Competition for resources during development drives allometric patterns in the grass *Setaria*

Renee Dale[1], Darshi Banan[2], Britney Millman[1], Andrew D.B. Leakey[3], Shankar Mukherji[4,*], Ivan Baxter[1,*]

[1] Donald Danforth Plant Sciences Center, St. Louis, MO, 63132
[2] University of Washington, Seattle, WA, 98195
[3] University of Illinois Urbana-Champaign, Champaign, IL, 61820
[4] Washington University in Saint Louis, St. Louis, MO, 63130
*Correspondence to: smukherji@physics.wustl.edu (S.M.), ibaxter@danforthcenter.org (I.B.)

Authors for correspondence: SM, mukherji@wustl.edu; IB, ibaxter@danforthcenter.org

Keywords: mathematical model, grass development, phenotyping, Setaria

Abstract

Grasses grow a series of phytomers during development. The distance between successive leaves is determined by internode lengths. Grasses exhibit genetic, developmental, and environmental variability in phytomer number, but how this affects internode length, biomass, and height is unknown. We hypothesized that a generalized mathematical model of phytomer development wherein between-phytomer competition influences internode length distributions would be sufficient to explain internode length patterns in two *Setaria* genotypes: weedy A10 and domesticated B100. Our model takes a novel approach that includes the vegetative growth of leaf blade, sheath, and internode at the individual phytomer level, and the shift to reproductive growth. To validate and test our mathematical model, we carried out a greenhouse experiment. We found that the rate of leaf emergence is consistent for both genotypes across development, and that the length of time spent elongating for the leaf and internode can be described as the ratio between the time of phytomer emergence and the elongation completion time. The validated model was simulated across all possible parameter values to predict the influence of phytomer number on internode length. This analysis predicts that different internode length distributions across different numbers of total phytomers are an emergent property, rather than a genotype-specific property requiring genotype-specific models. We applied the model to internode length only field data of *S. italica* accession B100, grown under both well-watered and drought conditions. The model predicts that droughted plants reduce leaf elongation time, reduce resource allocation to the internodes, and overall experience slower growth. Together, model and data suggest that allometric patterns are driven by competition for resources among phytomer and the shift to reproductive growth in *Setaria*. The resulting model enables us to predict growth dynamics and final allometries at the phytomer level.

Introduction

Genetically identical plants exhibit variability in their number of phytomers, influencing above-ground tissue growth. Variation in the number of phytomers within a species has been observed in the literature across grass species, including Setaria and Sorghum (Allen et al., 2018; Banan, 2019; Junqueira et al., 2020; Xue et al., 2012). However, the effect of variation in phytomer number on plant growth is poorly understood.

Phytomers are developmental units that make up the body of a plant, serially arranged with one stacked upon another. Grass phytomers consist of a leaf blade, leaf sheath, internode, and node. Internodes function as a sink for assimilates, influence whole-plant light distribution, and impact biomass and grain yields (Fournier, 2000; Kahlen and Chen, 2015; Mauro-Herrera and Doust, 2016; Sarlikioti et al., 2011; Xue et al., 2012).

Plant height integrates a variety of signals as development progresses, especially during the shift from vegetative to reproductive growth (Kawano et al., 2020). Stem length is an important yet highly plastic feature of plant architecture (Feldman et al., 2017), influencing the height of the panicle above the ground and grain yield (Peng et al., 2019; Serrano-Mislata and Sablowski, 2018). However, regulation of height is poorly understood in grasses, motivating the study of smaller components such as internodes that underlie height (Mauro-Herrera and Doust, 2016). Increasing internode lengths brings costs (McKim, 2020), and if the reproductive internode (peduncle) is too long, grain loss may occur due to lack of support (Li et al., 2023; McKim, 2019; Serrano-Mislata and Sablowski, 2018). Tillering, wherein off-shoots of the initial plant culm grow alongside the culm, further complicates the regulation of phytomer growth within the culm and between tillers. For grasses with a terminal inflorescence, the transition from vegetative to reproductive development shifts height growth from both phytomer emergence and elongation to only elongation of already emerged phytomers within a tiller. Understanding how developmental transitions are regulated and coordinated, and how biomass is allocated to individual organs and produces morphology of those organs are current challenges in plant developmental biology (Poethig, 2003, Zhang et al., 2016).

We hypothesize that competition between phytomers for resources drive internode allocation patterns. Competition between vegetative and reproductive growth has been put forward as a possible explanation of internode length distributions (Reddy et al., 1997). Phytomer sizes have been observed to reduce as the plant shifts to reproductive growth (Hodge and Doust, 2021), and higher yields are observed in plants with dwarfism, possibly reflecting the influence of competitive allocation between vegetative and reproductive growth (McKim, 2019). In a field experiment of *Setaria italica* B100, plants that had different numbers of phytomers (due to random variation) had different internode length distributions (**Figure 1A**) (Banan, 2019), indicating that variability in phytomer number may influence internode length and plant height. We test this hypothesis through developing a generalized model of above-ground grass development and determining its ability to capture internode lengths in two genotypes of Setaria. If such a model fails, we conclude that competition between phytomers is not sufficient to explain internode patterns.

Internode elongation has been modeled as responsive to a driver such as a hormone (eg, GA) (Buck-Sorlin et al., 2005; Schouten, 2002) or light (Kahlen and Chen, 2015; Kahlen and Stützel, 2011). The phases of internode elongation and growth dynamics have been modeled using non-linear regression in maize (Fournier, 2000), and with linear ordinary differential equations in maize (Vidal et al., 2018), using GLMS in cotton (Reddy et al., 1997), and a second-order function-value trait model using image-extracted metrics in Setaria (Hodge and Doust, 2021). However, these models typically focus on specific aspects of internode development, sometimes at the level of individual internodes. How the growth of individual internodes behaves in the context of multi-phytomer growth and the shift from vegetative to reproductive growth, features which are thought to influence internode growth and plant height is an existing knowledge gap (Hodge and Doust, 2021; Mauro-Herrera and Doust, 2016; Peng et al., 2019; Reddy et al., 1997; Serrano-Mislata and Sablowski, 2018).

To understand the underlying allocation rules that determine internode lengths, we take a mathematical modeling-based approach to apply our understanding of grass development and data on internode and phytomer growth. We consider resource allocation as a dynamic process, rather than the final allometry, which may lead to better understanding of the processes underlying allometry (Hodge and Doust, 2021). Here, we use ‘allocation’ in the modeling sense

to refer to the division of matter to component parts. We developed a parsimonious, phenomenological mathematical model describing resource allocation to phytomer components (leaf blade, leaf sheath, internode) and the shift from vegetative to reproductive growth. In our model we consider developmental time parameters such as the emergence rate of the phytomers, and how long each phytomer grows for. The model assumes that resources are evenly divided amongst currently growing phytomers. This model is validated and its parameters are simplified using experimental observations. The resulting model is able to capture dynamics in leaf growth and plant height, as well as the final lengths of the leaf blade, leaf sheath, and internodes, improving our understanding of developmental transitions and biomass allocation (Poethig, 2003; Zhang et al., 2016).

Results

Variation in internode lengths motivates development of a mathematical model of developmental resource allocation

Grasses grow a number of vegetative phytomers (N_{phyt}) that support leaves followed by a reproductive phytomer that supports the panicle. Each phytomer grows semi-sequentially, wherein the second phytomer emerges after some time, followed by the third, and so on (**Figure 1B**). This produces a system where the number of phytomers growing at a given time changes over the course of development. To capture the interplay between whole-plant biomass accumulation and resource allocation to simultaneously-growing phytomers and their component structures, we developed a phenomenological mathematical model of *Setaria* development. The mathematical model consists of 3 layers (**Figure 1C,D**).

First, to capture overall logistic plant growth, we use a Hill function model to capture growth dynamics. As the plant grows, it produces increasing amounts of biomass. We assume that the majority of biomass production comes from leaves, and therefore that the maximum biomass production will peak when total leaf area is greatest. Second, at the heart of the model, we consider the growth of each individual phytomer. Each vegetative phytomer has a leaf blade, leaf sheath, and internode, and the reproductive phytomer has a panicle and peduncle. The model contains two classes of developmental time parameters that describe phytomer growth: the emergence time τ and the duration of growth multiplier β. τ describes the delay between the initiation of one phytomer to the next, while β describes the length of time each phytomer component grows for. We use a different duration of growth multiplier for each tissue type. These parameters describe when each phytomer component will grow over the duration of plant development.

Finally, the model includes additional parameters describing relative allocation to phytomer components. Within each phytomer, allocation across the leaf blade, leaf sheath, and internode are described by relative allocation parameters, and allocation to the reproductive phytomer is described by the proportional degree of reproductive investment (ρ) parameter. The parameters describing sub-phytomer allocation are determined using parameter estimation. The model describes allocation dynamics to each phytomer across development, and integrating allocation dynamics provides the accumulated growth for each of the phytomer components (**Figure 1C**). This enables us to connect biomass growth over time with height over time, the relative allocation of biomass to individual phytomers and phytomer components, and the final relative height of phytomer components.

Different genotypes of *Setaria* show consistent emergence rates

To validate the model, we tracked the growth of *Setaria viridis* A10 and *Setaria italica* B100 in the greenhouse from 7 days after planting (DAP) until seed maturity and compared our results to the model (**Figure 2A**). We measured the emergence of leaves and elongation duration of phytomers (leaves and internodes). We found that the emergence rate, defined as the mean delay from initiation of one phytomer to the next, was not significantly different between *S. viridis*

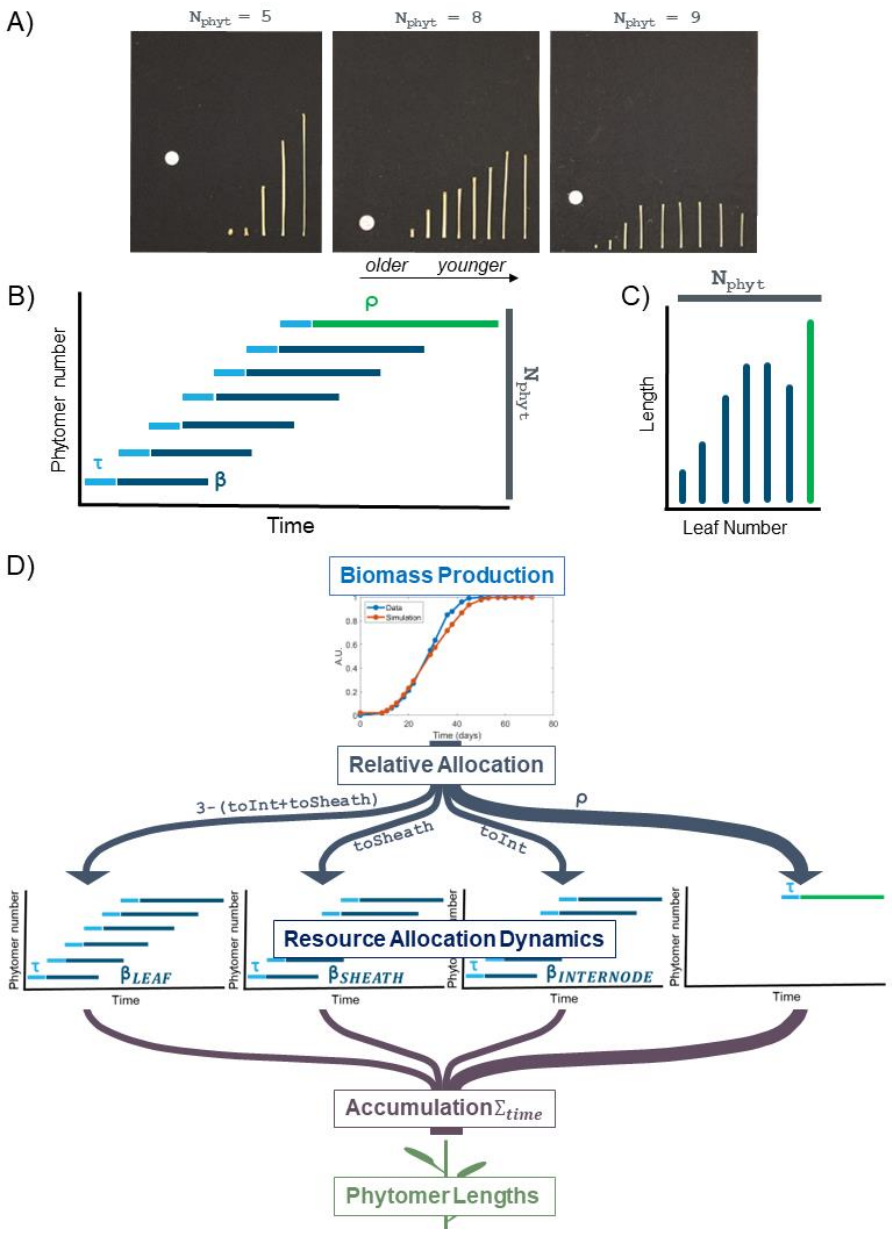

Figure 1. Plants with different numbers of phytomers display different internode length distributions. Three representative samples of dissected internodes from *S. italica* genotype B100 culms grown under field conditions are shown, in order from older internodes to the left and younger internodes to the right (A). To understand what processes may cause this, we developed a mathematical model of grass development based on the semi-sequential growth timing of phytomers (B). The model considers each phytomer as growing semi-sequentially, with (τ) time passing before the next phytomer initiates. Each phytomer grows for some time (β). Each phytomer takes a fraction of the total available biomass proportional to the number of currently growing phytomers, and reproductive phytomers take a larger fraction (ρ). Summing the resulting accumulation over time produces predicted internode length distribution as a function of phytomer or leaf number (C). (D) The model takes the biomass gain over time as an approximation of the biomass production over time. The biomass production is divided proportional to the relative allocation parameters which describe the relative amount of biomass allocated to each phytomer sub-component. The resource allocation dynamics of each phytomer sub-component are described by developmental time parameters. Together, this describes the amount of biomass allocated over time to each phytomer sub-component, as well as composite traits such as above-ground size and height.

A10 and *S. italica* B100 (B100 emergence rate 2.02 +/- 0.9 days, A10 emergence rate 2 +/- 0.4 days, t-test p-value 0.73) (**Figure 2B**). In a follow-up experiment, we tracked A10, B100, and five additional *Setaria* genotypes using automated phenotyping (see Methods). All seven genotypes had a mean emergence rate of between 2 and 2.6 days, and all genotypes were not significantly different from genotype A10 with a mean of 2 days (**Supplemental Figure 1**). This result allowed us to treat the emergence rate parameter (τ) as a constant in subsequent model simulations, and set it as 2 days.

Elongation duration was different between leaf, internode, and across genotypes in experiments

The duration of growth for each leaf and internode was tracked using the emergence day and the day when we observed the final length of that leaf or internode. We found that the duration of growth could be simplified by considering the relationship between the day each phytomer emerged and the day they completed elongation, which was well approximated as a linear relationship (**Table 1**, **Supplemental Figure 2**). Simplifying these relationships by setting the intercept to zero and taking the ratio of these two developmental time points improved R^2 (**Table 1**). In both A10 and B100, the growth duration was longer in internodes than in leaves (A10 leaf (β) 1.26 +/- 0.15, internode (β) 1.57 +/- 0.27, t-test p-value $2*10^{-20}$; B100 leaf (β) 1.29 +/- 0.14, internode (β) 1.4 +/- 0.14, t-test p-value $1*10^{-21}$) (**Table 1**, **Figure 2B**).

Table 1. Linear models describe the relationship between emergence time and growth completion time. The linear regression model is of the form *growth completion time = slope*emergence time + intercept*. The slope is the growth duration multiplier (β). The simplified linear model is of the form *growth completion time = slope*emergence time*. We use the simplified linear model in the following simulations.

	Linear Regression Model			**Simplified Model**	
Condition (β)	Intercept	Slope * emergence time	R^2	Slope * emergence time	R^2
A10 leaves	2.7	1.04	0.85	1.26 +/- 0.15	0.99
A10 internodes	-6.55	2.12	0.86	1.57 +/- 0.27	0.97
B100 leaves	2.55	1.15	0.95	1.29 +/- 0.14	0.97
B100 internodes	3.5	1.23	0.95	1.4 +/- 0.14	0.99

Summary of parameter simplifications in the model enabled by experimental results

The model's developmental time parameters were able to be simplified using experimental observations. The emergence time (τ) was found to be 2 days on average, and was conserved across genotypes (**Figure 2B**, **Supplemental Figure 1**). This enabled us to consider the emergence time as a fixed parameter. We also found that the duration of growth multiplier grow time (β) for each phytomer could be simplified as a linear relationship, where β=growth completion / emergence time, and therefore growth completion time is the emergence time multiplied by the duration of growth multiplier (β). We were therefore able to represent time spent elongating using the duration of growth multiplier (β) across the entire set of phytomer components, rather than individual elongation duration parameters. Generally, the N^{th} phytomer stops growing at $\beta(1+\tau(N-1))$, where β is a constant across each phytomer component (e.g., leaf blades, sheaths, or internodes), and τ and N are fixed parameters. Thus, later phytomers grow for extended times relative to initial phytomers when τ>1. For example, if the first phytomer starts growing on day 1, it stops at day 1*β. The 5th phytomer starts growing on day 1+4τ, and stops at day β(1+4τ). The shift to reproduction (the *N+1* phytomer) would occur at 1+τN.

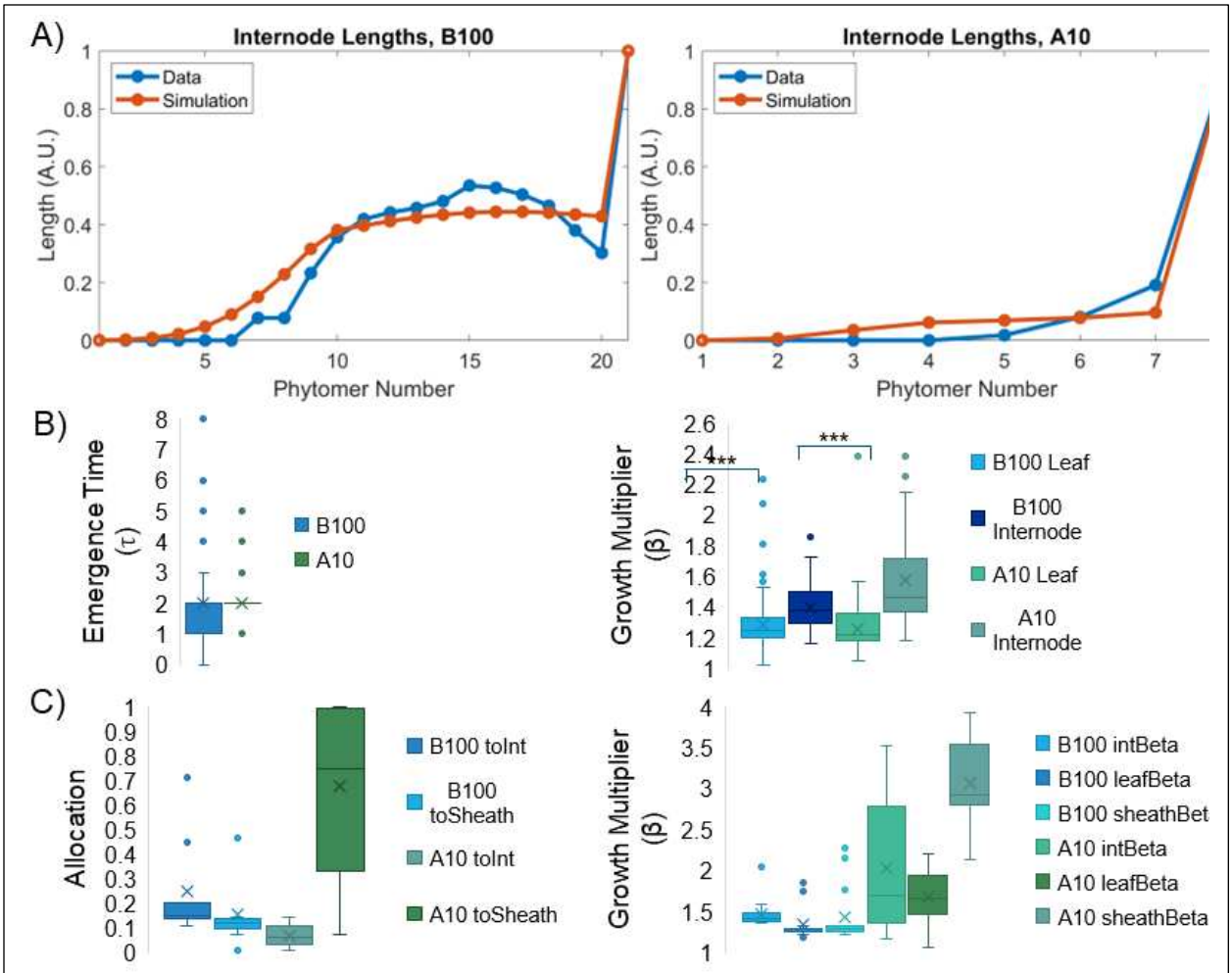

Figure 2. Experimental results and model validation. (A) The model is able to recapitulate internode length distributions for *Setaria* genotypes B100 and A10, which have a large difference in the average total number of phytomers. A representative fit to internode lengths from each genotype is shown (orange) compared to normalized internode length data (blue). Additional representative fits of growth dynamics, height dynamics, and sheath lengths for additional samples available as **Supplemental Figure 3**. (B) Both genotypes in our experiment had emergence rates around 2 days, with no significant difference across genotypes. This parameter was fixed in subsequent simulations and parameter estimations. Experimental results showed that the duration of phytomer growth was able to be estimated using the ratio of the time of elongation completion and the emergence time (**Supplemental Figure 2**). (C) Allocation parameters were estimated using parameter optimization. The model predicts that the proportional degree of reproductive investment parameter (ρ) is not significantly different between B100 and A10, that B100 has higher allocation to the internodes, and A10 higher allocation to sheaths relative to other phytomer components. The model predicts that all grow times are longer for A10 than in B100. Boxplots show the distribution of these grow times across each genotype. Leaf grow times are shorter than internode grow times. The parameter estimates for leaf and internode grow times captured the trends in the data, as internode grow times were longer than leaf grow times.

Experimentally, the duration of growth multiplier (β) was found to vary for leaf and internode, and typically the duration of growth for leaves were shorter than the duration of growth for internodes (**Figure 2B**). Our resulting model has 8 optimized parameters and 3 fixed parameters (**Table 2**).

Model-based estimation of reproductive allocation

The degree to which resources were allocated resources to the reproductive phytomer during the shift to reproductive growth was unable to be experimentally estimated. The proportional degree of reproductive allocation (ρ) was estimated using parameter optimization. The model predicts that A10 and B100 do not have significantly different proportional degrees of reproductive allocation. However, due to the larger size and phytomer number in B100, the absolute amount of resources allocated to the reproductive phytomer will be much greater, and the duration of reproductive growth much longer. The model predicts that B100 has higher allocation to the internodes, and A10 higher allocation to sheaths (**Figure 2C**).

Table 2. Summary of all parameters included in the model. The emergence rate was experimentally observed to be consistent across *Setaria* genotypes, and treated as a fixed parameter. The duration of growth multiplier was experimentally observed to describe the relationship between the time of phytomer emergence and the time of elongation completion for that phytomer component. The value of this multiplier was estimated for each phytomer component in the model (leaf blade, leaf sheath, and internode) using parameter optimization. The three relative allocation parameters (rho, toInt, and toSheath) describe the relative allocation of biomass within each plant, and are estimated by parameter optimization. The parameters k and H describe biomass growth over time, and are estimated using parameter optimization. The relative allocation within the reproductive phytomer to the peduncle (peduncleShare), relative to the panicle, was fixed to a reasonable value (0.01). The duration of growth multiplier for the reproductive phytomer was similarly fixed ($1.5*_{leaf}$).

Parameter	Description	Estimated
Tau (τ)	Emergence rate (constant)	Fixed to experimental median (2 days)
Beta ($_{leaf, internode,sheath}$)	Duration of growth multiplier	Parameter optimization
Rho (ρ)	Reproductive allocation	Parameter optimization
toInt	Relative allocation	Parameter optimization
toSheath	Relative allocation	Parameter optimization
k	Growth rate (biomass growth over time)	Parameter optimization
H	Cooperativity (biomass growth over time)	Parameter optimization
peduncleShare	Fractional allocation to the peduncle in the reproductive phytomer	Fixed to reasonable estimate (0.01)
Beta ($_{reproductive}$)	Duration of growth multiplier for reproductive phytomer	Fixed to reasonable estimate

The mathematical model predicts how the number of phytomers and the shift to reproductive growth influences competition for resources

We hypothesized that the internode patterns change as a function of phytomer number (**Figure 1A**) due to increasing competition between phytomers due to semi-sequential growth and the shift to reproductive growth. The *S. italica* B100 plants grown in the greenhouse did not exhibit the same degree of variability in the total number of phytomers as in the field experiment. The total number of phytomers for field growth *S. italica* B100 had an apparent number of internodes ranging from 5 to 12, which we estimate as a true phytomer count ranging from 9 to 19 (**Supplemental Figure 4**, linear regression between internode counts and phytomer counts in

two greenhouse experiments, R^2=0.99) (Banan, 2019). As early leaves often senesce early and are lost at harvest, and early internodes are compressed (non-elongated) and difficult to dissect, the number of apparent internodes (ie, larger than 0.5 cm) will be lower than the true number of phytomers (obtained via leaf count in greenhouse experiments). Despite this, we observed correspondence between internode length patterns in field-grown *S. italica* B100 with low phytomer number and *S. viridis* A10 (7 to 9 phytomers in greenhouse experiments), and between field- and greenhouse-grown *S. italica* B100 with high phytomer numbers (e.g., 19 to 21). This suggests that the phytomer number itself influences internode length patterns, outside of genotypic effects.

To determine how phytomer number influences phytomer growth we simulated our model across the entire parameter space. The parameters we included were the emergence rate τ, the growth duration multipliers β_{leaf}, $_{internode}$, and $_{sheath}$, the proportional degree of reproductive investment parameter ρ, and fractional allocation to the internodes and to the sheath. To characterize the degree of competition for resources we calculated the maximum degree of competition, which corresponds to the sum of the number of vegetative phytomers growing at a given time, plus the reproductive allocation parameter upon the shift to reproductive growth. We also tracked the length of time the *in silico* plants experienced the maximum degree of competition.

We found that changing the number of phytomers from 7 (e.g., A10 case) to 21 (e.g., B100 case) changes the maximum degree of competition by a median value of 1.8, and increases the median time under maximum competition by 2.5 times (**Figure 3**). The model predicts that this competition is primarily influencing the last vegetative phytomers to emerge, corresponding to the shift to reproductive allocation. This explains the reduction in internode length seen in the final group of vegetative phytomers for plants with high total numbers of phytomers (**Figure 1A**). In contrast, the final few vegetative phytomers in plants with low total numbers of phytomers are not reduced in length (**Figure 1A**). The model simulations support the idea that this phenomenon is an emergent property resulting from semi-sequential growth that occurs in *Setaria* development, rather than a genotype-specific property.

<u>The model is able to predict growth dynamics given only internode lengths</u>

To determine the ability of the model to accurately capture developmental patterns, we further applied the model to data from a field experiment measuring the influence of well-watered or drought conditions on *Setaria* B100 internode lengths. As this data consisted of only internode lengths, we first assessed the influence of reducing the amount of experimental data on model parameter estimates. We performed global and local identifiability analyses to determine the model's ability to estimate parameters given our full dataset (leaf length, internode length, sheath length, sum of leaf lengths over time, and height over time) and given internode length data alone (see Methods). Identifiability analysis measures the influence of changing model parameters on fit quality. An unidentifiable parameter has no effect on fit quality, meaning the value of an unidentifiable parameter does not influence the ability of the model to fit to our data, and therefore the model cannot be used to determine the proper value of such a parameter. We found that using the full dataset, all parameters were identifiable, and when using a reduced data set of internode lengths only, nearly all parameters were identifiable. Leaf growth duration was identifiable 40% of the time (6/15 plants in our experimental dataset), and sheath growth duration was identifiable 60% of the time (9/15 plants in our dataset).

<u>The model suggests that *Setaria* internodes grow for longer in drought than well-watered conditions, while plants increase allocation to leaves</u>

We applied the model to the existing field data of B100 to determine the model's ability to capture internode length patterns in this data (Banan, 2019), as well as to obtain model predictions on the influence of drought on B100 developmental parameters (**Figure 4A**). In

order to simulate these field data, we first needed to estimate the true number of phytomers, which is greater than the number of internodes long enough to be dissected by hand. In our greenhouse experiments we were able to observe both the true phytomer number (number of leaves) as well as the observed number of dissected internodes. A linear regression between actual phytomer number and observed phytomer number (number of internodes greater than 0.5 cm) described our data well ($N_{internode} = 0.7064*N_{phyt} - 1.38$, R^2 0.99, across two experiments and seven genotypes, **Supplemental Figure 4**). The resulting linear regression equation was used to estimate the true phytomer number for subsequent simulations on the field data.

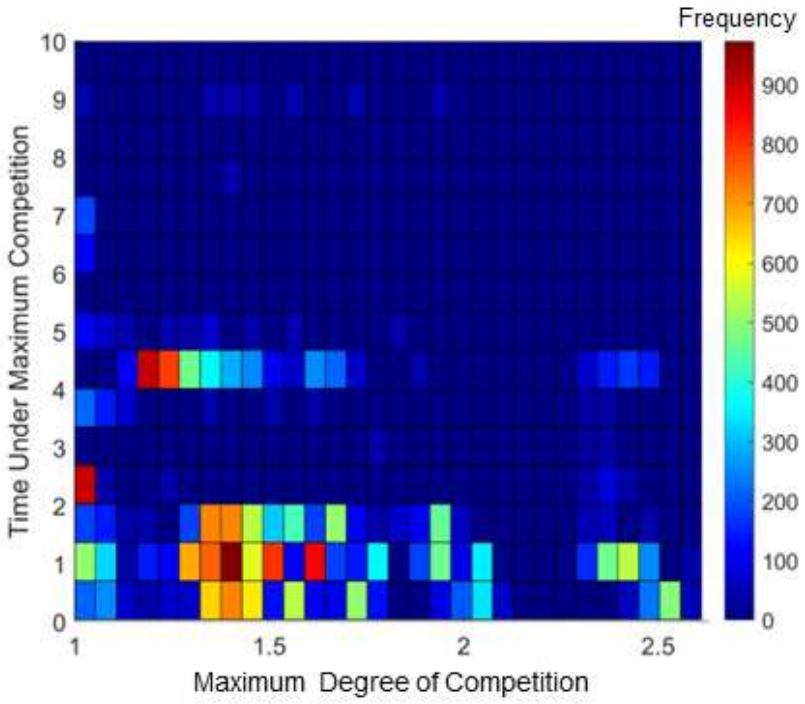

Figure 3. Increasing the number of phytomers increases competition for resources during growth. The model predicts that increasing the number of phytomers from 7 to 21 increases the degree of maximum competition (i.e., number of ways resources are divided at any point in time) by 1.45 times on average, while also increasing the duration of the maximum degree of competition by 1.34 times on average. To determine the general influence of the total number of phytomers the model was simulated across a wide range of values for each parameter (see Methods). The color represents the frequency of observation in the simulation conditions.

The model parameters were optimized on the field data of internode lengths for well-watered and droughted *Setaria* B100 plants. The influence of drought was assessed using t-tests on the parameter estimates. The model predicts that drought reduces the leaf growth duration multiplier (t-test p=0.022), decreases the relative allocation to the internode (t-test p=0.002), and reduces growth rate (t-test p<0.001) (**Figure 4B**). As no change is predicted in the proportional degree of reproductive investment, the model predicts that vegetative internodes will be relatively shorter under drought than well-watered conditions, and will take longer to reach their terminal lengths due to reduced growth rate and increased leaf growth duration multiplier, while the length of the reproductive internode (peduncle) will be similar across both conditions.

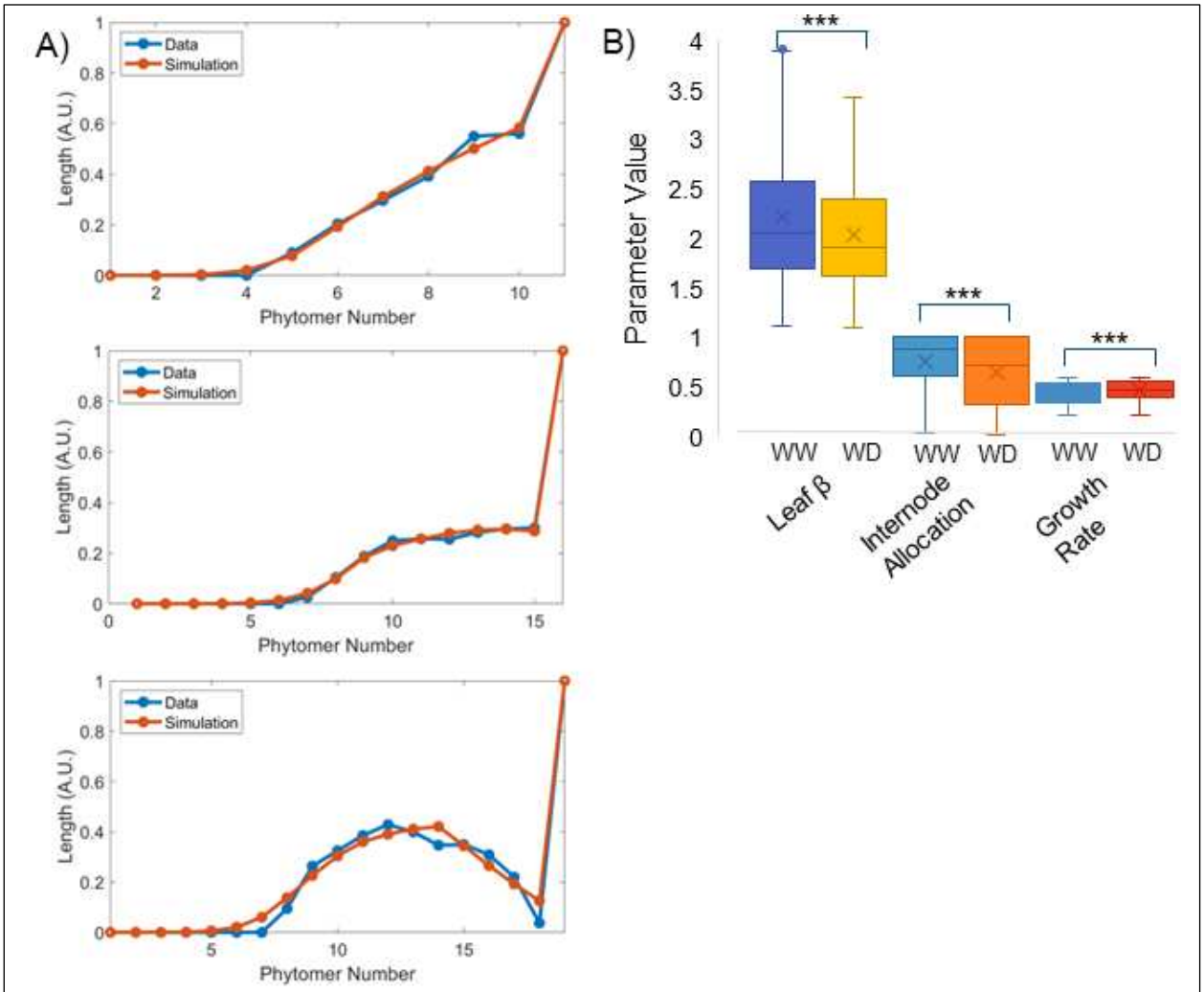

Figure 4. Model predicts a change in plant growth parameters under well-watered and drought conditions with field-grown *Setaria* B100. (A) Model is able to fit to field data, and 3 representative fits are shown across plants with three different internode numbers (from top to bottom, 10, 15, and 18 vegetative internodes). (B) The model predicts that, in response to drought conditions, significant reductions occur in leaf grow time, internode allocation, and growth rate (note, due to model formulation described in the methods, a larger value of k, the growth rate, corresponds to slower growth). Significance is determined by t-tests.

Discussion

In this paper we describe a mathematical model of resource allocation during grass development wherein phytomers emerge sequentially and grow semi-sequentially. The model uses developmental time parameters to describe phytomer emergence and how long each phytomer grows for, as well as the timing of the shift to reproductive growth. The model tracks leaves, sheaths, internodes, and the reproductive internode.

Experimental validation

We performed an experiment to validate and test the model. We measured the length and number of leaves and sheaths from 7 DAP until seed senescence for *S. viridis* A10 and *S. italica* B100 in the greenhouse. We made several observations that facilitated simplifications of model parameters. Experimentally we measured the emergence rate to be 2 days on average

for the genotypes in this study (not significantly different between genotypes). A constant emergence rate across development and genotype has been previously observed in *Setaria*, *Sorghum*, and maize (Fournier, 2000; Junqueira et al., 2020; Xue et al., 2012; Zhang et al., 2016), although genotypic effects have been observed in maize (Zhang et al., 2016). We found a constant linear relationship between when a phytomer emerged and when it completed elongation (the duration of grow multiplier parameter β). A linear relationship between phytomer emergence and growth completion times results in later phytomers growing for a longer time than early phytomers. Similar trends have been observed in *Sorghum* and maize (Fournier, 2000; Xue et al., 2012). We found the duration of growth was significantly longer in internodes compared to leaves for both A10 and B100 genotypes (t-test p-values <<0.05). These results enabled us to fix the model's phytomer emergence rate τ at 2 days, and use a single duration of growth multiplier parameter (β) to describe the relationship between emergence and growth completion for each phytomer in our simulations. We found that our resulting mathematical model is able to recapitulate the relative length distributions of leaf blades, leaf sheaths, and internodes, as well as biomass growth over time and height over time over time (**Figure 2A**, **Supplemental Figure 3**).

Phytomer number and competition for resources

By simulating across a range of reasonable values of the model parameters, the model predicts that increasing the number of phytomers greatly increases both the degree and duration of competition for resources (defined as the number of ways resources are divided) (**Figure 3**). This effect on competition explains the differences in internode length due to within-species phytomer number variability (i.e., **Figure 1A**), as well as the influence of between-species phytomer number differences and internode length patterns (**Figure 2A**). The model further suggests that this increased competition coincides with the shift to reproductive growth, which is supported by existing observations on the influence of the shift to reproductive growth on vegetative internodes and plant height (Hodge and Doust, 2021; Reddy et al., 1997).

Model predictive capacity

We performed identifiability analysis to determine the influence of using all data we collected (leaf lengths, internode lengths, sheath lengths, leaf growth over time, height over time), and tracking only internode length distributions on our model parameter estimates. Our analysis shows that the model is able to estimate nearly all parameters when using only internode length distributions. This predicts that internode length distributions are influenced not just by allocation to internodes, but also leaf growth and the overall growth dynamics of the plant.

Model prediction of the influence of drought stress on *Setaria* internodes

We used our model on internode length distributions from an existing field experiment to determine which parameters changed as a result of drought conditions (Banan, 2019). Model analysis of field data predicts that drought stress decreases leaf grow time, reduces allocation to internodes, and reduces growth overall. Previous studies have observed internode elongation being reduced in response to drought stress in tomato (Litvin et al., 2016). Leaf elongation rate has been observed to be sensitive to drought in the grass *C. squarrosa* (Yang et al., 2016), while leaf emergence rate has been observed to be insensitive to drought stress in maize (Zhang et al., 2016).

Expanding the model to other species

Here we have developed and validated a model with a highly generalizable representation of developmental growth in grasses. Here we optimized the model using *Setaria*, where the culm shifts to reproductive growth once. Other grass species have different number and timing of reproductive growth, as well as different numbers of phytomers, both factors that our model

predicts will strongly influence resource allocation and internode length patterns. Similar internode length distributions to those shown here in *Setaria* have been observed in maize (Birch et al., 2002; Fournier, 2000; Morrison et al., 1994), bamboo (Wei et al., 2019), soybean (Allen et al., 2018), and pea (de Saint Germain et al., 2013). Interestingly, cucumbers with few phytomers display a different length distribution, where each is subsequently smaller than the previous (Kahlen and Chen, 2015).

Conclusion

Our model allows us to integrate the growth of leaves, sheaths, and internodes, along with the shift from vegetative to reproductive growth, through considering resource allocation across developmental time. Integrating the growth of these parts into the whole enables us to connect phenotypes that are able to be obtained in high throughput (i.e., biomass growth and height over time) with phenotypes that are difficult to observe (i.e., internode lengths). Thinking in terms of developmental time allows us to consider the influence of time instead of growth rates. Developmental time parameters enable us to directly relate growth strategy and life history, as plants can be the same size at the same time through different combinations of phytomer number and phytomer grow times, allowing us to see signatures of domestication and selection. The ability of the model to estimate developmental time parameters, and growth dynamics, given endpoint length distributions will be powerful in reducing the amount of labor required to connect phenotype to growth strategy, and understand how organ initiation and growth is influenced by environment (Zhang et al., 2016).

Methods

Quantitative Analysis

Simulations of the mathematical model were performed in Matlab 2021a. Parameter estimates were performed by curve fitting the model to the leaf, sheath, and internode length data, as well as the total height and total length of all leaves over time. Curve fitting was performed in Matlab using global optimization function MultiStart across 50 starting points with least squares optimization (lsqcurvefit). To minimize the number of parameters by reducing the need for scaling factors, the data and simulation output was normalized by dividing by the maximum value of each data type (i.e., internode lengths). Linear regressions and t-tests were run in Microsoft Excel. A significance value of alpha=0.05 was used for the t-tests.

Identifiability Analysis

Global identifiability analysis was run in Matlab by fixing each parameter at one of each of 500 values across a range and optimizing the remaining parameters for each plant, using either all data (biomass over time, height over time, internode lengths, leaf blade lengths, and sheath lengths), or internode lengths only. Each optimization was run on data from a single plant, and our experiment contained 15 *Setaria* B100 plants. We found when using the full dataset, that most parameters were identifiable (for 14 to 15 of 15 plants), except for the reproductive investment parameter, the sheath allocation parameter, and the overall growth rate. When using internode length data only, we found that only the internode growth duration multiplier was globally identifiable.

We then performed local identifiability analysis in Matlab using the rank of the Fisher Information Matrix (FIM). We first optimized all model parameters for each of 15 plants as described previously, and then determined the identifiability by perturbing these optimized parameters by 0.001. The FIM was then constructed by calculating the difference between the model fits for the perturbation conditions, divided by *2*0.001*initial parameter estimate*. The rank of the FIM then determines the identifiability of each of the parameters. When using the full dataset for each plant, we found that most parameters are identifiable. The internode growth duration multiplier was identifiable for 6 of 15 plants, the leaf growth duration multiplier was

identifiable for 9 of 15 plants, and the sheath growth duration multiplier was identifiable for 12 of 15 plants. When using the internode length data only, we found that most parameters were again identifiable. Internode growth duration multiplier was identifiable 4 of 15 plants, leaf growth duration multiplier 6 of 15 plants, and the sheath growth duration multiplier 9 of 15 plants. Using the profile likelihoods obtained with global identifiability analysis, we observed that internode growth duration multiplier likelihood was often very flat around the minima for both the full dataset and the reduced dataset with internode lengths only. Thus, we infer that most parameters are identifiable in the region converged upon using global parameter optimization.

Experimental Approach

In the first experiment the domesticated *S. italica* genotype B100 was studied alongside its wild relative *S. viridis* genotype A10 in a greenhouse at the Donald Danforth Plant Science Center. This greenhouse was set to a 14 hour day length, with a 28°C day and 22°C night temperature. Relative humidity was maintained between 40-50%. 15 plants of each accession were grown in 1 gal pots. The plants were manually measured, as described below, 3 times a week from 7 days after planting (DAP) until 28 DAP, and then twice a week until dissection, when at least half of the seeds on every plant within each genotype were mature.

For the second experiment, B100 and A10 were grown along with accessions TB_12_0039, TB_12_0265, RIL-13, RIL-68, and RIL-183. The RIL are a recombinant inbred line derived from the cross of B100 and A10. Each line had 10 replicates except for S. italica and S. viridis which each had 5 replicates. These plants were grown in ¼ gal pots, first in a growth chamber, then moved into the Bellwether Foundation Phenotyping Facility (Bellwether Facility), and then into the same greenhouse as the previous experiment. From planting until 12 DAP, plants were in the growth chamber which had a 12 hour day length, day temperatures of 31°C, night temperatures 21°C, with light intensity of 400 (µmol/m^2/s) and relative humidity maintained near 40%. The plants were then loaded into the Bellwether Facility.

The Bellwether Facility allows for high-throughput phenotyping utilizing a controlled growth chamber environment with a conveyor belt system that moves plants to automatic weighing stations and through an imaging system. Every plant was weighed twice a day and watered to 85% of soil capacity. Imaging occurred once a day using both RGB visible imaging. Plants were photographed from above and at two side angles. The controlled environment was set to nearly the same parameters as the pre-loading growth chamber, with the only difference being the light intensity, set to 500 (µmol/m^2/s). Relative humidity also varied more in the facility due to plants moving between the controlled environment and the imaging station, sometimes raising relative humidity levels to above 60%. Plants were grown in the Bellwether Facility for 3 weeks before being moved into the same greenhouse as the previous experiment, where they were grown until seed maturity.

Manual measurements of phenotypic traits

For the first experiment, near daily measurements of when leaves emerged (defined as a visible leaf tip within the whorl) and total leaf number were taken to estimate tau (τ), the waiting time. To determine grow time of leaves and internodes, the lengths of leaves and sheaths were measured using a ruler. Sheath length was measured as the distance from one leaf collar to the next leaf collar, or from the soil to the first leaf collar. Sheath elongation was considered to be representative of internode elongation. Leaf length was measured as the distance from the leaf collar to the leaf tip. Each measurement was rounded to the nearest 0.25cm. Once a sheath and leaf measurement remained constant within +/- 0.5cm across three measurement days they were considered fully expanded and were no longer measured. Once emerged, the peduncle length was also measured, from the collar of the last leaf to the bottom edge of the panicle. Panicle length was measured. While *S. italica* has little to no tillering, *S. viridis* grows many tillers, and the main culm was tracked throughout the experiment. For the second experiment,

the emergence rate was estimated via visual inspection of Bellwether imaging to identify the emergence of new leaf tips, in addition to final leaf counts in the greenhouse prior to dissection.

Dissections

In all experiments plants were dissected after at least 50% of seeds were mature on all plants within a genotype. Plants were dissected manually using garden shears and razor blades. Leaves and sheaths were removed carefully by hand. Once the leaves and sheaths were removed, internodes were dissected and measured. A cut with shears was made at each node, which was clearly visible, to separate internodes from each other.

Model Implementation

As measurements began 9 days after planting in the first experiment, the exact date of germination wasn't observed. We estimate the germination date to be 5 DAP. Therefore, we assume the first phytomer emerged at 5 DAP, and phytomer 2 at $5DAP + \tau$, where τ is the emergence rate in days, and so on to estimate the emergence day of the remaining phytomers in the model. Panicle emergence is therefore N_{phyt} + *germination date*, where N_{phyt} is the number of phytomers. The first phytomer begins growing on day 1, and the second begins growing after the emergence time, on day 1+τ. Generally, the N^{th} phytomer begins growing on day $1 + \tau(N-1)$. The reproductive phytomer is treated as the $N^{th}+1$ phytomer, beginning on day $1+\tau N$. The time when each leaf, internode, or sheath is finished growing is set as the emergence date multiplied by the duration of growth multiplier (β) for that phytomer component (e.g., $\beta_{leaf,\ internode,\ sheath}$), which are all parameters in the model. Using these calculations we create the temporal allocation matrix, where the rows are the phytomer numbers and the columns are time in 0.01 day increments. One allocation matrix is created for each phytomer component being modeled (leaf, internode, sheath, reproductive growth). Each matrix element is either 0 for not growing or 1 for growing.

The growth matrices are then calculated by multiplying 1 by the relative allocation ratio. The relative allocation ratio for the internode and sheath is a model parameter between 0 and 1. The relative allocation ratio for leaves is 3 minus the sum of internode and sheath allocation ratios. The relative allocation ratio for reproductive components is ρ, where ρ is greater than or equal to 1.

The integral is then calculated by summing over the rows of the growth matrices, to obtain the allocation dynamics as a function of time. All growth integrals are then summed to obtain the total plant allocation dynamics over time. Dividing one by this vector gives us the fractional allocation matrix. The fractional allocation matrix is multiplied by the biomass production over time to give us the amount allocated over time. The resource allocation dynamics matrices are then multiplied by the relevant relative allocation parameter for each phytomer component to give us the total amount of biomass that is allocated to each phytomer component at each time point. The total allocation per component per phytomer is then calculated by summing this matrix over the columns, giving us the allocation amount per phytomer, for all 4 matrices that track the 4 phytomer components in the model (leaf, internode, sheath, and reproductive). The reproductive internode is allocated a fraction of the total reproductive allocation, fixed at 0.01.

After obtaining grow start and stop times, the phytomer identity matrix is created. The matrix has $N+1$ rows for each vegetative phytomer and one reproductive phytomer, and t timestep columns. In the matrix, elements set to 1 represent growing, and 0 not growing. Each vegetative phytomer gets a fraction of the current available biomass, such that if 4 vegetative phytomers grow, each gets ¼ of the available biomass. The reproductive phytomer will have an increased fraction ρ≥1 to represent increased allocation to reproductive structures. Thus, if 4 vegetative phytomers grow simultaneously to the reproductive phytomer, each vegetative phytomer gets $1/(4+\rho)$ fraction, while the reproductive phytomer obtains $\rho/(4+\rho)$ fractions. Collapsing across rows provides the vector of the number of phytomers growing as a function of time. The model

includes allocation to leaves, sheaths, internodes, and the reproductive structures (peduncle and panicle). The inverse of this vector provides the fractional allocation vector. A vector representing biomass production as a function of time is multiplied by the fractional allocation vector to get the amount allocated as a function of time. The leaf allocation dynamics are used to approximate growth, including the reduction in allocation to the leaves as the plant shifts to reproductive growth. The resulting vector describes the amount able to be allocated as a function of time, and is then multiplied by the phytomer identity matrix to obtain allocation to each phytomer component at each time point.

Here, we estimate biomass production experimentally using the sum of leaf lengths over time, which we use to track plant growth dynamics. We assume that biomass production is proportional to the amount of leaf a plant has. Our biomass production estimate is then fitted using a hill function model: $Size(t) = 1/(1+k*leafFinish / time)^H$, where k is the growth rate, *leafFinish* is the time when the leaves complete growing, i.e. $leafFinish = leafBeta*(\tau*(Nphyt-1)+startDay)$, and H is the cooperativity coefficient, which describes the shape of the growth curve. When the plant growth dynamics are not able to be measured experimentally, k and H are free parameters. In the model, additional reproductive growth time will not influence the internode, sheath, or leaf lengths if reproductive growth occurs without any vegetative growth, and so the reproductive phytomer grow time is set as $1.5*leafBeta$.

Model Simulation to Determine Influence of Phytomer Number on Competition

To understand the influence of increasing phytomer number on resource competition, we simulated the model across a wide range of possible parameter values. The model was simulated at each unique combination of parameter values, and each parameter was individually varied one at a time. Growth duration multipliers for each component (internode, leaf, and sheath) were varied from 1.01 to 3.51, the proportional degree of reproductive investment parameter was varied from 1 to 61, relative allocation to the internodes was varied from 0.1 to 0.4, relative allocation to the sheath was varied from 0.1 to 1, the overall growth rate was varied from 0.25 to 6, the and the growth cooperativity parameter was varied from 3 to 8.

Author Contributions

R.D. D.B. S.M. and I.B. conceived and developed the project; R.D. created the model; R.D. ran all simulations; R.D. and B.M. collected data; R.D. wrote the manuscript; R.D., D.B., B.M., A.D.B.L., S.M., and I.B. reviewed and edited the manuscript.

Acknowledgements

This work was supported by the National Science Foundation (IOS-2109790 to R.D.), the US Department of Energy (DE-SC0023160 & DE-SC0018277 to I.B. and A.D.B.L.), USDA-NIFA (2022-67013-36126 to I.B.).

References

1. Allen, L.H., Zhang, L., Boote, K.J., Hauser, B.A., 2018. Elevated temperature intensity, timing, and duration of exposure affect soybean internode elongation, mainstem node number, and pod number per plant. The Crop Journal 6, 148–161. https://doi.org/10.1016/j.cj.2017.10.005
2. Banan, D., 2019. Phenotypic and genetic variation in the architectural responses of a C4 grass to drought stress. University of Illinois at Urbana-Champaign.
3. Birch, C.J., Andrieu, B., Fournier, C., 2002. Dynamics of internode and stem elongation in three cultivars of maize. Agronomie 22, 511–524. https://doi.org/10.1051/agro:2002030
4. Buck-Sorlin, G.H., Kniemeyer, O., Kurth, W., 2005. Barley morphology, genetics and hormonal regulation of internode elongation modelled by a relational growth grammar.

New Phytologist 166, 859–867. https://doi.org/10.1111/j.1469-8137.2005.01324.x
5. de Saint Germain, A., Ligerot, Y., Dun, E.A., Pillot, J.-P., Ross, J.J., Beveridge, C.A., Rameau, C., 2013. Strigolactones Stimulate Internode Elongation Independently of Gibberellins. Plant Physiology 163, 1012–1025. https://doi.org/10.1104/pp.113.220541
6. Feldman, M.J., Paul, R.E., Banan, D., Barrett, J.F., Sebastian, J., Yee, M.-C., Jiang, H., Lipka, A.E., Brutnell, T.P., Dinneny, J.R., Leakey, A.D.B., Baxter, I., 2017. Time dependent genetic analysis links field and controlled environment phenotypes in the model C4 grass Setaria. PLoS Genet 13, e1006841. https://doi.org/10.1371/journal.pgen.1006841
7. Fournier, C., 2000. Dynamics of the Elongation of Internodes in Maize (Zea mays L.): Analysis of Phases of Elongation and their Relationships to Phytomer Development.
8. Hodge, J.G., Doust, A.N., 2021. Wheels within wheels, Nested Function-value Traits as a Tool for Modeling Ontogeny (preprint). Plant Biology. https://doi.org/10.1101/2021.03.16.435729
9. Junqueira, N.E.G., Bezerra, A.C.M., Cattem, M.V.O., Medici, L.O., Alves-Ferreira, M., Macrae, A., Ortiz-Silva, B., Reinert, F., 2020. Phenology of the genetic model Setaria viridis (Poaceae) according to the BBCH-scale of development. Botanical Journal of the Linnean Society 192, 224–241. https://doi.org/10.1093/botlinnean/boz070
10. Kahlen, K., Chen, T.-W., 2015. Predicting Plant Performance Under Simultaneously Changing Environmental Conditions—The Interplay Between Temperature, Light, and Internode Growth. Front. Plant Sci. 6. https://doi.org/10.3389/fpls.2015.01130
11. Kahlen, K., Stützel, H., 2011. Modelling photo-modulated internode elongation in growing glasshouse cucumber canopies. New Phytologist 190, 697–708. https://doi.org/10.1111/j.1469-8137.2010.03617.x
12. Kawano, T., Wallbridge, N., Plummer, C., 2020. Logistic Models for Simulating the Growth of Plants by Defining the Maximum Plant Size as the Limit of Information Flow. Plant Signaling & Behavior 15, 1709718. https://doi.org/10.1080/15592324.2019.1709718
13. Li, Q., Zargar, O., Park, S., Pharr, M., Muliana, A., Finlayson, S.A., 2023. Mechanical stimulation reprograms the sorghum internode transcriptome and broadly alters hormone homeostasis. Plant Science 327, 111555. https://doi.org/10.1016/j.plantsci.2022.111555
14. Litvin, A.G., van Iersel, M.W., Malladi, A., 2016. Drought Stress Reduces Stem Elongation and Alters Gibberellin-related Gene Expression during Vegetative Growth of Tomato. J. Amer. Soc. Hort. Sci. 141, 591–597. https://doi.org/10.21273/JASHS03913-16
15. Mauro-Herrera, M., Doust, A.N., 2016. Development and Genetic Control of Plant Architecture and Biomass in the Panicoid Grass, Setaria. PLoS ONE 11, e0151346. https://doi.org/10.1371/journal.pone.0151346
16. McKim, S.M., 2020. Moving on up – controlling internode growth. New Phytol 226, 672–678. https://doi.org/10.1111/nph.16439
17. McKim, S.M., 2019. How plants grow up. J. Integr. Plant Biol. 61, 257–277. https://doi.org/10.1111/jipb.12786
18. Morrison, T.A., Kessler, J.R., Buxton, D.R., 1994. Maize Internode Elongation Patterns. Crop Sci. 34, 1055–1060. https://doi.org/10.2135/cropsci1994.0011183X003400040040x
19. Peng, C., Wang, X., Feng, T., He, R., Zhang, M., Li, Z., Zhou, Y., Duan, L., 2019. System Analysis of MIRNAs in Maize Internode Elongation. Biomolecules 9, 417. https://doi.org/10.3390/biom9090417
20. Poethig, R.S., 2003. Phase Change and the Regulation of Developmental Timing in Plants. Science 301, 334–336. https://doi.org/10.1126/science.1085328
21. Reddy, K.R., Hodges, H.F., McKinion, J.M., 1997. Modeling Temperature Effects on Cotton Internode and Leaf Growth. Crop Sci. 37, 503–509.

https://doi.org/10.2135/cropsci1997.0011183X003700020032x
22. Sarlikioti, V., de Visser, P.H.B., Buck-Sorlin, G.H., Marcelis, L.F.M., 2011. How plant architecture affects light absorption and photosynthesis in tomato: towards an ideotype for plant architecture using a functional–structural plant model. Annals of Botany 108, 1065–1073. https://doi.org/10.1093/aob/mcr221
23. Schouten, R.E., 2002. Modelling of Temperature-controlled Internode Elongation Applied to Chrysanthemum. Annals of Botany 90, 353–359. https://doi.org/10.1093/aob/mcf196
24. Serrano-Mislata, A., Sablowski, R., 2018. The pillars of land plants: new insights into stem development. Current Opinion in Plant Biology 45, 11–17. https://doi.org/10.1016/j.pbi.2018.04.016
25. Vidal, T., Dillmann, C., Andrieu, B., 2018. A coordination model captures the dynamics of organ extension in contrasted maize phenotypes, in: 2018 6th International Symposium on Plant Growth Modeling, Simulation, Visualization and Applications (PMA). Presented at the 2018 6th International Symposium on Plant Growth Modeling, Simulation, Visualization and Applications (PMA), IEEE, Hefei, pp. 126–133. https://doi.org/10.1109/PMA.2018.8611569
26. Wei, Q., Guo, L., Jiao, C., Fei, Z., Chen, M., Cao, J., Ding, Y., Yuan, Q., 2019. Characterization of the developmental dynamics of the elongation of a bamboo internode during the fast growth stage. Tree Physiology 39, 1201–1214. https://doi.org/10.1093/treephys/tpz063
27. Xue, S., Han, D.Q., Yu, Y.J., Steinberger, Y., Han, L.P., Xie, G.H., 2012. Dynamics in elongation and dry weight of internodes in sweet sorghum plants. Field Crops Research 126, 37–44. https://doi.org/10.1016/j.fcr.2011.09.014
28. Yang, F., Gong, X.Y., Liu, H.T., Schäufele, R., Schnyder, H., 2016. Effects of nitrogen and vapour pressure deficit on phytomer growth and development in a C_4 grass. AoB PLANTS 8, plw075. https://doi.org/10.1093/aobpla/plw075
29. Zhang, Y., Liu, H., Li, J., Song, Y., Liu, X., 2016. Characterisation of maize organ development towards biomass allocation pattern at organ level, in: 2016 IEEE International Conference on Functional-Structural Plant Growth Modeling, Simulation, Visualization and Applications (FSPMA). Presented at the 2016 IEEE International Conference on Functional-Structural Plant Growth Modeling, Simulation, Visualization and Applications (FSPMA), IEEE, Qingdao, China, pp. 242–245. https://doi.org/10.1109/FSPMA.2016.7818313