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RESEARCH PAPER

A whole-plant analysis of the dynamics of expansion of individual leaves of two sunflower hybrids

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Abstract

Common features in the time-course of expansion of leaves which considerably differed in final area, due to phytomer position, growing conditions and genotype, were identified. Leaf development consisted of two phases of exponential growth, followed by a third phase of continuous decrease of the relative expansion rate. The rate and the duration of the first exponential phase were common to all phytomers, growing conditions and genotypes. Leaves differed in the rate and the duration of the second exponential phase. The decrease of the relative expansion rate during the third phase depended on neither genotype nor growing conditions. It was phytomer-dependent and was deduced from the rate of the second phase via a parameter common to all cases studied. Differences in final leaf area among growing conditions were linked to different expansion rates during the second exponential phase. The duration of the phases at any given phytomer position was the same for the two hybrids in different growing conditions. The dates of developmental events (initiation, end of the two exponential phases, full expansion), and the rate of the second exponential phase, were related to phytomer position, defining a strict pattern of leaf development at the whole plant level. Using this framework simplified the analysis of the

response of leaf expansion to genotype and environment.

Key words: Exponential growth, *Helianthus annuus* L., leaf expansion, phasic development, phytomer, thermal time, whole-plant-analysis.

Introduction

The next challenge assigned to plant biology, after the complete genome sequencing of model plant species, may well be to simulate the behaviour, in different environments, of genetically characterized phenotypes (Chory *et al.*, 2000). To achieve this, analysis of gene expression has to be combined with tools to analyse growth and development at the whole-plant scale and throughout the plant life cycle (Boyes *et al.*, 2001).

Descriptors of shoot development, or growth stages, are classically based on the repetitive activity of the shoot apical meristem, which sequentially produces phytomers (Lyndon, 1990). They consist of counting the number of leaves beyond a given stage (initiation, emergence, unfolding, threshold length) and have been developed for many species (Erickson and Michelini, 1957; Maurer *et al.*, 1966; Haun, 1973; Schneiter and Miller, 1981; Rickman and Klepper, 1995; Turc and Lecoœur, 1997) and, recently, *Arabidopsis thaliana* (Boyes *et al.*, 2001). The response of

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Abbreviations: PPFD, photosynthetic photon flux density; VPD, vapour pressure deficit.

growth stages to environmental factors such as temperature, day-length and light intensity has been extensively studied (Warrington and Kanemasu, 1983; Miglietta, 1989; Jamieson *et al.*, 1995; Kirby, 1995). Such descriptors are very useful in characterizing crop or plant development, but they often mismatch transitional events of individual leaf development (e.g. the beginning of linear growth or the end of leaf expansion) pertinent to the growth response to the environment (Turc and Lecoeur, 1997; Lafarge and Tardieu, 2002).

Analysing the relationships between plant growth and environment is made difficult by interactions between them (Sinoquet and Le Roux, 2000). For example, changes in leaf growth, resulting from the environmental conditions prevailing during early leaf development (Granier and Tardieu, 1999) or from genetic variation (Dolan and Poethig, 1997), modify the micrometeorological conditions within the canopy (e.g. the vertical distribution of light interception), which in turn affect the development of newly initiated leaves and the current microclimate. Three-dimensional models of plants, representing the canopy as a set of organs and yielding realistic computer-generated images of plant geometry through time (Díaz-Ambrona *et al.*, 1998; Fournier and Andrieu, 1998; Rey *et al.*, 2000) allow calculation of the radiative environment of each organ at each step of plant development (Dauzat and Eroy, 1997). These could, therefore, constitute pertinent tools to analyse ongoing interactions. However, the main difficulty of building virtual plants seems to deal with the mathematical expression of the genetic variability of plant responses to environmental conditions to include into the computer representation of plants (Tardieu, 2003).

A framework of analysis of leaf expansion of dicots, based on the time-course of the leaf relative expansion rate, provided a mathematical formalism, together with pertinent stages of leaf development, for the response of leaf expansion to environmental conditions (Granier and Tardieu, 1998a). Two phases were taken into account during leaf development to analyse these responses: during the first one, leaf growth was exponential and, during the second one, leaf relative expansion rate decreased rapidly to reach zero at the end of leaf expansion (Denne, 1966; Poethig and Sussex, 1985; Granier and Tardieu, 1998a). Relative expansion rate was affected by a reduction in absorbed light only if it occurred during the exponential phase (Granier and Tardieu, 1999). However, the validity of this approach, mainly obtained on leaf 8 of sunflower (*Helianthus annuus* L.) hybrid Albena, remains to be evaluated for all other nodal positions along the stem. Leaf development at other phytomer positions occurs under different environmental and endogenous (e.g. source-sink relationships, reproductive development) conditions that could interfere with leaf expansion and make inadequate a common pattern for all phytomers.

The objective of the present work was to establish a whole-plant pattern of leaf growth and development of sunflower consisting of a set of mathematical equations with identified parameters depending either on nodal position, genotype or environment. The dynamics of expansion of all individual leaves were analysed for sunflower plants grown under fluctuating environmental conditions (light and temperature). Time-courses were expressed on a thermal time basis (Granier and Tardieu, 1998b). The analysis consisted of determining, at all phytomer positions, the dates of occurrence, and the rate and the duration of the successive phases of leaf development relevant for leaf expansion. This was done on two sunflower hybrids differing in leaf number, grown at two different locations either as isolated plants, without competition for light, or in a plant canopy.

Materials and methods

Plant culture and growth conditions

Three experiments were performed to investigate the development of leaves originating from all successive phytomers of sunflower plants grown in the field under naturally fluctuating environmental conditions. Two experiments were carried out near Montpellier, France (43° 40' N, 3° 50' E). Sunflower (*Helianthus annuus* L., hybrid Albena) seeds were sown on 5 May 1998 and 11 May 1999 in a deep sandy loam soil (fluvio-calcaric Cambissol). Seedling emergence occurred a week after sowing in both experiments. Due to a poor soil N content, N fertilization was applied before sowing (80 kg ha⁻¹). Soil water potential was monitored with five tensiometers (DTE 1000 system, Nardeux, Saint-Avertin, France) placed every 0.20 m from 0.30 m to 1.10 m depth, and maintained by irrigation above -40 kPa in the first 0.5 m of soil during the whole period of leaf area establishment. This practice maintained available soil water content in a range where neither stomatal conductance nor leaf growth were affected (Turner, 1991). Two treatments were applied to plants. In the first one, carried out in 1998 and 1999, isolated plants were obtained by thinning to 0.06 plants m⁻² after seedling emergence to avoid light competition between neighbouring plants. In the second treatment, carried out in 1999, a plant canopy was obtained with a final plant density of 5.5 plants m⁻².

Seeds of Dekalb G100 hybrid were sown on 17 November 1997 at the INTA Balcarce Experimental Station, Argentina (37° 45' S, 58° 18' W), on a loamy clay typic Argiudol. Soil analysis indicated that neither N nor P fertilization were necessary as nutrient availability was adequate to obtain maximum yield of sunflower crops under non limiting water conditions (Sosa *et al.*, 1999; Andrade *et al.*, 2000). Soil water content was monitored every 5–7 d using a neutron probe (Troxler 4300, Troxler Electronic Laboratories, INC, Research Triangle Park, NC, USA). Irrigation was supplied to maintain soil water content above 40% of maximum available water content in the first 0.60 m of the soil profile during the entire growing season. This management avoided any decrease in stomatal conductance (Turner, 1991). After emergence (10 d after sowing), seedlings were thinned to obtain a plant density of 5.5 plants m⁻².

Incident radiation was measured at 2 m above the soil surface with a PPFD sensor (LI-190SB, Li-Cor, Lincoln, Nebraska, USA). Air temperature and relative humidity were measured at 2 m above the soil surface with a HMP35A capacitive hygrometer (Vaisala Oy, Helsinki, Finland) placed in a ventilated shelter. Leaf temperature was measured with 0.4 mm-diameter copper-constantan thermocouples. Thermocouples were placed at three locations along the

stem to characterize the gradient of temperature according to phytomer position. A thermocouple was inserted into the apical bud to measure apex temperature. A second one was pressed to the under-side of the lamina of a growing leaf near the shoot top, and a third one measured the temperature of a mature leaf, near the base of the plant. As the plant developed, thermocouples were progressively moved to upper leaves. All data were transferred every 20 s to a data logger (LTD-CR10 Wiring Panel; Campbell Scientific, Shepshed, UK and Delta-T DL2e Logger; Delta-T Devices Ltd, Cambridge, UK at Montpellier and Balcarce, respectively) and averaged every 600 s.

Daily mean values observed between emergence and first anthesis varied from 13.6 to 26.6 °C for air temperature, 12.9 to 25.7 °C for leaf temperature, 14.0 to 64.6 mol m⁻² d⁻¹ for incident PPFD, while daily maximum VPD varied from 0.05 to 4.6 kPa across dates and locations. Day-to-day meteorological variations in each experiment were greater than variations through year and location (not shown). Periods with low incident PPFD or high VPD were short in all cases: two consecutive days with PPFD lower than 30 mol m⁻² d⁻¹ never occurred, and periods with VPD higher than 2.0 kPa were limited to a couple of hours around midday, during few consecutive days (not shown). The effect of temperature on leaf expansion was taken into account by the use of thermal time (Granier and Tardieu, 1998b).

Stages of development and thermal time

Seedling emergence was defined as the date when the first leaf pair became just visible (3–5 mm length) between open cotyledons. It occurred about 100 °Cd (*c.* 6 d) after seed imbibition.

A leaf was considered as initiated when its primordium was visible (about 40 µm long) on the apical meristem under a microscope (Leica stereomicroscope, Wild F8Z, Wetzlar, Germany) at magnification ×80. Phytomers were numbered from the cotyledonary node. Each of the first four phytomers bear two opposite organs (cotyledons and three leaf pairs). Phyllotaxy is then alternate from phytomer 5 (corresponding to leaf 7) until the involucre bracts surrounding the capitulum. For example, leaf 16 corresponds to phytomer 14. Measurements concerned only the 'true' leaves. Cotyledons (phytomer 1) and sessile leaves (intermediate between leaves and bracts), generally present at the last two phytomers under the involucre bracts, were not measured.

The capitulum was considered as initiated when the shoot apical meristem broadened and became dome-shaped. Apex area comprised between 0.1 and 0.2 mm² at that stage, equivalent to Floral Stage 1.3 of Marc and Palmer (1981). First anthesis was reached when stamens became visible on the outer ring of flowers on the capitulum.

Thermal time was calculated by daily integration of leaf temperature and a base of 4.8 °C (Granier and Tardieu, 1998b), cumulated either from seedling emergence or from leaf initiation. For a given leaf, the calculation was made successively with apex temperature until the leaf emerged out of the apical bud, with temperature measured in the zone of growing leaves until full expansion and, subsequently, in mature leaves.

Growth measurements

A sampling procedure was designed to reduce variability in plant size due to different dates of seedling emergence (Granier *et al.*, 2002). A batch of ten plants close to the median of the distribution of length of the first leaf pair was selected after emergence and considered as reference plants. Length and width of all visible leaves (length >15–20 mm) were non-destructively measured with a rule three times a week on the reference plants until the end of expansion of the last leaf. A highly significant linear relationship ($r^2 > 0.99$, $n > 300$, $P < 0.0001$) was established in each experiment between the product length × width and leaf area measured using a planimeter

(Li-Cor 3100 Area Meter; Li-Cor Inc., Lincoln, Nebraska, USA) in Balcarce and a camera coupled to an interactive image analysis system (Optimas V 6.5; Media Cybernetics, Silver Spring, MD, USA) in Montpellier.

Three times a week in the Montpellier experiments and every 10 d in the Balcarce experiment, 4–6 plants having a developmental stage close to that of the reference plants were harvested to measure leaves enclosed in the apical bud. Leaf area of visible leaves was measured with an image analyser. The apical bud was dissected under a microscope (Leica MZ75, Wetzlar, Germany). Leaves were excised and area was measured through the microscope and a camera coupled with an image analyser.

For each leaf, relative expansion rate (R) was calculated at any given time j from leaf initiation to the end of leaf expansion as the local slope of the relationship between the logarithm of leaf area (A) and thermal time t :

$$R_j = [d(\ln A)/dt]_j \quad (1)$$

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

Growth curves fit

Leaf expansion was analysed as a function of thermal time by considering a succession of leaf developmental phases, characterized by the time-course of the leaf relative expansion rate. Two periods of exponential growth and a third one with an exponential decrease of the relative expansion rate were distinguished (see Results). For each leaf, the thermal-time-course of the logarithm of leaf area from leaf initiation to full expansion was fitted to a set of three equations. The parameters of the three equations, including the dates of shift between the successive phases, were all estimated simultaneously for each given leaf by least squares fitting, using an algorithm of generalized reduced gradient (Marquardt–Levenberg algorithm of software SIGMA PLOT 2001 V7.1; SPSS Science, Chicago, IL, USA).

Results

Considerable differences in kinetics of leaf growth and in final leaf area were observed

Final leaf area largely differed with phytomer position along the stem (Fig. 1). It varied from 3700 mm² for phytomer 2 to 120 000 mm² for phytomer 20 of isolated plants of hybrid Albena (Fig. 1A). Kinetics of leaf growth markedly changed from the plant base to the plant tip (Figs 2, 3). In particular, the duration of leaf expansion increased with phytomer number (Figs 2, 3): it equalled 420 °Cd for phytomer 2 and 746 °Cd for phytomer 26 of isolated plants of hybrid Albena (Fig. 2).

Competition with neighbouring plants reduced the final leaf area from phytomer 7 to phytomer 30 of plants grown in a dense canopy compared with isolated plants (Fig. 1A). This reduction increased with phytomer number and exceeded 70% for the highest phytomers (Fig. 1A). As a consequence, leaf area per plant largely differed between isolated plants and plants growing in a canopy (Table 1).

Hybrids Albena and G100 differed in their rates of vegetative development, as well as in reproductive development (Table 1). G100 initiated five leaves fewer than Albena (Fig. 1B): the first involucre bract appeared at

phytomer 28 for G100 versus phytomers 33 or 34 for Albena. A lower rate of leaf initiation by about 30% in G100 (Table 1) more than compensated for the difference in leaf number between both genotypes, resulting in a delay of about 60 °Cd of the dates of capitulum initiation

and first anthesis in G100 compared to Albena (Table 1). The final leaf area was very similar in hybrids G100 and Albena for phytomers 5 to 21 (Fig. 1B). It was slightly but significantly lower for phytomers 2 to 5, and sharply reduced above phytomer 21 in G100 compared to Albena (Fig. 1B). Final leaf area per plant was consequently lower by about 24% in G100 (Table 1).

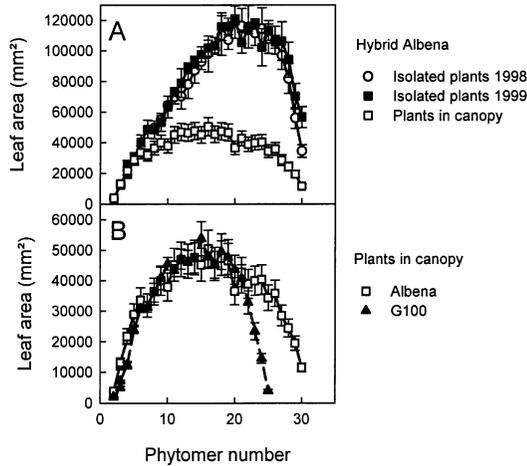


Fig. 1. Final leaf area as a function of phytomer number for isolated plants (open circles, 1998 and filled squares, 1999) or plants in a dense canopy (open squares) of field-grown hybrid Albena (A), and for plants of hybrids Albena (open squares) and G100 (filled triangles) grown in a dense field canopy (B). Vertical bars correspond to standard deviation.

The time-course of the relative expansion rate had common features in all cases

Nevertheless, the dynamics of growth of all leaves showed strong similarities, based on the kinetics of the relative expansion rate (Figs 2 and 3, bottom graphs). Provided it was expressed per unit thermal time, the time-course of the relative expansion rate had a general shape conserved across years, locations, genotypes, and phytomers (Figs 2, 3). In all cases, the relative expansion rate was high for all phytomers during the first 100 °Cd following initiation, rapidly decreased before 150 °Cd (except for phytomer 2, see below), then stabilized for a certain time, and subsequently continuously decreased to reach zero at the end of leaf expansion.

To account for this, the time-course of expansion of all individual leaves was divided into three periods (Fig. 4). The first two periods were assumed to be exponential, with rates equalling R_1 and R_2 , respectively. During the third

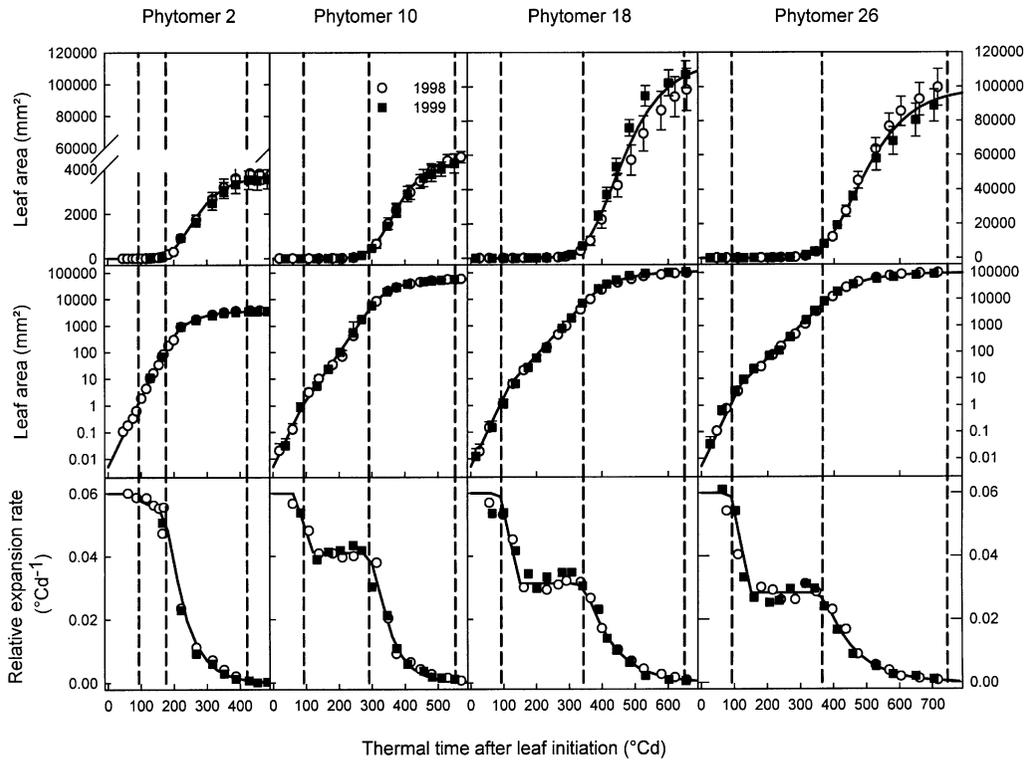


Fig. 2. Thermal-time-course of leaf area (linear and logarithm scale) and leaf relative expansion rate at phytomers 2, 10, 18, and 26 of isolated plants of hybrid Albena grown in 1998 (open circles) and 1999 (filled squares). Vertical bars correspond to standard deviation. The three successive vertical dashed lines on each graph correspond, respectively, to the end of the first exponential phase, the end of the second exponential phase and the leaf full expansion. Solid line corresponds to the three-phase fit (see text and Fig. 4).

period (rapid leaf growth) the relative expansion rate R was fitted for each leaf to a negative exponential function of thermal time t as follows:

$$R=R_3 \times \exp[-a \times (t-t_2)] \quad (2)$$

Change with time of leaf area was therefore fitted to the following set of three equations:

$$t_0 < t < t_1 \quad \ln A = \ln A_0 + R_1 \times (t-t_0) \quad (3)$$

$$t_1 < t < t_2 \quad \ln A = \ln A_1 + R_2 \times (t-t_1) \quad (4)$$

$$t > t_2 \quad \ln A = \ln A_2 + R_3/a \times \{1 - \exp[-a \times (t-t_2)]\} \quad (5)$$

where A was the leaf area at time t , t_0 the date of leaf initiation, A_0 the leaf area at initiation, R_1 the relative expansion rate during the first exponential period, t_1 the date of the end of the first exponential period, A_1 the leaf

area at the end of the first exponential period (calculated with equation 3), R_2 the relative expansion rate during the second exponential period, t_2 the date of end of the second exponential phase, A_2 the leaf area at t_2 (calculated with equation 4), a the parameter characterizing the exponential decrease of the relative exponential rate after t_2 , and R_3 the relative expansion rate at t_2 .

For consistency with data points, fitted relative expansion rates were averaged on periods of 60 °C (Fig. 4, solid line) and did not show the discontinuities at t_1 and t_2 obtained when calculated by deriving equations 3–5 (Fig. 4, dotted line). The succession of two exponential phases with different growth rates accounted for the decrease in relative expansion rate observed for all leaves between 50 °Cd and 150 °Cd after leaf initiation (Figs 2

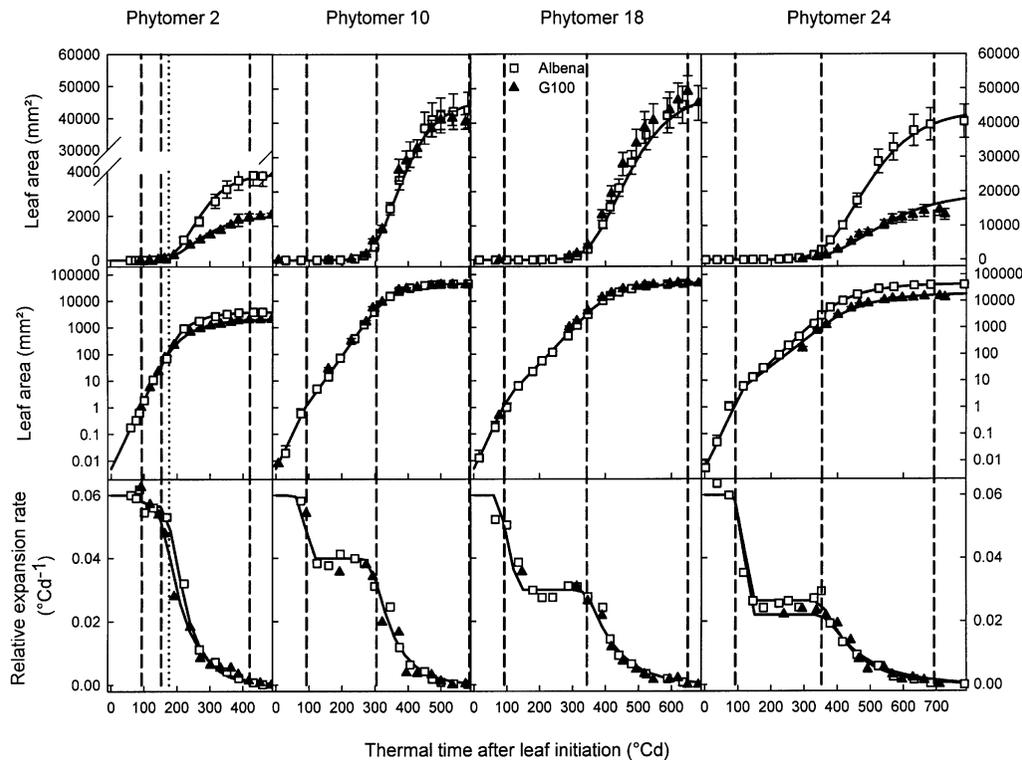


Fig. 3. Thermal-time-course of leaf area (linear and logarithmic scale) and leaf relative expansion rate at phytomers 2, 10, 18, and 24 for plants of hybrids Albena (open squares) and G100 (filled triangles) grown in a dense canopy. Vertical bars correspond to standard deviation. The three successive vertical dashed lines on each graph correspond, respectively, to the end of the first exponential phase, the end of the second exponential phase and the leaf full expansion. The dotted line corresponds to the end of the second exponential phase at phytomer 2 for Albena. All other dates were common to both hybrids. Solid line corresponds to the three-phase fit (see text and Fig. 4).

Table 1. Final leaf area per plant, rates of leaf development and dates of reproductive development for isolated plants of hybrid Albena and plants of hybrids Albena and G100 grown in a dense field canopy

Values are means ±SD.

Hybrid	Density	Final leaf area per plant (m ²)	Rate of phytomer initiation (°Cd ⁻¹)	Rate of leaf appearance (°Cd ⁻¹)	Capitulum initiation (°Cd)	Anthesis (°Cd)
Albena	Isolated	2.34±0.09	0.106±0.002	0.094±0.002	236±13	824±14
Albena	Canopy	1.06±0.06	0.106±0.002	0.090±0.002	236±13	832±16
G100	Canopy	0.82±0.06	0.071±0.002	0.058±0.003	297±14	902±15

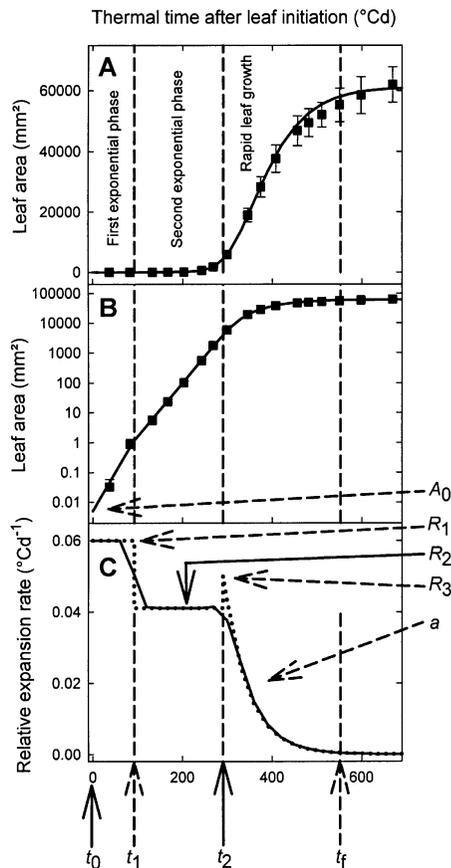


Fig. 4. A three-phase fit to describe the time-course of expansion of all leaves studied. The change with thermal-time of the logarithm of the leaf area (B) was fitted to two successive linear functions (first and second exponential phases) and to an exponentially decreasing relative expansion rate during the third phase (phase with rapid leaf growth on a linear scale (A)). Corresponding change with time of the relative expansion rate is indicated in C (dotted line). The solid line in C corresponds to the time-course of the relative expansion rate when averaged on periods of 60 °Cd to be consistent with experimental data (Figs 2 and 3, bottom graphs). Vertical dashed lines indicate the end of the phases. Solid arrows indicate the characteristic parameters of any given leaf: t_0 , the date of initiation, R_2 the rate of the second exponential phase, and t_2 , the end of the exponential growth. Dashed arrows indicate the parameters either common to all leaves (A_0 , the leaf area at initiation, t_1 and R_1 , the duration and the rate of the first exponential phase), or deduced from the preceding ones (R_3 , the relative expansion rate at the beginning of the third phase, a , the rate of exponential decrease of the relative expansion rate during the rapid leaf growth, and t_f , the date of full expansion).

and 3, bottom graphs). The results of fitting are presented as lines on Figs 2 and 3. They accounted for leaf growth in all cases ($r^2 > 0.999$, $P < 0.0001$).

Analysis of the relative expansion rates

The stability of the relative expansion rate during the first 100 °Cd following initiation was not clear for individual leaves (Figs 2 and 3, bottom graphs) because there were, at best, two values per treatment during this period owing to the method of calculation. Conversely, data processing of all leaves studied suggested that relative expansion rate

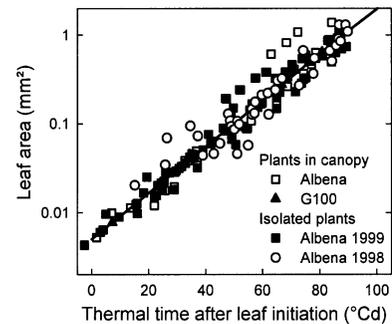


Fig. 5. Thermal-time-course of leaf area (logarithmic scale) between 0 and 100 °Cd after leaf initiation for phytomers 3 to 30 for isolated plants of hybrid Albenca grown in 1998 (open circles) and 1999 (filled squares), and plants of hybrids Albenca (open squares) and G100 (filled triangles) grown in a dense field canopy.

during this first period was common to all phytomers (Fig. 5). Data on leaf area versus thermal time after leaf initiation, gathered from three experiments and including phytomers 3 to 30, fitted to a common exponential function ($r^2 = 0.945$, $n = 171$, $P < 0.0001$). The slope of the exponential fit corresponded to the mean relative expansion rate during this first period and equalled 0.060 ± 0.001 °Cd $^{-1}$. The y-intercept of the exponential fit corresponded to the average area of the leaf primordium when initiated and equalled 0.0050 ± 0.0003 mm 2 . The relative expansion rate during the first phase was then calculated for each individual leaf (Fig. 6A) with the data from Fig. 5 by assuming that the initial area was common to all leaves. Individual rates were scattered around the mean rate (horizontal dotted line) with no significant effect of phytomer position or treatment (Fig. 6A). Therefore, early expansion of leaf primordium, from initiation to c. 100 °Cd later, appeared to be exponential and common to all leaves studied, irrespective of phytomer position, tested growing conditions and genotypes.

The relative expansion rate during the second exponential phase was lower than that occurring before 100 °Cd, except for phytomer 2, where the rate was maintained at a similar level (Figs 2 and 3, bottom graphs). It was highest for phytomer 2 (e.g. 0.057 °Cd $^{-1}$ for isolated plants) and decreased continuously with phytomer number (e.g. 0.028 °Cd $^{-1}$ for phytomer 26 of isolated plants, Figs 2, 6B). A similar trend was observed for isolated plants, plants grown in a dense canopy and for the two hybrids (Fig. 6B). For plants grown in a canopy, competition for light gave a greater decrease in expansion rate with phytomer number (Fig. 6B), without changing the general shape of the decrease. The difference between isolated plants and plants in a canopy increased from phytomer 6 upwards (Fig 6B and insert). The relative expansion rate during the second exponential phase was similar in both hybrids for phytomers 2 to 21 (Fig. 6B). It sharply decreased for the upper leaves of G100 corresponding to the last initiated

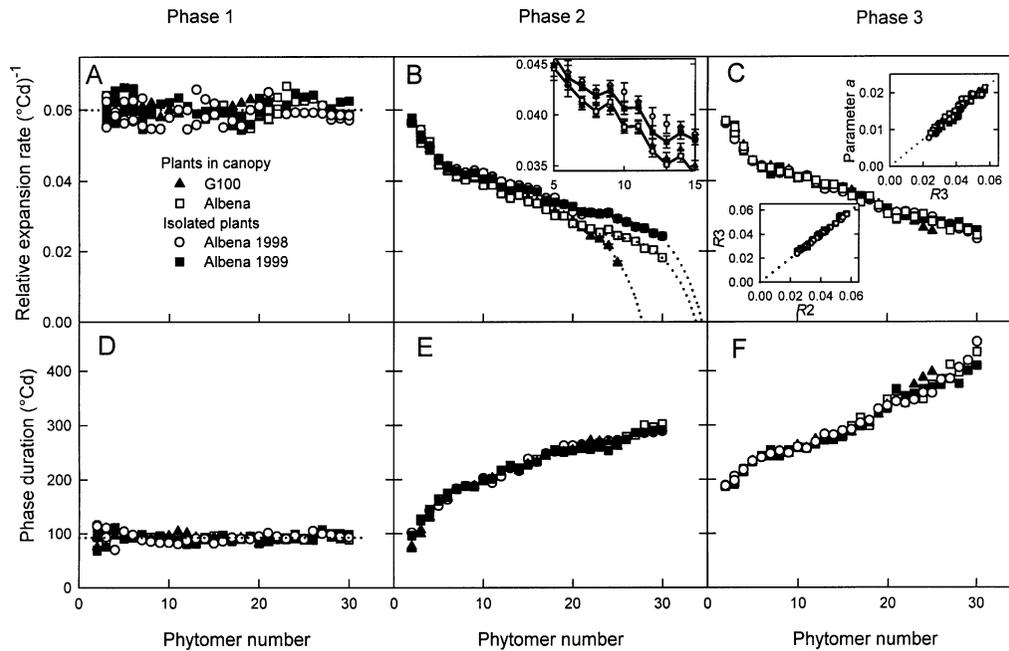


Fig. 6. Relative expansion rate (A, B, C) and duration (D, E, F) of each of the three phases of leaf development as a function of phytomer position for isolated plants of hybrid Albena grown in 1998 (open circles) and 1999 (filled squares), and plants of hybrids Albena (open squares) and G100 (filled triangles) grown in a dense field canopy. (B) The insert is a zoom of the main curve to make visible the differences between isolated plants and canopy plants for phytomers 5 to 15. Vertical bars correspond to standard errors and are generally smaller than symbol size on main figures. Dotted lines correspond to a negative exponential fit for the five upper ‘true’ leaves (equation 6 in text). The x -intercept corresponded to the phytomer position of the first involucre bract. (C) The main figure indicates the relative expansion rate at the beginning of the phase (R_3). The relative expansion rate then exponentially decreased with time (rate=parameter a) according to equation 2 (see text). Inserts illustrate the proportionality between R_3 and the parameter a (insert above the main curve) and the equality between R_2 (rate of the second phase) and R_3 for isolated plants (insert below the main curve).

phytomers (phytomers 21 to 25), and, for example, leaf growth of phytomer 24 of G100 did not fit with that of phytomer 24 of Albena (Figs 1, 3). A similar acceleration of the decrease in expansion rate with phytomer number was also observed for the last initiated phytomers of hybrid Albena (phytomers 25 to 30) either grown as isolated plants or in a dense canopy (Fig. 6B). This accelerated decrease was therefore more related to the proximity of the capitulum than to phytomer position counted from the plant base (Fig. 6B). It was fitted to the following equation:

$$R_{2(N)} = c \times \{1 - \exp[-b \times (N_b - N)]\} \quad (6)$$

where $R_{2(N)}$ was the second exponential rate for phytomer number N , and N_b the phytomer number of the first involucre bract. The same parameter b ($b=0.322$, $r^2=0.966$, $P < 0.001$) accounted for the decrease of R_2 on the five upper leaves for both hybrids grown either as isolated plants or in a dense canopy (dotted lines on Fig. 6B).

The relative expansion rate during the third period was characterized with two parameters, R_3 and a . For isolated plants, calculated R_3 did not differ significantly from R_2 , the rate of the second phase (Fig. 6C, insert below main curve, $R_3=R_2$ along the dotted line). For plants grown in a canopy, calculated R_3 was very close to that of isolated

plants at any phytomer position (Fig. 6C, main graph). Plotting a against R_3 for all leaves studied (Fig. 6C, insert above main curve) resulted in a strong linear relationship with a zero intercept (slope= 0.372 ± 0.002 , $r^2=0.955$, $n=123$, $P < 0.0001$), common to both hybrids and environmental conditions. So, neither R_3 nor a differed between isolated plants and plants in a canopy at any given phytomer position, showing that the time-course of the relative expansion rate was independent of interplant competition during this last phase. For plants grown in a canopy, R_3 progressively diverged from R_2 as phytomer number increased above 6 (compare Fig. 6B with C).

Stability of the duration of the phases

The duration of the first exponential phase (t_1-t_0) averaged 92.3 ± 1.7 °Cd, with no significant effect of phytomer position or treatment (Fig. 6D). Its variability was higher for phytomers 2 to 5, probably because the rates of the two successive phases were rather close for such leaves (e.g. phytomer 2, Figs 2 and 3, bottom graphs), making it difficult to date precisely a shift between them. For modelling purposes, the duration of the first phase was taken to be common to all leaves studied (Fig. 4).

The duration of the second exponential phase (t_2-t_1) increased with phytomer number (Fig. 6E) according to a

pattern common to plants of hybrids G100 and Albena, either isolated or grown in a canopy. It increased by about 30 °Cd and 7 °Cd per phytomer below and above phytomer 5, respectively. The duration of the second exponential phase was similar in isolated plants and plants grown in a canopy at any given phytomer (Fig. 6E). It was also similar in both hybrids for all alternate leaves (from phytomer 5 upwards, Fig. 6E). Duration was slightly shorter for leaf pairs of G100 at phytomers 2 to 4 (Fig. 6E).

The end of leaf expansion, t_f , was calculated with equation 5 as the date when leaf area A equalled 95% of its final value. The duration of the third phase ($t_f - t_2$) increased with phytomer number, but did not significantly differ between isolated plants and plants in a canopy, and between hybrids (Fig. 6F).

A whole-plant representation of leaf development to analyse the response of leaf expansion to environment and genotype

Four leaf developmental stages, namely leaf initiation, end of first exponential phase, end of second exponential phase and end of expansion (resp. t_0 , t_1 , t_2 , and t_f in Fig. 4) were required to characterize the time-course of leaf expansion. Representing the dates of occurrence of these four events for each phytomer illustrates the co-ordination of the development of the successive leaves (Fig. 7A for hybrid Albena and 7D for hybrid G100). Taken together for a given stage (e.g. initiation), these dates define the progression of this stage along the stem. For each hybrid, the number of initiated phytomers against thermal time was fitted to three successive linear regressions corresponding to (i) phytomers bearing leaf pairs (phytomer number <5), (ii) intermediate phytomers (phytomers 5 to 25 and 5 to 20 for Albena and G100, respectively), and (iii) the five upper phytomers bearing ‘true’ leaves (Fig. 7A, D). The rates of phytomer initiation of the three successive phytomer classes of Albena plants equalled 0.018, 0.106 and 0.245 phytomer °Cd⁻¹ ($r^2=0.993$, 0.997 and 0.987, respectively, $P < 0.0001$). Initiation rates of G100 were 30–50% lower: 0.014, 0.071 and 0.125 phytomer °Cd⁻¹ ($r^2=0.999$, 0.997 and 0.997, respectively, $P < 0.0001$). The progression of the other stages (Fig. 7A, D) was deduced from that of initiation and from the duration of the phases according to phytomer number described above (Fig. 6).

This whole-plant diagram (Fig. 7A, D) constitutes a framework of analysis of leaf expansion by positioning for each leaf the dates of development pertinent for its expansion. Differences in final leaf area between isolated plants and plants in a canopy concerned phytomers 6 and upwards (Fig. 7B), and were linked to different expansion rates during the second exponential phase (Fig. 6B). Leaf expansion was therefore affected by interplant competition for light between the end of exponential growth at phytomer 5 and that at phytomer 6. This occurred around

300 °Cd after plant emergence (dotted vertical line on Fig. 7A), long before any detectable effect on visible leaves (Fig. 7C). The growth rate of the second exponential phase of subsequent leaves was more and more affected (Fig. 6B), due to an increasing part of this exponential phase occurring during the constraint (Fig. 7A).

Differences between the two genotypes in the time-course of leaf area per plant (Fig. 7C) were mainly due to the slower initiation rate and to the lower final number of leaves of hybrid G100 compared to Albena (Fig. 7A, D). The delayed growth curve of G100 (Fig. 7C) was associated to delayed development of successive individual leaves. For example, leaf of phytomer 15 of G100 was initiated 172 °Cd after seedling emergence, i.e. 60 °Cd later than the equivalent leaf of Albena, and this delay was conserved through the successive stages of development of that leaf (vertical arrows on Fig. 7A, D) until full expansion (765 versus 705 °Cd). The lower final leaf area per plant of G100 (Fig. 7C) was mainly explained by the lower final leaf number (Fig. 7A, D). Changing the phytomer number of the first involucre bract (N_b) in equation 6 (28 instead of 33.5) was enough to account for the final area of leaves of G100 compared with Albena.

Discussion

A common pattern of development for all leaves of two sunflower hybrids grown under fluctuating conditions

The results suggest that, irrespective of phytomer position, tested environmental conditions and genotype, leaf development consists of the same succession of three phases characterized by the thermal-time-course of leaf relative expansion rate (Fig. 4 and Equations 3–5). Internode extension in maize was described with a similar three-phase framework (Fournier and Andrieu, 2000a).

The relative expansion rate was high (0.06 °Cd⁻¹) and common to all phytomers during a first short period (92 °Cd). It was followed by a second exponential phase with a different rate, while the relative expansion rate decreased continuously during the third phase. The earliest period was not mentioned in previous works on phytomer 6 (Granier and Tardieu, 1998a), probably due to a limited number of measurements during the few days following primordium initiation, and because it is not easily distinguishable from the next period for phytomers close to the plant base (due to similar relative expansion rates during the two phases), as is the case for phytomer 2 in the present work (Figs 2, 3). It was only evident when examining higher phytomers (Figs 2, 3), and gathering data from 30 phytomers (Fig. 5). Adding this first exponential period to the two-phase framework previously presented (Granier and Tardieu, 1998a) is not problematic because the parameters of this early phase (i.e. primordium area at initiation, and rate and duration of the phase) were

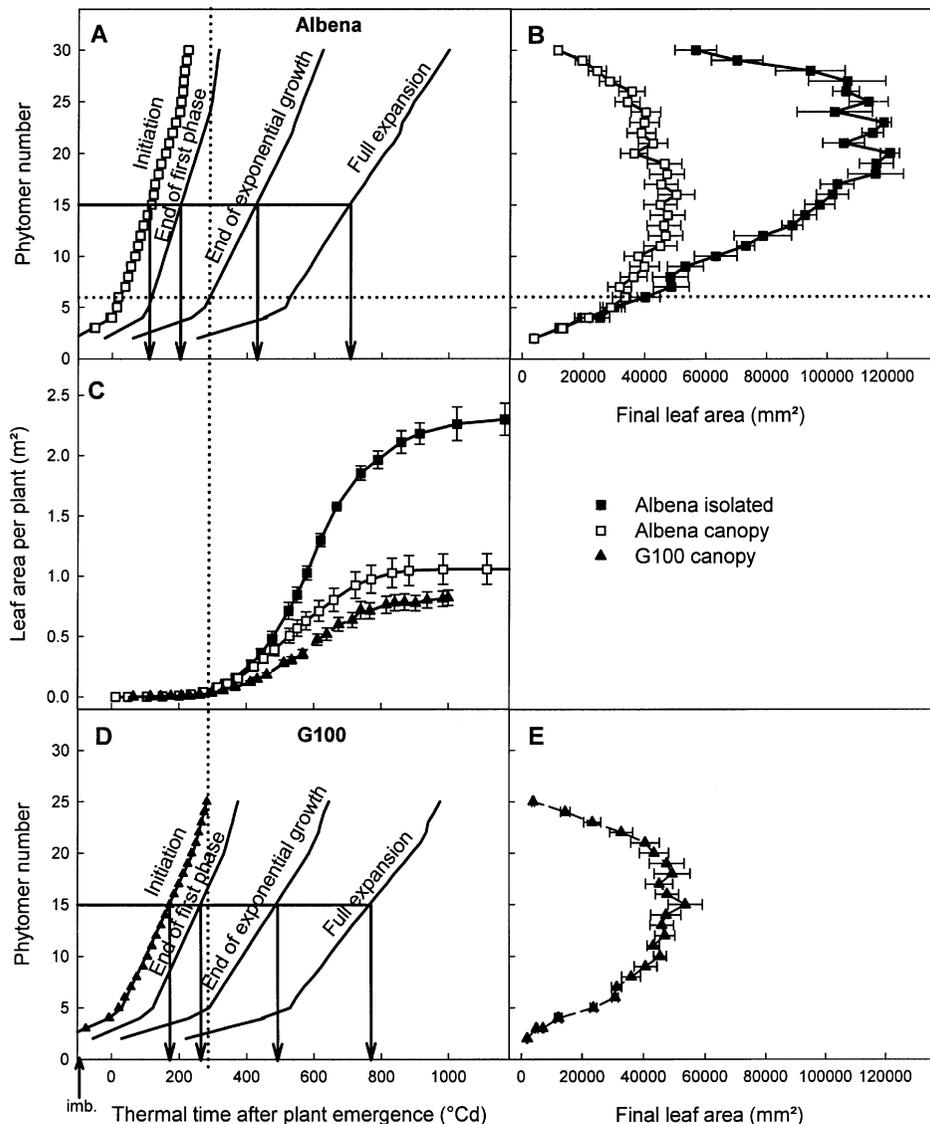


Fig. 7. A whole-plant framework to analyse leaf expansion in sunflower. Number of initiated phytomers, number of phytomers beyond first exponential phase, number of phytomers beyond exponential growth and number of phytomers with full expanded leaves as a function of thermal time after plant emergence in sunflower hybrids Albena (A) and G100 (D). Seed imbibition (imb.) occurred about 100 °Cd before emergence. Final leaf area according to phytomer number (B, E), and thermal-time-course of plant leaf area (C) for Albena isolated plants (filled squares) and plants of hybrids Albena (open squares) and G100 (filled triangles) grown in a dense canopy. For a given phytomer, for example, phytomer 15 in (A) (Albena) and (D) (G100), the diagram indicates the dates of occurrence of the four developmental stages characterizing leaf expansion (vertical arrows). At a given date, e.g. 300 °Cd (vertical dotted line), it indicates the number and the nodal position of the phytomers in each of the phases of development.

common to all leaves, and seemed to be insensitive to the environmental conditions tested.

This breakdown of leaf development allows a simple analysis of the effects of genotypes and growing conditions on leaf expansion. Leaf area strongly differed among the different situations described here. Nevertheless, these differences were accounted for by a limited number of variables of leaf growth and development. As shown for phytomer 6 (Granier and Tardieu, 1998*b*), the duration of the phases was stable for any given phytomer through different years and locations if expressed on a thermal time

basis. This stability was not observed for elongation of internodes in maize submitted to more severe light deficit (Fournier and Andrieu, 2000*b*). Irrespective of phytomer position along the stem, the response to a limitation of intercepted light per plant affected leaf expansion only during the exponential phase via a reduction of the relative expansion rate, as observed for phytomer 6 (Granier and Tardieu, 1999). The relative expansion rate was affected neither during the first exponential phase, nor during the third phase (rapid growth phase). As a consequence, in the studied cases, the final leaf area was determined as early as

the end of the second exponential phase, approximately half-time between initiation and full expansion.

Relative expansion rate decreased and exponential growth duration increased with phytomer number according to a pattern common to different genotypes and environments

Differences between phytomers concerned only the rate and the duration of the second exponential phase. These differences ranged according to a strict pattern common to the two studied hybrids and the different environmental conditions: the duration increased linearly, while the rate decreased continuously with phytomer position. This pattern resulted in the typical bell-shaped distribution of final leaf area along the stem (Kobayashi, 1975). Decreasing relative expansion rates versus phytomer number were observed on other species, including dicots (Cordero *et al.*, 1985; Granier *et al.*, 2002) and monocots (Gallagher, 1979; Lafarge and Tardieu, 2002). A linear increase in the duration of expansion with phytomer number was also described in pea (Turc and Lecoer, 1997) and sorghum (Lafarge and Tardieu, 2002). Conversely, Kobayashi (1975) considered that the duration from leaf emergence to full expansion was common to all phytomers, but its analysis was based on calendar time and not thermal time.

A first consequence of these results is that the notion of plastochron index, a continuous developmental scale based on leaf number (Erickson and Michelini, 1957), largely used in physiological studies in a wide range of species (Lamoreaux *et al.*, 1978; Gagnon and Beebe, 1996) should be used with great caution in sunflower. One of the three underlying assumptions, i.e. similar relative growth rates between successive leaves (Erickson and Michelini, 1957; Lamoreaux *et al.*, 1978) is not fulfilled, except during the earliest phase. Nevertheless, a leaf plastochron index could be acceptable if limited to a few consecutive leaves or to a relatively short period of plant development (Russin *et al.*, 1991; Gagnon and Beebe, 1996).

The involvement of reproductive development in leaf expansion rates has been shown, for example, on leaves of *Hedera helix* which exhibit specific morphology and anatomy depending on whether they were initiated before (juvenile) or after (mature) flower induction (Stein and Fosket, 1969). The relative expansion rate remains stable on successive juvenile leaves, whereas it decreases with phytomer number for mature leaves (Cordero *et al.*, 1985). Similarly, relative expansion rate remains stable until leaf 37 for short-day-grown *Arabidopsis thaliana* producing more than 40 leaves (Groot and Meicenheimer, 2000), while it decreases as early as leaf 6 for long day-grown plants producing 9 leaves (Granier *et al.*, 2002). In the present study, the effect of the reproductive development on the dynamics of the relative expansion rate seemed to be limited to the five last initiated leaves. For those leaves,

the same pattern, based on phytomer distance to the capitulum, was obtained for two hybrids differing in final leaf number, irrespective of absolute phytomer number (Fig. 6B). On the other hand, the continuous decrease in exponential rate observed for the two hybrids from plant base (phytomers 2 to 20) preceded reproductive events. It seemed to be ontogenetic, but not directly driven by reproductive development.

Other patterns of leaf development associated with phytomer position are frequently mentioned in the literature. For example, many plants produce different types of leaves during their development (Allsopp, 1967; Poethig, 1990), and some of these differences, concerning leaf shape and anatomy, are regular features of shoot development and are considered as components of a genetically regulated programme of shoot maturation (Poethig, 1997; Kerstetter and Poethig, 1998). The regular change with phytomer position of duration and rate of expansion observed here could be dependent on such a programme, modulated by reproductive events and environmental conditions.

Conclusion

The whole-plant framework established in this paper is a tool to structure the analysis of the response of leaf expansion to genotypes and environment. It identifies a set of relevant parameters and variables (rates and duration, and their pattern of change with phytomer number), the variability of which has now to be analysed and quantified in response to a wider range of genotypes and environmental conditions. Because it locates spatially and temporally the growth of the organs and the susceptibility of the expansion to environmental constraints (depending on organ developmental stage), this framework could constitute a pertinent phenological module for a computer-generated 3-dimensional representation of virtual plants responding to environment, thus simulating the phenotypes (Rey *et al.*, 2000).

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