Zeitschrift: Archives des sciences et compte rendu des séances de la Société
Herausgeber: Société de Physique et d'Histoire Naturelle de Genève
Band: 55 (2002)
Heft: 3

Artikel: Fluorescent light-independent computer-assisted imaging of shape changes and movements of Arabidopsis plants : with digital cameras and infra-red light

Autor: Degli Agosti, Robert / Greppin, Hubert / Quirici, Giovanni

DOI: https://doi.org/10.5169/seals-740299

Archs Sci. Genève	Vol. 55	Fasc. 3	pp. 149-160	Décembre 2002

FLUORESCENT LIGHT-INDEPENDENT COMPUTER-ASSISTED IMAGING OF SHAPE CHANGES AND MOVEMENTS OF *ARABIDOPSIS* PLANTS WITH DIGITAL CAMERAS AND INFRA-RED LIGHT

BY

Robert DEGLI AGOSTI*, Hubert GREPPIN & Giovanni QUIRICI

Communication présentée à la séance du 6 décembre 2001

ABSTRACT

Fluorescent light-independent computer-assisted imaging of shape changes and movements of *Arabidopsis* plants with digital cameras and infra-red light. - A high pass cut-off filter (850 nm) installed in the cameras eliminates fluorescent light (up to 750 nm) before reaching the image sensor. InfraRed (IR) Light Emitting Diodes (LED at 950 nm) provide the constant source of object (plants or part of them) illumination. With the system described, an image has a resolution of 582 x 752 = 437664 pixels with 256 gray levels and can be acquired/treated each second by a computer. Tests of performance show that a very small leakage of fluorescent light can occur (~0.48%) when a piece of paper covering almost the full range of gray levels (0-255) was examined, whereas no leakage was observed when objects were contrasted.

No apparent biological effect was observed with IR, as inferred by the typical etiolated aspect of *Arabidopsis* seedlings germinated in the absence of fluorescent light. In the same plant, a clear circadian rhythm is observed with the cotyledon movement. However, leaf surface kinetics examined during 3 $^1/_2$ days in adult plants cultivated in L:D (12:12 h) displayed a more complex "behavior" of growth and movements.

Key words: *Arabidopsis thaliana*, Infrared light, non-invasive method, digital cameras, biological rhythm.

INTRODUCTION

For a plant, and likely most of multicellular organisms, shape is the ultimate integrated result, from the subcellular to whole organism level, of all internal forces and their interactions with the environment. Shapes changes and movements are thus the «real time» kinetic expression of this fundamental fact. It may seem surprising that such an important aspect receives, in plant science, comparatively little scientific attention nowadays. Perhaps, a cause for this is, as quoted by HALLÉ (1999), that morphology is still lacking a quantitative approach and coherent theory. This may contribute to erroneously induce sometimes the idea that form is a secondary trait.

Besides the use of shape as a determinant factor in systematic, in plants, this parameter is reduced to elementary units of length, surface and volume, including biomass (weight, etc...) indicators. These latter's are extremely useful, but lack the precision to represent what is really going on at the plant level. A huge amount of information is lost in this way, and the general prejudice that humans have concerning plants as being

* Corresponding author, University of Geneva, Plant Physiomatics unit and L.B.M.P.S., Place de l'Université 3, CH-1211 Genève 4, Switzerland. E-mail: Robert.degliAgosti@bota.unige.ch

only static objects is reinforced. For example, high sensitivity and time resolution of the stem growth in *Arabidopsis* has revealed an unsuspected complex variability of its dynamical "behavior" (DEGLI AGOSTI *et al.*, 1997; JOUVE *et al.*, 1998, 1999, 2000a&b).

In such perspective and in the framework of whole-plant physiology approach by non-invasive methods (DEGLI AGOSTI *et al.*, 2000), kinetic digital imaging appears as a technique of first choice. Ruge (1961) has done an early historical analysis of the concept of snapshot sequences (time lapse) to characterize plant movements. It seems that the first idea has been presented in 1900 by PFEFFER. Since then, from original photography to nowadays digital imaging, important progress have been made. A known pitfall of this technique is the fact that it is necessary to obtain images also in complete darkness. In this respect, early studies have suggested the use of "non-photomorphogenetic and non-photosynthetic" green low intensity light ("safe light") as an equivalent of "dark" treatment for plants. However, the use of "safe light" will not allow to obtain the same image whether it is obtained in "darkness" (low green) or in the presence of light, due to the overlapping of the green and normal white light source spectra. The first suggestion to use infrared light for dynamic imaging of objects in this context seems to have been done by RIECK in 1953, using a conventional cinematography method.

Arabidopsis thaliana is nowadays a very useful and frequently used plant for basic research in genetics, biochemistry, molecular biology and also plant physiology (MEYEROWITZ, 1989; DEGLI AGOSTI *et al.*, 2000, see also http://www.arabidopsis.org/). Kinetic whole plant digital imaging has been realized with this plant in relation either with biological rhythms, using dim green "safe light" by SCHUSTER and ENGELMANN (1997), by luciferase bioluminescence and conventional light (MILLAR *et al.*, 1995; DOWSON-DAY & MILLAR, 1999), or for fine hypocotyl growth in darkness using infra-red light (PARKS and SPALDING, 1999). However none of these methods allowed kinetic digital image acquisition during light/dark transitions.

We present here a method and the corresponding installation to measure shape changes and movements independently of photosynthetic light (fluorescent tubes) conditions. Its principle is presented in Fig. 1. The actual continuous source of imaging light is provided by infrared LEDs (light emitting diodes at 950 nm). Conventional fluorescent light (ranging from 350 –700 nm) whether present or not is eliminated by a high pass filter in the digital cameras (RG 850 nm).

MATERIAL AND METHODS

Plant Material

For cotyledon movements, *A. thaliana* (C24) seedlings were grown in potting compost under L:D (16:8 h) for 6 days. Then they were individually transplanted in a new pot and cultivated in L:D (12:12 h) for 2 days further. At the age of 8 days (time 0) image acquisition was started. Measurements were done during 1 day in D:L (12:12 h) and then continuous light was applied. Light was provided by fluorescent lamps (Sylvania 36W Luxline-Plus) with an intensity of 75 µmol $m^{-2}s^{-1}$ (PAR). Growth and experiments were done in an environmental controlled chamber (ECC1) with a temperature of 22 ± 0.5°C and a relative humidity of 80 ± 6%.

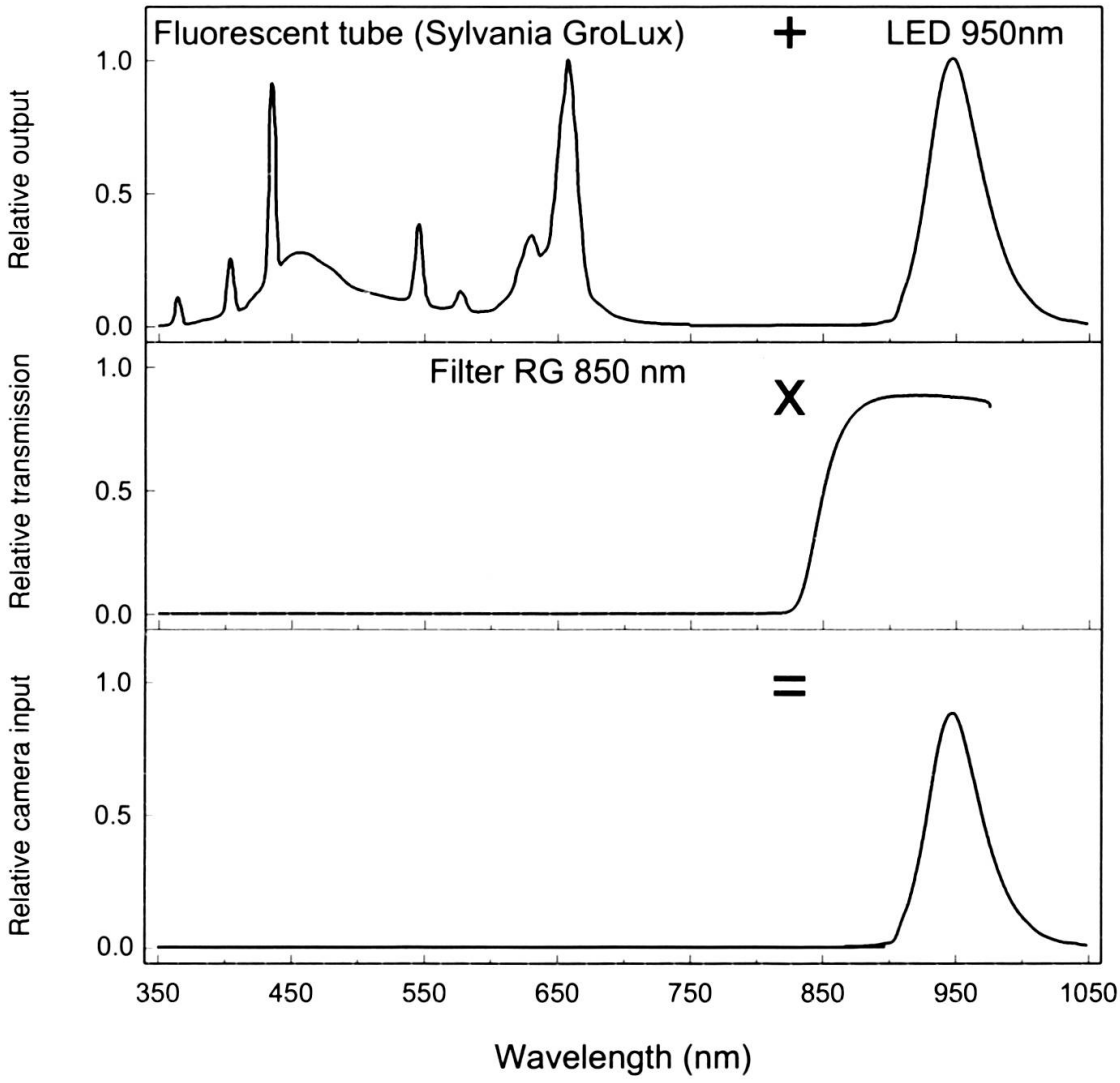

Fig. 1.

Principle for the continuous measurement of shape changes and/or movements in plants submitted to artificial light (fluorescent tubes). The plant material is continuously illuminated with a non-photosynthetic and non-photomorphogenetic light (LED at 950 nm, ~80 nm half bandwidth). When present, light is removed before reaching the sensitive part of the camera by RG filters (850 nm half-bandwidth cutoff). The sensitive cameras independently of the presence of fluorescent light could thus detect objects reflecting IR.

For leaf growth/movement *A. thaliana* (ler) was grown for ~3 weeks as above. Then transferred to an environmental controlled chamber (ECC2; 23 ± 1 °C, 60 ± 5% rH, Sylvania Gro-Lux F36W with PAR of ~30 µmol $m^{-2}s^{-1}$, 12:12h L:D).

For seedling growth in darkness, *A. thaliana* (C24) seeds were imbibed on distilled deionized water over a black filter paper (no 508, Schleicher and Schuell, D) in plastic boxes according to SCHUSTER and ENGELMANN (1997), for 2 days at 4°C in complete darkness (the box was warped with aluminum foil and put in a second box). Then the seeds were removed from 4°C and put in the environmental controlled chamber (ECC2; same conditions as above); light (same as above) was given for 30 min. During this period, the seeds were positioned with respect to the cameras with the IR LEDs sources on. Measurements were realized at 15 min interval in complete and carefully controlled

darkness. Time zero was defined at the transition from 4°C to 22°C. At the end of the experiment seedling's lengths were measured under a microscope.

Advanced infra-red 3D camera (AIR3D) installation

The installation has been partially described in a preliminary communication (DEGLI AGOSTI & GREPPIN, 1998) and is presented in Fig. 2. Two CCD cameras (B&W, KAPPA CF 8/1, 582x 752= 437664 pixels, 256 gray levels, VIDEAL, CH) together with 2 (15W) Infra-Red light emitting diodes (LED) as light sources at 950 nm (WFL-II/LED15 with a power supply NE-109/VT, Videor Technical, D), are mounted on an adjustable support (Combirohr, FOBA, CH). The presence of more than one camera, may potentially allow 3 dimensional (3D) reconstruction of movements, although this is in practice a challenging goal. The cameras are equipped inside with filters (RG 850 15x15, Balzers) to remove any light with a wavelength less than 850nm. For small plants (like *A. thaliana*) macrophoto lenses (35mm f/2.8, CANON, J) can be fitted to the cameras. Alternatively, standard lenses (Fujinon TV 1:1.4/16, FUJI OPTICAL LENSES, J) could be used. The cameras are connected to KTN-Y/C boxes (KAPPA, VIDEAL, CH) which process the images to the rest of the installation and powered the cameras. Images are grabbed to a computer (Pentium 75 MHz, 1.3 Gb, 16M RAM) by a framegrabber PCI card (Prysm PCI color Framegrabber, Synoptics, UK). A set of 2 images can be acquired each 2 s.

Three channels are available (R,G,B); 2 of them (R, G) being used each by one B/W camera. An application software developed with Semper for windows (Synoptics, UK) and Visual Basic (Microsoft) allows a controlled image acquisition and treatment if needed (Plant3D, Gloor Instr., CH). The software allows 3 modes of operation:

- "Time Lapse": images are stored for further treatments (image post-processing);
- "Little plants": Regions of Interest (ROI) are defined, after a procedure of thresholding, the object of interest in the ROI is defined. The area, the coordinates of the center of gravity and other parameters of each object, inside each ROI, are computed and stored without the images. If more than one object in one ROI is detected, the image is stored, for further manual post-processing.
- "Markers": high IR absorbing small markers are fixed on the object (plant); ROIs are defined at the beginning of the procedure around each marker the background (rest of the image inside the ROI) is eliminated with a threshold procedure. This allows the detection of the marker only, its center of gravity (position) is computed and stored. The XY coordinates of gravity center of the marker are then followed with time allowing a reconstruction of the trajectory of the objects (plant part). If more than one marker is inside an ROI, the unprocessed image is stored to be analyzed later. After each acquisition, each ROI is automatically moved such as to be centered around the new gravity center of the marker.

When an image is stored, a custom format (*.pic) is used by the Semper program. It needs to be converted into a more standard *.tif format, in order to be processed by other software (Photoshop, Adobe; MATLAB, Mathworks Inc.).

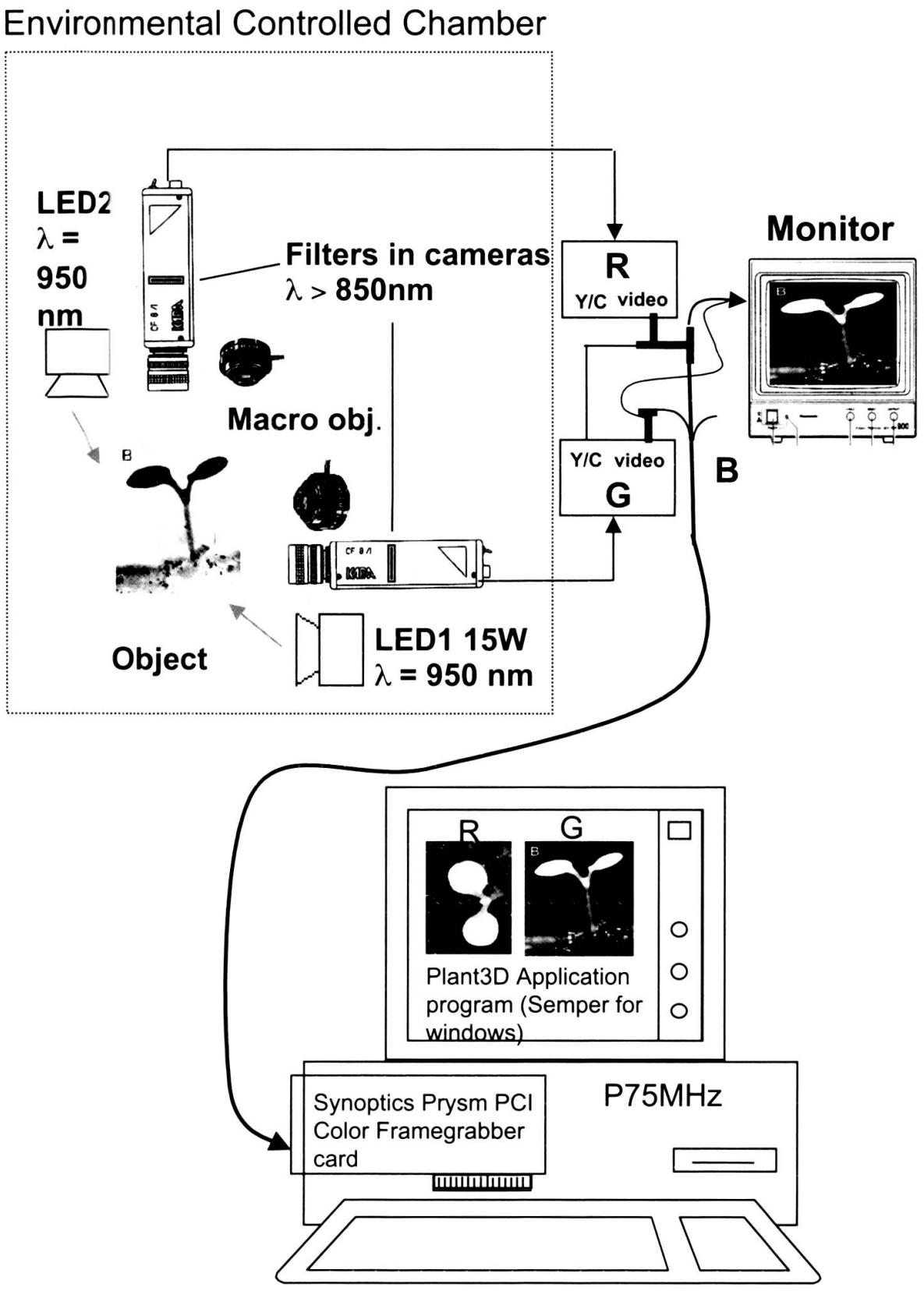

Fig. 2.

Installation for the non-invasive and continuous measurement of shape changes and movements in plants (see text for details).

RESULTS AND DISCUSSION

To examine the efficiency of the fluorescent light removal by the 850 nm high-pass filter, a piece of paper, with a progressive black to white picture, filling almost all the size of the acquisition image dimensions (i.e 582x 752= 437664 pixels), was scanned 10 min before and after a light-off 5 times each (see Fig. 3). The distribution (histogram) of pixels gray levels is from ~20 to ~230 covering a great part of the whole 0-255 range. The dark minus light image difference show a mean gray level diminished by 0.48%. The mean gray level before light-off is 116.245 ± 0.170 and after 115.691 ± 0.134 (n=5). A F-test for equal variances gives an F value of 1.62 (P=0.33) so that the hypothesis of equal variances could not be rejected. A Student t-test for the means gives t=5.73 (P=0.0002), the hypothesis of equal means can be rejected. Thus, in this situation, a very small leakage of fluorescent light is present. It is very likely that increasing the half cutoff of the filter to e.g. 900 nm, would completely remove this difference. However, if experimental changes are in the order of this value, the corresponding adequate controls should be done, otherwise this difference can be neglected. This is confirmed by an analysis of a white piece of paper over a black background examined over a period of minutes with repeated switching off the fluorescent tubes (see Fig. 4). In this case the thresholding procedure to detect the object over the background is sufficient to eliminate short-term differences in the evaluation of the surface. This is not surprising since the thresholding procedure diminishes greatly the gray level range. Another test consisted in examining a similar piece of paper, but over a long-term period of 24 h (Fig. 5). In this situation it is clear that a very small, but clear diminution of the surface is progressively detected in darkness. However, this difference is of ~12 over 17750 pixels (i.e 0.068%). The possibility that this very small change is due to other factors than fluorescent light leakage is not unlikely, since in darkness the diminution kinetic should have been more rapid. Overall, all these results point out to a very high efficiency of the system.

Although the demonstration should be ideally verified in each situation, we provide some evidence in Fig. 6, that the IR light (950 nm) we used is truly inactive photomorphogenetically. It is possible to observe 3 important criteria present in an etiolated plant. First, the length of the hypocotyl is rather long (i.e > 8 mm). This is typical of dark conditions. Indeed, in LL (continuous light) and in short days, DOWSON-DAY and MILLAR (1999) found ~4mm and 6-8 mm respectively. In dim green "safe light" length was higher (~10 mm) as reported by SCHUSTER and ENGELMANN (1997). Second, the segment situated between the upper part of the hypocotyl and the cotyledons is closed (i.e. hook shaped, see Fig. 6), this can also only be obtained in complete darkness (LISCUM & HANGARTNER, 1993). Third, the seedlings were apparently devoid of chlorophyll (visual inspection) whose appearance is a phenomenon triggered by the presence of light only (e.g. ARMSTRONG *et al.*, 1995). Moreover, a study has examined the effect of germination with IR LED sources at 880 nm and 935 nm (JOHNSON & STRYJEWSKI, 1995). They concluded to an effect with the 880 nm due to some leakage in the 730 nm region, whereas with 935 nm LEDs, no differences were observed in comparison to absolute darkness. Similarly, PARKS *et al.* (1998) concluded that no effect was observable with IR LED at 950 nm, in hypocotyl elongation experiments.

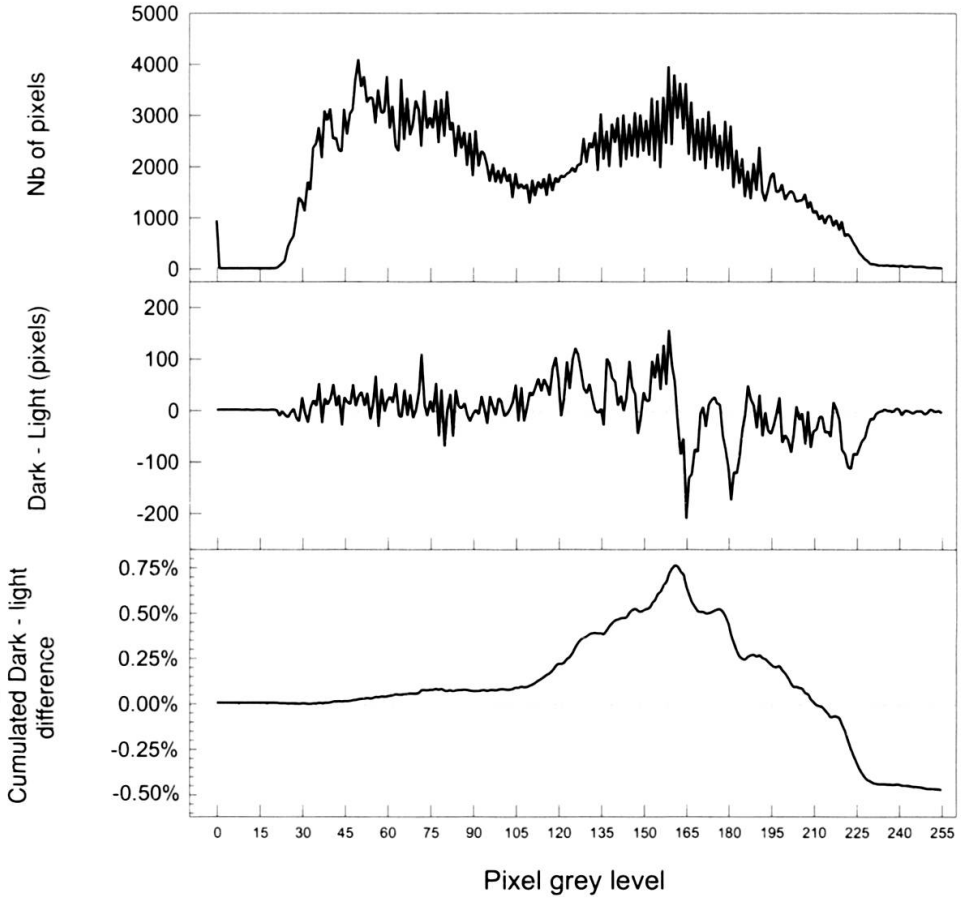

Fig. 3.

(A), Histogram of the gray levels (0-255) of the pixels in an image (piece of paper with a degraded printed gray), before (10 min) switching off fluorescent tubes. (B), Difference histogram of the gray levels between the image after (10 min) minus before (10 min) light-off. Small differences are visible with an excess of pixels from gray levels 105 to 165 and inversely from 165 and upper levels. (C), Relative cumulative difference histogram between dark and light images. The final difference is negative with respect to light by a significant 0.48% lower whole mean gray level of the image.

Fig. 7 shows the rhythmic cotyledons movement of *A. thaliana*. It is possible to observe that the oscillation persists in continuous conditions. This property is typical of a phenomenon under control of the circadian clock. Similar results have been obtained by MILLAR *et al.* (1995), but in constant illumination conditions only.

In-line automatic processing of shapes (leaves) is shown in Fig. 8. Different kinetics are obtained in details during $3^1/_2$ days in alternating L:D 12:12 h. A general positive trend line in ROI1 and ROI2 is representing a significant increase in size (growth), whereas, for leaf no 3, there seems to be no apparent growth. In all cases light-dependent movement is present together with some "anticipating" dynamic. In this situation it is interesting to observe that movements might be out of phase (compare leaf no 1 with no 2 & 3). Clearly more experiences are needed to separate growth and movement and endogenous movement with triggered responses. These are actually the subject of some research at the Plant Physiomatics unit at the University of Geneva.

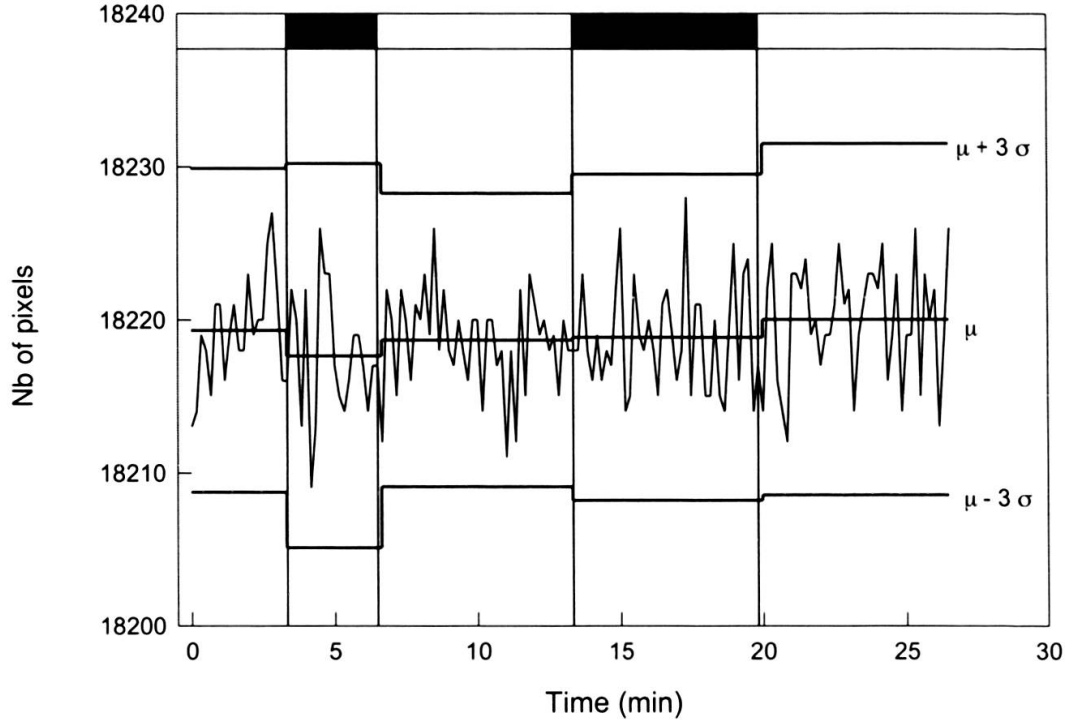

Fig.4:

Medium-term (min) evolution of a thresholded artificial surface (piece of white paper) during different fluorescent conditions (black stands for darkness). No significant differences are visible. μ is the mean nb. of pixels and σ: one standard deviation during the different periods.

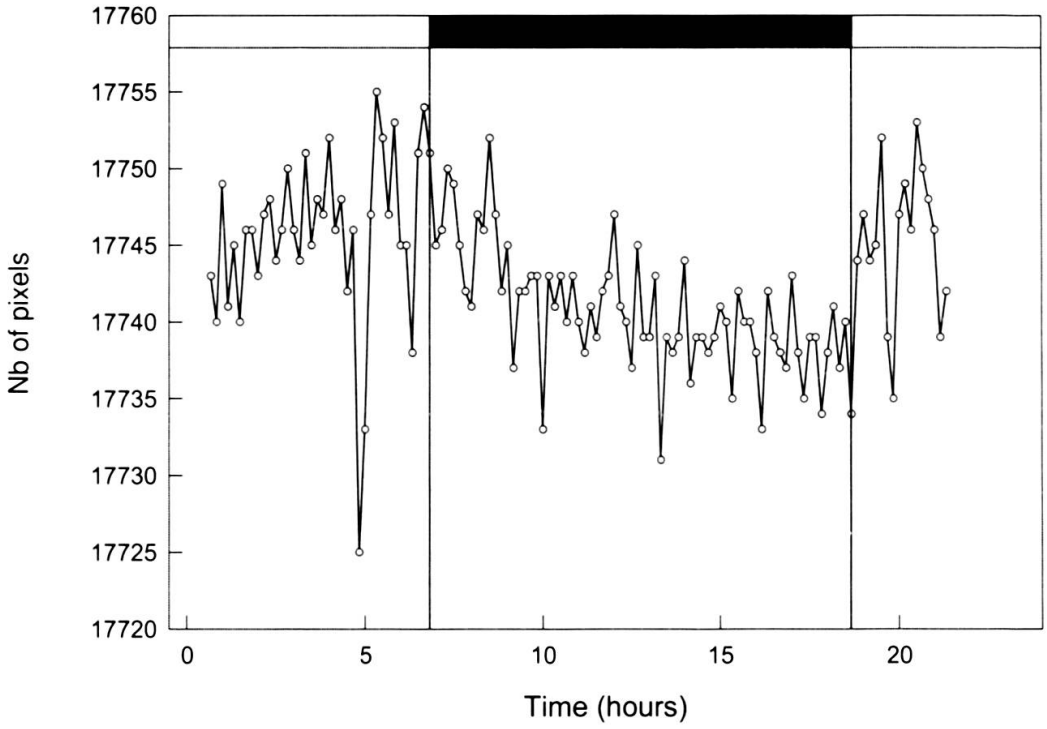

Fig. 5.

Long–term (day) changes of the evaluation of an artificial surface (piece of white paper) in an image in presence (white) or absence (black) of fluorescent light. A slightly higher surface (~12 pixels, i.e. ~0.07%) is observed in presence of fluorescent light.

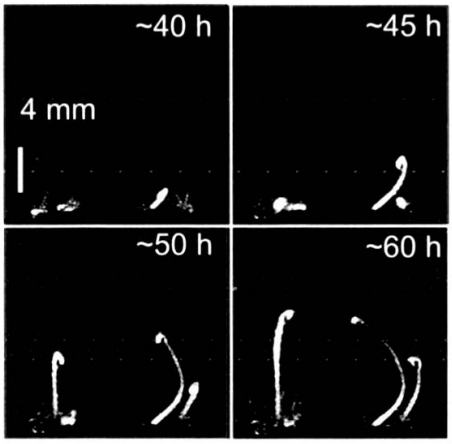

Fig. 6.

Germination of *Arabidopsis* and seedling development without fluorescent light at different times after sowing. Note the hook shaped cotyledons at the extremity of the relatively long hypocotyl, typical of plants in absence of light. IR illumination was continuously present.

CONCLUSION

"Intelligent" image analysis will increasingly be in the future a very important tool in general, and also in plant biology. Potentially, any shape change, either irreversible (growth, development, differentiation) or reversible (movements) might be addressed with the method of computer-assisted image analysis. However, some technical (software and hardware) developments are still needed. This in order for the computer to "intelligently" transform shape into a coherent aggregated human understandable and representative indicator. Theoretical efforts may also be needed in this respect as these parameters are developing in 5 dimensions (time = t & space = x, y, z and intensity/quality, i.e. gray level or color). The impact may be significant because this will allow detection of spatio-temporal dynamic of the plant with unprecedented resolution in relation to environmental fluctuations. For this, it is necessary that the system could be able to detect the plant at various dimensions scales and particularly, in darkness as well as in light conditions. We have described here such a prototype system for *Arabidopsis* which, in principle, might be used also for other plants growing under fluorescent tubes. However, more research and development is still necessary to design a system, which will be able to efficiently work in the natural environment, independently of light (i.e. sunlight) conditions.

RÉSUMÉ

IMAGERIE ASSISTÉE PAR ORDINATEUR DE CHANGEMENTS DE FORME ET MOUVEMENTS DES PLANTES D'*ARABIDOPSIS* AU MOYEN DE CAMERAS DIGITALES ET DE LUMIÈRE INFRA-ROUGE INDÉPENDAMMENT DE LA PRÉSENCE OU ABSENCE DE LUMIÈRE

Un filtre passe haut (850 nm) installé dans les caméras élimine la lumière fluorescente (jusqu'à 750 nm) avant d'atteindre le senseur d'image. Des diodes électrolumines-

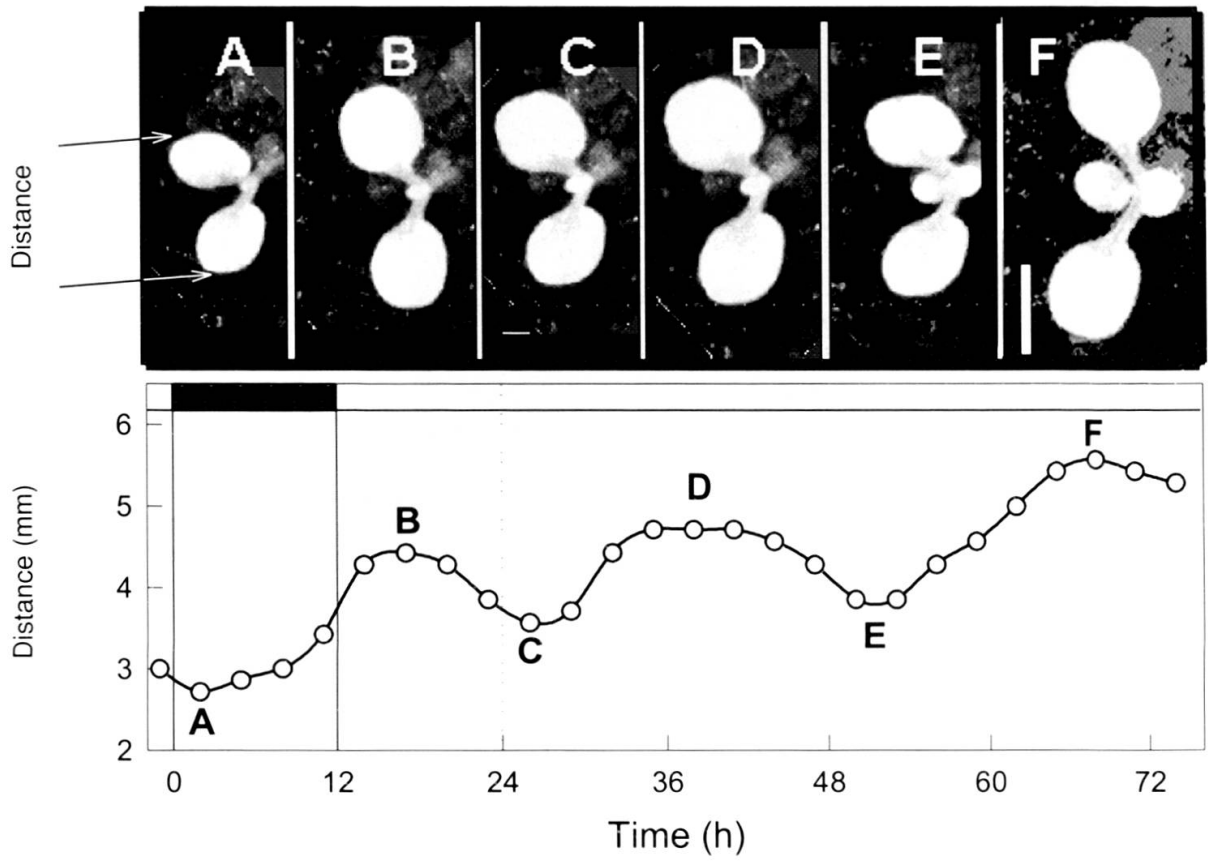

Fig.7.

Example of an image acquisition of *Arabidopsis* seedlings (upper view). The distance between the cotyledons has been post processed and its evolution is shown in the lower graphic. The black bar represents dark fluorescent conditions. The white bar in image F is 2.5 mm long. Acquisition of image was in the "Time lapse" mode.

centes (LED à 950 nm) infra rouge (IR) fournissent la source d'éclairement constant des objets (plantes ou leurs parties). Avec le système qui est décrit, une image possède une résolution de 582 x 752 = 437664 pixels avec 256 niveaux de gris et peut être enregistrée/traitée chaque seconde via un ordinateur. Des tests de performance montrent qu'il peut exister une très faible contamination par la lumière fluorescente (~0.48%) lorsqu'on utilise une image couvrant pratiquement l'ensemble de tous les niveaux de gris (0-255), alors que dans le cas d'images bien constrastées aucune contamination n'a été détectée.

Aucun effet biologique manifeste n'a été observé du aux sources IR lorsqu'on examine des plantules d'*Arabidopsis* qui possèdent un aspect typiquement étiolé lorsqu'elles sont germées en l'absence de lumière fluorescente. Chez la même plante, on observe un rythme circadien bien visible du mouvement des cotylédons. En revanche, la cinétique de surface de feuilles de plantes adultes cultivées en L:D (12:12h) examinée pendant $3^1/_2$ jours montre une dynamique plus complexe de croissance et mouvements.

Mots-clefs: *Arabidopsis thaliana*, lumière infra-rouge, méthodes non-invasives, camera digitale, rythme biologique.

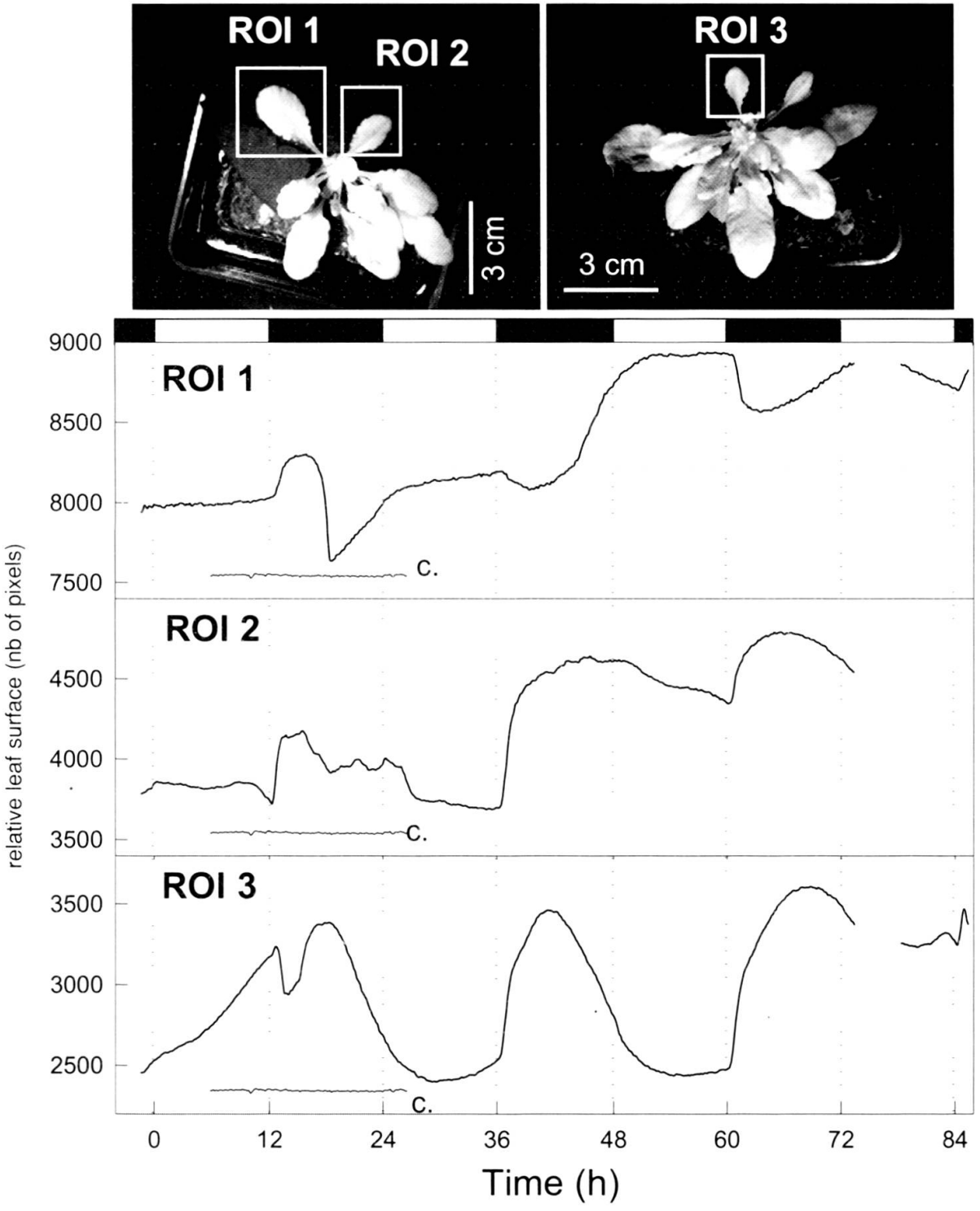

Fig. 8.

Leaf growth/movement of *Arabidopsis*. Different Regions of Interest (ROIs) including leaves were defined. An adequate threshold gray level was set for each camera in order to increase the contrast between the leaves and background. Each 15 min the ROIs were processed to calculate their total number of pixels above the defined thresholds (i.e. leaves) and stored. The different relative surface dynamics is shown in the time graphics below. c. is a control made with a piece of paper at the same relative scale as leaves (data is from Fig. 5).

ACKNOWLEDGEMENTS

With do thank O. Reverchon for his help in some experiments and Jurg Meyer (Gloor Instruments AG, CH).

BIBLIOGRAPHY

ARMSTRONG, G.A., S. RUNGE, G. FRICK, U. SPERLING & K. APEl. 1995. Identification of NADPH:protochlorophyllide oxidoreductases A and B: A branched pathway for light-dependent chlorophyll biosynthesis in *Arabidopsis thaliana*. Plant Physiol. 108: 1505-1517.

DEGLI AGOSTI, R., L. JOUVE, P. FAVRE & H. GREPPIN. 2000. Non-invasive whole-plant physiology in Arabidopsis. In: Integrated plant systems (H. Greppin et al. , eds.) University of Geneva, Geneva, pp. 55-77.

DEGLI AGOSTI, R. & H. GREPPIN. 1998. Computer-assisted monitoring of plant movements with Infra-Red (IR) cameras. USGEB98 5-6 march 98, Lausanne, Suisse, S4-21.

DEGLI AGOSTI, R., L. JOUVE & H. GREPPIN. 1997. Computer-assisted measurements of plant growth with linear variable differential transformer (LVDT) sensors. Archs. Sci. Genève 50: 233-244.

DOWSON-DAY, M.J. & A.J. MILLAR. 1999. Circadian dysfunction causes aberrant hypocotyl elongation patterns in *Arabidopsis*. The Plant Journal 17: 63-71.

JOHNSON, C.F. & E.C. STRYJEWSKI. 1995. Infra-red light-emitting diode irradiation affects *Arabidopsis* germination. Plant Physiol. 108 Suppl, no 259, p. 62.

JOUVE, L., H. GREPPIN & R. DEGLI AGOSTI. 2000a. Floral stem growth of *Arabidopsis* ecotypes. I. Differences during synchronized light regime and continuous light free run. Archs. Sci. Genève 53: 207-214.

JOUVE, L., H. GREPPIN & R. DEGLI AGOSTI. 2000b. Floral stem growth of *Arabidopsis* ecotypes. II. Short time scale events and evidence for ultradian rhythms. Archs. Sci. Genève 53: 215-224.

JOUVE, L, A. MENZIES A, H. GREPPIN & R. DEGLI AGOSTI. 1999. *Arabidopsis thaliana* floral stem growth: possible intervention of two different rhythms. In: Symbiosis and Eukaryotism. Endocytobiology VII. E Wagner et al (eds) University of Geneva Switzerland p 603-610.

JOUVE, L., H. GREPPIN & R. DEGLI AGOSTI. 1998. *Arabidopsis thaliana* floral stem elongation: evidence for an endogenous circadian rhythm. Plant Biochem. Physiol. 36: 469-472.

LISCUM, E. & R.P. HANGARTER. 1993. Light-stimulated apical hook opening in wild-type *Arabidopsis thaliana* seedlings. Plant Physiol. 101: 567-572.

MEYEROWITZ, E.M. 1989. Arabidopsis, a useful weed. Cell 56:263-269.

MILLAR, A.J. , I. CARRÉ, C.A. STRAYER, N.-H. CHUA & S.A. KAY. 1995. Circadian clock mutants in *Arabidopsis* identified by luciferase imaging. Science 267: 1161-1163.

PARKS, B.M. & E.P. SPALDING. 1999. Sequential and coordinated action of phytochromes A and B during *Arabidopsis* stem growth revealed by kinetic analysis. Proc. Natl. Acad. Sci. USA 96: 14142-14146.

PARKS, B.M., M. H. CHO & E. SPALDING. 1998. Two genetically separable phases of growth inhibition induced by blue light in *Arabidopsis* seedlings. Plant Physiol. 118: 609-615.

PFEFFER, W. 1900. Die Anwendung des Projektionsapparates zur Demonstration von Lebensvorgängen. Jb. Wiss. Bot. 35: 711-745.

RIECK, J. 1953. Kinematographie in der Dunkelheit. Naturw. Rundsch. H. 7: 289-293.

RUGE, U. 1961. Methoden der Wachstumsmessung. In: Handbuch der Pflanzenphysiologie (Ruhland W. ed.), Band XIV, Springer-Verlag, Berlin, pp.46-70.

SCHUSTER, J. & W. ENGELMANN. 1997. Circumnutations of *Arabidopsis thaliana* seedlings. Biological Rhythm Res. 28: 422-440.