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# 3D Architectural Modelling of Aerial Photomorphogenesis in White Clover (*Trifolium repens* L.) using L-systems

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The objective of this work was to construct a model of aerial development of clover that takes into account morphogenetic responses to the light environment, and to use it to analyse and understand these processes in terms of signal perception and integration. The plant model was interfaced with a Monte Carlo model that determines photosynthetically active radiation (PAR) and red/far-red ratio (R/FR) throughout the canopy, taking into account the absorption, reflection and transmission of light by individual leaves. Light intensity and quality were sensed by the plant model at discrete time intervals and at discrete sites of perception: apices, emerging internodes and petiole tips. This input regulated the final size of internodes and leaves, the vertical positioning of leaves, and the branching delay. The empirical relations (regression functions) quantifying this regulation were derived from data reported in the literature and original measurements. Simulations produced realistic visualizations and quantitative characterizations of the modelled plants for different light treatments. These results were in general agreement with observations of real plants growing under similar conditions, suggesting that the dependence of organ size and position on light treatments can be regarded as an integration of the responses of individual plant organs to their local light environment. The model is used to describe the regulation of branch appearance and the impact of self-shading on plant morphogenesis as a function of local light environment. 

**Key words:** Clover, *Trifolium repens* L, photomorphogenesis, plant architecture, L-system, modelling, Monte-Carlo method, competition for light, red : far-red ratio, irradiance, light quality, leaf size, self-shading.

## INTRODUCTION

In canopies, a plant is subjected to a heterogeneous light environment due to the interactions with neighbouring plants and with other parts of the plant itself (self-shading). Shading decreases the PAR, thus limiting the energy available for photosynthesis and, consequently, plant growth. Shading also modifies light composition due to different optical properties of leaves for different light wavelengths. With increasing depth in a canopy, the intensities of blue light (BL) and red light (R) decrease greatly, whereas the intensity of far-red light (FR) decreases more slowly (Holmes and Smith, 1977*b*). These changes in light environment are detected by plants inducing substantial responses which have the effect of reducing future shading, causing them to grow away from their neighbours (Novoplansky *et al*., 1990), and to position leaves in areas of full sunlight, thus playing a fundamental role in light interception. In return, changes in plant architecture will modify the local light environment. It is thus necessary to take into account the interactions between plant architecture and light environment when modelling plant growth and development (Aphalo and Ballaré, 1995).

Architectural models provide a useful tool to study the response of plants to environment, aiding in interpretation of observations and experiments in the field. Historically, construction of architectural plant models represents a progression from abstract models of generic branching structures to increasingly accurate models of specific plants (Měch and Prusinkiewicz, 1996). The model of photomorphogenesis of white clover presented in this paper is the next step in the quest for increasing faithfulness in the representation of light microclimate of individual plant organs. In addition to direct light from the sky, we consider indirect light, reflected and transmitted by the leaves. Furthermore, we consider the PAR, and R (660 nm) and FR (730 nm) wavelengths as the key indicators of light composition perceived by the plant. In contrast to R and FR, which can be assigned individual wavelengths, PAR is composed of a continuous range of wavelengths, which may interact with the surfaces in different manners. In principle, we could decompose PAR into a set of discrete wavelengths; however, the simulation of light distribution is then very slow on current computers. Consequently, we have estimated PAR on the basis of blue light distribution. This is in agreement with the correlation between the distribution of PAR and BL in a canopy (Baraldi *et al*., 1994) and the assumption that the blue light photoreceptor senses the change in PAR (Smith, 1982). This approach, however, does

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not allow us to consider photomorphogenetic responses specific to BL, which, consequently, are not included in the model.

Construction of an architectural model not only integrates our knowledge of the plant but, importantly, it also exposes missing elements in our knowledge. We believe that these aspects are representative of general methodological issues that should be addressed when constructing similar models of other plants. Our attention is limited to the morphogenesis of the above-ground parts, since very few data on the effects of light on the morphogenesis of clover roots are available.

## MORPHOLOGY AND DEVELOPMENT OF WHITE CLOVER

### *Data used to construct the model*

The spatial heterogeneity in light environment is particularly important for plagiotropic plants, such as white clover, in which secondary axes (stolons) spread horizontally. The development of a stolon is due to the sequential production of modules (phytomers) by the apical meristem (Thomas, 1987). A phytomer consists of a leaf, an internode, two root primordia, and an axillary meristem. An axillary meristem develops either into a flower or into a vegetative axillary bud, which in turn may develop to form a new axis, remain dormant, or die, depending on growing conditions.

The description of the development of white clover over time is based on the co-ordination between the initiation and growth of successive phytomers, and the co-ordination between different organs within a phytomer (Thomas, 1987; Belaygue *et al.*, 1996). In general, development can be expressed in plastochrons (time between the initiation of two successive leaves) or in phyllochrons (time between the emergence of two successive leaves). We arbitrarily choose to use the term 'phyllochron' in this paper, as in clover the phyllochron and the plastochron are equal. In clover, there are usually six phytomers hidden in the stolon tip and three visible growing phytomers in each (sufficiently old) stolon (Thomas, 1987; Belaygue *et al.*, 1996).

The growth of internodes, petioles and laminae is sigmoidal with time (Thomas, 1987; Belaygue *et al.*, 1996). Final size of organs is strongly affected by environment and genotype (Thomas, 1987). The duration of leaf growth increases under low irradiance and water deficit, but the co-ordination between leaf growth and leaf apperance is not significantly affected (Thomas, 1987; Belaygue *et al.*, 1996). Consequently, the model assumes that light environment has no major effect on phytomer development expressed in phyllochrons.

*Data analysis*. In this study, we consider the effect of PAR and R/FR on the final size of internodes, petioles and laminae and the formation of branches. The way clover integrates different light signals is not yet known, and thus the present model is based on the assumptions made by Turkington *et al.* (1991): each module size is independently regulated by the light environment perceived by the module itself, whereas module appearance rate is regulated at the plant level and assumed to be the same on every axis. The software Mathematica 3.0 (Wolfram, 1996) was used to fit polynomial functions to the data, and the quality of the fitting was estimated by calculating the coefficient of determination $R^2$ (Figs 1–3). The experimental data were obtained using three different types of treatment: changes in light composition applied throughout the photoperiod (Solangaarachchi and Harper, 1987; Thompson and Harper, 1988; expts 1 and 2 described below; control treatment in Gautier *et al.*, 1997); changes applied only at the end of the day (Moulia *et al.*, 1989; Robin *et al.*, 1992); and local irradiation of the apex applied continuously during the photoperiod (Robin *et al.*, 1994*b*). When PAR data were missing (Solangaarachchi and Harper, 1987), they were estimated from meteorological data and characteristics of the experimentation. We based our estimations of leaf area on the correlation between leaf area and petiole length (Barcikowska, 1976) from measurements of 183 leaves (Gautier *et al.*, 1997):

$$\text{individual leaf area (cm}^2\text{)} = 1{\cdot}39 \times \text{petiole length (cm)} \quad (R^2 = 0{\cdot}28) \tag{1}$$

As the canopy develops, actual branching decreases. Simon *et al.* (1989) linked this behaviour to light by showing that apices located within a clover canopy have a slower rate of branching than apices in sunlight. The present model quantitatively simulates regulation of branching by the light environment by considering that: (1) an apex shaded for three phyllochrons dies, which terminates the addition of new phytomers to its axis; and (2) shade decreases the number of branches by postponing their emergence.

The 'branching delay' (expressed in phyllochrons) was defined as the time between leaf emergence and the emergence of the corresponding axillary branch (Fig. 3). Since a new phytomer emerges every phyllochron, the branching delay was calculated as the number of visible phytomers without branches at the stolon tip (Robin *et al.*, 1994*b*; Gautier *et al.*, 1997). When this value was not specified directly (Solangaarachchi and Harper, 1987; Moulia *et al.*, 1989; our expt 2 described below), the branching delay was estimated by subtracting the number of branched nodes from the total number of nodes on the main axis, assuming that there was no flowering or aborted buds.

*Experiments*. Experiments 1 and 2 refer to data from two experiments on white clover (*Trifolium repens* L.) in growth chambers designed to determine the main morphogenetic responses induced by changes in the R/FR light ratio. Cuttings were prepared and grown as described by Gautier *et al.* (1997). Temperature was a constant 18°C (day and night) and the duration of the photoperiod was 10 h $d^{-1}$. The experiments were conducted for 4 weeks under metallic halide lamps (HQI, Osram, France). A low or high R/FR ratio was applied throughout the photoperiod. The low R/FR was obtained by a coloured plastic filter (Lee Filters, Andover, Hampshire, UK, filter HT118); PAR of both treatments was adjusted to 146 μmol $m^{-2}$ $s^{-1}$. At the end of

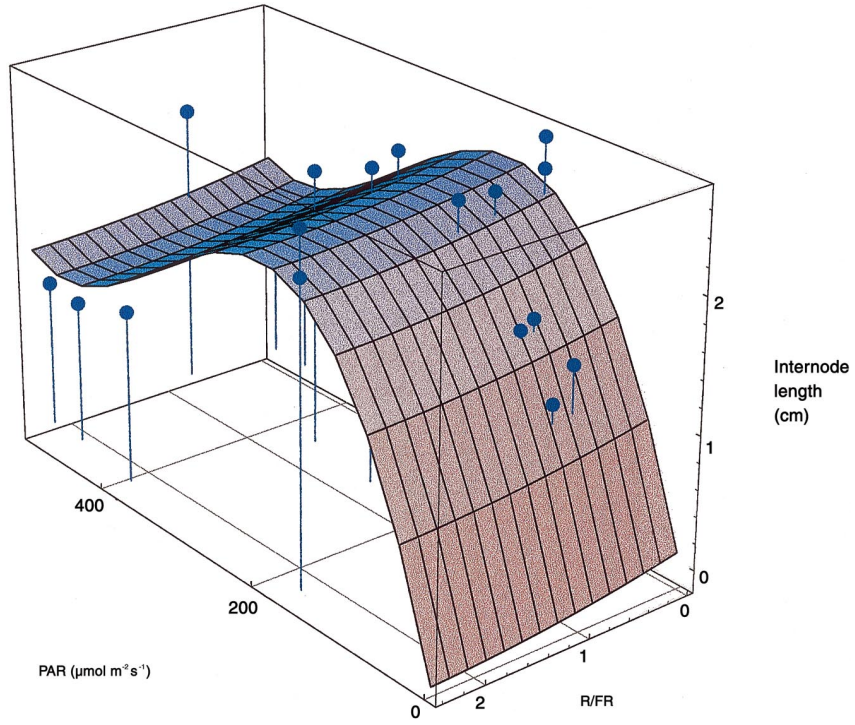


FIG. 1. Fitted response of internode length ($I$, cm) to light environment (PAR, µmol $m^{-2}$ $s^{-1}$ and R/RF) estimated for fully developed phytomers measured in different experiments. $I\,(\text{cm}) = 0{\cdot}107 + 27{\cdot}7 \times (\text{PAR}) - 101{\cdot}6 \times (\text{PAR})^2 + 104 \times (\text{PAR})^3 - 0{\cdot}148 \times (\text{R/FR}) + 0{\cdot}026 \times (\text{R/FR})^2$ $R^2 = 0{\cdot}54$.

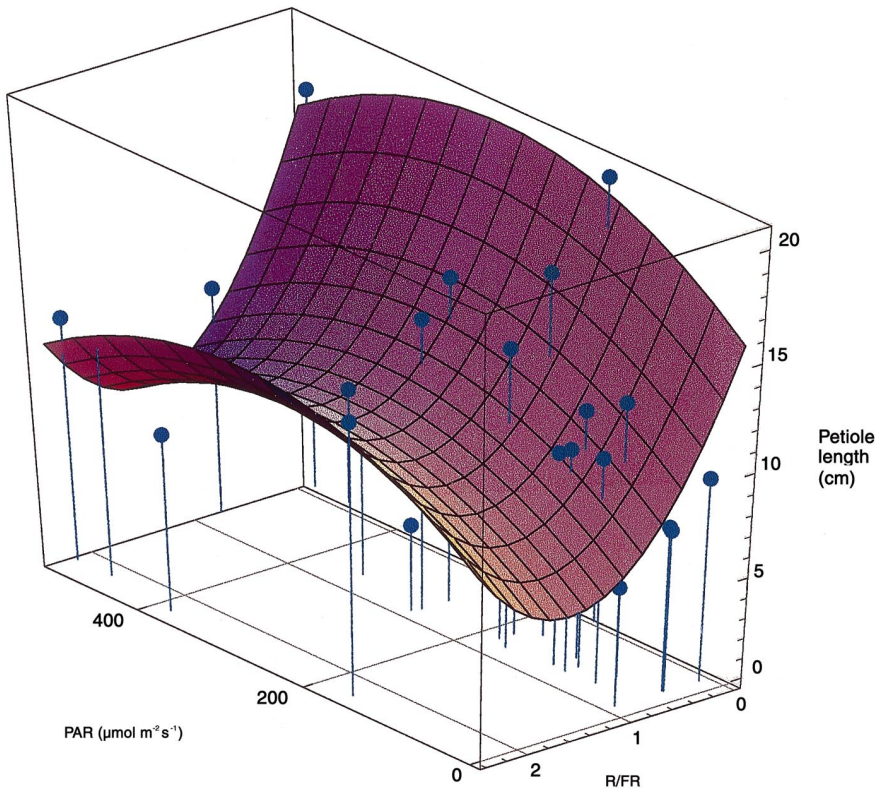


FIG. 2. Fitted response of petiole length ($P$, cm) to light environment (PAR, µmol $m^{-2}$ $s^{-1}$ and R/FR) estimated for fully developed phytomers measured in different experiments. $P = 15{\cdot}67 + 34{\cdot}39 \times (\text{PAR}) - 62{\cdot}53 \times (\text{PAR})^2 - 13{\cdot}44 \times (\text{R/FR}) + 4{\cdot}39 \times (\text{R/FR})^2 \quad R^2 = 0{\cdot}59$.

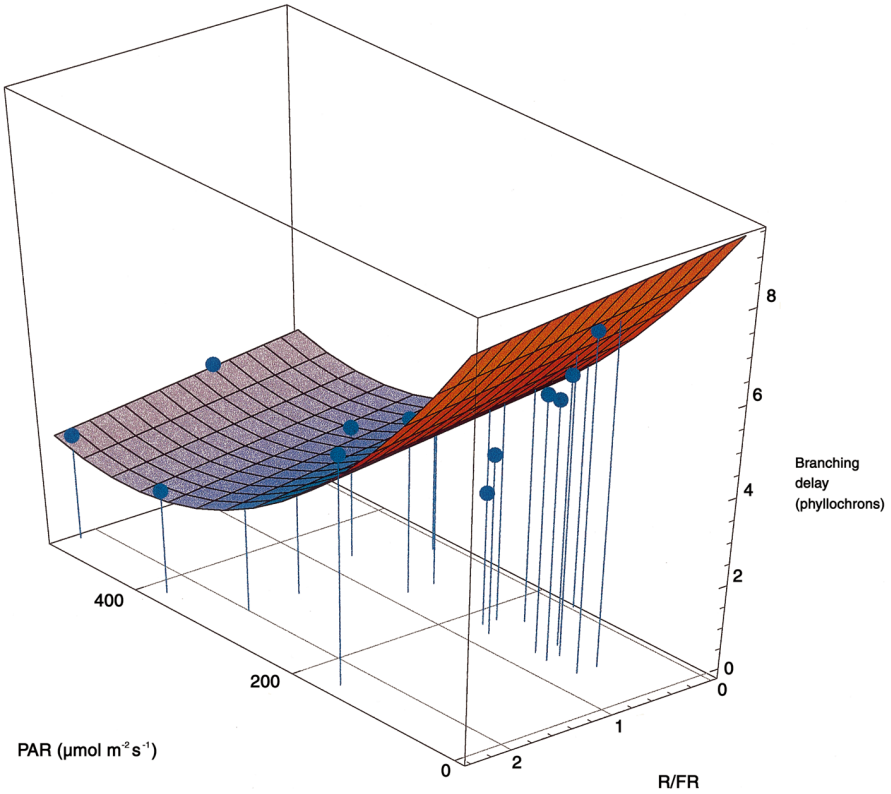


FIG. 3. Fitted response of branching delay (phyllochrons) to light environment (PAR, µmol $m^{-2}$ $s^{-1}$ and R/FR) estimated by the difference between the number of emerged phytomers and the number of emerged primary branches measured on the main axis. Branching delay (phyllochrons) = 9·34 − 31·84 × (PAR) + 38·5 × $(PAR)^2$ − 0·27 × (R/FR) $R^2 = 0{\cdot}88$.

the experiments the mean internode lengths were calculated as the ratios between the total stolon length and the number of visible internodes. In addition, the final size of the petiole and lamina of the last fully developed leaf was measured.

### *Data used to evaluate the model*

*Response to a decrease in R/FR*. Experiment 3 used a protocol similar to that used by Robin *et al.* (1992). Cuttings of white clover ('Huia') were grown in a controlled environment at 20°C and a relative humidity of 80% day and night, with a 9 h photoperiod. Plants were supplied with a complete nutrient solution. The day light conditions were similar between treatments (PAR = 315 µmol $m^{-2}$ $s^{-1}$ and R/FR = 2·3). Light treatments began when there were seven visible leaves per plant and lasted for 22 d. At the end of the day, nine plants received 1 h of FR light (resulting in a R/FR of 0·01; low R/FR treatment; $R_L$), whereas ten controls received no FR light ($R_H$). At the end of the experiment, the number of leaves, primary branches and total branches per plant were counted, and petiole length, lamina area and internode length were measured on every phytomer including the oldest ones which developed from the freshly made cutting. Hemispherical reflectance and transmittance of leaves were also measured with a spectroradiometer (LICOR, LI. 1800) and an integrating sphere, using the last fully developed leaf per plant. Outputs given by the models and experimental results were compared at the same number of emerged leaves on the main axis.

*Response to neutral or green shading*. The response to neutral or green shading was simulated for comparison with the data from Lötscher and Nösberger (1997). In their experiment, cuttings of white clover ('Ladino') were grown for 3 weeks in a growth chamber at 18/13°C day/night temperature, 75/85% relative humidity with a 16 h photoperiod, and a PAR of 175 µmol $m^{-2}$ $s^{-1}$. After 3 weeks of establishment, the main axis of the plant had developed eight phytomers; unfolded leaves were removed and light treatments were applied throughout the photoperiod: 'control' plants received 390 µmol $m^{-2}$ $s^{-1}$ PAR and a R/FR of 1·25 (high PAR and high R/FR: $F_HR_H$), 'neutral shading' plants received 175 µmol $m^{-2}$ $s^{-1}$ PAR and a R/FR of 1·12 (low PAR and high R/FR: $F_LR_H$), whereas 'green shading' plants received 175 µmol $m^{-2}$ $s^{-1}$ PAR and a R/FR of 0·32 (low PAR and low R/FR: $F_LR_L$). Simulations assumed that the plant was reduced to an apex at the beginning of the light treatment and were compared to experimental data at the same number of newly formed phytomers of the main axis.

*Visualization of plants grown under full light or green shading*. The development of the plant was simulated for 19 successive phyllochrons under PAR = 500 µmol $m^{-2}$ $s^{-1}$

and R/FR = 1·15 (high PAR and high R/FR: $P_HR_H$) or PAR = 150 μmol $m^{-2}$ $s^{-1}$ and R/FR = 0·1 (low PAR and low R/FR: $P_LR_L$). These conditions will be referred to as 'full sunlight' conditions and 'green shading', respectively. Vertical leaf positioning was determined every phyllochron and leaf area was calculated by multiplying the surface of a lamina by 0·5 while leaves were totally folded, and by considering the full area once they started unfolding.

*Impact of self-shading*. To determine the impact of using information about the intensity and composition of light at the sites of perception of successive phytomers (see below) in comparison to the use of the mean light environment above the plant, we compared outputs of simulations using locally computed light conditions or using a constant light throughout the simulated plant (PAR = 500 μmol $m^{-2}$ $s^{-1}$ and R/FR = 1·15 corresponding to high PAR and high R/FR: $P_HR_H$).

## THE MODEL

The complete clover model consists of two components: a structural-functional model of a developing plant, and a model of the light environment in which this plant develops. The plant model is implemented using an L-system-based plant simulator CPFG (Prusinkiewicz and Lindenmayer, 1990; Měch, 1997). The distribution of light in the canopy is simulated using the Monte Carlo method (Měch, 1997). The environment receives information from the plant model about the location, orientation, and optical properties of leaves and apical meristems and provides information about the local light environment (BL and R/FR ratio) sensed by these organs. This information exchange takes place every simulation step, set in our model to $\Delta t = 1/10$ of the phyllochron. The plant and environment simulators communicate by exchanging messages as described by Měch and Prusinkiewicz (1996). A similar technique was applied by Fournier and Andrieu (1998) to model the light environment (PAR) in a developmental model of maize.

### *The plant model*

The plant model was defined as a parametric L-system (Prusinkiewicz and Lindenmayer, 1990), extended with several constructs that facilitate model specification (Prusinkiewicz *et al*., 2000). According to assumptions of the L-system formalism (Lindenmayer, 1968), we regard a clover plant as a branching structure made of discrete units called modules. A set of up to six parameters is associated with each module:

$t$, the age of a module measured from the time of its creation. In the case of an active apical meristem $A$, it is the time from the production of the last phytomer. Time and age are expressed in phyllochrons;

$th$, the threshold age value, upon which a module produces other modules or dies;

*ord*, the order of the axis to which a module belongs. The main axis has order 0, its lateral axes have order 1, etc;

*pos*, position of a module within its axis. The module closest to the base of its axis has position 0, the subsequent modules have positions 1, 2, etc;

*dir*, a parameter indicating the direction at which a leaf and its axillary bud are issued: to the left ($dir = 1$) or to the right ($dir = -1$) of their supporting axis;

$x_{max}$, a parameter determining the maximum size of a fully developed organ (internode or leaf), or the final magnitude of a branching angle.

The initial structure consists of an apex $A$ with age $t = 0$ and threshold age $th_A = 1$. It is assumed that $A$ already contains six hidden phytomers. The remaining parameters specify axis order $ord = 0$, apex position $pos = 0$, and the direction of the first leaf and lateral bud $dir = 1$ (to the left). Parameter $x_{max}$ is omitted, as the apex, due to its small size, is not visualized in the model.

Plant development is described by *productions* that capture the fate of the modules as time advances. Productions are expressed using the syntax

$$predecessor : \{calculations\}condition \rightarrow successor \quad (2)$$

or

$$predecessor > context : \{calculations\}condition \rightarrow successor \quad (3)$$

The following production advances age $t$ of apex $A$ until the threshold value $th_A$ is reached (for simplicity, we omit other parameters):

$$A(t, th_A) : \{t' = t + \Delta t; \}t' < th_A \rightarrow A(t', th_A) \quad (4)$$

The exchange of information with the environment is implemented using *communication modules* (Měch and Prusinkiewicz, 1996). A communication module (referred to as a sensor, see below) returns information in the format *?E(blue, red, far-red)*, where the parameters *blue*, *red* and *far-red* represents light intensities for three different light wavelengths at the query point. If the amount of light perceived by the apex at time $t \geqslant th_A$ is greater than a threshold value *BlueMinApex* (corresponding to PAR = 30 μmol $m^{-2}$ $s^{-1}$), the apex differentiates into a phytomer $M$ and a new instance of apex $A$:

$$A(t, th_A) > ?E(blue, red, far\text{-}red) : \{t' = t + \Delta t; t'' = t' - th_A; \}t' \geqslant th_A \& blue \geqslant BlueMinApex \rightarrow M(t'', th_M)A(t'', th_A) \quad (5)$$

If the perceived light intensity is less than *BlueMinApex*, the sensor resamples it in one-phyllochron intervals for up to *DeathDelayApex* phyllochrons, waiting for the intensity to exceed the threshold. If this happens, the apex resumes the cycle of phytomer production; otherwise it dies:

$$A(t,th_A) > ?E(blue, red, far\text{-}red) : t + \Delta t \geqslant th_A + DeathDelayApex \ \& blue < BlueMinApex \rightarrow \varepsilon \quad (6)$$

TABLE 1. *Mean values of parameters used in the model*

| Growth function | $t_{min}$ (phyllochrons) | $t_{max}$ (phyllochrons) | $x_{min}$ | $x_{max}$ |
|---|---|---|---|---|
| Internode | 4·7 | 8·2 | 0 | Determined from Fig. 1 |
| Lamina | 5·5 | 9·5 | 0 | Correlated to petiole length [eqn (1)] |
| Petiole | 5·8 | 10·2 | 0 | Determined from Fig. 2 |
| Leaf angle β1 | 6·6 | 9 | 5° | 60° |

| | |
|---|---|
| Threshold age value for apex *A* to produce a phytomer | 1 phyllochron |
| Branching delay | Determined from Fig. 3 |
| Branching angle β2 | 44·8° ± 2·9° |
| Leaf senescence | 17·6 phyllochrons after phytomer initiation |
| *DeathDelayApex*, *DeathDelayBud*, *DeathDelayPetiole* | 3 phyllocrons |
| *BlueMinApex*, *BlueMinBud*, *BlueMinPet* | PAR = 30 μmol $m^{-2}$ $s^{-1}$ |

Standard deviation was fixed to 15% of the mean value unless specified.

Upon its initiation, a phytomer $M$ is decomposed into an internode $I$, a leaf primordium $K$ and a lateral bud $B$ using a *decomposition rule* (Prusinkiewicz *et al.*, 2000), which describes constituent elements of a compound module:

$$M(t, th_M) \xrightarrow{d} I(t, th_I)[K(t, th_K)][B(t, th_B)] \qquad (7)$$

The square brackets indicate that the leaf primordium and the bud are positioned laterally (as branches) with respect to their supporting internode (Lindenmayer, 1968).

The size of organs (internode, petiole and lamina) is dependent on light signals perceived at $t_{min}$ (Table 1: time when organ growth is initiated), which is in agreement with the greatest response following a switch to low BL observed on petioles still hidden within the apex (Gautier *et al.*, 1997). An internode $I$ elongates from the initial length $x_{min} = 0$ to its final length $x_{max}$ between the phyllochrons $t_{min} = 4{\cdot}7$ and $t_{max} = 8{\cdot}2$ (Table 1) according to the sigmoidal growth function defined by Prusinkiewicz, Hammel and Mjolsness (1993):

$$\text{growth}(t, t_{min}, t_{max}, x_{min}, x_{max}) = \begin{cases} x_{min} & \text{for } t \leqslant t_{min} \\ (-2\tau^3 + 3\tau^2)(x_{max} - x_{min}) + x_{min} & \text{for } t_{min} < t < t_{max} \\ x_{max} & \text{for } t \geqslant t_{max} \end{cases} \qquad (8)$$

where $\tau = (t - t_{min})/(t_{max} - t_{min})$, and $t$ represents the age of the module under consideration. The final length $x_{max}$ is calculated by the regression function that captures the impact of PAR and R/FR ratio measured at $t_{min}$ on internode length (Fig. 1). As reported by Turkington *et al.* (1991), the internode is a site of perception of the light environment. In the model, the light reaching an internode is assumed to be the same as the light reaching the apical sensor at the time of phytomer creation.

Qualitative observations indicate that stolons are not actually straight in a horizontal plane. In the model, we reproduced their course visually, using random values of the angle between consecutive internodes (5 ± 4°). The internodes were turned in the direction opposite to that of the petioles.

The petiole tip of a very young leaf is the site of light perception that regulates petiole growth (Thompson, 1995). Consequently, a leaf primordium, $K$, is associated with a light sensor in the model. If BL at phyllochron 5·5 exceeds the threshold *BlueMinPet* (corresponding to PAR = 30 μmol $m^{-2}$ $s^{-1}$), a leaf begins to grow. The final petiole length is estimated as a regression function that captures the impact of PAR and the R/FR ratio (Fig. 2). To take into account the lower size of the first and second leaves of an axis (Gautier *et al.*, 1997), the calculated size of leaves with $pos = 0$ and $pos = 1$ was reduced by 20%. The petiole elongates from the initial length $x_{min} = 0$ to its final length (Table 1) between the phyllochrons 5·8 and 10·2 according to the growth function [eqn (8)]. The final lamina area is assumed to be proportional to the final petiole length. The lamina expands in size sigmoidally between phyllochrons 5·5 and 9·5 (Table 1). Each leaflet is modelled as two surfaces, joined along the midrib. This makes it possible to simulate the unfolding of leaves, in addition to their expansion in size. The modelling method was described in the context of green ash leaves by Prusinkiewicz *et al.* (1994). In the model, a leaf is removed when its senescence begins (11 phyllochrons after its emergence, i.e. 17·6 phyllochrons after its phytomer initiation; Brougham, 1958).

The value of angle $\beta_1$ between an internode and the projection of the corresponding petiole on the horizontal plane was determined on clover plants in a growth chamber (Table 1). The angle α between the petiole and the ground is minimum (close to zero) when the plant grows in full light, and increases to the maximum of 90° in shade. This maximum value reflects the combined effect of the bending of the petiole (45°) and stolon (42°; Thomas, 1987). The bending of stolons is physically limited by the roots that keep the stolons close to the ground. Lifting of leaves is attributed entirely to the bending of petioles, since we did not consider rooting in the model, and described using a simple tropism model (Prusinkiewicz and Lindenmayer, 1990), which operates by reorienting consecutive line segments representing a petiole according to:

$$\varphi = e|\vec{H}x\vec{T}| \qquad (9)$$

Here φ is the angle between consecutive segments, $\vec{H}$ is the orientation of a segment before turning, $\vec{T}$ is the

direction of tropism (upwards in this case), and $e$ is a coefficient controlling the magnitude of the tropism effect. In the model, we assumed that this coefficient is a linear function of the PAR and R/FR perceived by the petiole tip:

$$e = 0{\cdot}05 + 0{\cdot}2(1 - PAR) + 0{\cdot}25(1{\cdot}15 - R/FR) \quad (10)$$

The light reaching the lamina is sensed every phyllochron, which makes it possible to simulate rapid adjustments of leaf elevation in response to changing local light conditions. Once the leaf stops growing, it maintains its last position until it becomes senescent and disappears.

The values of R/FR perceived by the oldest phytomer still growing within the apex affect the branching delay of its lateral bud *B* (Robin, Hay and Newton, 1994*a*). We assumed that both the R/FR ratio and PAR are perceived by the apex once per phyllochron and passed to the emerging phytomer. If BL is above the threshold *BlueMinBud* (corresponding to PAR = 30 μmol $m^{-2}$ $s^{-1}$), the bud *B* is transformed into an active apex *A*. The threshold age at which *B* will produce its first phytomer incorporates a branching delay, calculated as a regression function of PAR and R/FR ratio (Fig. 3). If BL is below the threshold *BlueMinBud*, the bud *B* waits for the light to improve for up to *DeathDelayApex* phyllochrons (Table 1). The lateral branch is positioned at the branching angle of approx. 45° ($\beta_2 = 44.8 \pm 2{\cdot}9$) with respect to the parent axis. This angle was measured on different axes of an isolated plant in early autumn 1997, on a white clover plant ('Huia') growing outdoors in Lusignan, France.

Although the above model description has been formulated in deterministic terms, selected parameters of the model were assumed to have normal distribution, with the standard deviation specified in Table 1 or set interactively to approximate the observed variability in the module sizes and branching angles. As discussed by Remphrey and Prusinkiewicz (1997), the inclusion of stochastic terms makes it possible to model the variability that is due to inherently random factors, or cannot be attributed to the mechanisms assumed in the model. Nevertheless, stochastic variation of parameters played only a minor role in the operation of the model, and did not have a substantial effect on the results. We ran simulations of every treatment eight times, using different initial values for the random number generator to obtain the mean values and standard deviations of the values of interest.

### *The model of light distribution in the canopy*

Our program 'MonteCarlo' for modelling light distribution in the canopy (Měch, 1997) is based on the path tracing approach (Sillion and Puech, 1994) adapted to take different light wavelengths into account. Due to the stochastic nature of the Monte Carlo method, we consider all wavelengths jointly, as attributes of individual rays, because this improves the accuracy of the estimates of the *ratios* between the intensities of light of different wavelengths (Rubinstein, 1981).

In general, rays can be traced from the sources of light towards the sensors, or from the sensors to the sources of light. The second approach was computationally more effective in our simulations because light intensity and spectral composition had to be known only at a relatively small number of plant points, assumed to be the sites of perception of the light environment. We used a small number of rays (20) per perception site, as trial simulations showed that increasing this number had a minimal effect on the simulated plant development.

The light source in the model was a hemispherical approximation of the sky, with uniform intensity and spectral composition of light coming from all directions. For natural full light, the value of R/FR was assumed to be 1·15 (Holmes and Smith, 1977*a*) and the mean daily PAR was 500 μmol $m^{-2}$ $s^{-1}$. This corresponds to 18 mol $m^{-2}$ $d^{-1}$ for 10 h photoperiod and, thus, to light conditions in early spring or late autumn. Other values occurred in experiments with artificial light and have been used in the simulations. It was assumed that the ground plane diffusely reflected 20% of the incoming light, irrespective of its wavelength.

Working with a simulation model of *Portulacca* seedlings, Měch (1997) deduced that apical meristems must be much more sensitive to red and far red light coming from the front of the apex than from behind in order to provide a good indication of the proximity of other leaves, branches, or plants. In the absence of this directional difference, the R/FR ratio is too strongly affected by the leaves and the stem produced by a given meristem itself. Consequently, in the clover model, each sensor is represented as a small 2 $mm^2$ square, characterized by two attributes: a vector $\vec{N}$ that specifies the direction of maximum sensitivity, and a sensitivity distribution function $f(\phi) = \cos^n \phi$, which describes the decrease in sensitivity for directions $\vec{J}$ that differ from $\vec{N}$ by angle $\phi$ ($\phi < 90°$). The parameter $n$ controls the rate of this decrease as in the Phong shading formula (Foley *et al.*, 1990). We assumed that $n = 20$, the direction of maximum sensitivity (vector $\vec{N}$) lies in the same vertical plane as the axis formed by the apex, and is raised 35° upward from the horizontal plane. The direction of maximum sensitivity of the leaves is perpendicular to the plane of the lamina. These assumptions are qualitatively plausible, but not supported by quantitative data.

From measurements made at the end of expt 3, hemispherical reflectance and transmittance of leaves were equal to 0·055, 0·053 and 0·426 for reflectance and 0·005, 0·020 and 0·405 for transmittance at 450, 660 and 730 nm respectively. Quantitative information about the angular distribution of the intensities of the reflected and transmitted light is lacking, so it is assumed that the reflected light is scattered uniformly in all directions (Lambertian reflection), whereas the transmitted light follows the direction of the incoming light.

## EVALUATION OF THE MODEL

### *Simulation of branching*

The sequence of appearance of primary branches and the total number of branches per plant observed in expt 3 were well predicted by the model under controlled conditions ($R_H$, Fig. 4B, C). However, primary branches and total

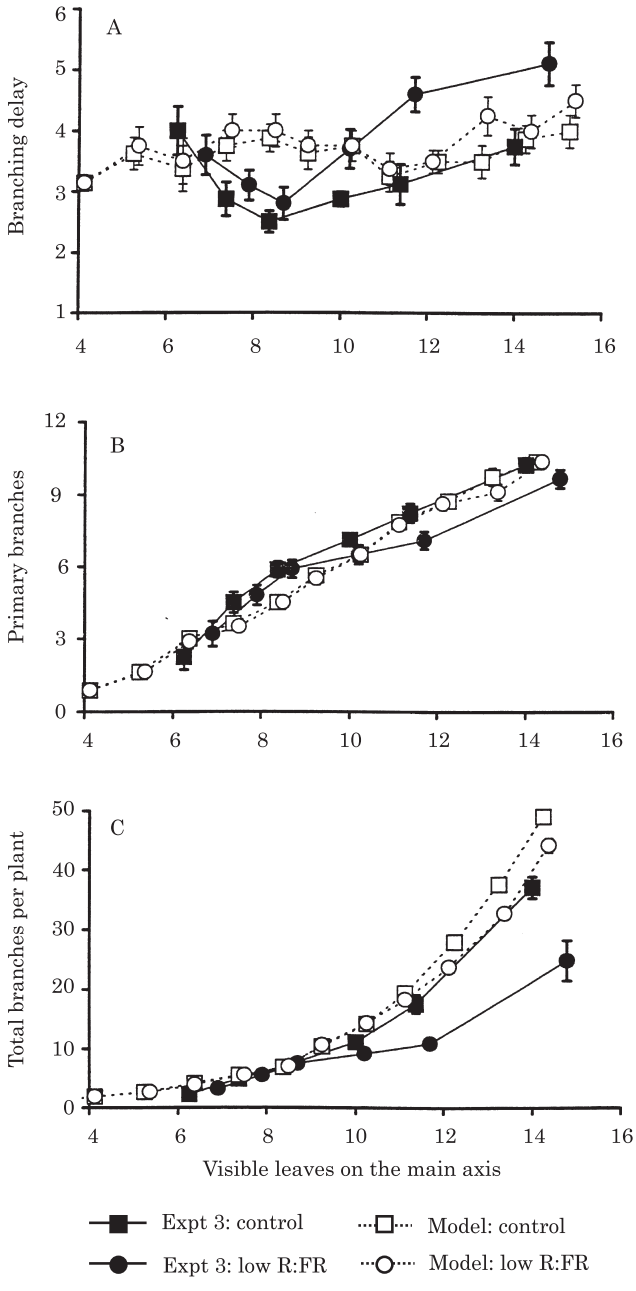


FIG. 4. Comparison of experimental data and simulations of the effects of a decrease in the R : RF light ratio (expt 3) on the branching delay (A), the number of primary branches (B) and total branches per plant (C). Data are means + s.e.

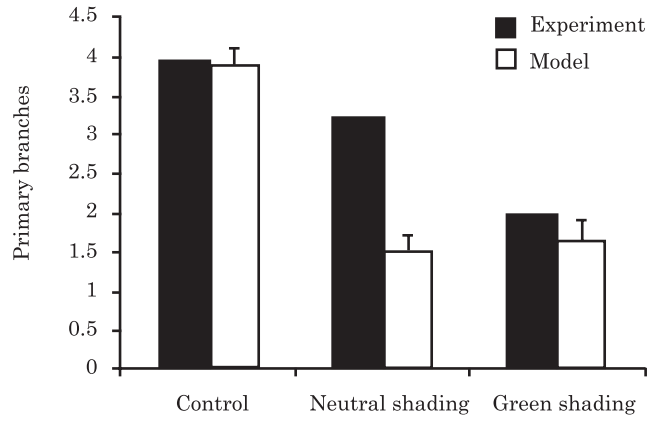


FIG. 5. Comparison of experimental data and simulations of the effects of neutral or green shading on the number of primary branches.

branches per plant were overestimated under low R/FR ($R_L$). This was due to the strong increase in the branching delay under low R/FR, which was not captured by the model (Fig. 4A).

Similarly, the simulation of primary branches under full light ($F_HR_H$) or green shading ($F_LR_L$) was very close to the data observed by Lötscher and Nösberger (1997), but the reduction of branching was overestimated under neutral shading ($F_LR_H$) (Fig. 5). Thus the model gave a good estimate of the total number of branches under control light or green shading. However, in treatments where decrease in PAR and decrease in R/FR were not correlated, it predicted too small a reduction in branch numbers for low R/FR, and too large a reduction in branch numbers for low PAR. These overestimations or underestimations, when considering the effects of R/FR and PAR separately, were related to the branching delay, which depended mainly on PAR and slightly on R/FR according to data used in the fitting (Fig. 3).

### *Simulation of final organ size*

The simulation of petiole length under control conditions ($R_H$) agreed with the measurements (Fig. 6A). The far-red treatment at the end of the day ($R_L$) increased petiole length both in the experiment and the simulation, but this increase was delayed with respect to the experimental data. This difference was due to the model's assumption that the final petiole length was entirely determined by the local environment at the beginning of petiole growth. In nature, petioles are responsive to light for a longer part of their development. Leaf area (Fig. 6B) and internode length (Fig. 6C) increased under low R/FR ($R_L$) in both the experiments and the simulations. The internode length of freshly-made cuttings decreased from internode 1 to internode 4 and then started to increase (Fig. 6C), whereas the internode length was similar between successive phytomers (Fig. 7C) when the cutting was established for 3 weeks before the beginning of measurements (see 'Data used to evaluate the model'). The decrease in internode length was linked to the lower growth potential of freshly made cuttings with no expanded roots and was not reproduced in the simulations.

Under green shading ($F_LR_L$), leaf area and petiole length increased in both experimental and simulated data (Fig. 7A, B). In contrast, the increase in leaf area and petiole length under the neutral shading ($F_LR_H$) observed in the experiment was not captured by the model (Fig. 7A, B), whereas internode length increased under neutral shading in experimental and simulated data (Fig. 7C).

The final size of organs simulated by the model was therefore in general agreement with that observed in

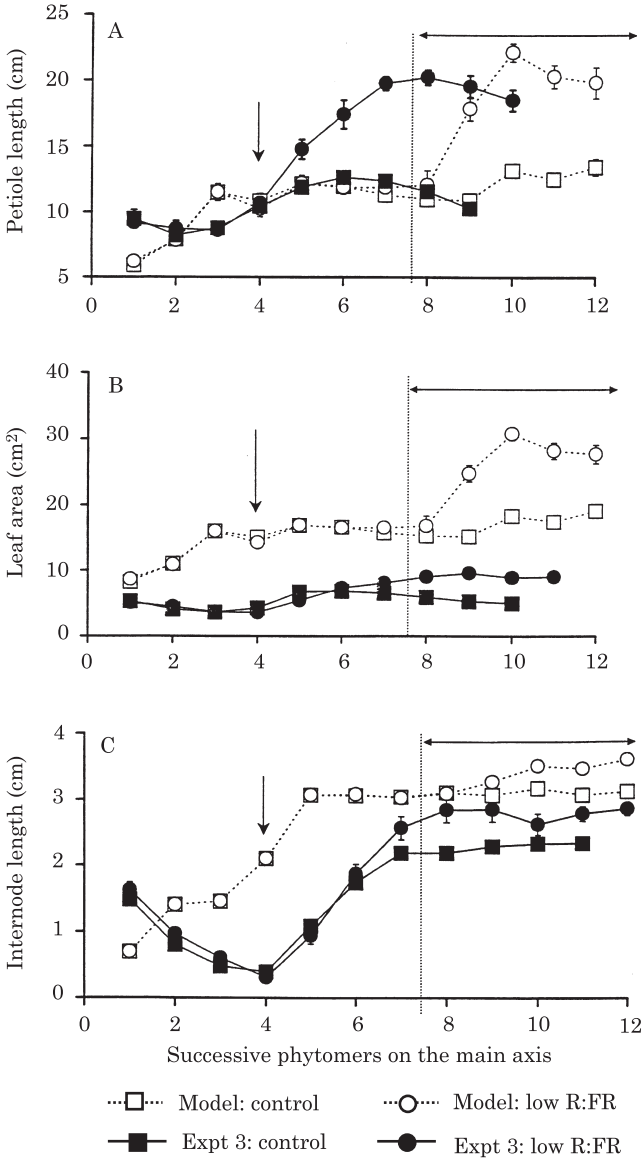


FIG. 6. Comparison of experimental data and simulations of the effects of a decrease in the R/RF light ratio (expt 3) on the individual size or organs of successive phytomers on the main axis. A, Petiole length; B leaf area; C, internode length. ⟷, Phytomers hidden in the stolon tip when the treatment was applied. The vertical arrow indicates the last fully expanded leaf when the treatment was applied.

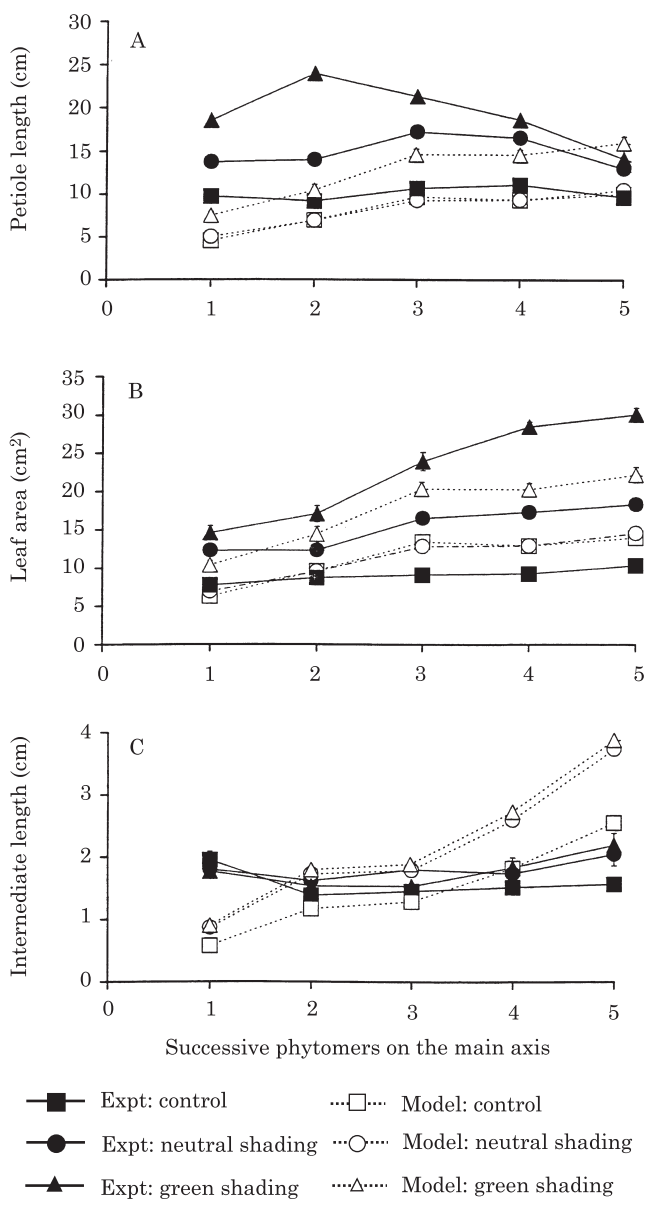


FIG. 7. Comparison of experimental data and simulations of the effects of neutral or green shading on the individual size of organs of successive phytomers on the main axis. A, Petiole length; B, leaf area; C, internode length.

experiments, although, depending on the experiment, either leaf area, petiole length or internode length were overestimated (Figs 6, 7). We conclude that the correlation between leaf area and petiole length is not precise enough to produce a reliable estimate of leaf area. Moreover, regressions relating light environment and morphogenetic responses were established from data from experiments on different genotypes (differing greatly in their leaf size) and lacked a sufficiently precise characterization of local light environment. Therefore, new sets of data on the response to BL and change in R/FR ratio under different PAR levels are needed to improve the regression fitting and consequently the accuracy of the model.

*Role of self-shading in phenotypic plasticity*

The inclusion of individual phytomer responses to their local light environment yielded structures with relatively larger mean values of leaf area, internode length and branching delay, compared to the models in which all plant parts were assumed to be exposed to constant full light ($P_HR_H$, Fig. 8). The differences between simulations with and without local light calculations increased over the time of simulations (within the assumed duration of 19 phyllochrons). This can be attributed to the gradual increase in the number of leaves that affected the sites of reception (in particular, cast shadows on other leaves and apices) in the plant simulated to self-shade.

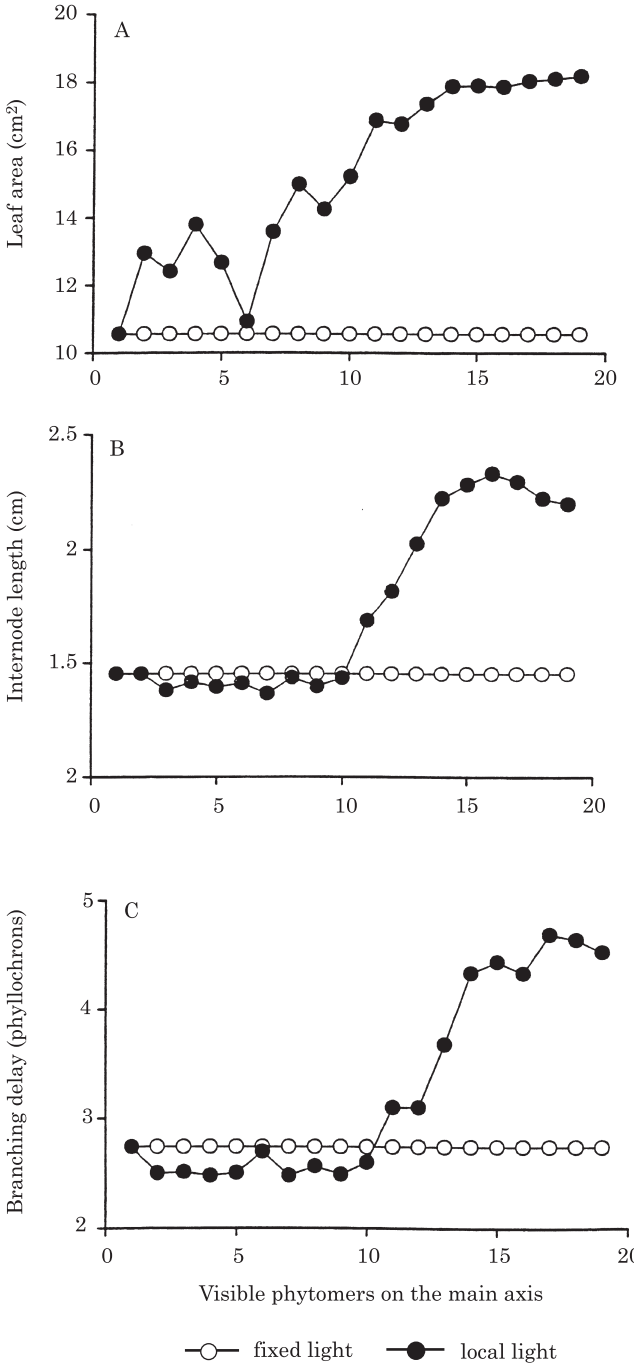


FIG. 8. Simulation of the final size of phytomers (A, leaf area; B, internode length) and of branching delay (C) determined from local light environment (● present model) or assuming a fixed light environment (○). The comparison of both simulations reveals the impact of self-shading. Above plant light environment is characterized by PAR = 500 µmol $m^{-2}$ $s^{-1}$ and R/RF = 1·15.


FIG. 9. Final stages of model development (19 phyllochrons) viewed from the side as a function of light treatment. White clover is grown under PAR = 500 µmol $m^{-2}$ $s^{-1}$ and R/FR = 1·15 (A, control conditions) or PAR = 150 µmol $m^{-2}$ $s^{-1}$ and R/FR = 0·1 (B, green shading).

The responses of individual plant phytomers to their local light environment, predicted by the model, can be used to explain the observed increase in the size of internodes and leaves of clover from one phytomer to the next during re-growth (Gautier *et al.*, 1997, 1998). Similarly, the appearance of smaller leaves after a cut (Hart, 1987) could be attributed in part to the changes in light environment (increase in PAR and R/FR) due to the cut. Thus, the responses of the plant to self-shading make it possible to explain some of clover morphogenetic characteristics as mediated by the phenotypic plasticity of the plant.

### *Simulation of plant shape*

In full light ($P_HR_H$), the development of stolons in different directions confers an approximately circular shape on the patch (Fig. 9A). In contrast, the delayed development of lateral branches under green shading ($P_LR_L$) slows down development away from the main axis, yielding an elongated patch (Fig. 9B). These results are consistent with experimental data obtained by Solangaarachchi (1986) where clover changed from a 'richly branched habit' to a 'largely linear form' as the stolons entered canopies of neighbouring plants.

Moreover, under green shading ($P_LR_L$), the leaves are positioned higher up (Figs 9, 10). The gradual increase in mean leaf height under both treatments (Fig. 10) reflects the fact that new leaves are elevated until they reach the top of the canopy, above the existing leaves. This behaviour is the result of two phenomena: the upward bending and the increase in length of petioles that grow under green shading.

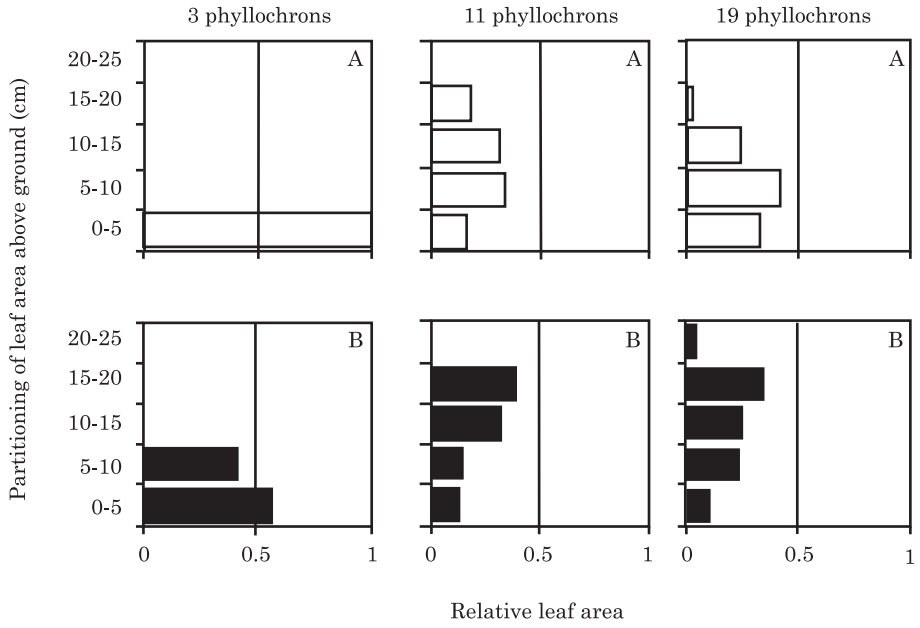


FIG. 10. Vertical partitioning of leaf area above ground simulated by the model as a function of time and light treatments. A, control PAR = 500 μmol $m^{-2}$ $s^{-1}$ and R/FR = 1·15); B, green shading (PAR = 150 μmol $m^{-2}$ $s^{-1}$ and R/FR = 0·1).

The placement of young leaves above older ones is a behaviour consistent with experimental data (Brougham, 1958) and is involved in the 'shade avoiding strategy' of clover. The model is, thus, able to reproduce the vertical and horizontal spread of white clover in response to its light environment.

## CONCLUSIONS

The formalism of L-systems and its implementation in the plant modelling program CPFG provided a convenient conceptual framework and software environment for constructing a simulation model of clover according to experimental data. The resulting model advances the state of the art in plant modelling methodology by considering both the local light intensity (PAR) and the spectral composition of light (R/FR ratio) as morphogenetic factors.

Comparison of simulation results with experimental data reveals some discrepancies, indicating that the model can be further improved by acquiring more precise data for a specific genotype. However, even in this preliminary form, the model shows that the responses to self-shading induce strong changes in phytomer morphogenesis over time (increase in internode size and leaf size from one phytomer to another, the gradual increase in mean leaf height, and the reduction in leaf size after a cut), and explain these morphogenetic characteristics as emergent phenomena, mediated by the phenotypic plasticity of the plant. Moreover, the introduction of the 'branching delay', a parameter dependent on light environment, offers an additional control when simulating plant branching, and makes it possible to predict the development of axillary buds and leaf area according to plant management. By integrating these responses over the whole plant structure, the model predicts global characteristics of the plant (the shape and vertical spread of the patch) under different light treatments.

Our eventual goal is to use well calibrated models of plants, including clover, for predictive management of multi-species crops, such as a grassland. We expect that a combination of developmental models of different species and clones will make it possible to characterize their balance in the crop, taking the competition for light into account. Consequently, it will be possible to analyse the effects of different strategies of management (e.g. frequency and intensity of cutting, and density of planting) on the structure of the crop (development and disappearance of species), and select persistent genotypes according to their prospective utilization and the management.

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