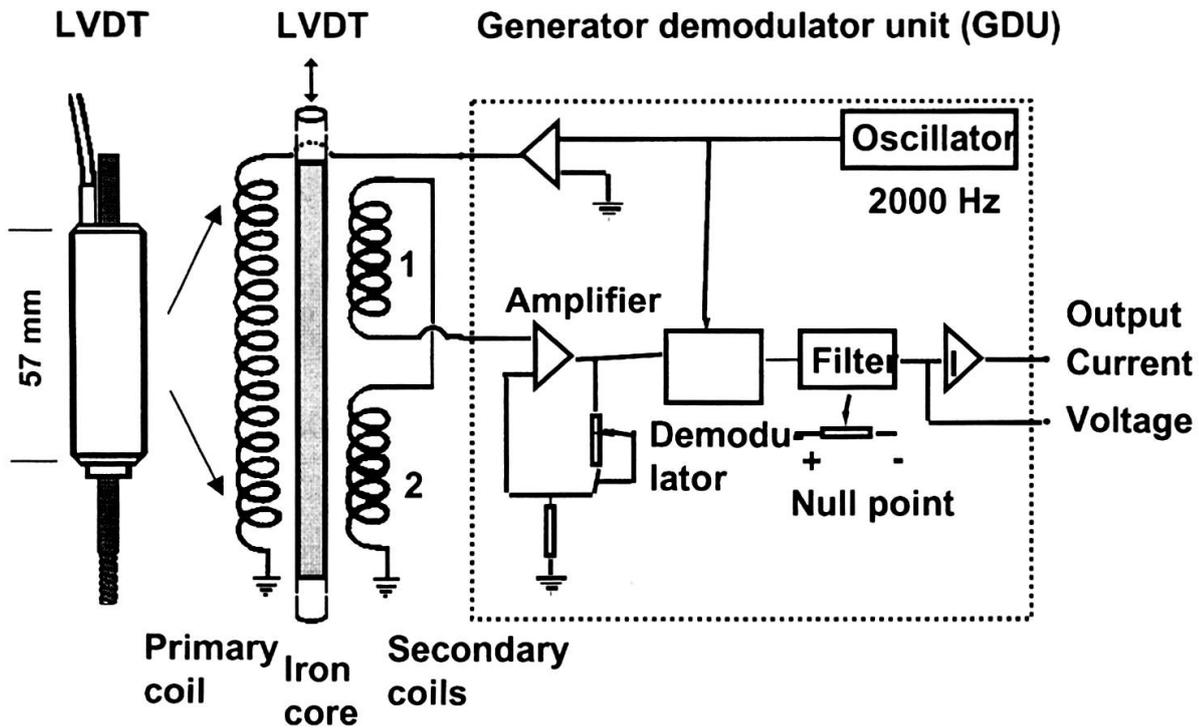


FIG. 1.

Physical aspect of a LVDT sensor (L20 from Chauvin-Arnoux, France) together with its electrical circuit. The iron core moves within a primary and two secondary coils. An oscillating current is sent by the GDU to the primary coil and induces voltages in the secondary coils. These voltages depend on the position of the iron core and are further processed by the GDU to deliver a DC output current or voltage precisely indicating the position of the iron core to which the measuring object is connected.

From Faraday's law, the voltage  $U$  induced in one secondary coil is:

$$U = -N \frac{d\phi}{dt} = -M \frac{dI}{dt} \quad (1)$$

Where,

$N$  = number of coil windings

$\Phi$  = magnetic flux

$M$  = mutual inductance between the primary and secondary coils

$I$  = primary current

With 2 secondary coils:

$$U = U_2 - U_1 = M_2 \frac{dI}{dt} - M_1 \frac{dI}{dt} \quad (2)$$

$$U = (M_2 - M_1) \frac{dI}{dt}$$

$M_1$  and  $M_2$  are functions of the  $x$  position of the iron core. Thus

$$\begin{aligned}
 M &= M_2 - M_1 = M(x) \\
 U &= M(x) \frac{dI}{dt} \\
 \text{or} & \\
 M(x) &= \frac{U}{\frac{dI}{dt}}
 \end{aligned}
 \tag{3}$$

The amplitude of the secondary voltage is proportional to the position of the iron core. The phase displacement between the primary and secondary signals indicates the position of the core relative to the zero position. Indeed, if the core is in the center of the two secondary coils, the voltages are identical (same amplitude) and they are 180° out of phase: their sum is zero (DECKER & KOSTKA, 1989).

#### *General set-up of the installation*

The general set-up of the installation is shown in Fig. 2. The plant is connected to the moving part of the LVDT sensor with a cotton thread fixed to a support with a spring (mean tension is less than 1 g). The position of the mobile iron core of the LVDT can be continuously monitored with the LVDT and its GDU. The voltage delivered is converted by an analogic to digital converter card (A/D) (A/D-14bit, 3a, Germany) and a computer (386 SX-16). The support and interfaces have been constructed by the workshop of the University of Freiburg (Biological Institutes I & II, University of Freiburg, Germany). Twelve sensors are installed allowing for 12 plants to be almost simultaneously measured. The A/D card is also connected to environmental sensors for monitoring light intensity (Silicon-PN-Planar photodiode BPW20, Telefunken, Germany), temperature and relative humidity (HT-732-M-00, Pewatron, Switzerland). The A/D card is a 14-bit (16384 resolution) and the LVDT has a 20 mm corresponding measuring range, thus the theoretical resolution of the system is 1.22 μm. However, this depends strongly on the number of measurements which are done. Indeed, data treatment and statistical methods may enhance this resolution considerably. On the other hand, this situation is complicated and limited by the inherent mechanical and electronical noise of the system. Overall, and in standard conditions, the precision of the system is of about 2 μm (RUIZ-FERNANDEZ & WAGNER, 1995).

When the stem of a plant elongates, it will happen, if the elongation is greater than the measuring range of the LVDT, that the iron core will leave the measuring range of the sensor. To avoid this problem, the coils (the rest of sensor without the iron core), are lifted up by a motor. The motor is controlled by an appropriate interface unit connected to an I/O card (I/O 48 Kanäle 8255, 3a, Germany). The I/O card is driven by a control and data acquisition software which is described later. Although the iron core is not in contact with the coils, some friction may eventually occur during this movement. This

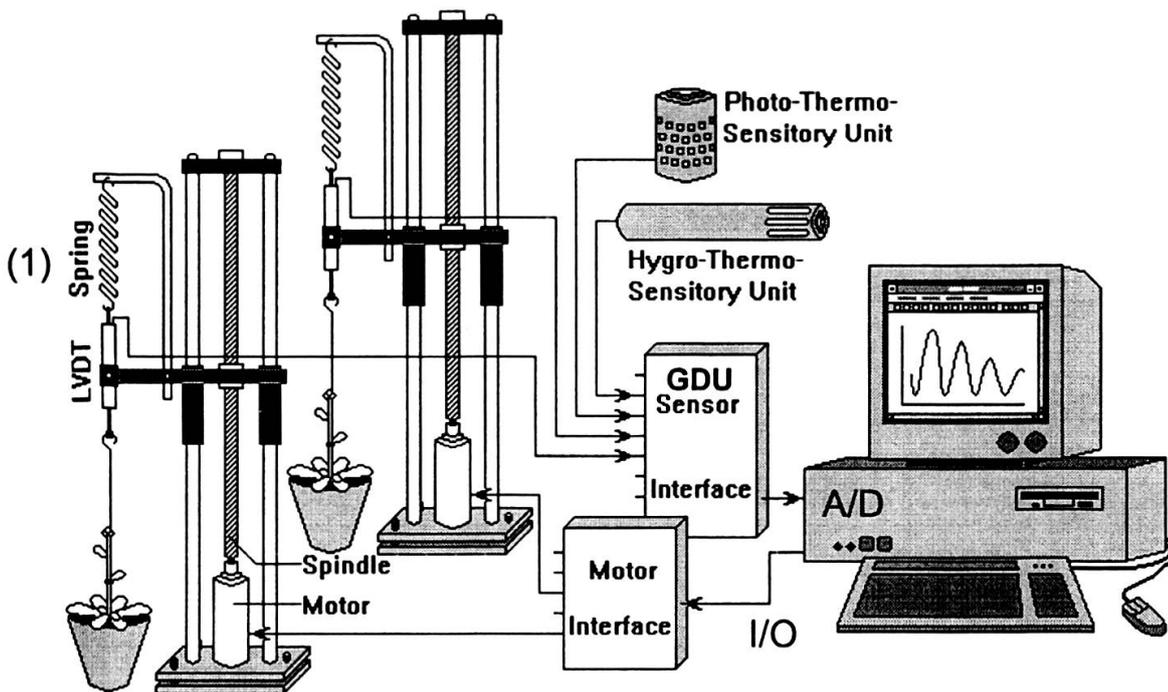


FIG. 2.

General diagram of the LVDT based measuring system. The plant is connected to the iron core of the LVDT. A small traction is maintained with a spring linked to a support (1). The LVDT signal is converted to a DC signal by the GDU units (Sensor interface) converted to digital number by an A/D card in a computer and finally sampled by a specific software. Twelve LVDT sensors are present. Temperature, light and relative humidity sensors signals are processed (amplified, filtered and scaled in the sensor interface), they are also sent to the computer via the A/D card. When needed or when the plant growth is such as the iron core is not optimally positioned within the LVDT sensor, the control software individually and specifically triggers an I/O card which controls the motors on the supports. The rotation of a spindle repositions the plant-connected core and its LVDT sensor accurately.

would lead to a sudden increase in the tension on the plant generating a potential stress effect. The present system has been carefully tested for this phenomenon, no stress effect (temporary decrease in growth rate) has been observed during the motor readjustments.

#### *Software: dedicated computer control with immediate out-line analysis*

A specific custom made software has been developed (G. Genève). It has been written in visual C++. It controls the data acquisition and motor lifting automatically. Its main features are: intelligent automatic management of motors, automatic journal (any important detectable external factor that may alter the exactness of the measuring system is notified), data display (either the numerical digitized values of the sensors, or a time series graph may be shown), export at any time (since after each measurement, or access to a data file, the corresponding file is always immediately closed, it is possible to export the data file to analyze it on a standard spreadsheet software such as Excel®). A dedicated computer is the host of this program. The advantage of the present system

as compared to an on-line analyzer (RUIZ-FERNANDEZ & WAGNER, 1994) is a more simple, robust and efficient software less prone to interruptions (crash) by other simultaneously running computer operations, together with very efficient and almost immediate data treatment by commercially powerful available software.

#### *Data acquisition and treatment*

The control program is able to sample and store in the computer all signals (LVDTs and environment sensors) in less than 1 sec as long as requested (until hard disk memory saturation). For the present work the sampling rate was fixed to 1 minute. These crude numerical values were converted into positions and after taking account of the motor's shifting and artefacts created by electronic spikes into elongation. In order to reduce mechanical and electronic noise, a first basic treatment is performed as following: 3 successive point are averaged. From the resulting time series a moving mean over 3 points is calculated on it. Sampling is thus reduced to 1 point per 3 min. The resulting time series represents the elongation of the plant, and to obtain the stem extension rate, measurements are differenced.

## RESULTS

To illustrate the basic features of the installation an experiment is presented in which the sensor was attached to a fixed 15 cm long cotton thread (Fig. 3). Rates of length change are expressed as a function of time. Since measurements are very narrow (3 min), small electronics or mechanical noise yield to a significant corresponding noise. The latter is very stable and fluctuates around the zero value without any drift. Moreover, during the alternating light to dark conditions we can observe an almost total independence of the signal, either to the light or, more importantly, to temperature and relative humidity conditions.

Fig. 4 shows the result obtained after measurement of an *Arabidopsis* floral stem in synchronizing (L:D 12:12) and release in free-run (constant light) conditions. Fig. 4A shows the crude result delivered by the LVDT sensor and sampled by the computer after conversion to displacement values. The curve is not regular since the motor was driven to readjust the sensor position. This can be observed as sudden drops in the elongation. If we cumulate the latter values we obtain a typical plant stem growth (Fig. 4B). The floral stem internode elongates progressively with a more rapid growth phase between 3 to 5 days and then reach a maximum after approximately 7 days. The time span displayed is the result of more than 3500 points with a high precision. As a consequence, it is already possible to observe that the growth dynamics is not a monotonous continuous phenomenon. During the L:D 12:12, slow phases of growth during dark alternate with higher growth rates during light. In continuous light, growth appears also to be irregular. In this situation it is however more difficult to observe the details of growth.

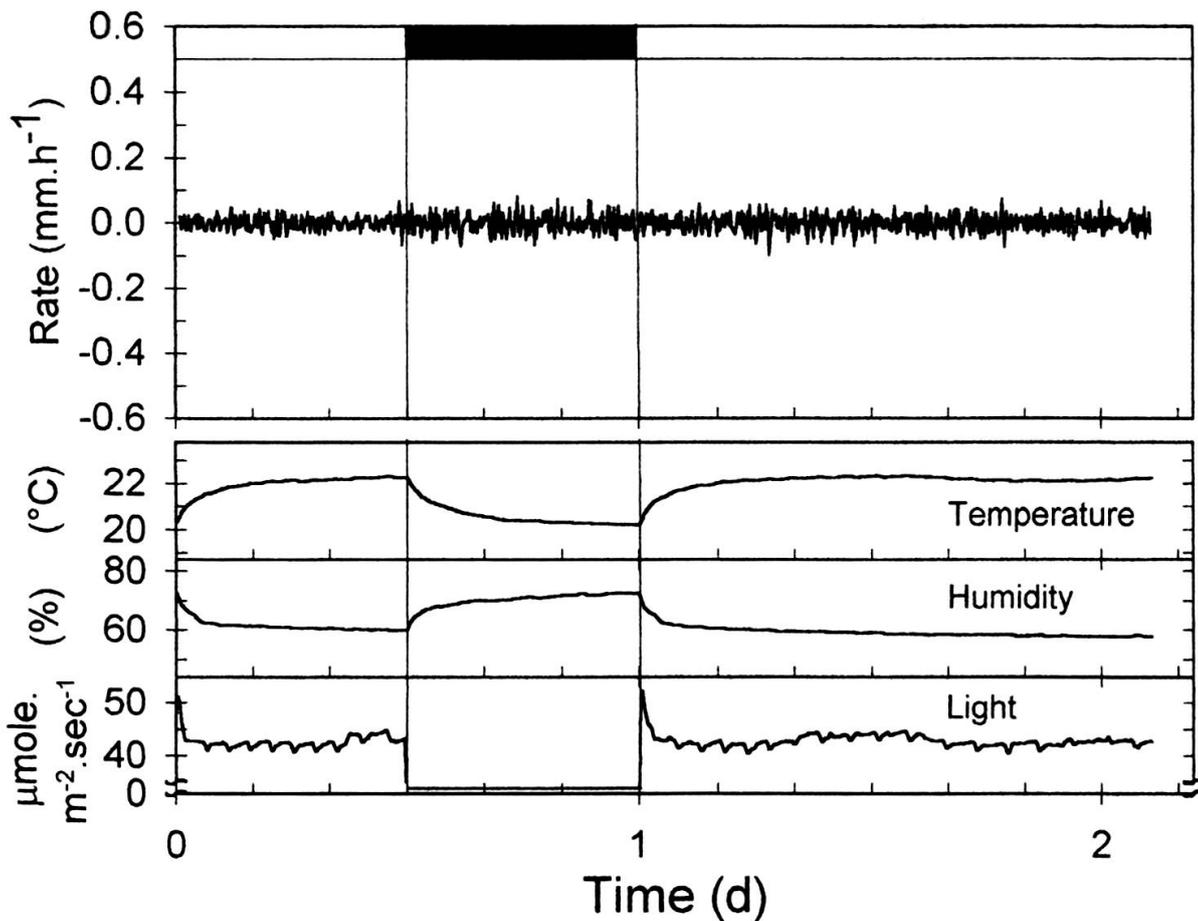


FIG. 3.

Measurement of a fixed 15 cm long cotton thread attached to the LVDT sensor in alternating light dark conditions. Upper curve: rate represents changes of position by unit of time. The lower curves represent the monitoring of temperature, relative humidity and light intensity during the same light dark transitions.

The details of the elongation dynamics of the floral stem are clearly visible when the rate of elongation is represented as a function of time (Fig. 5). The same data as in Fig. 4 is represented in Fig. 5 after differencing it according to the time scale. It is clear that there are very important fluctuations ranging from 0.2 mm.h<sup>-1</sup> to 0.9 mm.h<sup>-1</sup>. The whole curve (from 0 to 7 days) has the aspect of a "bell" shape modulated by more rapid changes of a daily range. During L:D 12:12, growth rate is lower in the night than in the light phase. When the same plant is maintained in continuous light, fluctuations persist, but are more complex. Main peaks and valleys could be observed at regular intervals of ~24 h. Other secondary peaks appear at 4.2 and 5.2 days.

An important noise is present, which could be filtered by the following mathematical treatment of the time series: 8 points are averaged to produce one point, since the original measurements were at 3 min interval, this means they now are separated each by 24 min. The resulting time series is then further filtered by a moving average technique over 5 points. The result is presented in Fig. 5B. The treatment maintains the

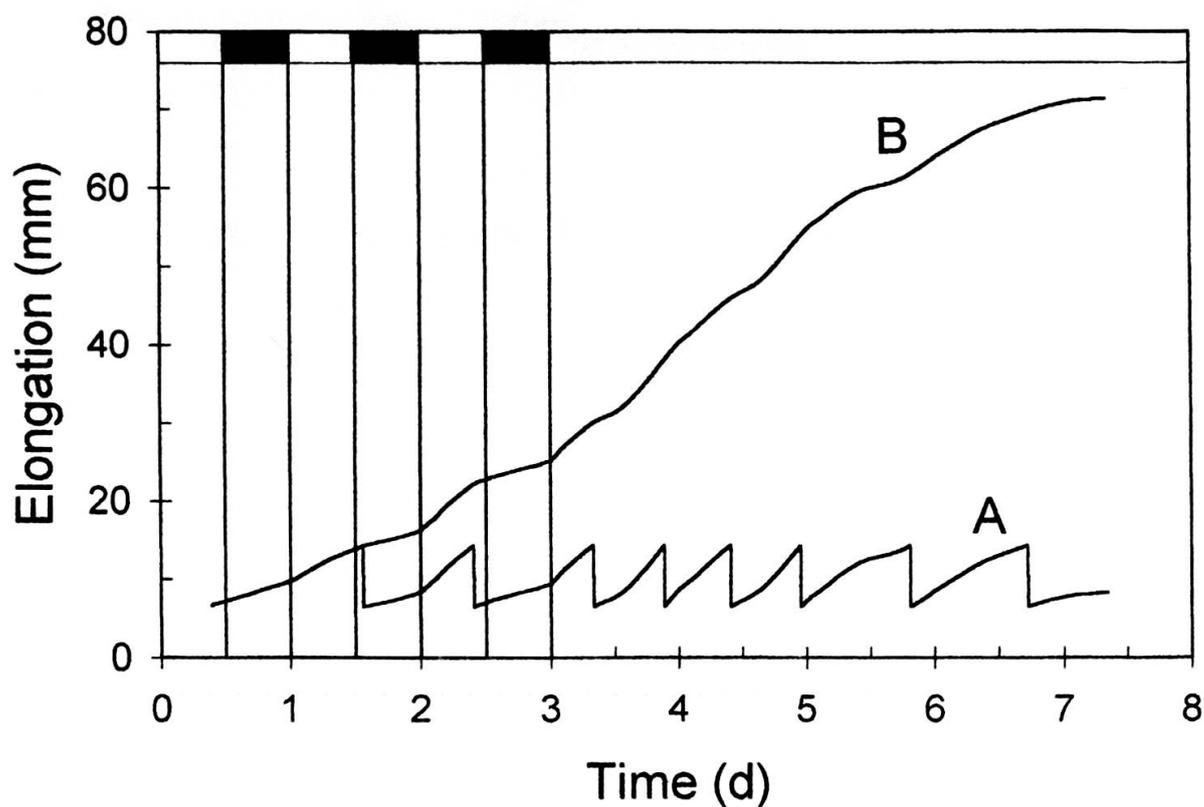


FIG. 4.

Elongation of the first floral stem internode in *Arabidopsis thaliana* measured by the LVDT sensor in L:D 12:12 then in continuous light conditions. A: original data with readjustment of the position of the sensor by the computer controlled motor. B: Same data after removing the motor uplifting (curve A cumulated).

core of the observations previously described on the unfiltered time series, but with considerably less noise.

The comparison of Fig. 5A and Fig. 3 (Rate curve) is interesting. Indeed, the noise range with only the cotton thread is about  $0.05 \text{ mm.h}^{-1}$ , whereas with the plant it is about  $\pm 0.1 \text{ mm.h}^{-1}$ . This difference could eventually be attributed to a higher mechanical instability of the system with a plant. Alternatively, it could also be due to some other signals generated by the plant itself. A zooming in the stem elongation rate measurements at the 5th day is presented in Fig. 6A, together with a signal generated by only a cotton thread (Fig. 6B). It is clear that some deterministic signal is present in the trace created by the plant. Intermediate high and low extension rate values which repeat themselves are present, their mean period evaluated by a visual inspection is in the order of 30 min.

## DISCUSSION

The floral stem growth of *Arabidopsis thaliana* exhibits a large range of dynamics. Over the period of 1 week, a sigmoidal like evolution of elongation is obtained. When we look at the extension rate, a clear daily rhythm is visible. After entertainment by an

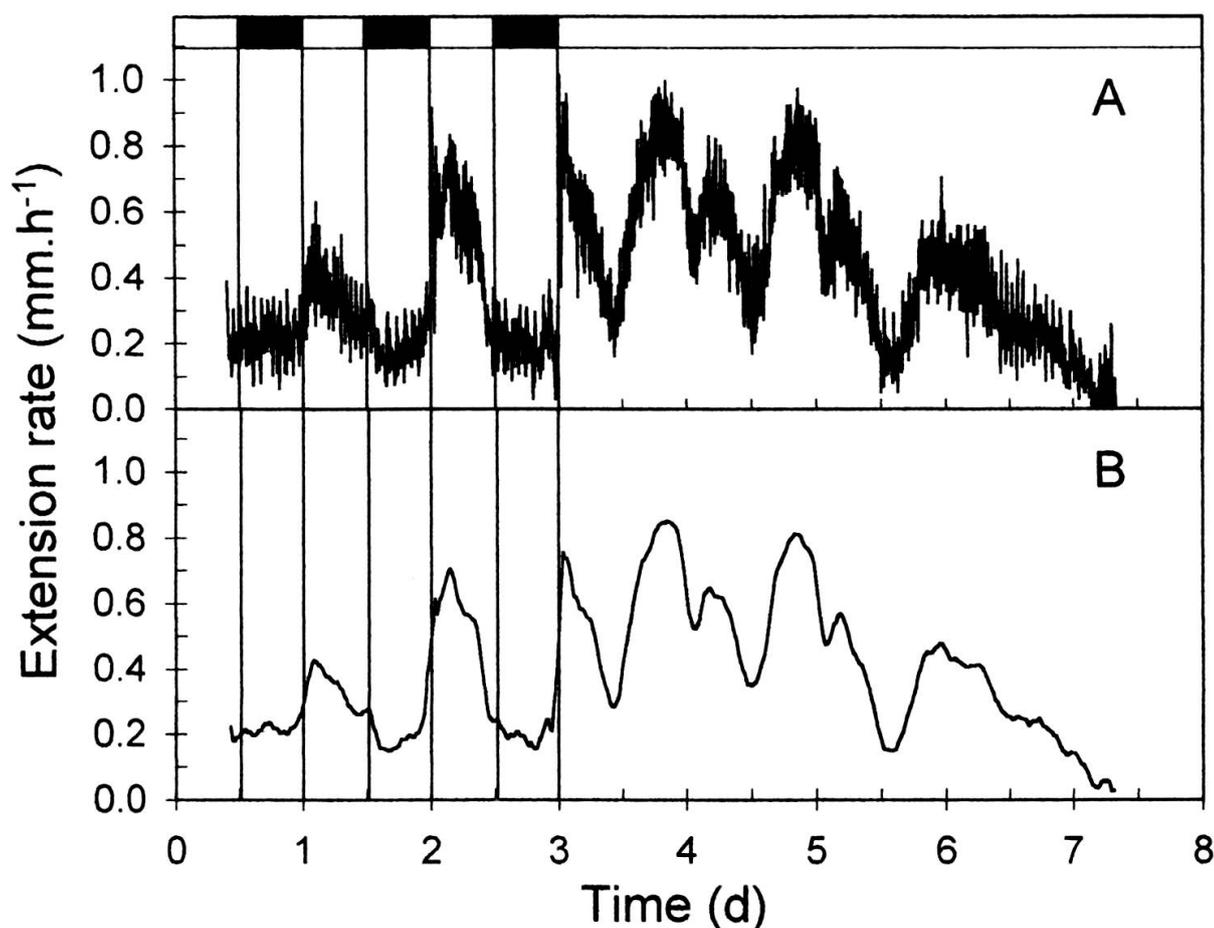


FIG. 5.

Extension rate of the first floral stem internode in *Arabidopsis thaliana* measured by the LVDT sensor in L:D 12:12 then in continuous light conditions (same data as in Fig. 4). A: unfiltered time series. B: same data as in A, but with data filtered to remove high frequency noise.

alternating light-dark (L:D) 12:12 photoperiod, this phenomenon persists in continuous light. This is characteristic of processes under the control of a biological clock (EDMUNDS, 1988). More elaborate studies have been done in this plant which fully demonstrate that the floral stem growth is a circadian rhythm (JOUVE *et al.*, 1998). Circadian rhythm in stem growth are not uncommon since they have already been observed in other plants such as tomato (ASSAD-IBRAHIM *et al.*, 1981), *Chenopodium* (LECHARNY & WAGNER, 1984) and soybean (OGIER *et al.*, 1987).

At a lower time scale (hours), we observe the presence of clear rapid oscillations (~30 min). This is a typical ultradian periodicity. It is known that plant movements display ultradian rhythms and particularly with the stem. For this latter the phenomenon is called circumnutation (BAILLAUD, 1962). It consists of a more or less, depending on the plant species, regular stem rotation. It may be or not linked to the growth process itself, but its main feature is its reversibility unlike growth, which is a cumulative process. In our situation, the bending of the stem may be sufficient to create changes in

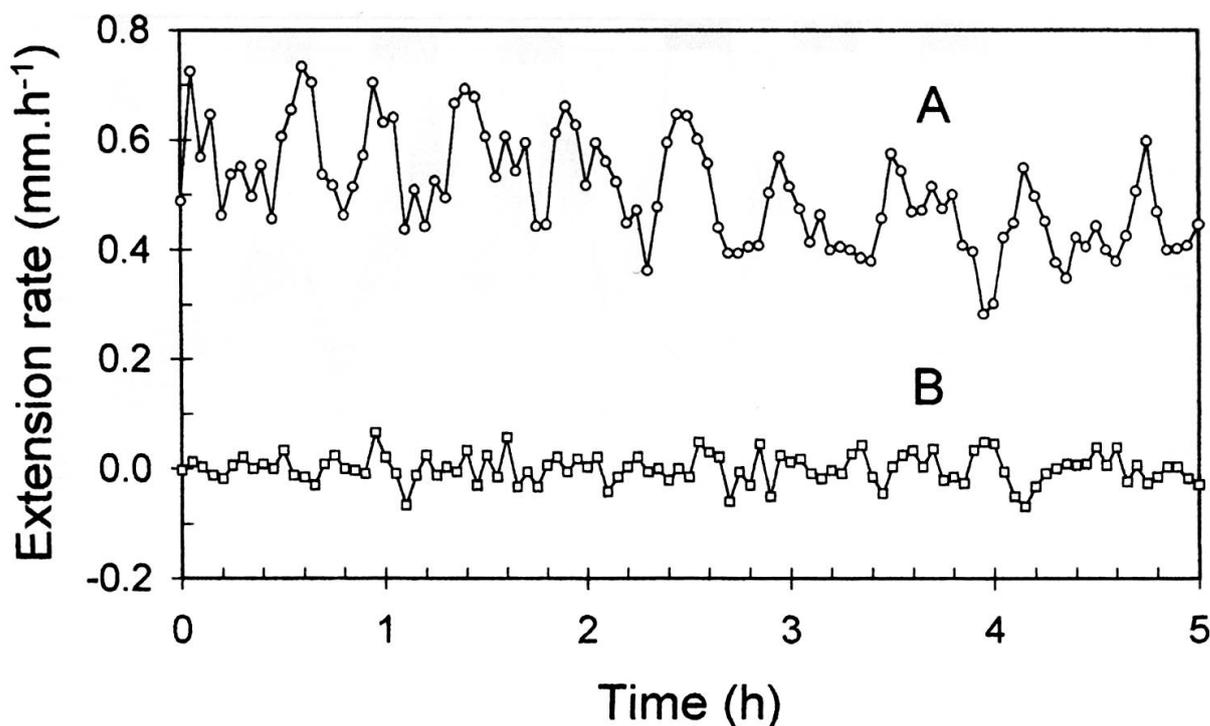


FIG. 6.

Floral stem extension rate in *Arabidopsis thaliana* with high frequency oscillations obtained by zooming in the time series of Fig. 5A (5th day). A: each point is sampled at 3 min. intervals. B: a control cotton thread without any plant attached.

the LVDT sensor position and could thus explain the presence of such oscillations. Nevertheless, its true nature deserves further investigations.

The presence of a circadian and ultradian rhythm in stem is interesting since this organism is widely used as a rosetta stone for investigations in plant molecular biology in general, and also more precisely for understanding the mechanisms of the biological clock (MILLAR & KAY, 1997; MILLAR *et al.*, 1995). The physiological aspects illustrated here could thus be now investigated together with molecular biology manipulations (transgenic plants or mutants).

The large dynamics range of phenomenon's observed are the results of the high performances achieved by the LVDT sensor-based system developed and presented here. The utilization of the concept of computer dedicated with immediate out-line analysis allows a rapid monitoring and treatment of experimental data, which is indispensable to correctly conduct experiments. Moreover, this simplify considerably the control software on the dedicated computer by minimizing interference's with other programs. The presence of 12 LVDT channels which could be monitored starting at a frequency of one measurement per second is also an important feature. We have illustrated here the stability and resolution of the system with *Arabidopsis* plants in these respects. The computer assisted measurement of plant growth system is part of a more important project (Plant Physiomatics: GREPPIN & DEGLI AGOSTI, 1997) which will

address to high space-time resolution plant physiology on whole intact organisms in order to understand their internal network organization and analyze the significance of subtle signals emitted by the plant with respect to this internal organization. The use of high technology sensor-based methods together with computer acquisition and data treatment will be an indispensable tool for this purpose.

## RÉSUMÉ

### MESURES ASSISTÉES PAR ORDINATEUR DE LA CROISSANCE CHEZ LES VÉGÉTAUX A L'AIDE DE TRANSDUCTEURS LINÉAIRES DE DÉPLACEMENT (LVDT)

Un système permettant de mesurer la croissance chez les végétaux avec une grande précision ( $\sim 2 \mu\text{m}$ ) et à haute fréquence (1 mesure par seconde) est décrit. Il est construit avec des capteurs de déplacement LVDT et commandé grâce à un ordinateur qui lui est dédié. Celui-ci possède un logiciel qui lui permet de faire "hors ligne" des analyses et des traitements de données de manière quasi immédiate. Ce logiciel contrôle automatiquement la position de chaque senseur sur une distance d'élongation de 15 cm au moyen du déclenchement de moteurs déplaçant les LVDT pour une mesure optimale. L'ensemble est constitué de 12 senseurs LVDT avec 4 autres canaux qui mesurent les paramètres environnementaux (température, humidité relative et intensité lumineuse). La croissance de la hampe florale d'*Arabidopsis thaliana* a été suivie en conditions photopériodiques L:D (12:12) suivie par de la lumière continue. Les alternances synchronisent un rythme de croissance avec maximum pendant la phase lumineuse. En conditions constantes, ces fluctuations de faible et forte croissance persistent avec une période de  $\sim 24\text{h}$ . Ces propriétés sont typiques des phénomènes sous contrôle d'une horloge biologique. A une autre échelle de temps (heure), l'enregistrement de la vitesse de croissance montre des oscillations possédant une périodicité ultradienne ( $\sim 30 \text{ min}$ ). Ces exemples illustrent la grande capacité dynamique et la résolution de l'installation mise au point.

**Mots-clés:** *Arabidopsis thaliana*, croissance, hampe florale, LVDT.

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