# L-GrassF: a new model for simulating the genetic environment interactions on the reproductive phenology of grasses

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## Abstract

Predicting the reproductive phenology in perennial grasses is a major concern because it determines the quantity and quality of forage. It varies a lot depending on site, year and cultivar. Projections of future climates suggest significant changes in seasonal temperature pattern, with new combinations of temperature and photoperiod, whose consequences on the floral induction of perennial grasses are unknown. L-GrassF is a new Functional Structural Plant Model simulating genetic variability of the phenology of perennial ryegrass in order to better understand the perenniality of grasslands and better anticipate the effects of climate change. L-GrassF stems from a previous model (L-Grass) and now simulates the reproductive stages by integrating the interactions between vegetative growth, floral induction and reproductive organ development. The sensitivity analysis of a set of parameters was studied in the range of oceanic temperate climate conditions, on several European cultivars. It was further calibrated and validated on two independent datasets from the French Variety and Seed Study and Control Group (GEVES), which include the observations of heading dates for seven cultivars of *Lolium perenne* grown in six French locations between 2001 and 2017.

## Introduction

In temperate regions, grasslands produce throughout the year albeit with large seasonal variations directly and indirectly linked to climate. Grasses dominate the grasslands and their foliage production rate changes with temperatures, daylength, and water availability. From germination onward, each grass tiller undergoes a series of phenological stages (Heide, 1994, Rouet et al 2021). The rate at which the different steps in that cycle are achieved is under genetic and environment controls. Genetically, and compared to other physiological processes in plants, reproductive phenology is indeed highly variable and heritable. Conversely the phenological stage of each tiller has a major impact on its functioning, growth potential and relationships with neighbour plants. Heading plants for instance show much faster growth rates than vegetative plants of the same size under the same conditions. Following flowering, only vegetative tillers survive and insure the persistency of the grassland. The percentage of flowering tillers in a grassland is therefore of primary order to understand and manage the long term variations of its productivity and genetic composition.

Climate change is expected to change the correlations between day length and temperature, two variables, which are crucial in determining the floral transition in grass apices. Although, the roles of temperature, both cold days in winter and warm days in spring, as well as photoperiod was qualitatively analysed for grasses, the quantitative assessment of the genetic variability of the tiller response to climate remains to be made.

Numerical modelling can help both to integrate the complex relationships between the plant responses to environment at tiller scale and the changes in composition and production of grasslands with different climate conditions and different cultivars. Such models could also help designing new ideotypes adapted to furture climate conditions. We present here a new individual-based model, L-GrassF, that simulates the phenological changes occurring for each tiller of a plant in response to its local environment (temperature and photoperiod). The model stems from the model L-Grass (Verdenal et al 2008), which only dealt with vegetative growth. New functions describing the morphological and developmental transition at the apex were implemented. A sensitivity analysis of parameters related to these functions was performed and the identification of the most sensitive parameters was made in order to characterize a set of cultivars tested for different years and sites.

## Methods

### *Hypothesis and model description*

The initial L-Grass model described the growth of individual ryegrass plants in 3D using the L-System formalism (Verdenal et al 2008). It represented the vegetative production series of leaves characterized by their shape. The kinetics of leaf elongation was coordinated with the its appearance to light and also determined the start of the next leaf. In that vegetative stage, one new primordium was produced at each leaf appearance. Leaves went through two phases of elongation until full unfolding. In a first phase and as long as the leaf was shorter than the sheath of the previous one, the growth equation was a beta function of thermal time. In a second phase and following the appearance of leaf tip, a new elongation regime was also described by a beta function of thermal time but using new parameters with a potential final length, which depended on the time spent elongating within the sheath. The new tillers were produced from the axillary apical meristem at the basis of each leaf, also in coordination with the length of axillating leaf, and depending on canopy closure.

The new ryegrass model (Rouet et al 2022) is based on the first hypothesis that each shoot apical meristem is independent and receives signals only from the environment, irrespective of the status of other apices of the same plant.

The second set of hypothesis assumes two developmental phases, strictly sequencial (Heide 1994, Rouet et al. 2021). During the first phase, the apical meristem of each tiller reaches a primary induction stage under the influence of cold temperatures. Only once primary induction is reached, and when the photoperiod is longer than a minimum duration (in hours), the second development phase of the tiller starts under the influence of the photoperiod. When the secondary induction (adimensional) is completed, the apex achieves the floral transition. Heading, defined as the appearance of the ear out of the sheath of the last unfolded leaf, therefore occurs once all leaves produced during the induction phases have fully unfolded.

The third set of hypothesis is that once the secondary floral induction step starts, *i.e.* under a minimum daylength, the rate of primordia production as well as the leaf elongation rate increases. Following floral transition, the primordia accumulated at the apex level will form spikelets instead of leaves, at the same rate until the last leaf appears out of the previous leaf's sheath. In L-GrassF, the elongation of the ear and peduncle are not simulated and the heading date was set to the day when the last leaf was fully elongated.

### *Model evaluation*

Among the 28 parameters of the model, most of parameters were given the value from the initial L-Grass model (Verdenal et al. 2008, Rouet et al. 2022). The sensitivity analysis of the model outputs was made on the seven new parameters introduced to simulate the floral transition and its consequences on tiller growth: (1) the minimum number of leaves in vegetative stage allowing for a maximal rate of secondary induction, (2) the maximum daily rate of the primary induction, (3) the minimum daylength for secondary induction, (4) the maximum rate of secondary induction, (5) the number of primordia initiated at each leaf emergence, (6) the potential maximum leaf length before leaf emergence and (7) the acceleration factor of leaf elongation when the apex enters in the secondary induction phase. The variables tested were the heading date and the leaf length. Parameter values were explored by 6 steps each, using the fractional factorial design of Morris (1991). That sensitivity analysis was repeated in three site x year situations contrasted for their winter and spring air temperatures, unabling to evaluate the dependency of parameters sensitivity to conditions.

To test the ability of the model to simulate the real variations of a heading dates under natural conditions, we used a data base collected by the GEVES, which is the agency that makes the official test for new cultivars in France. Fifty control varieties were used between 2000 and 2017 in six locations spread over the country, covering a large domain of genetic and environmental variability. Under severe water limiting conditions of certain springs in certain locations, water deficit altered the phenology and those situations were identified and excluded from the analysis because the new functions implemented did not yet include all relationships with water status. To determine the situations where water deficit could have been limiting a simple water balance was used, using the local soil and meteorological data. Following that filter, 7 cultivars exhibiting more than 23 independent site x year conditions and covering the whole range of heading dates were used for a numerical optimization of the most sensitive parameters. Half of the available data set was used to identify the parameters and the other independent half was used to evaluate the precision of the simulations. The quality of prediction was evaluated computing the root mean square error over the data set.

## Results and Discussion

### *Sensitivity analysis*

The seven new parameters introduced for simulating the reproductive phenology exhibited contrasted impacts on heading dates. The minimum number of leaves and the rate of cold temperature accumulation had a lesser impact. The minimum daylength triggering secondary induction, as expected given the small interval in daylength within France, and the rate of primordia production during the secondary induction both had a moderate impact. By contrast, the simulation of heading date appeared highly sensitive to the three other parameters: Within the range and conditions tested, the third most sensitive parameter was the maximum rate of secondary induction. The second and first most sensitive parameters were the potential leaf length in the initial phase of vegetative leaf growth rate and the acceleration of leaf elongation triggered by the secondary induction photoperiodic conditions. Hence, and somewhat unexpectedly, the two most important parameters to explain the heading date variations in LGrass F were directly involved in the growth of leaves. The classification of these sensitivities revealed largely independent of the temperature conditions tested.

### *First quantitative assessment of the model precision.*

The data set obtained from the official test network (Geves) was used for a first quantitative assessment of the model. Within that cultivars set, the variation of heading date in perennial Ryegrass was about 30 days on average (Rouet et al. 2021) with an average date of 140 days after January 1st (5 May). For early cultivars, that was also the variation observed from one year and one site to the other. Hence, the environmental range was equivalent to the genetic range as observed in other plant species (e.g.: Papper and Ackerly, 2021 for oak). For later varieties however, the environmental variation was less, which might be adaptative (Keep et al 2021). The number of observations largely varied between cultivars because of the periodic, yet irregular renewal of control varieties in the test network. For the validation study, the seven cultivars chosen were Bronsyn, Indiana, Lactal, Bargala, Milca, Carillon and Escal, ranging from 126 days up to 156 days, approximately.

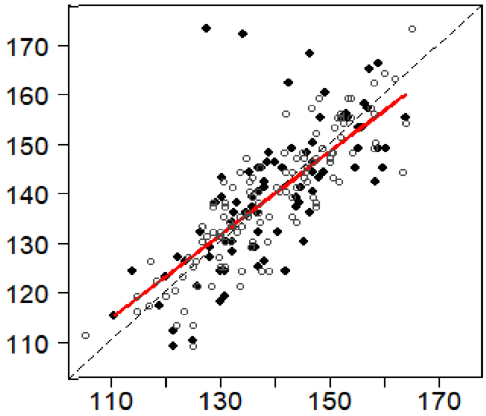

Figure 1. Relationships between the observed and simulated heading date for seven cultivars grown in six locations in France between 2000 and 2017. Open and closed symbols correspond to observations used for calibration and validation, respectively. For validation data: $R^2 = 0.48$. RMSE = 10.2

For those seven cultivars, fixing all other parameters to average values, it was possible to determine a set a values for the three most impacting parameters and each cultivar, using half of available data. Over the whole conditions and cultivar set (Fig 1), the general precision of the heading date was about 10.2 days (relative root mean square error of 7.2%, approximately), which was similar to what was found in early phenological empirical models (e.g. Jouven et al 2006).

### *Simulation of shape and percentage of reproductive tillers.*

The 3D L system formalism used in L-GrassF with its appropriate geometrical interpretation (Boudon et al. 2012) also enabled the visual and realistic representation of the plant as it grew from seed (Fig 2). This was important to test the consistency of the simulations in terms of architecture and relative length of leaves. It illustrated how all developmental stages can be found on the same plant, as observed in the field.

Figure 2. 2 D projections of simulated ryegrass plants using LGrass F, at four stages of their development. 1: young seedling in early fall, 2 and 3: vegetative stages (before primary induction) in mid fall then winter, 4: aged plant with four eared tillers and three vegetative tillers in summer. Green: leaves on the vegetative tillers. Red: leaves on reproductive tillers. Purple: ears.

## Conclusions

The new model exhibited promising performances in terms of plant geometry, development rate and phenology. It suggested a strong involvement of the vegetative growth parameters in the determination of developmental stages, opening questions for future research in the physiology of flowering in grasses. That first quantitative assessment of the prediction of genetic x environment interaction, proved the new model to perform well.

Further developments will be made to test the ability of L-GrassF to simulate the proportion of reproductive tillers in spring and subsequent regrowth potential, as well as repeat-flowering in summer. With its ability to interact with light water and nitrogen, L-GrassF also has the potential to contribute to long term studies of grassland dynamics responses to climate change .

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