SPATIAL AND TEMPORAL PATTERNS OF CELL DIVISION AND TISSUE EXPANSION AS AFFECTED BY TEMPERATURE AND WATER DEFICIT IN SUNFLOWER LEAVES.

Christine Granier and François Tardieu, Institut National de la Recherche Agronomique,
Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux,
2 Place Viala, 34060 Montpellier, France.
Fax 33 4 67 52 21 16 ; e-mail granier@ensam.inra.fr;

Summary :
Patterns of cell division and tissue expansion were followed in individual sunflower leaves from initiation to the end of expansion, in a large range of environmental conditions including temperature, radiation and soil water status. Final area of individual leaves at a given position on the stem can have 10-fold variation without changes in epidermal cell area. Time courses of relative leaf expansion rate and of cell division rate are well conserved among a large range of environmental conditions, provided that durations and rates are expressed in thermal time. Maximum relative rates, observed during exponential phases, are common to all zones of a leaf and several leaves of a plant. They are also common to a range of experiments performed at contrasting temperatures, in the absence of water, nutrient or light deficits. A short water deficit which causes neither symptom nor reduction in photosynthesis have considerable effect on final leaf area, especially when imposed at the beginning of leaf development. An apparent 'delayed effect' is observed after rewatering, but can be analysed in a simple way if relative rates of expansion and cell division are taken into account instead of absolute rates. A reduction in PPFD causes no effect on leaf expansion during the rapid growth period, while the leaf exports photosynthates, but considerably reduces the relative expansion rate during early leaf development. As in the case of water deficit, a transient reduction in relative rates of expansion and division results in a permanent reduction in absolute rates.

Introduction :

The leaf area of sunflower can vary several-fold depending on environmental conditions (see Dosio et al. same issue). We have dissected leaf development in order to analyse and model these environmental effects. Leaf expansion can be analysed as a two-phase process Granier and Tardieu 1998). A period with exponential increase in area (constant relative expansion rate) is followed by a period with a decrease in relative expansion rate. No 'linear' phase is observed. The same two-period pattern applies to different leaf strips perpendicular to the midrib, thereby accounting for leaf shape and spatial gradients in the leaf. Strips near the leaf tip are smaller than those near the leaf base because the exponential period is shorter. The same time course and patterns apply to cell division. In this framework of analysis, variables which describe leaf development are (1) relative expansion rate (new mm^2 per mm^2 of leaf and per day), (2) the duration elapsed from leaf initiation to end of leaf expansion, (3) relative cell division rate (number of cells formed per cell and per day) and (4) the duration elapsed from leaf initiation to end of cell division.

The objective of this paper is to present the way in which this pattern of leaf development is affected by environmental conditions, based on previously published papers (Granier and Tardieu 1998ab, 1999ab). This was done on sunflower plants grown in the field, in the greenhouse and in the growth chamber under contrasting conditions of temperatures, available soil water and intercepted light. Spatial and temporal analyses of tissue expansion and cell division were carried out in leaves at two positions on the stem in order to test whether responses to environmental conditions were common to both leaf positions.

Material and Methods :

Plant culture and growth conditions.
Sunflower (*Helianthus annuus* L., Albena) plants were grown in a field near Montpellier (southern France) during 4 growing periods in 1995 and 1996. They were also grown in a greenhouse for 6 growing periods in 1995 and 1996 and in a growth chamber during 4 growing periods in 1997. In all experiments, light was measured continuously using a PPFD sensor. Air temperature and RH were measured every 20 s. Leaf temperature was measured with a copper-constantan thermocouple (0.4 mm diameter) appressed to the under side of the lamina. Environmental conditions during these experiments are presented elsewhere (Granier and Tardieu, 1998b). Water deficit was imposed during 6 experiments in the greenhouse (Granier and Tardieu, 1999a). Variability in intercepted light was imposed either by shading plants or by covering part of the leaf area (Granier and Tardieu, 1999b).

Growth measurement.
Three plants were harvested every second day from germination to the end of leaf expansion (leaf 8 and 16), and observed after dissection under a microscope. A leaf was considered as initiated when its primordium was visible on the apical meristem with the microscope at magnification x10x80. Leaf age was then calculated in days after initiation. Areas of three leaves were measured every second day from initiation to emergence by dissecting the apex under the microscope, excising the studied leaf and measuring its area with an image analyser. When the leaf was 25 mm long , five leaves were photographed with a video camera every day at 12.00 h (solar time) during the expansion period, and areas were determined with the image analyser. Each picture was calibrated with a mark of known length on the leaf. Cell area in the adaxial epidermis of three leaves was measured every second day from five days after initiation until the end of leaf expansion. A transparent negative film of the adaxial epidermis was obtained after evaporation of a varnish spread on the upper face of the leaf. Films were placed under a microscope coupled to the image analyser. The areas of 50 epidermal cells were measured in three to eight transects perpendicular to the midrib. Cell number was estimated as described earlier (Granier and Tardieu, 1998a).
Leaf relative expansion rate (*RER*) at time j was calculated from initiation to the end of expansion as the slope of the relationship between the logarithm of leaf area (A) and time :

$$RER_{leaf,\,j} = [\, d\,(\ln A) / d\,t \,]_j \qquad (1)$$

It was calculated by linear regression on the three coupled values of *A* and t corresponding to times j-1, j and j+1. Relative cell division rate (*RDR*) of the whole leaf was calculated as :

$$RDR_j = [\, d\,(\ln N_{leaf}) / d\,t \,]_j \qquad (2)$$

taking into account cell number per leaf (N_{leaf}) on days j-1, j and j+1.

Results and discussion :
Division and expansion have a two-phase time course which is common to all zones of a leaf and several leaves of a plant.
During the first part of leaf development, absolute increases in leaf area and in cell number are low (Figure 1A), but relative increases in leaf area and in cell number are maximal and nearly constant (exponential increases in both cell number and in leaf area, Figure 1B). The duration of the exponential phase for expansion is longer than that for cell division, but the lag between both processes is short compared to the whole duration of leaf development. Cell division rate and relative expansion rate are uniform in the whole leaf during the first third of leaf development. They cease to be constant first at the tip of the leaf and then progressively towards the base. As a consequence zones at the base of the leaf have a larger final area and larger final cell number than those at the leaf tip (Granier and Tardieu, 1998a).

Time courses of division and expansion rates are common to a large set of conditions with contrasting temperature if expressed in thermal time.

If expressed in clock time, rates and durations of leaf expansion and cell division largely differ between experiments carried out at 14, 18.5 and 26°C (Figure 2A, B). In contrast, they are unified if expressed in thermal time, regardless of the temperature imposed to leaves (Figure 2 C,D). This is due to the fact that the rates of cell division and of leaf expansion increase linearly with leaf temperature, with relationships which applied to all studied conditions (field, greenhouse, growth chamber). In the same way, the reciprocal of the durations of leaf expansion and of cell division are linearly related to leaf temperature with relationships which apply to all studied conditions. Because all these relationships have a common x-intercept of 4.8°C, all processes can be expressed in thermal time with a unique threshold temperature (Granier and Tardieu, 1998b). Time courses of leaf expansion and of cell division have proven to be extremely well conserved for a given genotype in a wide range of conditions, provided that plants experience no stress.

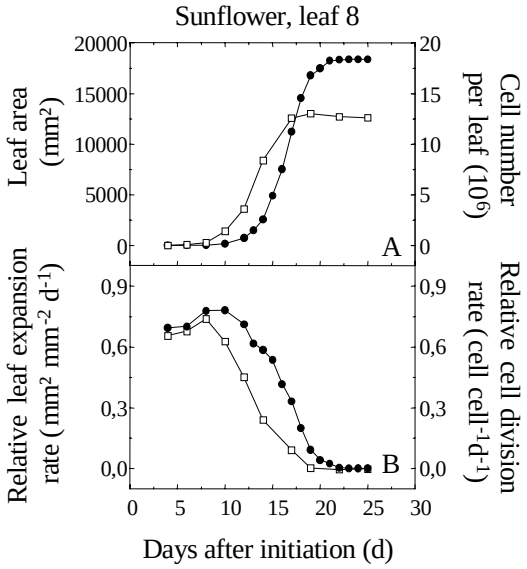

Figure 1. Changes with time in leaf area (A, •), and in epidermal cell number per leaf (A,▫) in sunflower for a greenhouse experiment in July 1996 (experimental conditions are described in Granier and Tardieu, 1998b). Corresponding changes with time in relative leaf expansion rate and in relative cell division rate are presented in B.

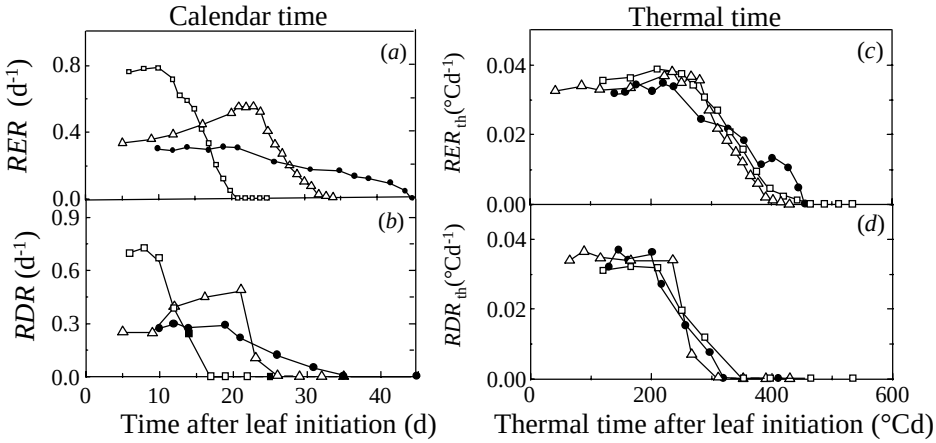

Figure 2. Change with time in relative expansion rate (*RER*, *a*) and in relative division rate (*RDR*, *b*) in leaves 8 of sunflower grown during 3 experiments at contrasting temperatures : in the greenhouse with fluctuating temperature, mean temperature 26°C (—□—), in the field with fluctuating temperatures, mean temperature 18.5°C (—△—) and in the growth chamber at a constant temperature of 14°C —●—. Each point represents the mean calculated from 5 leaves. The same data are presented in panels (*c*) and (*d*) as a function of thermal time. Redrawn from Granier and Tardieu 1998b.

Short water deficits decrease cell number and leaf area without affecting the durations of both processes. Absolute rates of expansion and division are still affected after rewatering but relative rates are not.

The final area of a leaf is largely affected by a five-days period of water deficit which cause neither symptoms nor decrease in photosynthesis (Fig. 3). Early water deficits, imposed when the area of the leaf is of some mm^2 only, have a greater effect than deficits imposed later in leaf development. The duration elapsed from leaf initiation to end of cell division, and that to end of leaf expansion are not affected by these mild water deficits. Only in case of very severe deficits, rarely observed in agricultural conditions (leaf predawn water potential lower than -1 MPa), they are increased by several days.

Early water deficits apparently cause a considerable delayed effect on both expansion and division because the absolute increases in cell number and in leaf area are still affected after rewatering (Figure 3). In opposition to a common idea, this cannot be attributed to a primary effect on cell division during the deficit, which in turn affects leaf expansion after rewatering. In effect, cell division and tissue expansion are affected with similar time courses and both show after effects (Granier and Tardieu 1999a). The apparent delayed effect disappears if the processes of expansion and of cell division are expressed using the framework of analysis presented in Figures 1 and 2. Relative rates of expansion and of cell division are reduced during the deficit only (Fig. 3), but absolute rates are permanently reduced, even after rewatering. Absolute expansion rate observed on a given day is the product of relative expansion rate and of leaf area on the same day. Leaf area is smaller in droughted plants at the end of the water deficit period, so absolute expansion rate remains smaller, even after rewatering. The same reasoning applies to the increase in cell number, which is permanently affected in droughted plants although relative cell division rate recovers shortly after rewatering. The apparent delayed effect of water deficit is therefore a consequence of the exponential characteristic of leaf expansion and cell division.

Early deficits, imposed during the period with both tissue expansion and cell division cause a decrease in both final leaf area and final cell number. Individual cell area is not affected by water deficit in this case because cell division rate and tissue expansion rate are affected by the same extent. Later deficits, imposed when cell division rate has already declined, has a greater effect on leaf area than on cell number, thereby resulting in a smaller individual cell area. Cell area therefore appears as the result of the processes of leaf expansion and cell division, rather than an autonomous variable.

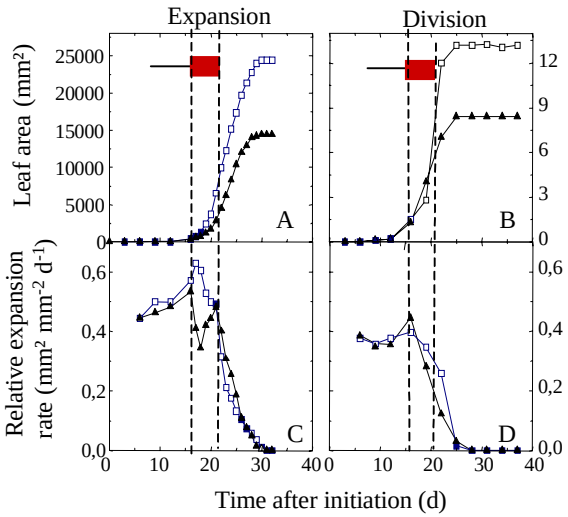

Figure 3. Change with time in leaf area (A) and in cell number per leaf (B) of leaves 8 of control plants (▫) and of plants with early moderate water deficit (▪) during an experiment in the greenhouse in April 1995 (described in Granier and Tardieu, 1999a). Corresponding changes with time in relative leaf expansion rate and relative cell division rate are presented in C and D. Horizontal thin bars, periods with declining soil water content ; horizontal thick bars, periods during which available soil water was maintained at 23% of maximum.

Changes in intercepted light affects leaf expansion rate and cell division rate during early leaf development, but have no effect after the leaf begins to export photosynthates.

A reduction in intercepted light imposed when the leaf is visible has no effect on the area of this leaf (Fig. 4a,c). This is observed when reductions are caused either by shading or by reducing photosynthetic leaf area. In contrast, an early reduction considerably reduces both the final leaf area and cell number (Fig. 4b,d). This is probably due to a carbon effect, because a reduction by 40% in incident light had the same effect as a reduction in photosynthetic leaf area by 40%. As in the case of water deficit, relative expansion rate is reduced for a short period but absolute expansion rate is permanently affected (Granier and Tardieu 1999b).

The leaf is sensitive to change in intercepted light during the first third of leaf development, i.e. when the leaf imports photosynthates. It ceases to be sensitive when cell division ceases to be exponential, i.e. when the leaf begins to export photosynthates. A close relationship was found between final leaf area and the amount of light intercepted during the first period (Fig. 5). A similar relationship was observed with final cell number. As in the case of early water deficits, a reduction in intercepted light has similar quantitative effects on cell division rate and on leaf expansion rate. Individual cell area was therefore not affected by changes in light.

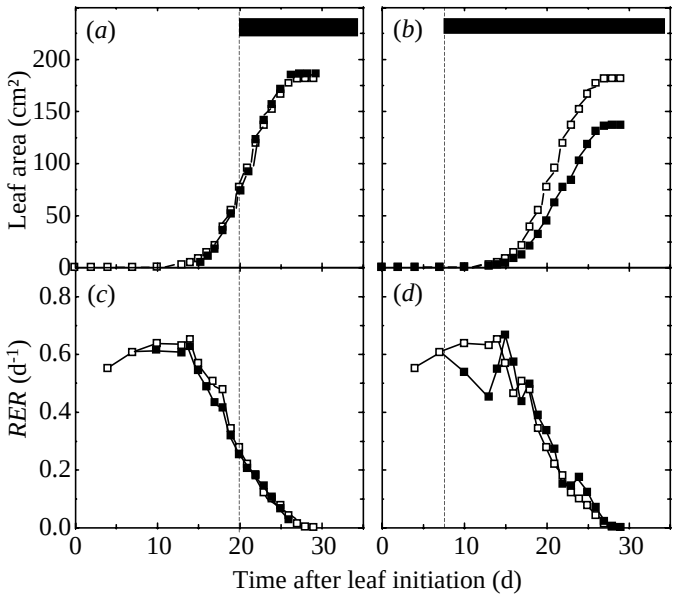

Figure 4. Change with time in leaf area (*a*, *b*) and in relative leaf expansion rate (*RER*, *c*, *d*) of leaves 8 of sunflower for control plants (□) and for plants with late (*a*, *c*) or early (*b*, *d*) reduction in intercepted PPFD (■). Plants were grown in the field with a mean incident PPFD of 45 mol m^{-2} d^{-1} and mean leaf temperature of 21°C during leaf development. Leaves 1, 3 and 5 were covered with aluminium foil, reducing photosynthetic leaf area by 40%. Horizontal thick bars represent the periods with reductions in intercepted PPFD.

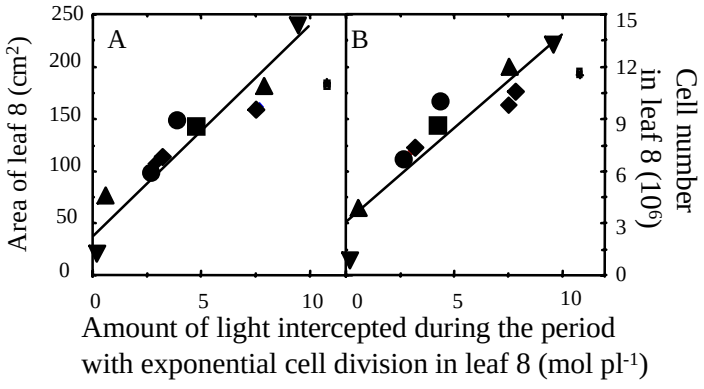

Figure 5. Final area (A) or cell number (B) of leaf 8 as a function of the amount of light intercepted by the plant (leaves 1 to 6) during the period with exponential cell division. Variability in intercepted light was imposed by either a reduction in incident light or by covering a part of the photosynthetic leaf area

Conclusion :

The time course of leaf development and the effects of environmental conditions are relatively straightforward and easy to model when analysed with the framework of analysis presented in the introduction and in Figure 1. This is not the case with other frameworks of analysis are used, for instance (i) with the method of Trapani & Hall (1996) where the duration of expansion is characterised as the period from 'leaf emergence' to end of expansion and leaf expansion rate is characterised by its value during the apparently linear period or (ii) with the method of Rawson et al (1980) where the duration of expansion is approximated by the period for the leaf to grow from 5 to 95% of its final area, and leaf expansion rate is characterised by its mean value during the same period. When any of these methods were used, the effect of temperature appeared complex (Granier and Tardieu 1998b) and those of early water deficit or light reductions could not be analysed. We therefore believe that the model of leaf development presented here helps for analysing environmental effects. It is now used in our laboratory to model plant architecture (Rey et al 2000) and to compare the development of all the leaves of a plant (Dosio et al 2000).

References :

Guillermo Dosio, Thierry Lefeuvre, Benoît Suard, Natalia Izquierdo, Luis Aguirrezabal, Olivier Turc. Stability of sunflower leaf development in France and Argentina. This issue.

Granier, C. and Tardieu, F. (1998a) Is thermal time adequate for expressing the effects of temperature on sunflower leaf development? *Plant Cell. Environ.* **21,** 695-703.

Granier, C. and Tardieu, F. (1998b) Spatial and temporal analyses of expansion and cell cycle in sunflower leaves. A common pattern of development for all zones of a leaf and different leaves of a plant. *Plant Physiol.* **116,** 991-1001.

Granier, C. and Tardieu, F. (1999a) Water deficit and spatial pattern of leaf development. Variability in responses can be simulated using a simple model of leaf development. *Plant Physiol*. **119,** 609-620.

Granier, C. and Tardieu, F. (1999b) Leaf expansion and cell division are affected by reducing absorbed light before but not after the decline in cell division rate in the sunflower leaf. *Plant, Cell Environ*, **22**, 1365-1376.

Rawson H.M., Constable G.A. & Howe G.N. (1980) Carbon production of sunflower cultivars in field and controlled environments.II Leaf growth. *Australian Journal of Plant Physiology*, **7,** 575-86.

Trapani N. & Hall A.J. (1996) Effects of leaf position and nitrogen supply on the expansion of leaves of field grown sunflower (*Helianthus annuus* L.) *Plant and Soil*, **184,** 331-340.

Rey H, Dauzat J, Barczi JF, Tardieu F, Lecoeur J. Numerical sunflower plants responding to light and temperature. This issue.

This work was supported by I.N.R.A and C.E.T.I.O.M grants.