Incorporation of 3D Plant Structures in Genetic and Physiological Models

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Abstract

The recently developed virtual plant modelling approach has strongly increased the potential of model applications in crop sciences. Virtual plants are based on a new modelling concept and are generated in a 3-dimensional (3D) virtual space. The technique facilitates the incorporation of 3D environmental effects on plant growth and development. The methodology to generate virtual plants is described for *Arabidopsis* flower mutants and for Chrysanthemum plants. The profiling method was used to create 3D images of existing plants by merging 2D digital pictures of the plant silhouette to a 3D object. The data from the digitised plants were used to calibrate an architectural model for *Arabidopsis*, based on the L-systems algorithm. This architectural model was able to simulate the morphological differences between a number of plant genotypes. On the basis of L-systems, a prototype architectural model was made for Chrysanthemum. The L-system calculated temperature driven growth and light interception on the basis of radiosity. A method is presented to link this 3D model to a physiological growth model to incorporate effects of carbon dynamics. The first results show that the combined strength of both models may help to understand and visualise plant growth and appearance.

INTRODUCTION

Few studies have incorporated the visualisation of 3D plant structures in genetic and -growth models (e.g. Sievänen et al., 2000). The number of possible applications for this is rapidly increasing as a consequence of powerful 3D tools that can facilitate the incorporation of 3D functionality. The L-system formalism is able to generate detailed and realistically visualised 3D plants on a computer screen, referred to as 'virtual plants' (Prusinkiewicz et al., 1999). The L-system is used in this paper as a basis for two case studies.

Effects of Genes on Morphology

To understand the way genes affect plant form, many studies have used mutations of the gene in question to show its effect. For *Arabidopsis* this research resulted in much information on its genetic pathways (Blázquez et al., 1998). There is a vastly increasing genetic database which requires an easy method of accessing the information concerning specific genotypes. This method may be realised in the form of these new 3D tools. The visualisations used up to now were restricted to schematic 2D representations of the plant or organ form. If the reported effects of genes on morphology could be modelled in 3D, this may contribute to our understanding of the spatial occurrence and outgrowth of tissues and organs. An example is modelling the form of *Antirrhinum* flower petals which could only be done with the aid of a 3D dynamic model on petal shape development (Rolland-Lagan et al., 2003). The MADS-box genes A, B and C control the flower morphology in *Arabidopsis* and many other species. These floral organ identity genes are responsible for the formation of sepals, petals, stamens and carpel of the *Arabidopsis* flower (Coen and Meyerowitz, 1991). Blocking the expression of one or more of these

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genes will result in a flower mutant. Our approach consists of a digitisation of 'wild type' *Arabidopsis thaliana*, incorporation of the digitised data in an architectural model and adding the effect of gene expression by adaptation of the growth rules. The approach may illustrate the capabilities of 3D digitisation and modelling tools to incorporate effects of gene expression on plant form.

Linking of an Architectural Model to a Physiological Model

An architectural model of Chrysanthemum is built and linked to an existing, process-based physiological model. Several modelling studies have shown the impact of the 3D structure on growth resulting from acquisition for light by the canopy (Fournier and Andrieu, 1999), nutrients by roots (Mech and Prusinkiewicz, 1996) or from 3D based allocation of assimilates between organs (Drouet and Pagès, 2003). Recent studies show that the full incorporation of structures and functions in a single model may be too complex and error prone (Sievänen et al., 2000). The combination of an architectural and a physiological model, executed simultaneously, seems more promising (Hanan and Hearn, 2003). In this paper, such a combination is studied with respect to linkage of light interception and growth calculations. Both models are parameterised separately for a similar growth trial and outcomes are mutually compared. The possibility to link both models to benefit from 3D functionality as well as physiology is discussed.

MATERIAL AND METHODS

3D Digitisation

The spatial properties of the growing plant, geometrical as well as topological, have to be acquired to parameterise and validate 3D plant models. A profiling system has been used to automatically acquire and store 3D data of each scanned plant in a digital format. The system was based on a 3D Scanstation (Scanbull Software GmbH, Nürnberg), consisting of a computer connected to a digital camera and a turntable, on which a plant was placed. Digital pictures (3.15 MB each) were taken from 24 sides of the plant. From each digital picture a plant silhouette was generated by 'keying out' the plant from its background. The Scanstation software merged the silhouette images of the 24 viewpoints together to a 3D volume, digitally stored as a wireframe, using an iterative calculation procedure (© Scanbull). The colour and texture of the different object parts were automatically extracted from the taken pictures. This texture information was mapped onto the polygons that form the 'building blocks' of the wireframe. The data on length, width and position of each organ were extracted from the 3D digital image by listing their pixel coordinates. These coordinates were translated to sizes in cm and angles in degrees.

For *Arabidopsis*, at days 0, 1, 2, 5 and 8 after the start of generative growth, the same two plants were digitised in the 3D Scanstation. Plant height and internode elongation were also measured with a ruler. The 3D scans resulted in a series of wireframes and fully textured 3D images of all plant stages up to seed formation. Due to its limited focus distance (≥15cm), the scanning technique was not used to generate 3D images of flower details. Instead, existing detailed 2D images were used for definition of shape and organ position.

For Chrysanthemum, digital scans were made at week 2 and week 7 after planting. Plants were scanned to record the geometry and position of leaves, the internode length, and the phyllotactic angle.

Arabidopsis

1. Plant Growth Measurements. *Arabidopsis thaliana* wild type was grown in a climate chamber at 22°C and 19°C (day/night setpoint) at a photoperiod of 16 hours per day, at a setpoint of 70% air humidity. Water and nutrient supply was non-limiting. Measurements started at the onset of flowering. Thus, appearance and growth of rosette leaves, occurring in the vegetative period, was not measured and assumed equal to the meristem appearance

rate during generative growth. During the observed period the rosette leaves did not appear to grow. The length-width ratio of the rosette leaves was derived from the scanned images. Shoot growth and flower appearance were deduced from the scanning data of 4 days during generative growth.

2. Growth Modelling. The architectural model of *Arabidopsis* was constructed in L-studio (version 3.1 © University of Calgary, see Prusinkiewicz et al., 1999), a software package that uses L-systems grammar in combination with a graphical interface. In the *Arabidopsis* 3D model, three distinct phases occur: vegetative growth in the rosette stage, development of the flowering shoot and flower formation. The 3D characteristics were derived from the scanned images: spiral phyllotaxy (assumed equal for rosette leaves, side shoots and main stem flowers), position, size and branching angle of the leaf at each node. Length growth of leaves and internodes was related to thermal time using a logistic function and an equal final length for all nodes and programmed in the L-system model.

The appearance of side shoot and floral meristems was related to thermal time using the measured plastochron of 5 d°C.

3. Modelling of Flower Development of Wildtype and Mutants. The development of the flower meristems was modelled as follows:

Three classes of genes determine organ identity (Coen and Meyerowitz, 1991) and operate individually (A, C) and/or in combination (B with A or C):

A : sepals, A & B : petals, C & B: stamens, C : carpels.

The floral organs are formed from the outside to the inside of the meristem in four concentric rings, called ‘whorls’. In the wildtype, A genes are active in the outer whorls 1 and 2, and C genes in inner whorls 3 and 4. Gene B is only active in whorls 2 and 3, thereby limiting the formation of petals or stamens to these whorls.

The user can specify the value of a Boolean operator to express (=1) or 'knock out' the gene. Genes A and C are antagonists: if A is blocked, C is expressed and vice versa. The ‘knock out’ of A, B or C gene results in A, B or C flower mutants respectively (Coen and Meyerowitz, 1991). In Table 1 the expression or knock out (-) of genes is shown.

The gene combinations result in different floral organs per whorl between the mutants. Shape of the floral organs was derived from commonly available electron microscope images. In the model, sepal, petal, stamen and carpel have their own, unique shape described in corresponding architectural production rules. If the organs are formed at the different whorls due to gene actions, they keep the same shape. In the present version of the model, it is assumed that all the organs appear at the same moment per flower.

Linking of an Architectural Model to a Physiological Model of Chrysanthemum

Cut Chrysanthemum (*Chrysanthemum* Indicum ‘Discovery’) plants were grown on sandy soil in a greenhouse at an air temperature of 20°C and at non-limiting water and nutrient supply. Plant density was 47.2 plants m^{-2}. After planting (4 April 2002), a ‘long day’ period of 20 days with natural light was followed by a ‘short day’ period with 9.5 hours of daylight per day, using screens. The flowering plants were harvested 70 days after planting. At regular time intervals five plants were harvested for determination of biomass of stem, branches and flowers, as well as length, width and surface area of all the individual leaves. Digital scans (2 replicates) were made once in the long day and once in the short day growth period.

A generic, mechanistic model of crop growth (Gijzen et al., 1994) was accommodated to simulate cut Chrysanthemum growth by parameterisation of carbon dynamics on the basis of data from other literature sources (Spaargaren, 1996). The Fortran written model requires hourly meteorological data (Table 1). The crop model calculates daily increments of root, stem, leaf and flower biomass at the crop level. The maximum rate of photosynthesis (J_{max}) was adjusted to provide a good fit between simulated and observed growth. Water and nutrient supply were assumed optimal.

The architectural model has been programmed in L-studio (Prusinkiewicz et al., 1999) and describes potential growth as a function of day and night temperature.

Appearance rate of internode, associated leaf and axil was related to average daily temperature according to experiments on Chrysanthemum 'Reagan improved' by Carvalho et al. (2002). Also according to Carvalho et al. (2002), internode final length was related to temperature. Two types of leaves are distinguished: small leaves on the first 10 nodes, and bigger leaves with a stronger indentation higher on the plant. Geometry and elongation of the leaves is derived from the digital scans and surface area measurements with a LICOR device. The architectural model calculated light interception of the canopy using the nested radiosity model of Chelle and Andrieu (1998). The model is based on radiosity calculations from one plant and four neighbouring plants and a turbid medium approach for the surrounding canopy. The reflection coefficient was calibrated on the observed light extinction at diffuse light at three moments during the growth. Transmission of light through the leaves was assumed negligible.

Common features in both models were the calculation of the leaf appearance rate on the basis of temperature and, at ten leaves, start of the generative phase (short day period).

On the basis of the functionalities of both 3D and physiological model (Table 2), we tested the following linkage of the models:

1 – The number of appearing flowering shoots is calculated in the physiological model by: NoS = 1.938 * plant biomass (in g) – 2.34 (Carvalho and Heuvelink, 2003) and sent to the 3D model, where flower formation is initiated;
2 – the ratio between calibrated and current biomass growth (at other lighting conditions than during calibration) is calculated with the physiological model. Leaf expansion and stem width growth in the 3D model is multiplied with this ratio, to realise current growth. Specific leaf area and tissue densities are assumed equal in all situations. Only current increments, during the time step (e.g. day or week), are corrected proportionally;
3 – the light extinction is calculated for the actual 3D canopy structure and passed to the physiological model, which then calculates absorbed light and carbon assimilation.

This linking has been carried out for one scenario, where plant density was increased by 50% relative to the calibrated situation. At weekly time steps, parameters in both models were updated manually. An automatic linkage procedure is foreseen in the near future.

RESULTS

3D Modelling of Wildtype and Flower Mutants of *Arabidopsis*

Growth of the wildtype could satisfactorily be modelled with L-studio, starting from two rosette leaves until beginning of seed formation. The accumulated shoot length was 14.5 cm after 7 days at 20°C, with lower internodes of approximately 4.3 cm length. After this period a total of 21 lateral meristems was formed, consisting of 5 side shoots and 16 flowers. So, on average 3 flower meristems appeared per day, giving a plastochron of 5 d°C at an assumed base temperature of 5°C. The phyllotaxy of the lateral meristems decreased from 160 ± 20° (lateral shoots) to 137 ± 10° (flower meristems).

Elongation of the side shoots was observed after the first flowers on the main inflorescence matured (see wildtype in Fig. 1 for this stage). The period between flower appearance and pollination was approximately 4 days. The measurements ended at the start of seed formation.

The effects of blocking A, B or C genes on flower formation were adequately simulated. Simulated total plant appearance is not much affected by mutation, but close-ups of the flowers show distinct differences (Fig. 1).

Linking Light Interception between 3D and Physiological Model for Chrysanthemum

The physiological model correctly calculated the harvested plant biomass of 10.8 g DM per plant owing to the calibrated photosynthesis parameter. Observed leaf thickness

(SLA,, specific leaf area, and standard error) was 40.8 ± 2.5 m^2 kg^{-1} for higher and 32.5 ± 0.7 m^2 kg^{-1} for lower leaves and was made input to the model. For the physiological model, simulated interception of PAR was closely fitted (R^2 = 0.97) to observed data by tuning the extinction coefficient. In the 3D model, extinction in the canopy was tuned by changing the value of the reflection coefficient to 40%.

Without coupling, the physiological model predicted a negligible increase (<1%) in light extinction and leaf area at increased plant density. Owing to the results of the physiological model, growth of individual plants in the 3D model was less at higher plant density. During the first three weeks, this depressed growth resulted in 16% less light extinction in the 3D model relative to the more open crop. After increasing the leaf/stem ratio, reported for a crop of similar density (Spaargaren, 1996), the physiological model asked the 3D model for 10% more leaves per m^2 soil and thus leaf area. The resulting extinction rates increased 9% at 16 (after approximately 3 weeks) and 5% at 26 leaves per plant relative to the calibrated plant density, which resulted in a 8% increase in total above ground biomass at harvest according to the physiological model.

Simulated biomass (7.9 g DM) and number of flowers (13) of individual plants were lower in the denser crop relative to those simulated for the open crop, having 10.8 g DM and 19 flowers per plant.

DISCUSSION

The automatic procedure of the 3D Scanstation to generate 3D images of plants facilitates the production of a large library of digitised plants. Yet, the images show a lack of detail for thinner structures (<1 mm on a 10x10cm image) are missing. These problems may be alleviated with an improved chroma keying technique that is currently being developed. Like in most scanning techniques, hidden structures (e.g. the main stem behind many leaves) are not detected. Missing elements in the 3D image could be added with 3D editing software by using information of the detailed 2D images.

The *Arabidopsis* L-system model represents a closed cybernetic system without influences from climatic effects. The software is capable of incorporating growth response with fluctuating air temperatures. The calibrated parameters of the L-systems of *Arabidopsis* and Chrysanthemum only hold for the given growth conditions. With other humidities, soil status and pre-treatment, the parameters should be re-calibrated.

The expressions of the A, B and C classes of genes are activated outside the 3D model: an activated gene will generate a specific set of architectural rules, being purely descriptive. The gene activation grammar can easily be extended to respond to climatic conditions like light level, photoperiod and temperature. Such a response would be relevant for expression of genes such as *LFY*, which regulates the activity of the A, B and C flower organ identity genes, and is sensitive to climate and plant physiological status (Blázquez et al., 1998). These environment-induced gene expressions give possible explanations of the flowering process in a model that combines gene expression with physiological processes (Parcy et al., 1999). If most pathways are known for *Arabidopsis*, most probably for many other dicots 3D models may be developed to simulate the effects of genetic expression on flower shape. This may eventually enable virtual breeding experiments with, commercially viable ornamental plants.

We have shown one possibility to link an L-system to a physiological model and visualise effects of source-sink relations on plant form, i.e. leaf size and flower number. The results on the 3D light extinction simulations at different plant densities suggest more model tests and experimental observations in 3D are necessary. The combination of existing physiological and new architectural models increases our insight in to the interaction between plant structure and growth processes (Hanan and Hearn, 2003).

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Tables

Table 1. The expression of knock-out genes in *Arabidopsis* flowers

Genotype:	wildtype	A mutant	B mutant	C mutant
Whorl:				
1	A	C	A	A
2	AB	CB	A-	AB
3	CB	CB	C-	AB
4	C	C	C	A

Table 2. Characteristics of 3D and physiological model and data exchange.

	3D model	Exchange	Physiological model
Input	Temperature		Temperature
	Radiation		Radiation
			CO_2
			Air humidity
Output	Geometry and position of stems,	Leaf area growth, stem size and no. of flowers	C-assimilation
	buds, leaves, flowers	←	Transpiration
			Biomass
			Nr. of leaves
	Light level per leaf	→	Leaf area
		Light extinction of crop	Light absorption
Time step	Plastochron		Hour
Spatial scale	Group of individual plants		Crop per m^2

Figures

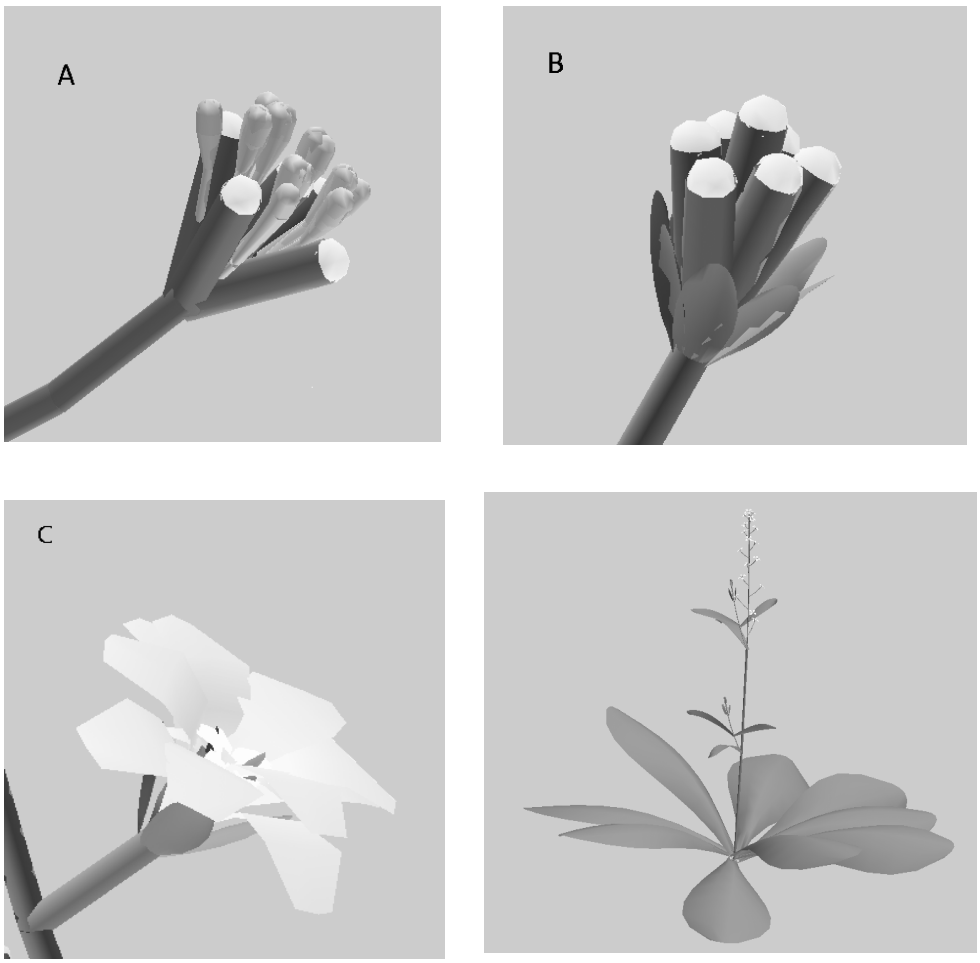

Fig. 1. Simulated flowers of *Arabidopsis* mutants A, B and C, and the wildtype plant.