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Co-Ordination of Cell Division and Tissue Expansion in Sunflower, Tobacco, and Pea Leaves: Dependence or Independence of Both Processes?

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Abstract

Temporal analyses of cell division and tissue expansion in pea, tobacco, and sunflower leaves reveal that both processes follow similar patterns during leaf development. Relative cell division and relative tissue expansion rates are maximal and constant during early leaf development, but they decline later. In contrast, relative cell expansion rate follows a bell-shaped curve during leaf growth. Cell division and tissue expansion have common responses to temperature, intercepted radiation, and water deficit. As a consequence, final leaf area and cell number remain highly correlated throughout a large range of environmental conditions for these different plant species, indicating that cell division and tissue expansion are co-ordinated during leaf development. This co-ordination between processes has long been explained by dependence between both processes. Most studies on dicotyledonous leaf development indicate that leaf expansion rate depends on the number of cells in the leaf. We tested this hypothesis with a large range of environmental conditions and different plant species. Accordingly, we found a strong correlation between both absolute leaf expansion rate and leaf cell number. However, we showed that this relationship is not necessarily causal because it can be simulated by the hypothesis of independence between cell division and tissue expansion according to Green's theory of growth (1976).

Key words: Leaf development; Cell division; Tissue expansion; Dicotyledonous; Kinematic analyses

INTRODUCTION

Dicotyledonous leaf development has often been described as a two-phase process (Eq. 1): one phase during which the leaf grows mainly by cell division (Eq. 2, phase 1), from initiation to leaf emergence, and one phase during which growth is by cell expansion only, from emergence to the end of leaf expansion (Eq. 2, phase 1, Clough and Milthorpe 1975; Maksymowych 1963; Terry and others 1971). This description is formalized in Eq. 2. In this theory, pertinent variables for analysis of leaf growth are cell division rate and cell expansion rate, both of which

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contribute to absolute leaf expansion rate. However, this two-phase formalism does not fit with at least three observations: (1) cell division and tissue expansion are simultaneous during most of leaf development, from initiation to the time the leaf reaches 75% of its final size, (2) 90% of the total number of cells in a leaf form after it has emerged (Granier and Tardieu 1998a; Milthorpe and Newton 1963), and (3) inhibition of cell division by chemical treatments does not affect whole tissue expansion (Haber 1962; Haber and Foard 1963).

$$A_t = N_t * C_t \quad (1)$$

$$dA_t/(dt) = C_t * dN_t/(dt) + N_t * (dC_t/(dt)) \quad \text{phase 1} \quad \text{phase 2} \quad (2)$$

$$dC_t/(C_t.dt) = dA_t/(A_t.dt)\ /(N_t.dt) \quad (3)$$

This theory was rejected by Green in 1976 because an increase in cell division per se cannot generate growth of the tissue. In Green's theory, leaf expansion and cell division are two independent processes: as the whole tissue is growing, cell division allows cell partitioning, and cell expansion results from cell division and tissue expansion at each time during organ development (Eq. 3). In this theory, a pertinent variable for analysis of growth is relative leaf expansion rate, which is independent of relative cell division rate and relative cell expansion rate. According to Eq. 3, (1) decreases in the relative cell division rate do not alter the relative organ expansion rate but can cause an increase in the relative cell expansion rate, (2) cell size increases when the relative leaf expansion rate is greater than the relative cell division rate, (3) conditions that affect tissue expansion more than cell division would cause a decrease in cell size, (4) cell size is not affected by conditions that reduce to the same extent the relative cell division rate and relative tissue expansion rate.

Equations 2 and 3 are both correct mathematically, but each supports a different theory of growth (Jacobs 1997). To our knowledge, there is no proof to confirm one or the other growth theory. In this review, we will consider both cell division and tissue expansion in sunflower, pea, and tobacco leaves. We will first show that both processes are highly coordinated during leaf development and that they have similar responses to temperature, water deficit, and light. As a consequence, final leaf area and final cell number per leaf remain highly correlated under a large range of environmental conditions. Finally, the co-ordination between both processes will be discussed with one or the other theory.

Material and Methods

Plant Culture and Growth Conditions.

In all experiments, light was measured continuously with a PPFD sensor (LI-190SB, LI COR, Lincoln, Nebraska). Air temperature and RH were measured every 20 s (HMP35A Vaisala Oy, Helsinki, Finland). Leaf temperature was measured with a copper-constantan thermocouple (0.4-mm diameter) appressed to the underside of the lamina.

Sunflower (*Helianthus annuus* L., hybrid Albena) plants were grown in a field near Montpellier (southern France) during four growing periods in 1995 and 1996. They were also grown in a greenhouse for six growing periods in 1995 and 1996 and in a growth chamber during four growing periods in 1997. Variability in environmental conditions during these experiments have been described in Granier and Tardieu (1998b). Water deficit was imposed during six experiments in the greenhouse as described in Granier and Tardieu (1999a). Variability in light interception was imposed as described in Granier and Tardieu (1999b).

Tobacco plants were grown in 7.5-L pots in a growth chamber and in a greenhouse during six growing periods between 1996 and 1997. Environmental conditions were measured as explained for sunflower experiments. Two types of treatments were imposed during three growing periods: (1) either a reduction in intercepted PPFD obtained by covering part of the photosynthetic leaf area or by shading whole plants, or (2) plants intercepted full light but "control" plants differed by natural variations in incident PPFD between growing periods. Treatments also differed by the times at which plants were shaded or covered.

Pea (*Pisum* sativum L., cv Messire) plants were grown in 35-L pots in a greenhouse from February to April 1995. Growing and environmental conditions are described in Turc and Lecoeur (1997). Water deficit was imposed by maintaining soil water potential around −80 kPa from full expansion of leaf 5 until full expansion of the last leaf. Available soil water corresponding to this water potential was calculated as described in Lecoeur and others (1995) and equalled approximately 40% of its maximum value.

Growth Measurement

A leaf was considered as initiated when its primordium was visible (about 40 μm in height) on the flank of the apical meristem when examined with a microscope (Leica stereomicroscope, Wild F8Z, Wetzlar, Germany) at magnification ×80. Leaf age was

then calculated in days after initiation. To measure leaf area, the apex was dissected under the microscope, the studied leaf was excised (leaf 8 and 16 for sunflower, leaf 6 for tobacco, all leaves for pea), and its area was measured with an image analyzer (Bioscan-Optimas V 4.10, Edmonds, WA). Three plants were harvested every second day from germination to the end of leaf expansion. When they reached 25-mm long, sunflower and tobacco leaves were photographed with a video camera every day at 12.00 h (solar time), and areas were determined with the image analyzer. The same five leaves per treatment were measured until their full expansion. Each picture was calibrated with a mark of known length on the leaf.

Measurements of Cell Area and Calculations of Cell Number Per Leaf

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 (LEICA- Leitz DM RB, Wetzlar, Germany) coupled to the image analyzer. The areas of 50 epidermal cells were measured in three to eight (depending on leaf length) transects perpendicular to the midrib. Cell area was measured every second day on three leaves from 5 days after initiation until the end of leaf expansion. Prints were generally made on the leaves that were harvested for determination of leaf area. Because leaf area was measured with a nondestructive method after leaf emergence in sunflower and tobacco, three leaves were sampled every second day for determination of cell area.

Cell number in the whole leaf was estimated by first calculating the mean cell area in different transects of the lamina. The proportion of leaf area corresponding to each transect was then calculated as the area of a trapezoid whose sides are leaf edges and lines located at midpoint between transects (Granier and Tardieu 1998a).

$$A_{i,j} = W_{i,j} * (y_{i+1,j} - y_{i-1,j})/2 \quad (4)$$

where $W_{i,j}$ is leaf width at the y coordinate of transection day j, and y_{i-1} and y_{i+1} are y coordinates of transects i–1 and i+1 on day j. Cell number of the leaf on day j ($N_{leaf,j}$) was calculated as:

$$N_{leaf,j} = \Sigma A_{i,j}/a_{ij} \quad (5)$$

where a_{ij} is the mean cell area in transect i on day j. Summation was carried out over all the transects analyzed on day j.

In pea, cell area was measured on stipules. Because there was no gradient in cell size along the midrib (data not shown), cell number per stipule on day j was calculated as:

$$N_{stipule,j} = A_{stipule,j}/a_{stipule,j} \quad (5')$$

where $A_{stipule,j}$ and $a_{stipule,j}$ are the mean stipule area and mean cell area on day j, respectively.

Calculations of Absolute Leaf Expansion Rate, Relative Leaf Expansion Rate, and Relative Cell Division Rate

Absolute leaf expansion rate at time j was calculated from initiation to end of expansion as the local slope (at time j) of the relationship between the leaf area (A) and time.

$$LER_j = [d(A)/dt]_j \quad (6)$$

It was calculated by linear regression on the three coupled values of A and t corresponding to times j–1, j and j+1. Maximal absolute leaf expansion rate (LER_{max}) was the maximum value reached by LER during leaf development. The leaf relative expansion rate (RER) at time j was calculated from initiation to the end of expansion as the slope (at time j) of the relationship between the logarithm of leaf area (A) and time:

$$RER_{leaf,j} = [d(\ln A)/dt]_j \quad (7)$$

It was calculated by linear regression on the three coupled values of A and t corresponding to times j–1, j and j+1.

The relative cell division rate (RDR) of the whole leaf on day j was calculated as:

$$RDR_j = [d(\ln N_{leaf})/dt]_j \quad (8)$$

taking into account cell number per leaf (N_{leaf}) on days j–1, j and j+1 in the same way as in Eq. 6.

Results and Discussion

Cell Division and Tissue Expansion : A Similar Pattern During Leaf Development

Kinetics of cell division and tissue expansion in the whole leaf. In sunflower and pea, during the first part of leaf development, absolute increases in leaf area and cell number were low (Figure 1A, Figure 2A). During this same period, relative increases in area and in cell number were maximal and quasi-constant (Figure 1B, Figure 2B), suggesting that this phase corresponds to an exponential increase in both cell number and leaf area. Total duration of expansion was longer than total duration of cell division and the differences in duration between pro-

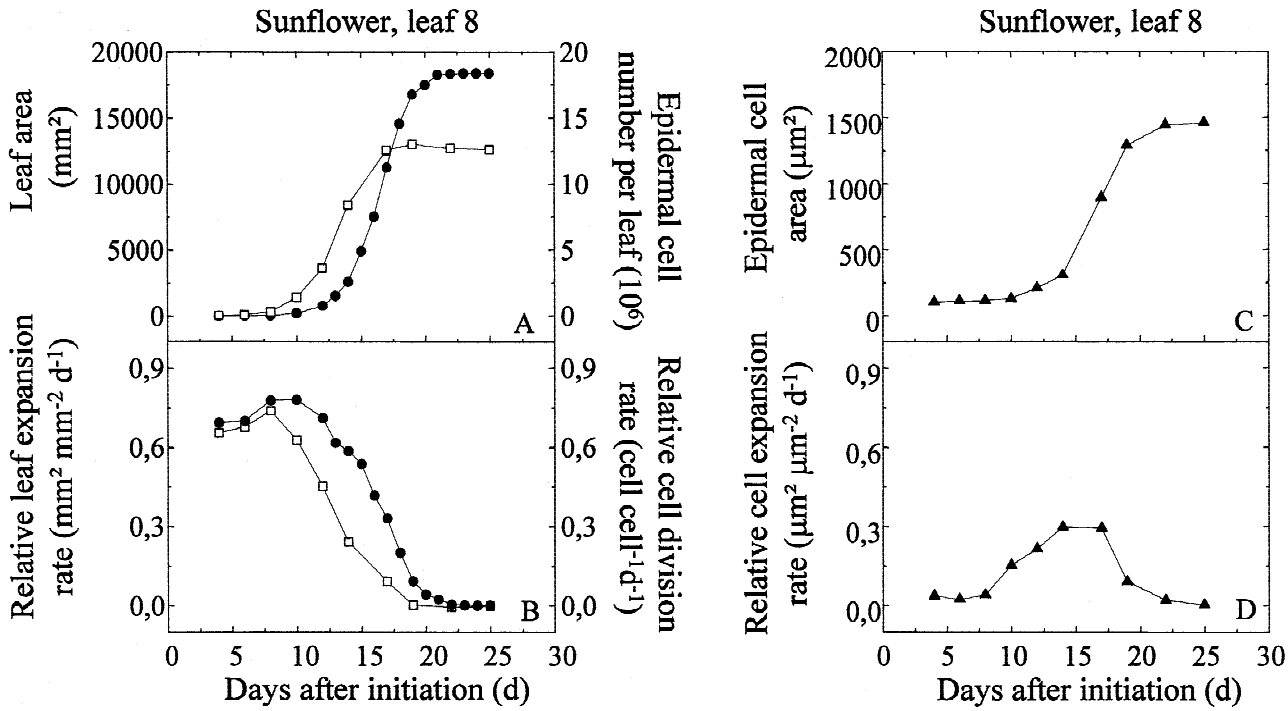

Figure 1. Changes with time in leaf area (**A**, ●), in epidermal cell number per leaf (**A**, □), and in epidermal cell area (**C**, ▲) in sunflower for a greenhouse experiment in July 1996 (experimental conditions are described in Granier and Tardieu 1998b). Corresponding changes with time in relative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

cesses was short compared with the duration of whole leaf development (less than 30% of total duration of leaf development).

Cell area increased slowly during the first part of leaf development, whereas increases in both cell number and leaf area were exponential (Figure 1C, Figure 2C). During this period the relative increase in cell area was low and quasi-constant (Figure 1D, Figure 2D). It was followed by a considerable increase to reach a maximum value and then a decrease with time (Figure 1D, Figure 2D). This bell-shaped curve of the relative cell expansion rate contrasts with the pattern of changes over time in the relative cell division rate and relative tissue expansion rate in the leaf (see also Maksymowych 1963 on Xanthium).

Kinetic changes in leaf area and in cell number per leaf in pea and sunflower are similar to what is described in many dicotyledonous leaves (Clough and Milthorpe 1975 for tobacco, Maksymowych 1963, for Xanthium, Milthorpe and Newton 1963 for cucumber, Sunderland 1960 for Lupin).

Spatial variability of both cell division and tissue expansion within the leaf. Spatial distribution of cell division and tissue expansion rates are not uniform within the leaf. Spatial analysis of tobacco (Avery 1933), spinach (Saurer and Possingham 1970), grapes (Wolf and others 1986), or sunflower (Granier and Tardieu 1998a) leaves growth, revealed tip-to-base gradients in tissue expansion and cell division rates in dicotyledonous leaves. In our study on sunflower leaf development, kinetics in relative tissue expansion rates and relative cell division rates are similar in all leaf zones and to the whole leaf (Granier and Tardieu 1998a). However, relative tissue expansion rate and relative cell division rate cease to be constant first at the tip of the leaf and then progressively toward the base. As a consequence, at the end of leaf development, zones at the base of the leaf have a larger area and larger final cell numbers than those at the tip.

Kinetics of cell expansion are similar among different zones of the leaf and similar to what is observed at the whole leaf level (Granier and Tardieu 1998a; Maksymowych 1963). However, as in the case of cell division and tissue expansion there is a time lag between each zone: cell area increases rapidly first at the tip of the leaf and then gradually to the base. As a consequence, during leaf development, cell area can greatly differ among the different zones of a leaf (Granier and Tardieu 1998a; Maksymowych 1963; Pyke and others 1991). However,

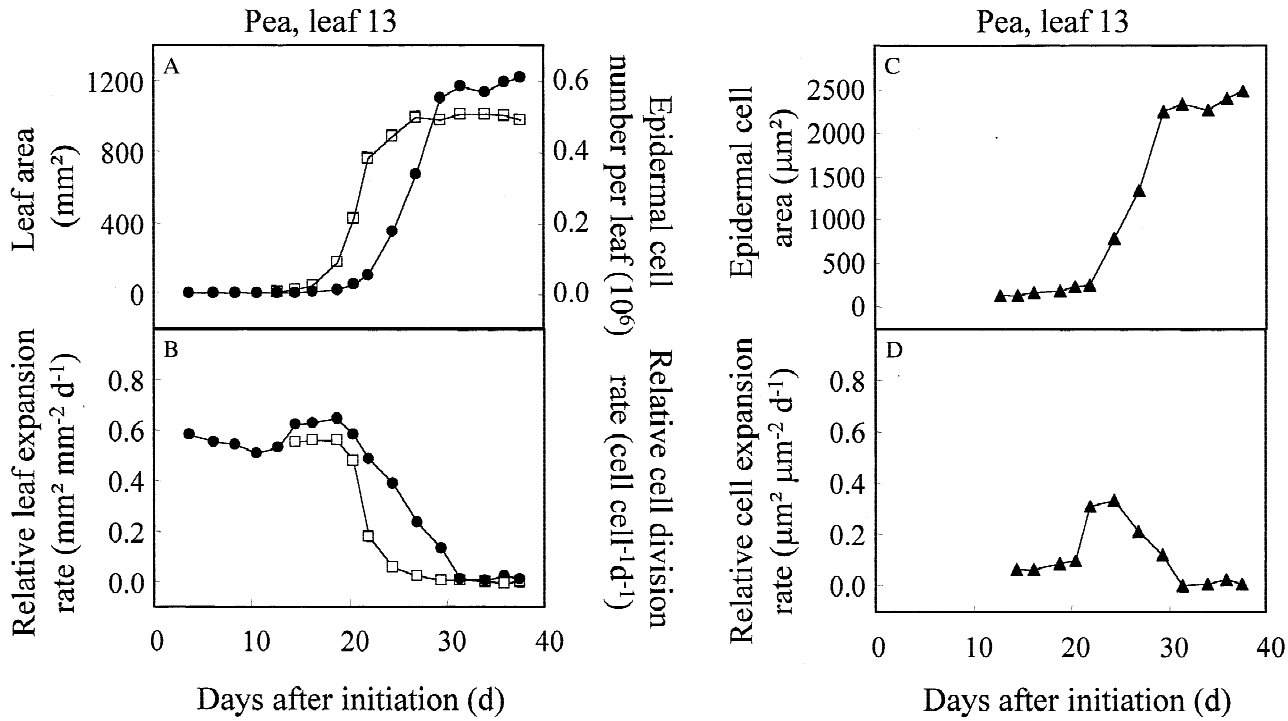

Figure 2. Changes with time in stipule area (**A,** ●), in epidermal cell number per stipule (**A,** □), and in epidermal cell area (**C,** ▲) in pea for a greenhouse experiment from February to April 1995. Growing and environmental conditions are described in Turc and Lecoeur (1997). Corresponding changes with time in relative stipule expansion rate (●), in relative cell division rate (□), and in relative cell expansion rate (▲) are presented in **B** and **D.**

within the same zone, cell area varies within a narrow range and its distribution remains normal (Granier and Tardieu 1998a; Pyke and others 1991). At the end of leaf development, cell area is uniform in the whole leaf (Maksymowych 1973; Pyke and others 1991; Granier and Tardieu 1998a).

Cell Division and Tissue Expansion: A Similar Response to Environmental Conditions

Large variability in final leaf area and cell numbers can be found in leaves of the same genotype at the same position on the stem, depending on environmental conditions. Most abiotic stresses cause a reduction both in final cell number and in final leaf area by the same proportion. Accordingly, final leaf area is highly correlated with final cell number for a large range of environmental conditions (Figure 3A, C).

Effect of temperature. Durations of cell division and leaf expansion depend on temperature. The reciprocals of the durations of both processes were positively related to leaf temperature by a common relationship (Granier and Tardieu 1998b). Expressed in thermal time, these durations were stable for a large range of environmental conditions, including differing temperatures, water deficits, and light intensities (Granier and Tardieu 1998b, 1999a, 1999b). Furthermore, the rates of processes involved in leaf development such as leaf initiation, leaf emergence, and leaf expansion are positively correlated with temperature (Granier and Tardieu 1998b on sunflower, Turc and Lecoeur 1997 on pea). We recently showed that this was also the case for the epidermal cell division rate (Granier and Tardieu 1998b). Changes in temperature affected both cell division rate and relative tissue expansion rate to the same extent (Granier and Tardieu 1998b), and final cell area in a leaf was not affected by changes in temperature. Linear relationships between rates of processes involved in plant development and temperature have been found in other plant species (Ben Haj Salah and Tardieu 1995 on maize; Gallagher 1979 on wheat; Lafarge and others 1998 on sorghum; Ong 1983 on pearl millet).

Effect of water deficit. Final leaf area was affected by a short period of water deficit whenever the deficit was imposed between leaf initiation and the end of expansion (Granier and Tardieu 1999a on sunflower; Lecoeur and others 1995 on pea). Periods of moderate water deficit caused reductions in final

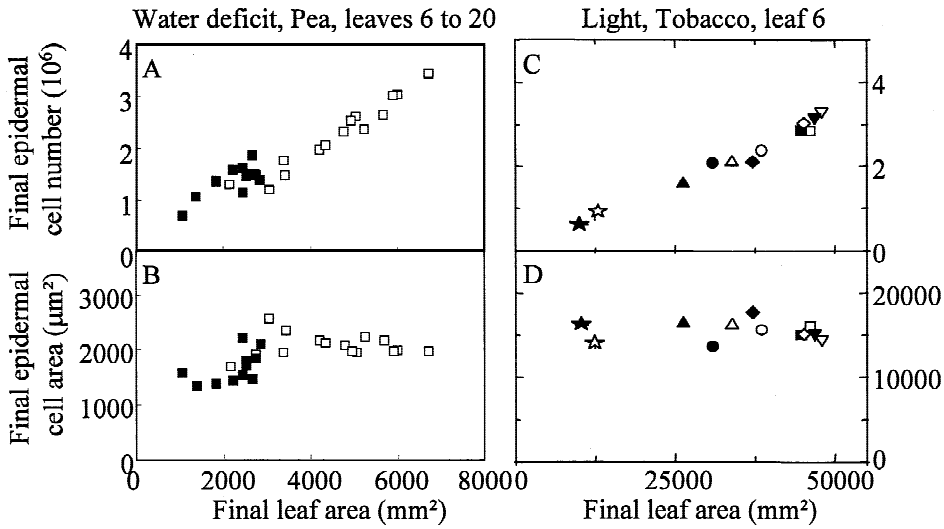

Figure 3. Relationship between final area of leaves 6 to 20 in pea and final epidermal cell number per leaf **(A)** or final epidermal cell area **(B)**. Plants subjected to a moderate water deficit in a greenhouse in March 1995 (■) are compared with well-irrigated plants grown in the same conditions (□). Relationship between final area of leaf 6 and final epidermal cell number **(C)** or final epidermal cell area **(D)** for tobacco plants grown in different light interception. Variability in light interception was obtained either by shading plants or by covering part of the photosynthetic leaf area *(closed symbols)* or by natural variability of incident light *(open symbols)*.

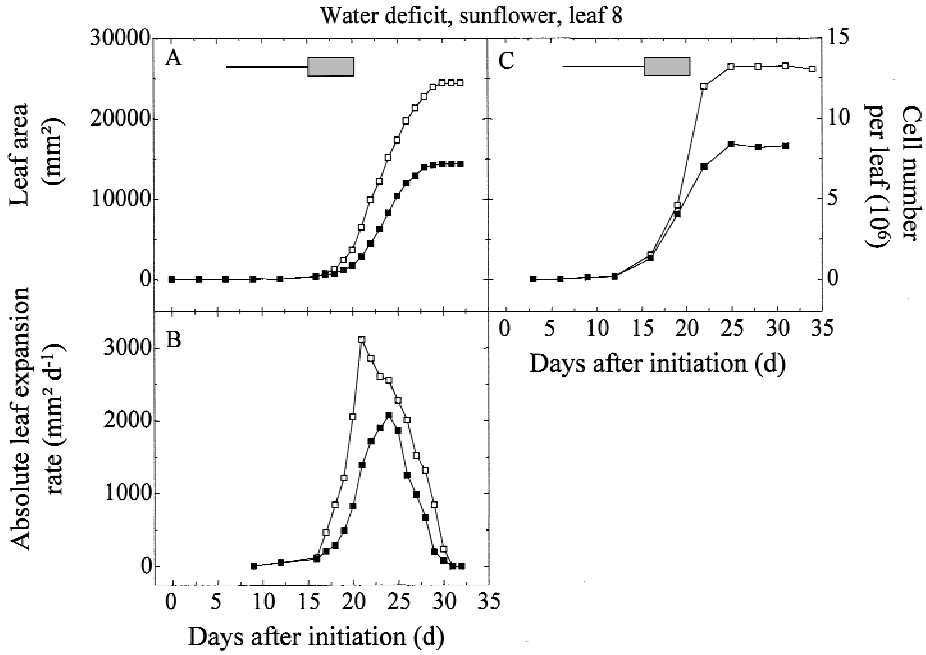

Figure 4. Change with time in leaf area **(A)** and in cell number per leaf **(C)** of leaf 8 of control plants (□) and of plants with early moderate water deficit (■) during an experiment in the greenhouse in April 1995 (described in Granier and Tardieu 1999a). The change in absolute leaf expansion rate with time is presented in **B.** Horizontal thin bars, periods with declining soil water content; horizontal thick bars, periods during which available soil water was maintained at 23% of maximum.

leaf areas and final cell numbers of more than 40% without affecting the duration of either process (Figure 4A, C for sunflower, Lecoeur and others 1995 on pea). Early water deficits, imposed during the period of tissue expansion and cell division, caused a decrease in final leaf area and final cell number (Figure 4A, C for sunflower). Late water deficits imposed during the leaf development period without cell division caused reductions in final leaf areas and final cell areas without affecting final cell numbers (Granier and Tardieu 1999a on sunflower; Lecoeur and others 1995 on pea; Randall and Sinclair 1981 on *Phaseolus vulgaris;* Yeggapan and others 1982 on sunflower). However, because the noncell division period is short compared with the duration of leaf development, final leaf areas remained highly correlated to final leaf cell numbers in plants subjected to a period of water deficit (Figure 3A, B in pea).

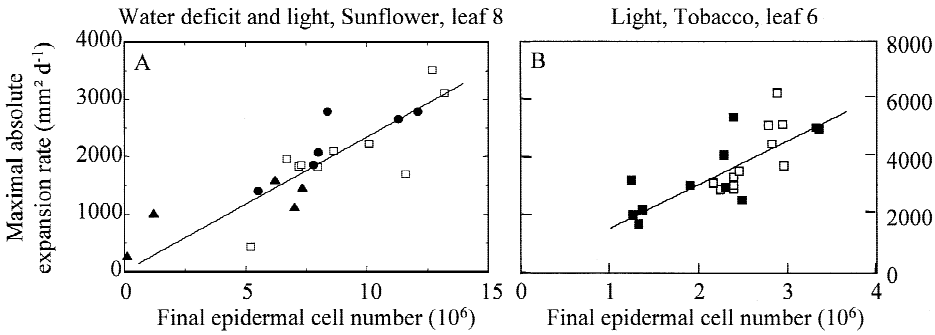

Figure 5. Relationship between maximal absolute expansion rate and final epidermal cell number in leaf 8 of sunflower grown in different conditions of water availability and light **(A)** and in leaf 6 of tobacco plants grown in different light conditions **(B).** Solid lines are linear regressions fitted on the data. Open symbols, plants grown without water or light restriction; closed symbols, plants grown with water or light restriction.

Effect of light. Reduction in incident light caused a reduction in final cell numbers and final leaf areas in dicotyledonous leaves (Granier and Tardieu 1999b on sunflower, Figure 2C, D on tobacco). We showed recently that the effect of reduced incident light was equivalent to a reduction in light interception (Granier and Tardieu 1999b in sunflower). This was shown by comparing the effect of a 40% reduction in incident light imposed by shading and a 40% reduction of intercepted light imposed by covering part of the photosynthetic leaf area. Both treatments affected the relative cell division and relative tissue expansion rates to the same extent without affecting the durations of the processes. As a consequence, final leaf area and final cell number remained highly correlated in leaves of plants grown in various light conditions (Figure 3C, D in tobacco, see also Granier and Tardieu 1999b in sunflower).

Similar tendencies have been found in other plant species by different groups (Dale 1964 in *Phaseolus* vulgaris, Dengler 1980 in sunflower, Wilson 1966 in Xanthium). Cell area is rarely affected by shading in dicotyledonous leaves except in the work of Verbelen and DeGreef (1979), who reported a reduction in cell size in leaves of *Phaseolus vulgaris* grown in complete darkness.

Which Theory of Growth Can Be Used to Take into Account the Effect of Environmental Conditions on Leaf Development?

Does tissue expansion rate depend on cell number? When cell number is reduced by early water stresses, the subsequent leaf expansion rate is reduced even when favorable growth conditions are restored (Lecoeur and others 1995 on pea, Figure 4A, B on sunflower). This after-effect was taken into account in the model of leaf expansion from Lecoeur and others (1996) by considering that after leaf emergence leaf expansion rate was equal to the product of cell number and individual cell expansion rate (see Eq. 2). In this view, early water deficit caused a reduction in the cell division rate during the first phase of leaf development, and the resultant reduction in cell number caused a reduction in the leaf expansion rate after rewatering, even if the cell expansion rate was re-established. Leaf expansion was predicted according to this theory in a large range of water deficit treatments (Lecoeur and others 1996).

If this formalism was correct, any reduction in cell number would be accompanied by a reduction in the absolute leaf expansion rate. We tested the robustness of this hypothesis by imposing different treatments known to affect cell number (water deficit and reduction in light interception) to different plant species (sunflower and tobacco). For the same genotype and for leaves at the same position on the stem, reductions in cell numbers were accompanied by a similar reduction in the maximum absolute leaf expansion rate (Figure 5A, B). Correlation takes into account the variability of both variables caused by temperature, light, and water deficit.

The maximal leaf expansion rate is poorly correlated with environmental conditions in sunflower (Figure 6A, see also Granier and Tardieu 1998b for temperature, 1999a for water deficit). It is clearly shown in Figure 6A, that at least part of the noncorrelation can be linked to the variability in cell number. Even if they grow at similar temperatures, leaves with more cells have a higher maximal absolute leaf expansion rate than those with few cells. When cell number is fixed in the leaf, the ratio between maximal absolute expansion rate and final cell number is equivalent to maximal cell expansion rate. This ratio is closely related to leaf temperature

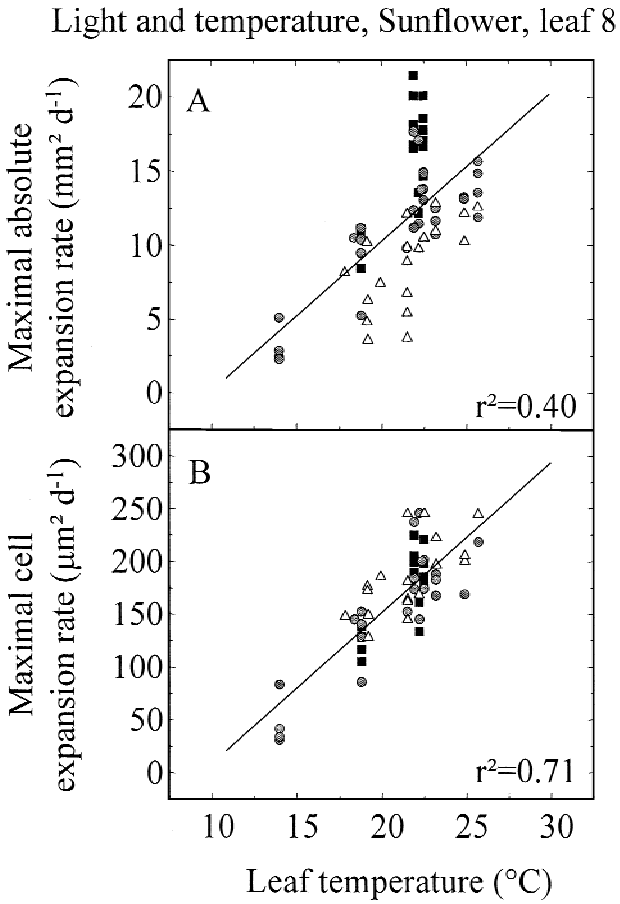

Figure 6. **(A),** Relationship between maximal leaf expansion rate and temperature for leaf 8 of sunflowers grown in different light and temperature conditions. Each point represents an individual leaf. Different symbols represent differences in final epidermal cell number (Nc) per leaf (■, Nc ≥ 8 10^6; ●, 5 10^6 ≤ Nc ≤ 8 10^6;△, Nc ≤ 5 10^6). **(B),** Relationship between maximal cell expansion rate (ratio between maximal absolute expansion rate and final cell number) and temperature for leaf 8 of sunflowers grown in different conditions of light interception and temperature. Solid lines are linear regressions fitted on the data.

by a linear relationship (Figure 6B). We suggest that this ratio can be used to test the effect of environmental conditions for a given genotype and similarly positioned leaves on the stem.

As suggested by Eq. 2, co-ordination between cell division and tissue expansion in sunflower, pea, and tobacco leaves seems to result from the relationship between the absolute expansion rate and cell number. This relationship is unique for a range of environmental conditions and suggests that at each time after leaf emergence, the absolute leaf expansion rate depends on the cell expansion rate and on the number of cells formed during the first phase of leaf development.

Can we account for the effect of environmental conditions on leaf development by considering tissue expansion and cell division as independent processes? We tested this hypothesis by simulating the effect of periods of abiotic stresses on both tissue expansion and cell division (Figure 7). These simulations were based on the following assumptions:

1. Expansion and cell division in "control" leaves were simulated (simulation 1) by considering the time courses of relative expansion rate and relative cell division rate obtained in the experiment presented in Figure 1A and in other experiments presented in Granier and Tardieu (1998b).

2. The effects of abiotic stresses (either water deficit or reduction in light interception, simulations 2 and 3) were simulated by uniformly reducing relative expansion rate and relative cell division rate during the period of stress without affecting durations of either process. According to previous experimental results (Granier and Tardieu 1999a; Sacks and others 1997), relative cell division rate was affected by a higher proportion (39%) than relative expansion rate (36%).

3. Kinetic changes in leaf area and cell number per leaf (Figure 7B, D, F) were calculated from values of relative tissue expansion rate and relative cell division with initial values of 0.7 mm^2 and 18,000 cells, respectively, for leaf area and cell number at time 0. Changes with time in absolute leaf expansion rate were deduced from changes with time in leaf area (Figure 7, *insets*).

The temporary reduction in relative expansion caused by the stresses (Figure 7B, C) resulted in a permanent reduction in the absolute expansion rate (Figure 7D, E, F, and insets). These reductions in absolute expansion rate were simulated independently of the reduction in final cell number caused by the same water deficit. They resulted from a characteristic of exponential processes that expansion rate at each time is proportional to leaf area at that time. This suggests that the observed after-effect of water deficit on absolute leaf expansion rate can be explained by a temporary reduction in relative expansion rate during the stress (see also, Granier and Tardieu 1999a) and is not necessarily linked to the concomitant reduction in cell number. As shown by the comparison of Figure 7E, F and corresponding insets, the stress that has a greater effect on absolute leaf expansion rate is the one that has a greater effect on final cell number. These results have been simulated with the hypothesis of independence between cell division and tissue expansion and indicate that the observed relationship in Figure 5A and

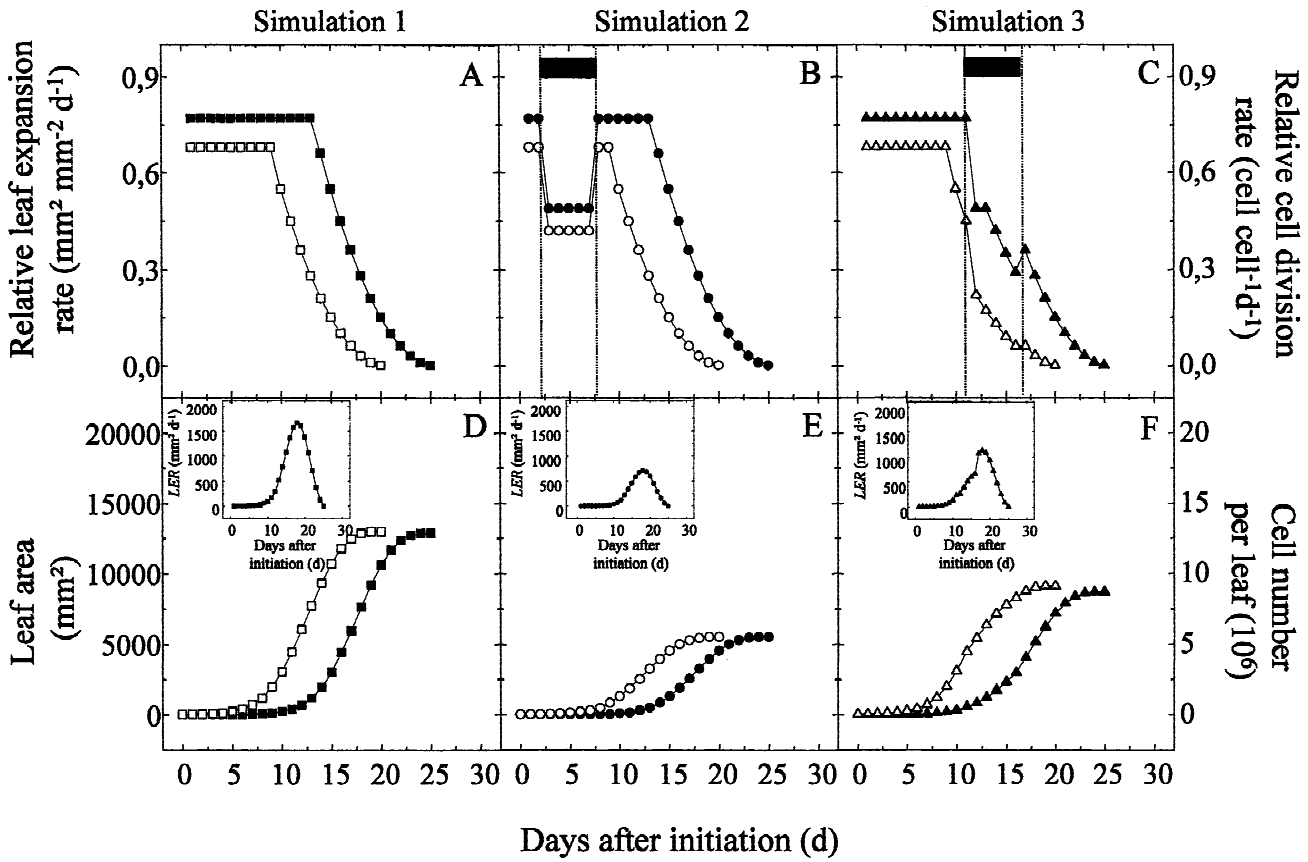

Figure 7. **(A–C)** Simulated time courses of relative expansion rate (closed symbols), relative cell division rate (open symbols) in the leaf of control plants **(A)** or plants subjected to a period of abiotic stress **(B, C). (D–F)** Resulting simulations of leaf area *(closed symbols)*, cell number *(open symbols)*, and absolute leaf expansion rate *(insets)* are presented in panels **D–F** and are deduced from time courses presented in panels **A–C** (see text). Positions of the two periods of deficit are represented by the thick black horizontal line. Simulations of relative expansion rate and relative cell division rate of control plants are based on data presented in Figure 1A (experiment at 26°C) and on the model presented in Granier and Tardieu (1998b). A period of abiotic stress lasting 5 days is simulated by imposing a reduction in relative expansion rate by 36% and relative cell division rate by 39% (see text).

B does not prove that leaf expansion rate depends on cell number.

Conclusion

Cell division and tissue expansion are well co-ordinated during dicotyledonous leaf development. As a consequence, final areas of a zone of a leaf or final leaf area remains correlated to final cell number, respectively, per zone or per leaf over a range of environmental conditions. This co-ordination could result either from a dependence between both tissue expansion and cell division or from a similar but independent response of the processes to environmental factors. The observed correlation between absolute tissue expansion rate and cell number could indicate that the two processes depend on one another. However, we showed that this correlation could be simulated by considering that the processes are independent but affected to a similar extent by abiotic stresses. It is suggested, therefore, that the observed correlation is not necessarily causal.

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