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Parameter Optimization and Field Validation of the Functional–Structural Model GREENLAB for Maize

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• *Background and Aims* There are three reasons for the increasing demand for crop models that build the plant on the basis of architectural principles and organogenetic processes: (1) realistic concepts for developing new crops need to be guided by such models; (2) there is an increasing interest in crop phenotypic plasticity, based on variable architecture and morphology; and (3) engineering of mechanized cropping systems requires information on crop architecture. The functional–structural model GREENLAB was recently presented that simulates resource-dependent plasticity of plant architecture. This study introduces a new methodology for crop parameter optimization against measured data called multi-fitting, validates the calibrated model for maize with independent field data, and describes a technique for 3D visualization of outputs.
• *Methods* Maize was grown near Beijing during the 2000, 2001 and 2003 (two sowing dates) summer seasons in a block design with four to five replications. Detailed morphological and topological observations were made on the plant architecture throughout the development of the four crops. Data obtained in 2000 was used to establish target files for parameter optimization using the generalized least square method, and parameter accuracy was evaluated by coefficient of variance. *In situ* plant digitization was used to establish 3D symbol files for organs that were then used to translate model outputs directly into 3D representations for each time step of model execution.
• *Key Results and Conclusions* Multi-fitting against several target files obtained at different growth stages gave better parameter accuracy than single fitting at maturity only, and permitted extracting generic organ expansion kinetics from the static observations. The 2000 model gave excellent predictions of plant architecture and vegetative growth for the other three seasons having different temperature regimes, but predictions of inter-seasonal variability of biomass partitioning during grain filling were less accurate. This was probably due to insufficient consideration of processes governing cob sink size and terminal leaf senescence. Further perspectives for model improvement are discussed.

Key words: Plant architecture, competition among sinks, source–sink relationships, functional–structural models, Zea *mays*, model parameterization.

INTRODUCTION

It is increasingly recognized that plant architecture and topology are important determinants of crop performance and agro-ecological adaptation, and should thus be taken into account in crop modelling (Yan *et al*., 2004). This is not the case for most models designed to answer agronomic questions (e.g. Jones *et al*., 1998; Brisson *et al*., 2002), which essentially describe the flux of external resources through the apparatus 'plant' and into a pool called yield. The plant is thereby seen as a set of invariable (genetic) rules that govern resource acquisition and conversion on a field area basis, while aggregating morphological entities into larger compartments [e.g. big-leaf concept as in Allen *et al*. (1998) or Jones and Kiniry (1986)].

Although this approach is useful in many situations, it is quite insensitive to the morphological and architectural traits breeders seek to improve crop adaptation and productivity, and in particular to the study of alternative plant type concepts that may guide efforts to develop new crops. For example, Dingkuhn *et al*. (1991) proposed an alternative rice plant type adapted to high population densities, based in part on modified assimilate partitioning patterns among organs. The crop model used in that study divided the leaf canopy into horizontal strata, instead of successively appearing leaves. Consequently, partitioning patterns were forced by the model instead of resulting from demand functions generated by organogenetic processes. In fact, if modified architectural plant types are to be biologically plausible, they need to derive the phenotype from interactions among structural dynamics, external resources and the physiological processes that govern inter-organ competition and stress responses (Dingkuhn *et al*., 2005*a*). For example, breeders have been trying for some time to develop grain sorghum varieties for West Africa that combine photoperiod sensitivity with 'modern' crop architecture and high harvest index, a plant type concept that needs to consider the complex interactions between photoperiod response and crop plant architecture (Clerget *et al*., 2004; Dingkuhn *et al*., 2005*b*).

Another shortcoming of non-architectural crop models is their inability to simulate adaptive, phenotypic plasticity (Dingkuhn *et al*., 2005*a*; Luquet *et al*., 2005) that allows

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TABLE 1. *Growing period (calendar dates, duration in days and thermal duration) and mean atmospheric conditions for four consecutive maize crops*

Year	Sowing–harvest (dd mm^{-1})	Duration (d)	Mean temp. (air, °C)	Thermal time (*Tb* = 8 °C)	Mean solar radiation (MJ m^{-2} d^{-1})	Mean relative humidity (%)
2000	8 May–5 August	89	26·3	1629	24·7	61·7
2001	20 April–12 August	114	23·5	1767	23·6	62·5
2003A	19 May–13 August	86	25·9	1539	19·0	61·0
2003B	29 June–13 September	75	25·8	1335	17·1	67·2

Tb, Base temperature.

the plant to adjust its morphology to edaphic or atmospheric constraints, or to the presence of weedy competitors. Lastly, modern plant production systems are increasingly shaped by genetic, agronomic and environmental engineering seeking specific structural features of the crop (Prakash, 2001; McFerson and Pierce, 2003), requiring models that provide plant architectural detail and provide for compensatory responses to interventions such as pruning.

In a previous study, a new model GREENLAB that combines the dynamic simulation of the complete plant architecture with simple algorithms of biomass assimilation, for which the growing organs compete, was presented. This model marks a major progress from architectural models (Lindenmayer, 1968; Smith, 1984; Prusinkiewicz *et al*., 1988) and their derivatives that are now commonly used in townscaping, landscaping, advertising and computer graphic games (www.greenworks.de, 2001; www.OnyxTREE.com, 2003; www.xfrog.com, 2003), but so far are poorly suited as tools in agro-ecological research (de Reffye *et al*., 1988; Dauzat, 1994; Dauzat and Eroy, 1997; Chelle and Andrieu, 1998). In contrast to some recent, functional–structural plant models such as LIGNUM (Perttunen *et al*., 1996; Sievanen *et al*., 2000), AmapHydro (de Reffye *et al*., 1999) and GroGra (Kurth and Sloboda, 1997), which simulate discrete events based on conditional algorithms (e.g. if . . . then loops), GREENLAB is a mathematical model. Discrete-event simulators are affected by a number of inherent problems, namely (*a*) high computational time requirements, particularly for complex plants such as trees; (*b*) difficult calibration of a large number of parameters, frequently by optimization when they cannot be measured directly; and (*c*) opaqueness of the simulation process from a mathematical point of view. As a result, bug proofing is difficult, and it is quite possible to achieve perfect fit with a flawed model if optimization techniques such as genetic algorithms are applied to a large number of parameters. This problem is bound to be aggravated if models are to simulate plastic morphogenesis resulting from multiple internal feedbacks, as opposed to biomass partitioning among organs forced by rigid rules that can be calibrated directly. Mathematical models such as GREENLAB are more transparent and their parameters easier to optimize because they behave linearly, but parameters do in most cases not correspond to measurable properties of the plant and thus need to be calibrated by optimization. Furthermore, plant behaviour can profoundly change in the course of ontogeny, and may thus sometimes require departure from pure mathematical concepts.

This study addresses the problem of parameter optimization for the GREENLAB model using target files containing measured variables that correspond to model simulation outputs. A new multi-fitting method is presented that permits simultaneous optimization for several independent target files and allows evaluating the statistical accuracy of parameter values. The advantages of multi-fitting as compared to single fitting are evaluated on the basis of a maize field experiment, and the resulting model is validated with 3 independent data sets. Lastly, a new feature of the model, permitting dynamic 3D representation of simulation results, is presented.

MATERIALS AND METHODS

Field experiments

Field measurements were conducted at the China Agricultural University (CAU) (39°50′N, 116°25′E). Maize cultivar ND108 (*Zea mays* L., DEA cultivar) seed was sown 0.6 m apart in north–south-oriented rows that were 0.6 m apart. The resulting plant population (28 000 plants ha^{-1}) is about half that commonly used by local farmers and was chosen to minimize competition among plants, the aim here being to analyse growth and organogenesis of individual plants. Plants were sown to emerge on 18 May in 2000 (Exp. 2000), 1 May in 2001 (Exp. 2001), 26 May in 2003 (Exp. 2003A) and 4 July (Exp. 2003B) (Table 1). The experiments had four replications in 2000 and 2001, and five replications in 2003A and 2003B, following a randomized complete block design. One plant was collected per replication and sampling date. The soil was a sandy clay loam (Aquic Cambisol) previously managed as a meadow. The plots were irrigated and fertilizer inputs were such as to avoid any mineral and water limitation to plant growth. Weeds were removed by hand to avoid any herbicide effects on plant growth. No plant disease, pest or stress symptoms were observed.

Field measurements on plants

Throughout crop development, destructive sampling was periodically done on individual plants in order to characterize growth and organogenesis. Only above-ground organs were collected. Samples were taken on 12 dates in 2000, seven in 2001, 14 in 2003A and 18 in 2003B. In order to prevent water loss during measurements, plants were dug out with roots and soil and transported to the

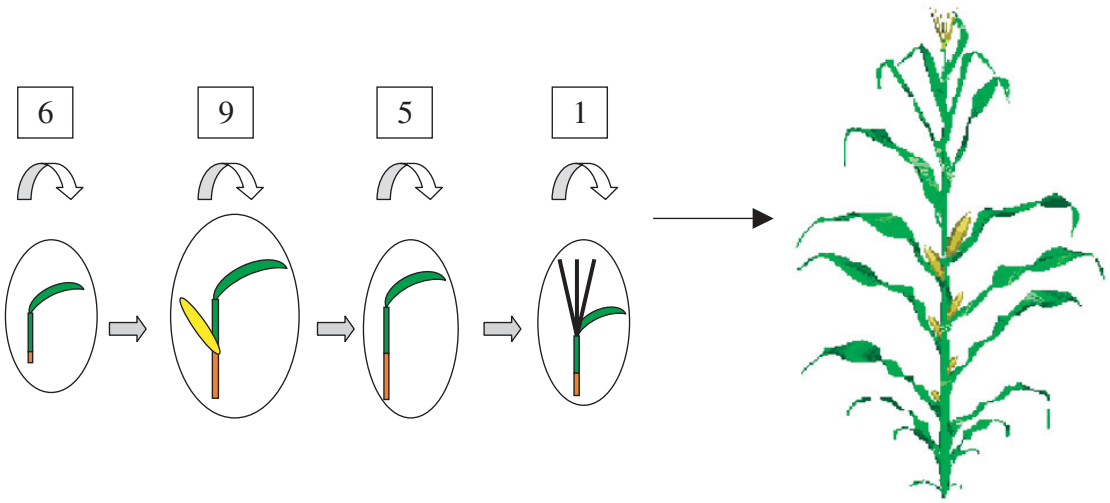

FIG. 1. An automaton theory for maize development. Organogenesis is simulated by a simple automaton that successively creates four types of metamers. The first six metamers are vegetative and have short internodes, the next nine metamers have elongating internodes and may produce cobs, the next five metamers remain vegetative, and the last metamer carries the tassel.

laboratory for measurements (width, length, and fresh weight (f. wt) of leaf sheaths; length, width, area, and f. wt of leaf blades; diameter, length and f. wt of internodes; dimensions and f. wt of cob and tassel). These measurements were done on all existing metamers on the plants samples. The date of onset of senescence was recorded for each metamer in order to estimate leaf life span.

GREENLAB model and version used in this study

The GREENLAB model has been previously described in detail (Yan *et al*., 2004). Only the main principles and specific options used here for maize will be presented.

Modelling concepts. The GREENLAB model dynamically represents the morphogenesis and architecture of a plant based on a few recurrent mathematical equations and generic metamorphic rules. It is executed at time steps corresponding to organogenetic growth cycles (GC), corresponding to the thermal time it takes to generate a new metamer (the architectural unit comprising a node, internode and leaf, or the metamorphic variations of these organs). The plant architecture is generated by an automaton (Yan *et al*., 2004), providing compartments (organs; Fig. 1) that represent sinks for biomass in the course of their development which can span several GCs. Biomass acquisition is simulated by applying atmospheric, evaporative demand (potential evapotranspiration PET according to FAO guidelines; Allen *et al*., 1998) to the exposed green leaf area, and by linearly converting the resulting transpiration rate into fresh biomass assimilation using an empirical value for transpiration efficiency. No soil water limitations are considered.

The model is parameterized by optimization procedures using botanical and morphological observations measured on a sample plant at maturity (case of single fitting) or in the course of its development (multi-fitting), and subsequently is able to construct identical or divergent phenotypes by implementing the same rules and parameters for the same or different environments. It is thereby capable of simulating some of the phenotypic plasticity of a genotype, as far as the architectural and morphological modifications result from fluctuations in biomass acquisition. The model, however, does not claim to be fully mechanistic with regards to physiological processes and fluxes involved in plant growth. In fact, it is empirical and some of its underlying rules, such as a non-linear relationship between leaf surface and assimilation rate, are intuitive. The model was developed to explore the potential to mimic with a small set of mathematical rules not only a complex plant architecture, but also its morphogenesis and resource dependent variability.

Metamers are initiated at regular intervals of thermal time as observed in the field. In maize, metamers 1–6 produce leaves (consisting of blade and sheath) with short internodes, metamers 7–15 produce leaves with long internodes and may carry cobs, metamers 16–20 produce leaves with long internodes but no cobs, and metamer 21 (the last) produces a leaf, an internode and a tassel (Fig. 1). Although these metamers are initiated sequentially, their periods of growth and life spans overlap considerably, resulting in parallel development of these organs and consequently, competition among them for a shared pool of incremental biomass. The model is not implemented with daily time steps, as mostly done in crop models, but with time steps equal to the thermal time elapsing between the appearance of two metamers (growth cycle GC, similar to phyllochron). This rhythm of model execution is maintained after the initiation of the last metamer, in the case of maize the one carrying the tassel. Plant development ends at the 33th cycle. The last leaf (21th metamer) therefore has an age of 12th GC at crop maturity (33th GC). Leaf expansion time and longevity, expressed in GC and therefore temperature dependent, were either measured directly or adapted from Lizaso *et al*. (2003), who provided complete reference

data sets for maize. Thermal time is computed as the additive accumulation of mean, daily, air temperature minus a crop specific base temperature (8 °C in this study as recommended for maize by Ritchie and NeSmith, 1991). Other cardinal temperatures such as T_{opt} and T_{max} are not considered.

The biological assumptions underlying the model can be summarized as follows.

Crop transpiration (T) is driven by PET and the exposed fraction of green leaf area. In order to take into account mutual shading of leaves, Beer–Lambert's extinction law (Vose *et al*., 1995) is used:

$$T = \mathrm{PET} \times [1 - \exp(-k\mathrm{LAI})] \quad (1)$$

where LAI is the leaf area index adjusted to the soil surface available to the individual plant and k is the extinction coefficient.

Crop transpiration is calculated with a resistance term that resides in the leaves. This resistance term is considered leaf size dependent using a function that is empirically parameterized by optimization (Yan *et al*., 2004). A second resistance term is derived from LAI using Beer–Lambert's law.

Fresh biomass assimilation is considered to be proportional to crop transpiration, resulting in an incremental reserve pool available to all growing organs (sinks). Only fresh biomass is considered in the model. For all green leaves and for each GC, the following equation is implemented:

$$Q_m(i) = \frac{E(i)S_\mathrm{p}}{r_1 r_2}\left(1 - \exp\left(-r_2\frac{\sum_{j=1}^{n(i)} S_j}{S_\mathrm{p}}\right)\right) \quad (2)$$

where $Q_m(i)$ is the biomass production during GC(i); $E(i)$ is the average, potential of biomass production during GC(i) which is the product of PET and transpiration efficiency (TE) in this study, but can be sensitized to other environmental variable as needed; $n(i)$ is the number of green leaves during GC(i); S_j is the blade surface of the jth leaf; S_p is the ground projection area of the leaf surface, which takes into account its inclination; r_1 and r_2 are empirical resistance parameters that are parameterized by optimization as described in Yan *et al*. (2004), with r_1 setting leaf size effects on transpiration per unit area and r_2 setting the effect of mutual shading of leaves according to Beer–Lambert's law.

Sinks receive an incremental allocation of biomass that is proportional to their relative sink strength. The sink strength for each type of organ denoted by o is defined as a function of its age in terms of GCs:

$$P_\mathrm{o}(j) = p_\mathrm{o} f_\mathrm{o}(j) \quad (3)$$

where o = indices for organ type (leaf blade, b; sheath, s; internode, e; cob, f; tassel, m); p_o is the coefficient of sink strength associated with organs of type o. For leaf blade $P_\mathrm{b} = 1$ is set as normalized reference; $f_\mathrm{o}(j)$ is an organ type specific function of sink variation in GC(j). A normalization constraint $\sum_{j=1}^{t_\mathrm{o}} f_\mathrm{o}(j) = 1$ is set, with t_o the expansion duration of organ o for the rank k.

TABLE 2. *Crop parameters optimized using a target file of field observations*

Parameters	Description
P_b	Blade sink strength; P_b is set to 1
P_s	Sheath sink strength
P_e	Internode sink strength
K_e	Secondary sink strength of internode pith growth
P_f	Cob sink strength
P_m	Tassel sink strength
B_b	Blade sink variation (parameter for the beta function of blade expansion)
B_s	Sheath sink variation (parameter for the beta function of sheath expansion)
B_e	Pith sink variation (parameter for the beta function of internode expansion)
B_f	Cob sink variation (parameter for the beta function of cob expansion)
r_1	Coefficient for leaf size effect on leaf resistance
r_2	Canopy extinction coefficient derived from Beer–Lambert's law

Other parameters, such as leaf blade area to fresh weight (f. wt) ratio, seed f. wt and leaf longevity, were directly measured and input in the model.

The beta function f_o is formulated as follows:

$$f_\mathrm{o}(j) = \begin{cases} g_\mathrm{o}(j)/M_\mathrm{o} & (1 \leqslant j \leqslant t_\mathrm{o}) \\ 0 & (j > t_\mathrm{o}) \end{cases}$$
$$g_\mathrm{o}(j) = (j - 0.5)^{a_\mathrm{o}-1}\cdot(t_\mathrm{o} - j + 0.5)^{b_\mathrm{o}-1} \quad (4)$$
$$M_\mathrm{o} = \sum_{j=1}^{t_\mathrm{o}} g_\mathrm{o}(j)$$

The parameters a_o and b_o vary with organ type. According to this function the shape of the sink variation is flexible and can be fitted to data by optimization.

In the course of the development of an organ, its relative sink strength varies according to a beta function parameterized by optimization. All organs of the same type behave according to this function, regardless of crop developmental stage or resources. During the general parameterization of 12 model parameters by optimization (Table 2), only one parameter (B_o) is optimized to define the beta function for each organ type, and its two parameters a_o and b_o are subsequently derived from B_o by iteration using the constraints $a_\mathrm{o} + b_\mathrm{o} = 5$ and

$$B_\mathrm{o} = \frac{a_\mathrm{o}}{(a_\mathrm{o} + b_\mathrm{o})}$$

These constraints are empirical and generally yielded good results for different organs and plants.

Wherever meaningful, empirical, allometric rules are used and parameterized manually to simplify the model and the optimization process. Examples are length and weight ratios of leaf sheaths to blades, leaf blade area and weight ratio, root to shoot weight ratio (GREENLAB is able to simulate root sink dynamics but no reference measurements were available in this study), and dimensional ratios describing the shape of various organs such as cobs or internodes. Angles for branching and phyllotaxy complete the set of geometrical parameters, permitting 3D

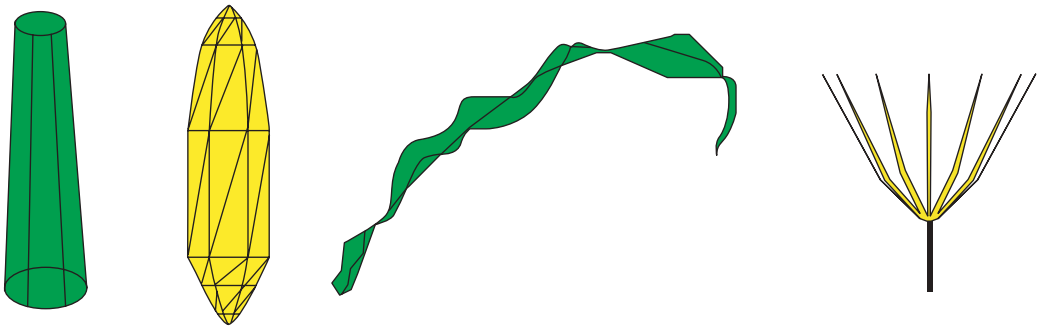

FIG. 2. Normalized, 3D architectures of internode, cob, leaf blade and tassel of maize as derived from *in situ* digitization and transformed to symbol files for 3D representations of model outputs.

organ representation (Fig. 1, simulated appearance of the whole plant; Fig. 2, geometry of individual organs).

As a consequence of these model concepts, organ size is variable because it depends on resources and the number and strength of sinks that share these resources at a given time. The model is deterministic (final organ number and crop duration are fixed), although model versions exist that use stochastic organogenetic principles as well as resource feedbacks of organogenesis, such as tiller production. Photoperiodic responses of phenology, which affect the number of metamers produced by the plant, have not yet been implemented. A model version simulating dry matter (which is then 'hydrated' to simulate fresh matter) is in progress.

Summary of crop parameters used in the model. Twelve crop parameters (Table 2) that are not accessible to direct measurements are optimized by statistical procedures. For the sink strength of specific organ types, one parameter sets sink strength relative to other organ types, and another sets the shape of the beta function defining sink strength variation in the course of organ development. For leaves, a metamer-specific parameter sets leaf longevity, either on the basis of direct observations or, alternatively, using values derived from the empirical function proposed by Lizaso *et al*. (2003), adapted here to fit the different number of metamers produced. Two parameters govern leaf resistance as a function of leaf size and shading within the canopy.

Input variables. The model version used here, with its extreme simplification of assimilation and growth processes, only uses daily mean air temperature and PET as environment inputs.

Optimization of crop parameters

GREENLAB, in contrast to most crop models, is mathematical in the sense that it uses one single, aggregate, recurrent equation that generates, in combination with the automaton system, the entire plant structure. No non-linear behaviour resulting from 'if–then loops' and no changes in parameter values occur during the simulation. As a consequence, the equation uses a relatively small number of crop parameters whose values are 'hidden' (not accessible to direct measurements because they do not describe a specific physiological process or morphological feature) but are well suited to statistical optimization procedures (exceptions are parameters involved in the topological structure, allometric rules and leaf longevity that are set manually before the optimization process).

Optimization is performed simultaneously for all relevant parameters against a target file (Table 3) of morphological plant observations (dimensions and weight of organs). In a previous study, results obtained with optimization against a single plant observed at a specific phenological stage were presented. In the present study, a new, multi-fitting procedure allowing optimization of the same parameters against many plants was used, with the objective of evaluating the robustness of crop parameters across growth stages and environments. Twelve parameters are optimized to assume a generic value (common to all metamers on the plant that carry the organ to which the parameter applies), enabling the simulation of one aggregate entity (the plant). There can be an unlimited number of test cases (observed targets) for parameter optimization, which correspond to the individual metamers observed on many plants. These plants may be of different development stage, different replications or treatments, or grown in different seasons.

The generalized least square method. Parameter optimization of the model uses the Generalized Least Square Method (GLSM), an approach suited to curvi-linear parameter behaviour (Press *et al*., 1992). The application of this method to GREENLAB was described by Zhan *et al*. (2003). Suppose there are n measurements of y that correspond to model outputs (organs weights or dimensions for specific metamers) and m crop parameters P:

$$\begin{aligned} y_1 &= f_1(P_1, P_2, \ldots P_m) \\ y_2 &= f_2(P_1, P_2, \ldots P_m) \\ &\ldots\ldots \\ y_n &= f_o(P_1, P_2, \ldots P_m) \end{aligned} \tag{5}$$

The function f_i is the model itself. The number of functions corresponds to the number of organ types (leaf, internode, cob, etc.), whereas y stands for measured variables.

TABLE 3. *Target file of observations for parameter optimization (Exp. 2000, means of four replications)*

Metamer no.	Internode			Leaf sheath			Leaf blade		Cob	Tassel
	Diameter (cm)	Length (cm)	Fresh weight (g)	Width (cm)	Length (cm)	Fresh weight (g)	Fresh weight (g)	Area (cm^2)	Fresh weight (g)	Fresh weight (g)
1	1·91	0·65	2·01	0·60	4·80	0·09	0·09	5·70	0	0
2	1·91	0·65	2·01	1·04	6·22	0·19	0·23	12·80	0	0
3	1·91	0·65	2·01	1·50	6·60	0·34	0·41	14·34	0	0
4	1·91	0·65	2·01	2·62	7·91	0·95	1·03	45·26	0	0
5	1·91	0·65	2·01	5·95	16·01	2·75	4·50	178·38	0	0
6	2·84	3·73	26·23	7·87	18·83	7·76	8·22	315·23	0	0
7	2·85	6·33	44·21	8·82	19·95	10·29	11·46	409·82	0	0
8	2·78	8·88	52·53	9·85	20·53	13·23	15·46	535·42	0	0
9	2·65	10·43	53·34	10·30	20·43	15·26	19·78	670·34	0	0
10	2·53	11·75	50·71	10·23	19·35	16·24	23·58	792·32	0	0
11	2·42	12·73	44·32	10·75	18·23	17·17	26·45	908·06	0	0
12	2·31	13·75	39·92	10·93	17·7	17·63	28·38	977·94	0	0
13	2·17	14·18	34·48	10·53	16·93	17·25	28·11	1007·87	0	0
14	2·05	14·58	26·82	10·05	16·80	17·14	26·75	987·71	0	0
15	1·86	14·2	20·86	9·30	16·88	15·84	24·77	912·65	895·40	0
16	1·62	14·23	16·20	9·08	16·75	14·36	22·22	855·31	0	0
17	1·43	14·55	12·43	8·15	16·50	12·42	18·86	739·27	0	0
18	1·24	14·08	8·94	7·50	15·95	10·10	14·35	597·45	0	0
19	1·05	14·48	6·70	6·20	15·58	7·65	9·25	421·99	0	0
20	0·81	11·53	3·88	4·30	10·98	3·99	4·18	205·85	0	0
21	–	–	1·11	–	–	1·12	0·20	9·11	0	29·11

A Jacobian matrix M is then defined by:

$$M = \begin{bmatrix} \frac{\delta f_1}{\delta P_1} & \frac{\delta f_1}{\delta P_2} & \cdots & \frac{\delta f_1}{\delta P_m} \\ \frac{\delta f_2}{\delta P_1} & \frac{\delta f_2}{\delta P_2} & \cdots & \frac{\delta f_2}{\delta P_m} \\ & \cdots\cdots & & \\ \frac{\delta f_0}{\delta P_1} & \frac{\delta f_0}{\delta P_2} & \cdots & \frac{\delta f_0}{\delta P_m} \end{bmatrix} \quad (6)$$

This matrix is computed by numerical approximations. Optimization starts from an arbitrary set of values for the parameters P_i representing one possible solution.

Then it can be written:

$$\begin{aligned} y_{1,0} &= f_1(P_{1,0}, P_{2,0}, \ldots P_{m,0}) \\ y_{2,0} &= f_2(P_{1,0}, P_{2,0}, \ldots P_{m,0}) \\ &\cdots\cdots \\ y_{n,0} &= f_0(P_{1,0}, P_{2,0}, \ldots P_{m,0}) \end{aligned} \qquad \Delta y = \begin{bmatrix} y_1 - y_{1,0} \\ y_2 - y_{2,0} \\ \cdots\cdots \\ y_n - y_{n,0} \end{bmatrix} \quad (7)$$

The difference between the target and the computed value is given by the vector Δy. The adjustment made for each parameter P_i will then be given by the equation:

$$\Delta P = (M^T M)^{-1} M^T \Delta y \quad (8)$$

The process is repeated until the solution is stabilized and an optimal solution is found for parameter values. Advantages of the method are its rapid convergence (ten iterations are often sufficient) and that it provides the standard deviation linked to the parameter values thus indicating the accuracy of the solution. As maize has a simple architecture, the problem of multiple possible solutions (multiple minima of the error term in optimization) was not encountered. However, for more complex architectures (and more complex system behaviour), it will be necessary to couple the gradient-descent approach with a scan to identify zones containing minima.

Fitting the plant architecture. Once the plant architecture is defined for each metamer, it provides the format of a target file that can be filled with observed y_i values. Fitting the architecture consists of achieving the minimum value for $\Sigma[y_i - f_i(P_1, P_2, \ldots P_m)]$. This can be done on a single plant for a given phenological stage (single fitting). Several plants of different phenological stage and/or experiments can also be fitted at the same time for the same set of parameters, along the trajectory of the dynamic system (multi-fitting). In this study, target files established from observations at 8th GC (eighth-leaf stage, vegetative), 18th GC (roughly, flowering stage) and 33th GC (maturity) were used for multi-fitting. At these growth stages, eight, 18 or 21 metamers, respectively, were present on the plant.

Three-dimensional representation of plants

In order to permit a dynamic, 3D representation of GREENLAB simulations of maize, a library of symbol files is needed that describe the shape and appearance of each organ type. An electromagnetic digitizer (3Space Fastrak Long Ranger; Polhemus) was used to measure the 3D co-ordinates of organs *in situ* (Sinoquet *et al.*, 1998) (Fig. 2). Points were selected to delineate the dimensions of each organ. Each leaf was characterized by a set of points along its midrib and its two margins. On cobs, points were digitized along two lines from the base to the tip on opposite sides of the cob, one line being along the uppermost surface and the other along the lowermost surface. In tassels, the 3D co-ordinates of the base, middle and tip of each branch were recorded, as were diameters of three representative branches

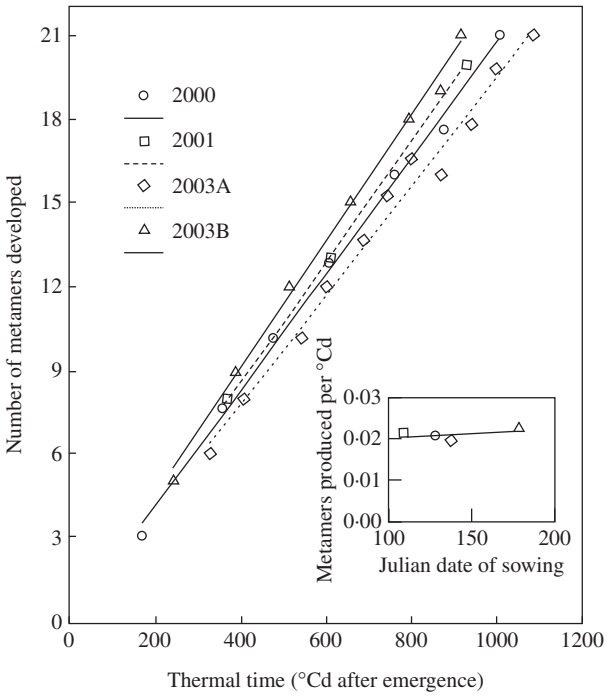

FIG. 3. Number of metamers on maize plants developed as a function of thermal time. The inserted graph shows that the production rate of metamers was not affected by sowing date.

at the base, middle and tip of each tassel using calipers. The stem was represented by a succession of truncated cone-shaped tubes having hexagonal sections, each tube corresponding to one internode. The nodes at the ends of each internode were taken as the basal positions of the corresponding leaf blades. The diameter of the node at the end of each section of stem was set to the diameter directly measured in the field. Each internode was represented by a set of 12 triangles, two per hexagon face, which produced a realistic representation while minimizing the number of triangles and computations used. Cobs and tassels were represented by ellipsoids.

The objects represented in the library are characterized by their shape, relative dimensions and volume. This permits adjusting their size according to simulated organ fresh weights and known tissue density. Angles for branching and phyllotaxy are also defined, and the orientation of each leaf and cob are defined by inclination and azimuth values. Stochastic methods were used to create natural variability of appearance.

RESULTS AND DISCUSSION

Field observations and establishment of target files for modelling

Regardless of season and year, the maize crop produced nearly the same number of metamers (20–22 at maturity). Initital analyses on the basis of 0 °C for T_b gave variable metamer production rates per unit thermal time among sowing sates (0.0135–0.018 metamers $°Cd^{-1}$), but choosing T_b = 8 °C gave nearly constant metamer production rates (0.020–0.022 $°Cd^{-1}$; inserted graph in Fig. 3), indicating that this value proposed by Ritchie and NeSmith (1991) is accurate and generic.

Fresh, above-ground biomass produced by the crop was greatest for the earliest sowing dates (2000 and 2001) and smallest for the latest sowing date (2003B), possibly because organogenesis was more rapid and crop duration shorter in the latter (Fig. 4), due to higher temperatures (Table 1). Conversely, crop duration was longest for the earliest sowing date (Exp. 2001).

Cobs were produced on several metamers but those carried by metamers 14 and 15 were the most productive, contributing >80 % to cob yield (data not presented). To simplify simulation, henceforth cob production is attributed to metamer 15 only.

Plant observations were used to build target files for the optimization of model parameters. An example for Exp. 2000 is given in Table 3. In this case, a nearly completely filled target file was used. Incompletely filled target files (structured for the complete architecture but containing measurements for only some metamers) can also be used but may result in loss of accuracy of simulations.

The field observations also served to determine allometric relationships that permit estimating sheath f. wt and leaf blade area from leaf blade f. wt (Fig. 5). Leaf blade to sheath f. wt ratios were not constant and it was therefore decided to simulate blades and sheaths as independent entities. On the other hand, the leaf blade area to f. wt ratio was about constant and this allometric relationship was used in the model to convert simulated leaf f. wt to surface area.

Modelling results using single and multi-fitting

Single fitting. For a single fitting exercise of the model, observations on mature plants of Exp. 2000 were used (Fig. 6). According to model time steps (thermal time elapsing between the production of two metamers), the crop age at maturity is 33th GC. The observations at 33th GC permit reconstructing the entire morphogenetic process of the crop because, at maturity, almost all organs ever produced are still present, and their time and sequence of initiation are determined by the known plant architecture and the duration of the crop cycle, assuming that metamers were produced at regular intervals of thermal time (Fig. 3).

The calibrated (optimized) model reproduced well the data in the target file used for parameter optimization (Table 3). This is noteworthy because the model generates the variability among metamers of organ size and f. wt on the basis of a single set of parameters applied to all metamers alike (Fig. 6). For example, the increase and subsequent decrease of leaf f. wt is entirely generated by competition among sinks and by assimilate supply, and not forced with growth stage-specific functions.

Table 4 presents the corresponding parameter values, their standard deviation and their coefficient of variation (CV), which will subsequently serve to compare these results with those of a multi-fitting exercise. Six out of 12 crop parameters carried a high CV (>10%), indicating low accuracy of their respective values.

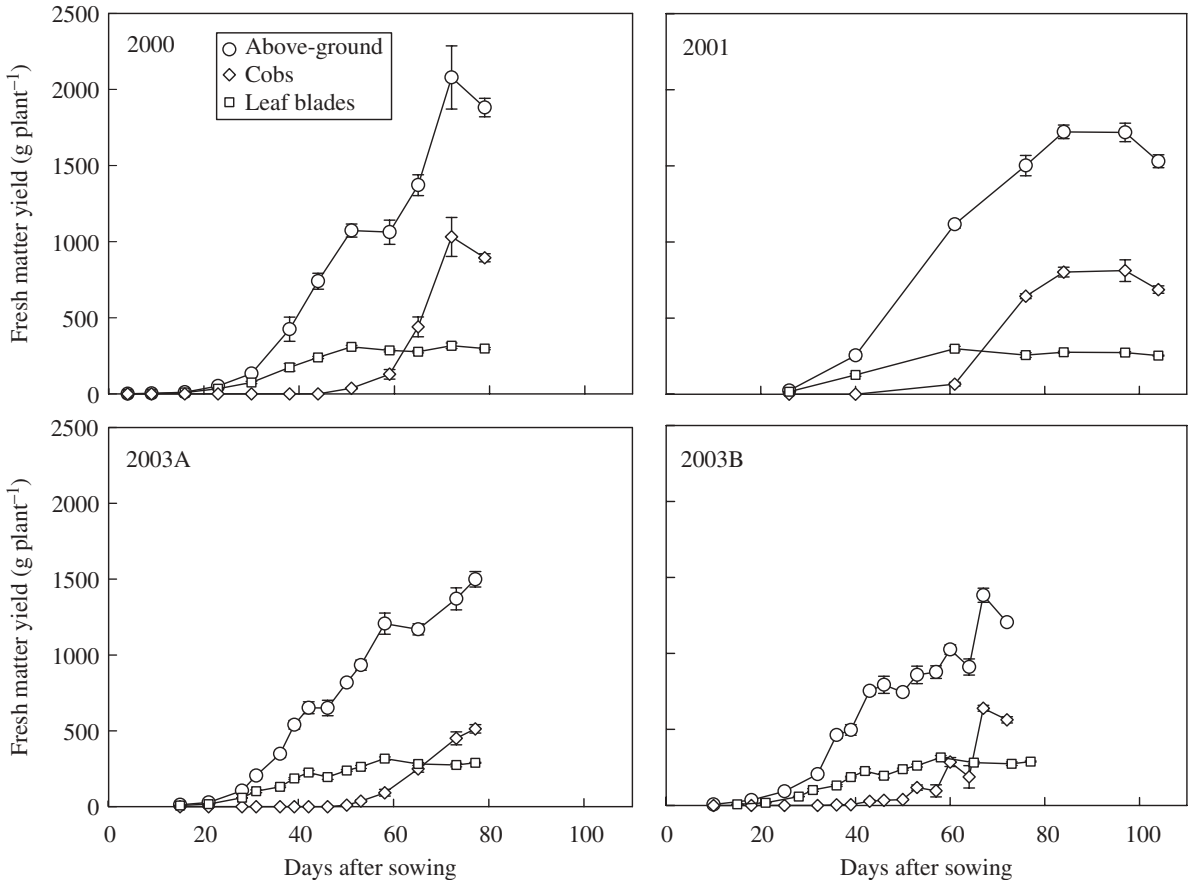

FIG. 4. Kinetics of maize fresh weight. Means ± standard error of four (2000, 2001) or five (2003AB) replications.

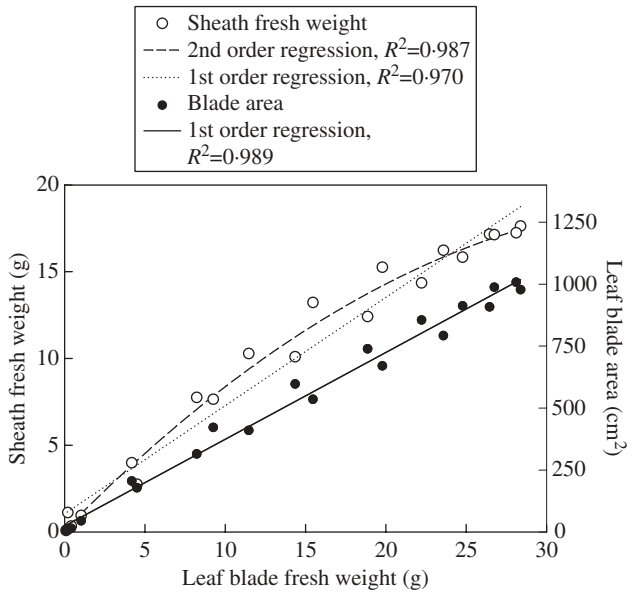

FIG. 5. Allometric relationships between leaf sheath and blade fresh weight and between blade fresh weight and area for individual metamers of maize plants as in Table 3. Since the sheath : blade weight ratio was not constant, the two organs were simulated separately. The blade area : weight ratio was constant and therefore simulated using an allometric rule.

Multi-fitting on several growth stages. For multi-fitting, three target files of the type as presented in Table 3 were established, representing observations made at vegetative stage, flowering stage and grain maturity. Figure 7 presents simulation results analogous to Fig. 6, but here, intermediate situations with some organs still growing and others not yet appeared are shown. Although the fit is not absolute, observations are well approximated.

One advantage of multi-fitting is that the accuracy of parameter estimation is better (Table 5, columns on left-hand side). Only three out of 12 crop parameters had a high CV (>10 %, compare with Table 4 for single fitting). The CV remained high for parameters P_f and P_m, which set the sink strength of cobs and tassels. This can be explained by the fact that these organs are not replicated in the plant architecture and, thus, have poorly documented behavioural norms. The high CV for K_e, the sink strength parameter for secondary pith growth, may be due to inconsistent data on this organ.

Parameter optimization exercises described up to here were based on model forcing with observed durations of organ expansion and life span. As an alternative, the reference data published by Lizaso *et al.* (2003) can be used (Table 5, right-hand side). Resulting parameter values and CV were similar, except for strongly increased CV for P_f (cob sink strength), indicating that the reference data are

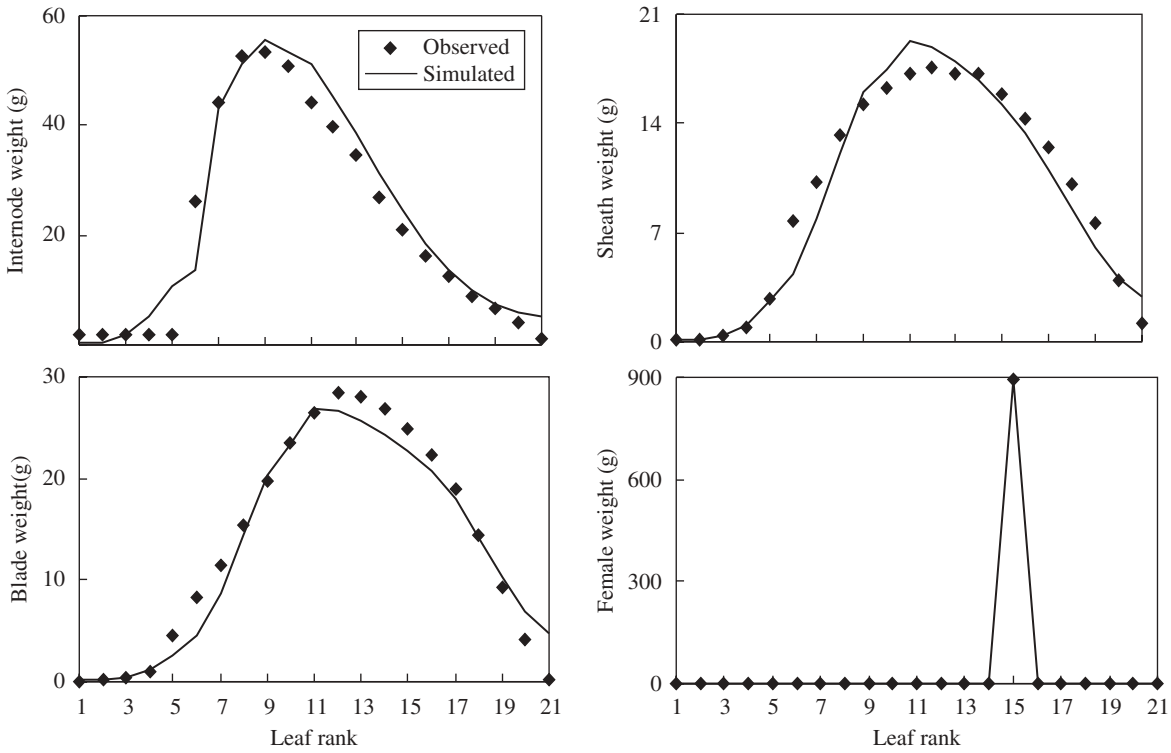

FIG. 6. Results of a single-fitting exercise using observations on mature plants.

TABLE 4. *Estimated parameter values, standard deviation and coefficient of variation with single fitting*

Parameters	Value	s.d.	CV (%)
P_b	1	0	0
P_s	0·73	0·03	4·11
P_e	2·38	0·12	5·04
K_e	0·44	0·12	**27·27**
P_f	499·85	128·69	**25·75**
P_m	8·26	1·06	**12·83**
B_b	0·14	0·05	**35·71**
B_s	0·21	0·04	**19·05**
B_e	0·51	0·03	6·00
B_f	0·48	0·03	6·25
r_1	190·95	15·45	8·09
r_2	7·91	0·84	**10·62**

The sink strength of leaf blades (P_b) is set to 1 as reference because all sink strength parameters carry relative values. This parameterization is based on forced organ expansion duration and longevity using field observations.

CV values larger than 10 % in bold print for comparison with Table 5.

Parameter definitions as in Table 2.

generic for vegetative organs of maize and, thus, can help reduce the experimental effort required for model calibration.

Another advantage of multi-fitting as compared with single fitting emerges from the analysis of simulated sink variation kinetics. In the course of the parameter optimization process, the shape of organ sink kinetics is set by calibration of a beta function that can assume vastly different forms. If single fitting is conducted on the basis of the final plant morphology, no information on the expansion laws governing organ development is available. The model thus picks the function shape that equilibrates best biomass assimilation with the succession of (temporally overlapping) demand functions. If several developmental stages of the crop are considered in a multi-fitting process, however, data on expanding organs are available and are taken into account in the fitting of expansion laws. In fact, multi-fitting dramatically changed leaf blade and internode sink kinetics (Fig. 8), particularly for leaf blades where a descending curve was transformed into a physiologically more probable bell-shaped curve (Fournier and Andrieu, 1999). The change in sink kinetics for expanding leaf blades was associated with a decrease of the CV for parameter B_b from 36 % (Table 4, single fitting) to 3 % (Table 5, multi-fitting). Although the resulting kinetics still remain hypothetical and thus need to be validated with detailed measurements, it seems that the multi-fitting process is potentially able to extract relevant information on plant morphogenetic processes from descriptive, morphological data.

This heuristic method of extracting 'hidden' information from descriptive data has obvious limitations (Hammer *et al*., 2002). It depends on (*a*) the assumption that the model is accurate with respect to its underlying hypotheses, and (*b*) that no major, third processes escape the modeller's attention. Since crop models based on economic principles (source budgets competed for by sinks, feedbacks on the source) generally provide for compensatory processes, and since statistical parameter optimization generally forces accurate end results even if intermediate results are inaccurate, a good simulation result is no proof of the model's

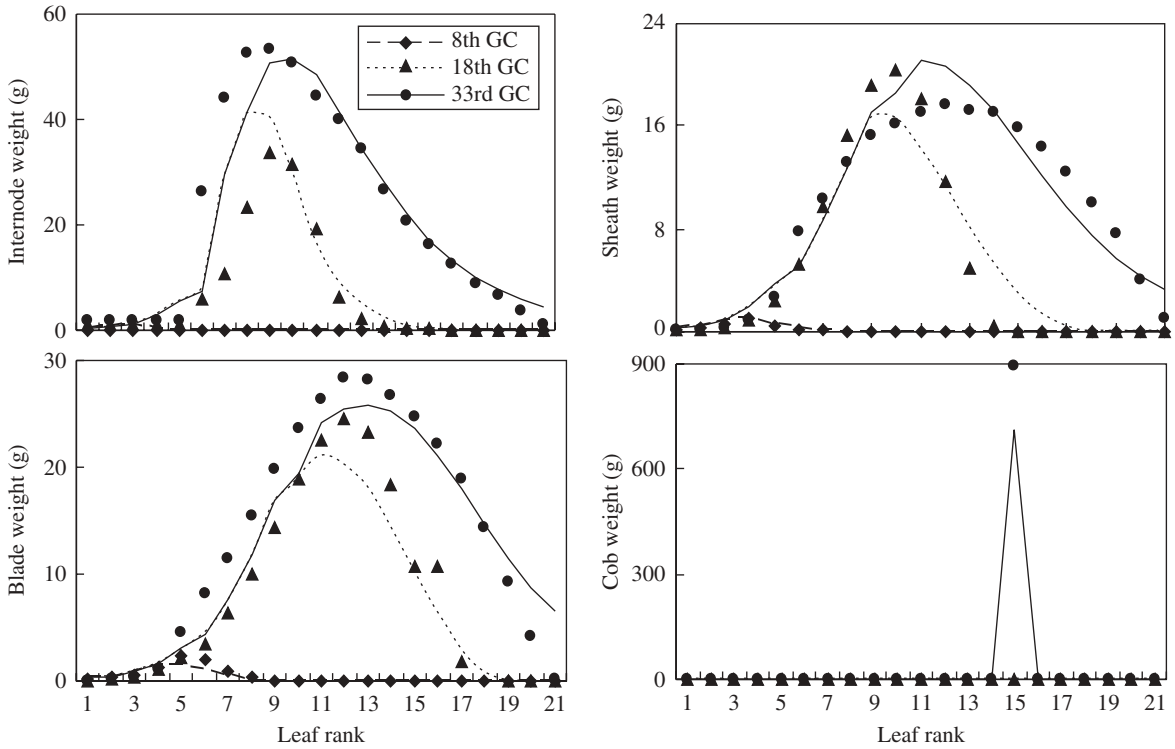

FIG. 7. Results of a multi-fitting exercise using observations on plants observed at three different growth stages (eight, 18 and 33 growth cycles).

TABLE 5. *Estimated parameter values, standard deviation and coefficient of variation with multi-fitting for field observations at three growth stages*

	Calibration using empirical organ expansion duration and life span			Calibration using generic maize parameters (Lizaso *et al*., 2003)		
Parameters	Value	s.d.	CV (%)	Value	s.d.	CV (%)
P_b	1	0	0	1	0	0
P_s	0·76	0·02	2·63	0·84	0·02	2·38
P_e	2·19	0·06	2·74	2·10	0·07	3·33
K_e	0·21	0·07	33·33	270·01	216·45	80·16
P_f	343·71	63·24	18·40	2·82	0·57	20·21
P_m	2·91	0·74	25·43	0·43	0·07	16·28
B_b	0·31	0·01	3·23	0·38	0·01	2·63
B_s	0·45	0·01	2·22	0·52	0·01	1·92
B_e	0·66	0·01	1·52	0·7	0·01	1·43
B_f	0·59	0·03	5·08	0·62	0·05	8·06
r_1	342·31	14·82	4·33	303·66	13·99	4·61
r_2	3·27	0·32	9·79	4·64	0·37	7·97

The three columns on the left are based on empirically forced leaf longevity as in Table 4. The three columns on the right use references in the literature for organ duration.

The parameter definitions are as in Table 2.

biological (mechanistic) accuracy. With respect to GREENLAB in its present version for maize, major uncertainties remain, notably because no distinction is made between fresh and dry biomass (thus not taking into account true carbon budgets) and because intermediate reserves in vegetative organs are not considered (thus ignoring the plant's capacity to buffer assimilate shortages through mobilization (Dingkuhn *et al*., 2005*a*; Luquet *et al*., 2005). The heuristically obtained information on organ expansion laws as shown in Fig. 8 is therefore only indicative of the potential residing in this approach that remains to be validated.

Crop growth prediction

Biomass partitioning and simulation of biomass gain of plant compartments. Simulated crop growth rate (aboveground, fresh biomass gain per GC simulated with the multi-fitted model) in 2000 increased exponentially after

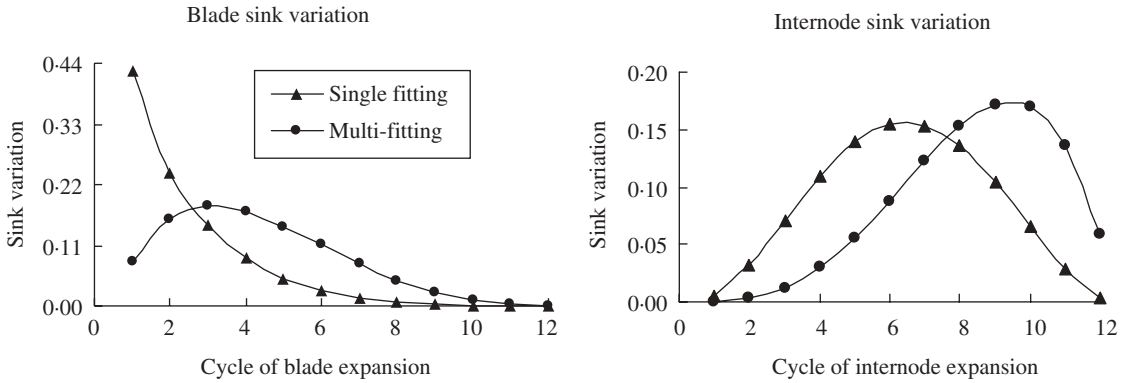

FIG. 8. Generic functions (across metamers) for sink strength variation of leaf blades and internodes obtained with parameter optimization using single and multi-fitting.

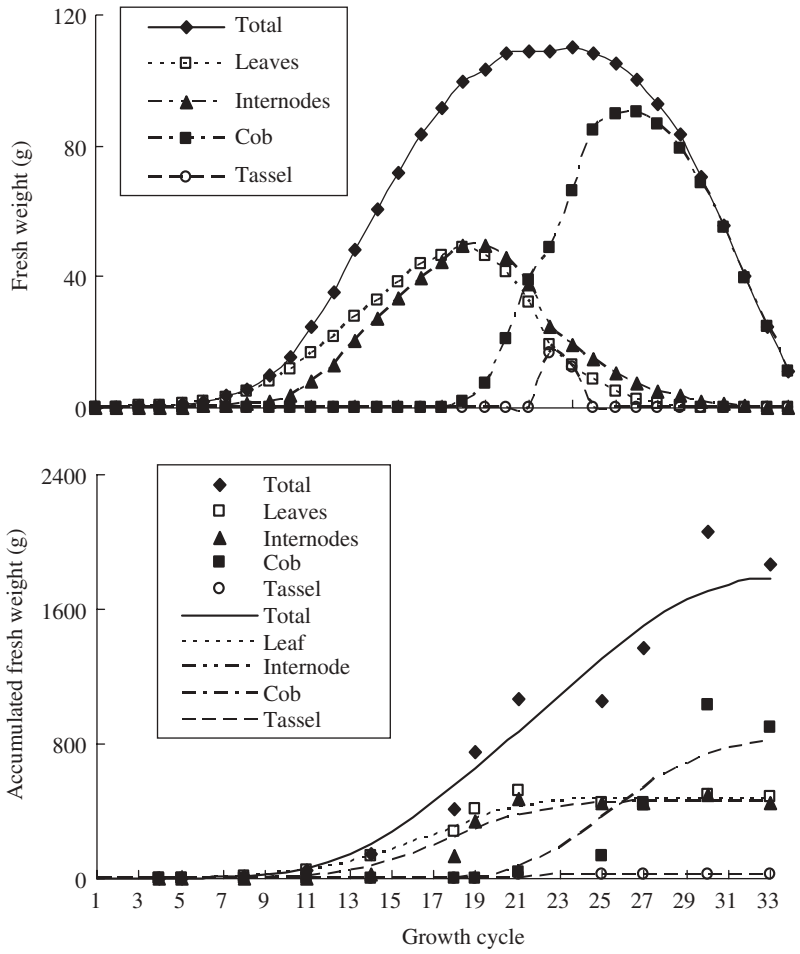

FIG. 9. Above: incremental, fresh biomass gain and partitioning during per organogenetic growth cycle (thermal time between production of two metamers) of maize as simulated with the multi-fitted model. Below: observed and simulated, cumulative, fresh biomass dynamics for organ compartments. Exp. 2000.

crop establishment, attained a maximum at the onset of grain filling (about 20th GC) and decreased thereafter (Fig. 9, top graph). During pre-grain filling stages, biomass was partitioned mainly to leaves initially, then at similar proportions to leaves (blades + sheaths) and internodes. After the production of the last metamer (21th GC), the cob sink became dominant and only a small proportion was allocated to the other organs, produced during early grain filling stages. The decrease in crop growth rate after 21th GC was due to leaf senescence, simulated on the basis of a finite life span for each individual leaf blade.

This partitioning pattern reproduced satisfactorily the observed growth kinetics of internodes, leaf sheaths, leaf blades and the cob (Fig. 9, bottom graph). Note that

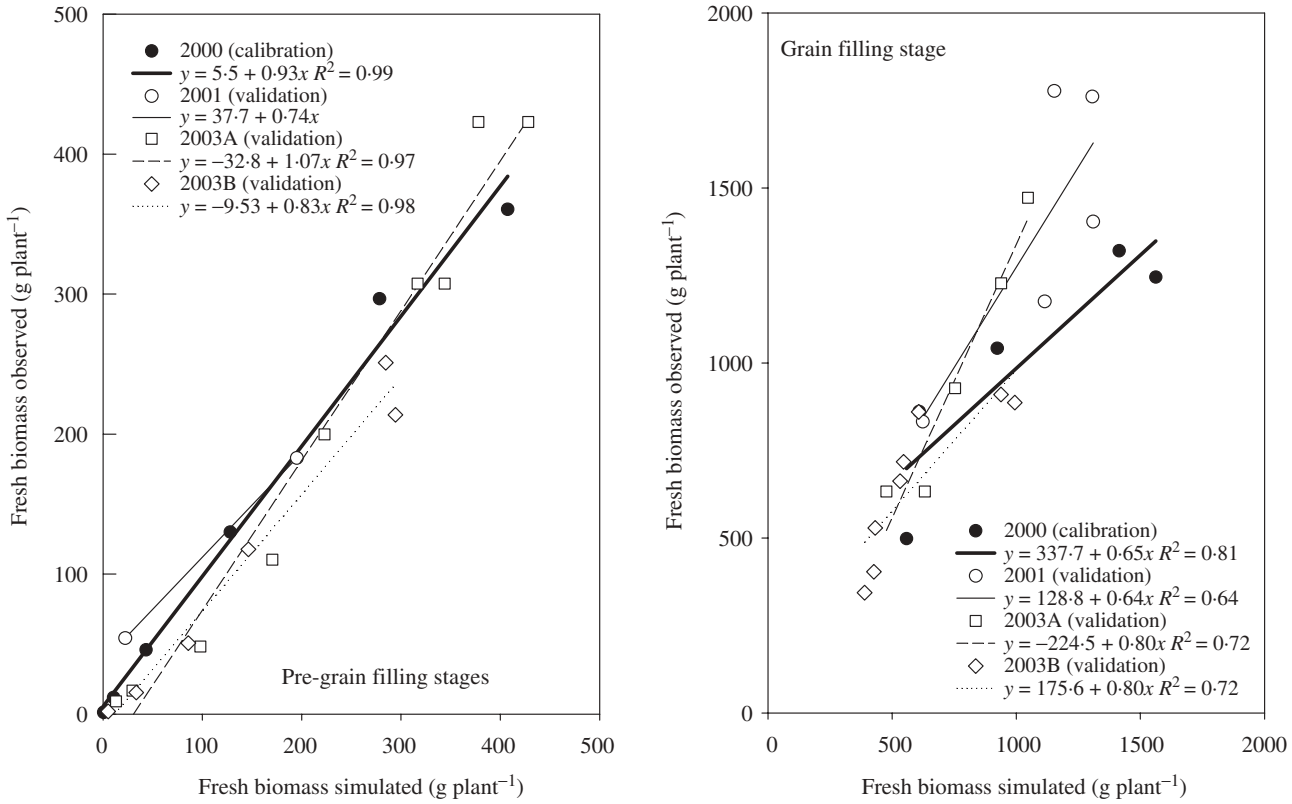

FIG. 10. Prediction of above-ground biomass production in Exp. 2001, 2003A and 2003B using crop parameters from Exp. 2000. Left: observations during vegetative growth (pre-grain filling). Right: observations made during ripening stages (grain filling). Poor predictions during later stages of grain filling (>800 °C.d) are due to inaccurate prediction of cob sink capacity and leaf senescence.

parameter optimization targeted only observations made on individual metamers and not cumulative biomass per compartment.

Extrapolation to other seasons. The crop parameters optimized by multi-fitting for Exp. 2000 were used to predict observed, above-ground biomass for Exp. 2001, 2003A and 2003B (Fig. 10). Excellent predictions were made for vegetative growth (pre-grain filling) but predictions for the last growth stages, during which cobs developed and leaves senesced, were less good. It appears that the model in its present version is robust for growth stages during which the plant architecture expands and vegetative organs grow, but misses important processes that cause variable cob sink capacity across among seasons.

Cob sink capacity may depend on numerous endogenous and environmental factors. Kernel number can be reduced by heat stress (Cantarero *et al*., 1999) and assimilate source limitation (Andrade *et al*., 1999; Gambin *et al*., 2004), whereas kernel size is affected by source–sink relationships at post-flowering stages (Borras and Otegui, 2001). The green leaf longevity (Lizaso *et al*., 2003), affected by genotype and nitrogen supply, also influences cob yield in maize (Martin *et al*., 2005). GREENLAB simulates assimilate supply effects on cob yield only during grain filling (when the sink is active), but does not take into account source effects on potential sink size at earlier stages, such as silking (when the future sink size is set). This, as well as the model's inability to simulate heat stress effects on sink size, probably explains the relatively poor predictions of cob yield across seasons (T_{max} sometimes exceeded 40 °C during late crop growth stages). Nevertheless, the demonstrated capability of the model to predict crop architecture, growth and biomass partitioning from germination to the early stages of grain filling (0–800 °Cd; Fig. 10) is encouraging and, as far as is known, unique for architectural plant models.

Three-dimensional representation of plant architecture

Using a library of symbol files for the different organs of maize previously established from *in situ* digitization, the GREENLAB model outputs were automatically converted to 3D representations. In Fig. 11, results are shown for three developmental stages of the Exp. 2000 crop (12th GC, end of the vegetative growth period; 21th GC, end of organogenesis; 33th GC, maturity). Such image files are output for each simulation time step (GC) and can thus be used for animated representation. Apart from didactic applications, the 3D representations can be used to calculate precise light interception and energy balances, for single plants as well as for uniform or mixed plant populations that grow under conditions of inter-plant competition for light and, ultimately, for water.

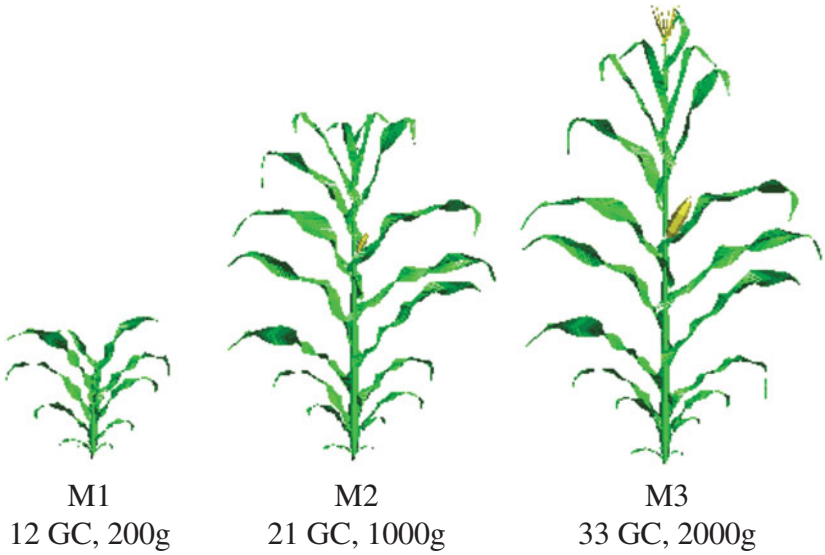

FIG. 11. 3D model output for maize plants simulated at 12th, 21th and 33th growth cycles (M1, end of vegetative phase ; M2, end of organogenesis; M3, maturity) for Exp. 2000.

Perspectives for model improvement and applications

Although GREENLAB represents a major step forwards towards the detailed simulation of virtual plants developing and responding to environment as real plants do, more development work is needed to enable its efficient application to a range of purposes. Some of these improvements are of general importance and others would be specific to the modelling objectives.

Among the necessary, generic improvements, as highlighted by this study, are the development of a true carbon balance and a water balance. A new version is in progress that simulates biomass dynamics on the basis of dry matter, which is then 'hydrated' to give organs their eventual mass and shape, including bending effects caused by gravitation. A simple soil water balance interacting with the root system already exists in the model (although not used here due to absence of field observations), but it will be physiologically meaningful only after both the carbon and hydrological architectures of the plant are implemented.

With regards to agronomic applications emphasizing yield, this study clearly demonstrated that GREENLAB simulates well the sink variations of vegetative organs among metamer positions and seasons, but fails to predict inter-seasonal variation of the reproductive sink (cobs). A future version of the model thus needs to predict cob weight not only on the basis of a fixed sink coefficient (parameter P_f) and source limitations during filling, but also a predimensioning process of sink capacity that is sensitive to carbon resources and stresses (e.g. extreme temperatures and drought) during a specific phenological period.

Although classical, agronomic, field applications of GREENLAB appear to be within reach, they do not require much of the structural detail this model provides. Its comparative advantage would therefore reside in applications requiring virtual plants responsive to environment, either for technical objectives (e.g. town/landscaping; management of specific crop architectures in horticulture) or for the exploration of new crop ideotypes in a breeding context. Furthermore, the detailed plant and canopy architecture simulated with models such as GREENLAB can be used to improve the accuracy of water and energy balances. The model improvements required by these and other applications are quite specific to the purpose and thus are beyond the scope of this discussion.

Beyond practical simulation purposes, this study demonstrated that modelling a plant's body plan and its different, environment dependent expressions can provide new information on emergent system properties, such as parameters that are common to different organs or organs that are replicated by different metamers. Further studies on parameter stability and system sensitivity to parameters are in progress.

CONCLUSIONS

This paper presented the adaptation, calibration and validation of the functional–structural model GREENLAB for field grown maize. New methodologies for crop parameter optimization using a multi-fitting approach and for 3D representation of simulation results were developed. Using target files of morphological field observations and the generalized least square method for 12 crop parameters, model algorithms describing source–sink relationships in the plant were calibrated. Two main advantages of multi-fitting for several growth stages, as compared with single fitting using descriptors at maturity only, were identified. First, multi-fitting improved considerably the accuracy of most parameter values, and secondly, generic sink kinetics for the different organ types were generated that converge with our current understanding of organ development. More research is needed to validate such behavioural rules extracted from static plant descriptors, but it appears that this modelling

approach has good potential for heuristic analyses of ontogenetic processes that would be difficult to observe directly on the plant.

Model validation for different seasons was successful for pre-grain filling growth stages, during which the plant architecture and morphology develop. Predictions of growth and partitioning during grain filling stages, however, were less accurate, indicating that the model simulates insufficiently processes related to cob sink development and leaf senescence. Furthermore, the model needs to be equipped with a true carbon and energy balance and a full water balance to permit agronomic applications. The development of such features is in progress.

ACKNOWLEDGEMENTS

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