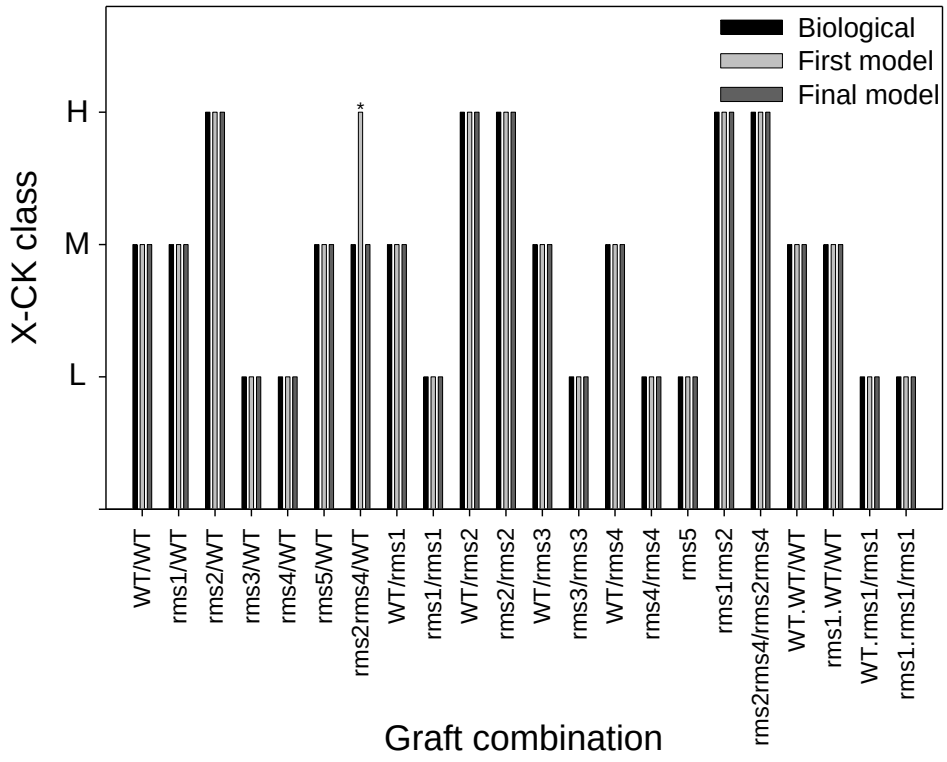
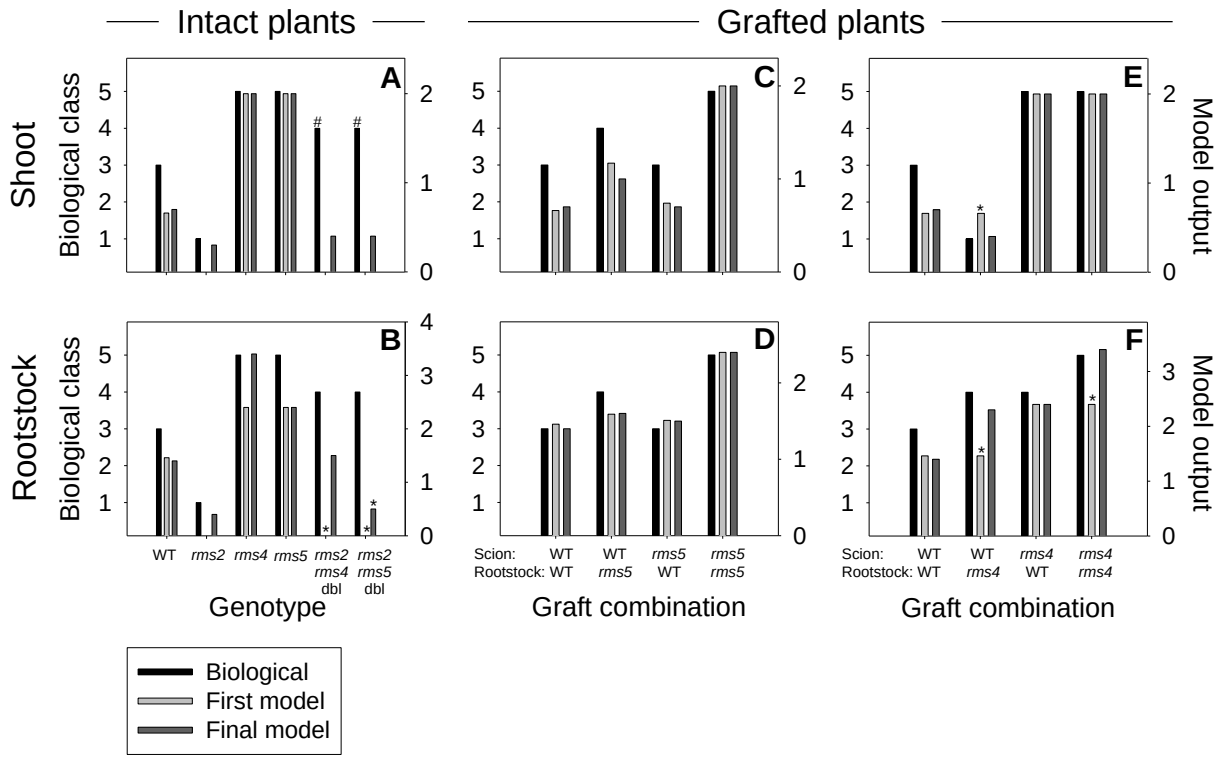
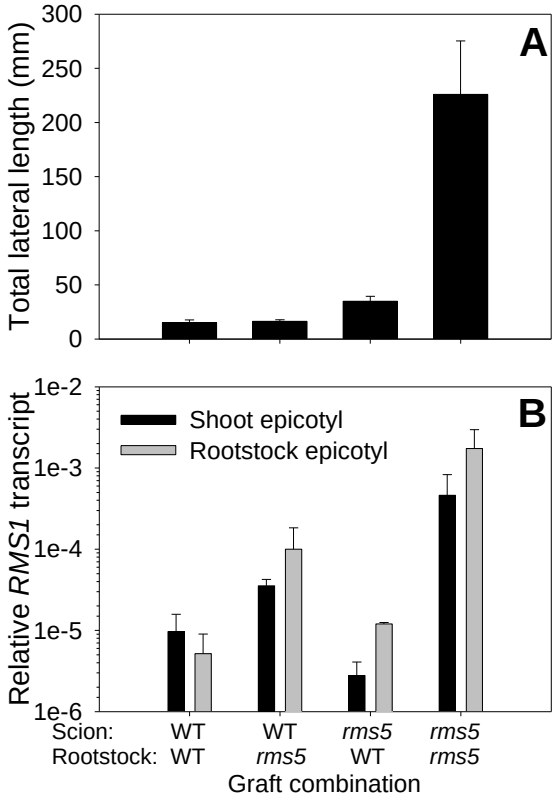
Supplemental Data. Dun et al. (2009). Computational modelling and molecular physiology experiments reveal new insights into shoot branching in pea.



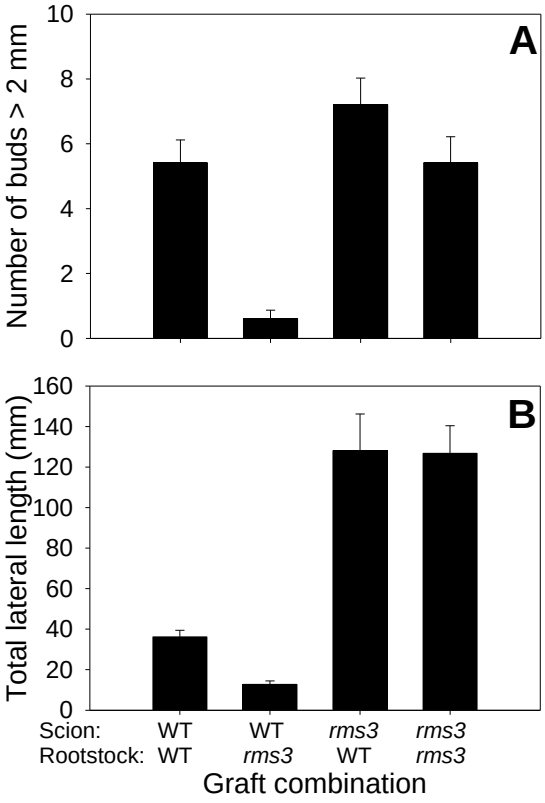
Supplemental Figure 1. Model and biological classes of rootstock X-CK. Rootstock xylem sap cytokinin (X-CK) levels separated into three classes: L, low X-CK; M, WT X-CK; H, high X-CK based on previously published data (biological data), or from output of models. * indicates biological data points that are not captured by the model. Notation is scion/rootstock for I-grafted plants, and scion.cotyledonary shoot/rootstock for Y-grafted plants. Biological data for *rms5* and *rms1 rms2* are for intact plants; data from Beveridge et al. (1997a), Beveridge et al. (1997b), Beveridge (2000), Morris et al. (2001) and Foo et al. (2007).



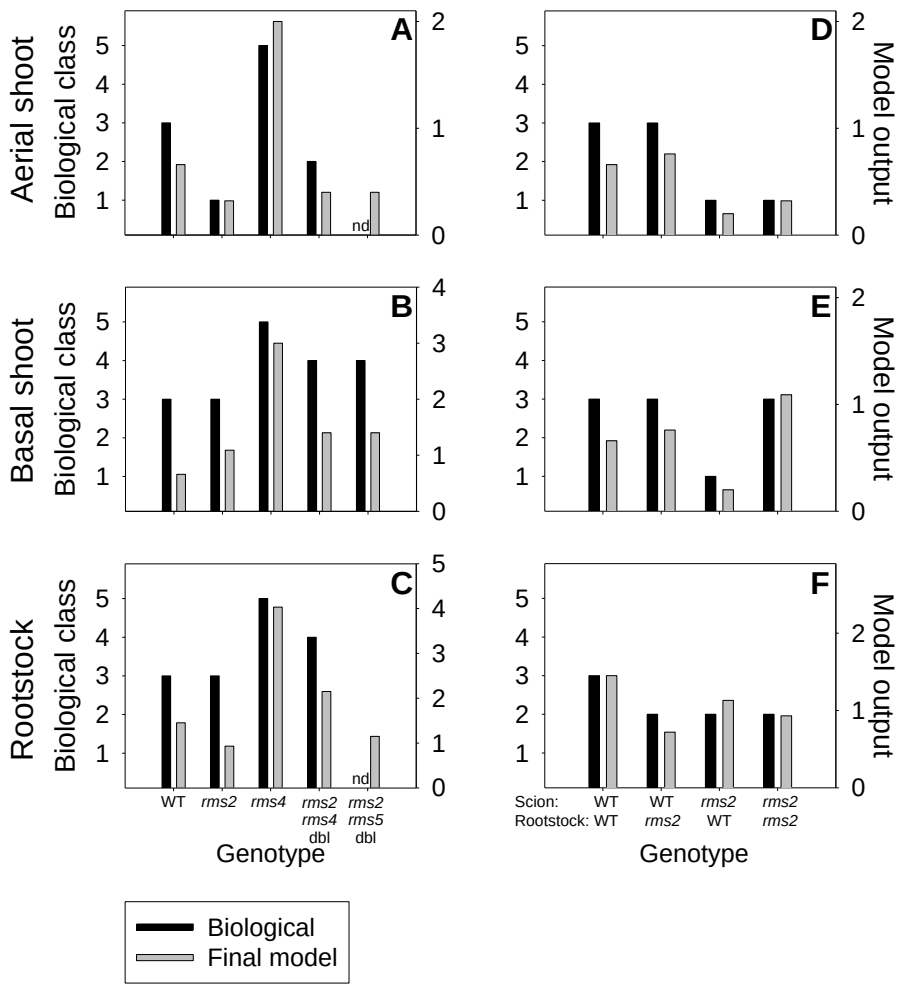
Supplemental Figure 2. *RMS1* expression model output and biological data prior to branching. *RMS1* expression in shoot (**A**, **C** and **E**) and rootstock (**B**, **D** and **F**) of intact (**A** and **B**) and grafted (**C**, **D**, **E** and **F**) plants. Data presented are biological classes (left axis) and output from the computational models (right axis). Results for intact and grafted plants with *rms4* single or double mutant shoot and/or rootstock also apply to *rms3* shoot and/or rootstock **(A, B, E and F)**. Note that the biological class presented for the shoot and rootstock of intact plants **(A and B)** is derived from epicotyl tissue only. dbl indicates double mutants. * indicates biological data points that are not captured by the model. # indicates data where expression in the epicotyl of intact plants was initially incorrectly assumed to represent expression in the shoot (see Figure 4). Biological data are from Foo et al. (2005) **(A, B and F)**, Johnson et al. (2006) **(F)** and this study **(C, D and E)**.



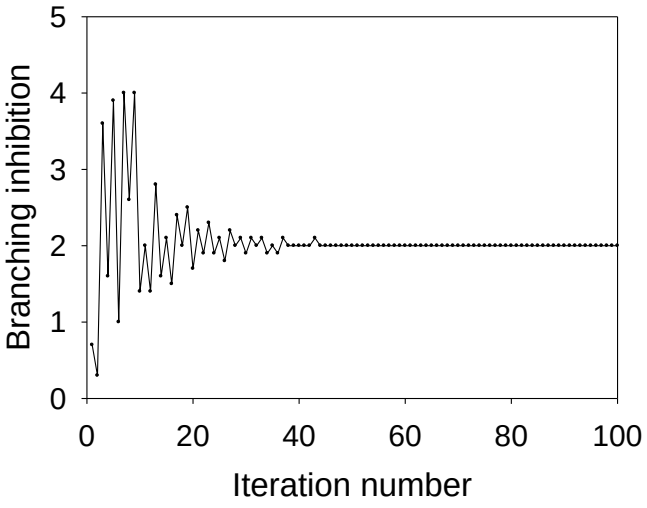
Supplemental Figure 3. *RMS1* expression relative to *18S* in grafts between WT and *rms5*. Total lateral length (mm) **(A)** and *RMS1* gene expression relative to *18S* in the epicotyl of the scion and rootstock **(B)** in grafts between *rms5-3* (HL298) and WT seedlings on a Torsdag background was measured 27-d and 28-d after grafting respectively. *RMS1* gene expression was measured using Taqman real time RT-PCR as per Johnson et al. (2006). Values are mean ± SE of **(A)** 13 to 18 plants and **(B)** of two biological replicates of 6 to 9 plants.



Supplemental Figure 4. Branching phenotypes of grafts between WT and *rms3*.
Branching phenotype in grafts between *rms3-4* (T2-30) and cultivar Térèse WT seedlings grown under short-day conditions (12 h photoperiod) measured 40-d after grafting. Values are mean ± SE of 10 to 12 plants. **(A)** Number of buds and branches >2 mm in length. **(B)** Total lateral length (mm).



Supplemental Figure 5. *RMS1* expression model output and biological data after branching. *RMS1* expression in the aerial (**A**, **D**) and basal portion (**B**, **E**) of the shoot, and in the rootstock (**C**, **F**), of intact (**A-C**) and grafted (**D-F**) plants. Data presented are biological classes (left axis) and output from the final computational model (right axis). dbl indicates double mutants. nd indicates biological data that are not yet determined. Biological data are from Foo et al. (2005) **(A-C)**, and this study **(D-F)**.



Supplemental Figure 6. Output of branching inhibition over 100 iterations for a WT/WT I-grafted plant from final computational model. Parameter values used are listed in Supplemental Table 1 online.

Supplemental Table 1. Model parameters. Range of values listed yield appropriate branching phenotype output from models for data that is already published or new data resulting from previous models, while values used in figures are example values that yield appropriate output from models for *RMS1* gene expression trends and rootstock X-CK trends.

Parameter name in models	Parameter meaning	Graft type applicable		Value of parameter	
				First model	Final model
DOWN	Proportion of feedback signal arriving in rootstock from shoot	All	Values used for figures	0.700	0.675
			Range	0.05-0.95	0.05-1.00
UP	Proportion of stigolactones arriving in shoot from rootstock	All	Values used for figures	0.750	0.750
			Range	0.05-1.00	0.05-1.00
LEAKY	% functional RMS2 product produced in *rms2*	All	Values used for figures	0	0.20
			Range	-	0-0.55

Supplemental Table 2. Branching phenotypic classes of epicotyl-epicotyl I-grafted plants. 0 represents no inhibition of branching; 0.5 represents partial inhibition of branching; 1 represents total inhibition of branching; - represents graft not done. * indicates data point from an intact, rather than grafted, plant. Data from different alleles and genetic backgrounds of the *rms* mutants from [a]Beveridge et al. (1994); [b]Beveridge et al. (1996); [c]Beveridge et al. (1997a); [d]Murfet and Symons (2000a); [e]Murfet and Symons (2000b); [f]Morris et al. (2001); [g]Foo et al. (2001); [h]Johnson et al. (2006); [i]Foo et al. (2007); Fig. refers to figure in this paper, SFig. refers to Supplemental Figure online.

	Scion									
Rootstock	**WT**	***rms1***	***rms2***	***rms3***	***rms4***	***rms5***	***rms1rms2***	***rms1rms5***	***rms2rms4***	***rms2rms5***
WT	1[a,b,c,f,h,Fig. 7]	1[c,f]	1[a,b,c,f,i,Fig. 7]	0[b,f,SFig. 4]	0[b,c,f,h,i]	1[f]	1[f]	1[f]	0[i]	-
rms1	1[c,f]	0[c,f]	0[c,f]	0[f]	0[c]	0[f]	0[f]	0[f]	-	-
rms2	1[a,b,c]	0.5[c,f]	0[a,b,c,f,i, Fig. 7]	0[b]	0[b]	0.5[f]	0[f]	-	-	-
rms3	1[b, SFig. 4]	1[f]	1[b]	0[b,f, SFig. 4]	0[b]	1[f]	-	-	-	-
rms4	1[b,h,Fig. 7]	1[c]	1[b, Fig. 7]	0[b]	0[b,c,f,h,i,Fig. 7]	1[f]	-	-	-	-
rms5	1[f]	0[f]	0[f]	0[f]	0[f]	0[f]	-	0[f]	-	-
rms1rms2	1[f]	0[f]	0[f]	-	-	-	0[f]	-	-	-
rms1rms5	1[f]	0[f]	-	-	-	0[f]	-	0[f]	-	-
rms2rms4	1[Fig. 7]	-	1[Fig. 7]	-	-	-	-	-	0[d*,h,Fig. 7]	-
rms2rms5	-	-	-	-	-	-	-	-	-	0[e*]

Supplemental Table 3. Branching phenotypic classes of two-shoot grafted plants. 0 represents no inhibition of branching; 0.5 represents partial inhibition of branching; 1 represents total inhibition of branching; - represents graft not done; *indicates data point from a two-scion grafted plant; S represents the scion (Y-grafts) or scion 1 (two-scion grafts) branching phenotype; C represents the cotyledonary shoot (Y-grafts) or scion 2 (two-scion grafts) branching phenotype. Data from different alleles and genetic backgrounds of the *rms* mutants from [a]Foo et al. (2001); [b]Morris (2001); [c]Foo (2003); [d]Foo et al. (2007); Fig. refers to figure in this paper, SFig. refers to Supplemental Figure online.

Rootstock and Cotyledonary Shoot/Shoot 2		Scion				
		WT	***rms1***	***rms2***	***rms4***	***rms5***
WT	S	1[a,c,d,Fig. 8*]	1[a,d]	1[c]	-	1[b]
	C	1[a,c,d,Fig. 8*]	1[a,d]	1[c]	-	1[b]
rms1	S	1[a,d]	0[a,d]	-	-	-
	C	0[a,d]	0[a,d]	-	-	-
rms2	S	1[Fig. 8*]	-	0[Fig.. 8*]	0[Fig.. 8*]	-
	C	0.5[Fig. 8*]	-	0[Fig. 8*]	1[Fig.. 8*]	-
rms4	S	-	-	-	0[Fig.. 8*]	-
	C	-	-	-	0[Fig.. 8*]	-
rms5	S	1[b]	-	-	-	0[b]
	C	0[b]	-	-	-	0[b]

Supplemental Table 4. Branching phenotypic classes of two-rootstock grafted plants. 0 represents no inhibition of branching; 1 represents total inhibition of branching; - represents graft not done. Data from different alleles and genetic backgrounds of the *rms* mutants from [a]Foo et al. (2001); [b]Morris (2001).

		Scion		
Rootstock A	**Rootstock B**	**WT**	***rms1***	***rms5***
WT	**WT**	1[a]	1[a]	1[b]
WT	***rms1***	-	1[a]	-
WT	***rms5***	-	-	1[b]
rms1	***rms1***	-	0[a]	-
rms5	***rms5***	-	-	0[b]

SUPPLEMENTAL METHODS

A regulatory network model (e.g. Bolouri and Davidson, 2002) with multiple compartments is used to capture the dynamic interactions of long-distance signals and genetic regulatory networks controlling branching. In computational terms, this model is also deterministic (not probabilistic) and continuous (Haefner, 2005). The following describes the initial and final models (both in Figure 1) and their operation.

Model inputs

The inputs to the models are the graft type (I-graft, two-shoot graft or two-rootstock graft) and the genotypes of each of the five *RMS* genes in the shoot(s) and the rootstock(s) for each graft combination. For each gene, genotypes are expressed as 1 for the wild-type (WT) state, 0 for the mutant state that results in negligible functional product, and slightly greater than 0 for a leaky mutation, such as *rms2* that produces some functional product.

Model components

The components of the models are the level of expression of each of the five *RMS* genes, the level of strigolactones, feedback signal and branch-derived feedback signal (final model only) in each shoot and rootstock, xylem-sap cytokinin (X-CK) content in the rootstock(s), and the extent of shoot branching inhibition in the shoot(s). The levels are expressed as continuous values , allowing for more detailed comparison of the biological data and model output than could be achieved with a two-state (0 or 1) approach. In the computational models, the expression of each *RMS* gene was assumed to be directly correlated to the protein level since protein levels have not yet been directly measured.

Conversion of hypotheses to algebraic rules

Algebraic rules were derived based on the hypotheses listed in Table 1 and are presented below for each model. Since we were aiming for output that corresponded easily with the classes for branching inhibition, the algebraic rules were derived such that each component of a WT plant would have a steady state value of 1 prior to contribution from other plant parts.

The algebraic expressions describe the state of each component as a function of the states of components in the previous iterations. Component states are updated synchronously. Time delays of one step for the signals moving between the shoot and rootstock were implemented (Table 1: *hyp24*). For example, where the state of a shoot component was

dependent on a signal from the rootstock, the equation utilised the state of the rootstock component from two iterations prior to the current iteration. Equations are written in general terms such that they could be applied to I-graft, two-shoot (Y-graft and two-scion) and two-rootstock graft types (as indicated by subscripts). For each equation, the hypotheses from Table 1 that were used to create that equation were listed next to the equation. This assisted in the progression between models; if a hypothesis was changed, the equations were easily updated accordingly by searching for all equations that utilised that hypothesis, thus maintaining consistency in the equations.

Where two or more components were together required to positively affect the level of a third component, the states of the required components were multiplied to form the conjunction of the required components, analogous with the Law of Mass Action (e.g. Equation 1.4). Therefore, if any of the required components were absent (state equal to 0) then the level of the third component was also equal 0. Where two or more components independently affected the state of a third component positively, for example when a signal is moves from the shoot to the root, but is also made in the root, the levels of the two components were summed (e.g. Equation 1.10).

Unlike Boolean and other generalised logical descriptions where regulation results in on or off states (e.g., Thomas and D'Ari, 1990), negative regulation is modelled as a 'limiter' which decreases hyperbolically as the negative regulator increases (e.g. Equation 1.1).

In the final model, subtraction is used to describe the negative influence of RMS3 and RMS4 on *RMS1* and *RMS5* gene expression independent to the positive influence of the feedback signal (e.g. Equation 2.11).

Five parameters are used in the equations. The ranges of appropriate values for each model and graft-type, including the values used to generate the output reported in Supplemental Figures 1, 2 and 5 online, are reported in Supplemental Table 1 online. The *DOWN* parameter describes the proportion of feedback signal produced in the shoot that arrives in the rootstock(s) via the phloem (Table 1: *hyp26*) while the *UP* parameter describes the proportion of strigolactones produced in the rootstock(s) that arrives in the shoot(s) via the xylem (Table 1: *hyp26*). Variation in the values for the parameters *UP* and *DOWN* did not lead to significant alterations in the trends for the branching inhibition output between graft combinations. Parameters *splitU* and *splitD* describe the proportion of upward-moving and downward-moving signals shared between rootstock and shoot compartments in two-shoot

and two-rootstock graft types (Table 1: *hyp18*, *hyp19*, *hyp22*, *hyp23*). The *LEAKY* parameter, utilized in the final model, describes the leakiness of the *rms2* mutation, that is, how much functional RMS2 product was made in the *rms2* mutant (Table 1: *hyp27*).

Algebraic Rules

The notation below is used in the following lists of algebraic rules.

x = iteration number
$RMSX$ = pertaining to the genotype or transcript of the *RMSX* gene, as indicated by subscript
FS = $RMS2$ - mediated feedback signal
SMS = shoot multiplication signal (strigolactones)
BI = branching inhibition
BIP = branching inhibitor (strigolactone) perception
$BDFS$ = branch - derived feedback signal
$AerialBI$ = branching inhibition in aerial zone of shoot
$AerialBIP$ = branching inhibitor (strigolactone) perception in aerial zone of shoot
$Branchlength$ = length of growing buds

The following parameters are used in the algebraic rules.

$$Ygraft = \begin{cases} 1 \text{ if Y - graft or two - scion graft} \\ 0 \text{ otherwise} \end{cases}$$

$$Tworoot = \begin{cases} 1 \text{ if two - rootstock graft} \\ 0 \text{ otherwise} \end{cases}$$

$$splitU = \begin{cases} 0.5 \text{ if two - rootstock graft} \\ 1 \text{ otherwise} \end{cases} \qquad hyp23$$

$$splitD = \begin{cases} 0.5 \text{ if two - shoot graft} \\ 1 \text{ otherwise} \end{cases} \qquad hyp22$$

$$\left.\begin{matrix} DOWN \\ UP \\ LEAKY \end{matrix}\right\} = \text{values listed supplemental table 1 online}$$

The following subscripts indicate locations and identities (genotype, transcript) of the components in the grafted plant.

s = scion
c = second shoot (cotyledonary shoot of Y-grafts or second scion of two-scion grafts)
$r1$ = rootstock of Y-grafts and I-grafts, or the first rootstock of two-rootstock grafts
$r2$ = second rootstock of two-rootstock grafts
gs = scion genotype
gc = second shoot genotype
$gr1$ = rootstock genotype
$gr2$ = second rootstock genotype
ts = scion transcript
tc = second shoot transcript (cotyledonary shoot of Y-grafts or second scion of two-scion grafts)
$tr1$ = rootstock transcript (Y-grafts and I-grafts), or first rootstock transcript (two-rootstock grafts)
$tr2$ = second rootstock transcript (two-rootstock grafts)
st = scion total
ct = second shoot total (cotyledonary shoot of Y-grafts or second scion of two-scion grafts)
$r1t$ = rootstock total (Y-grafts and I-grafts), or first rootstock total (two-rootstock grafts)
$r2t$ = second rootstock total (two-rootstock grafts)

Algebraic rules: first model

The hypotheses (*hyp#*) that were utilised to construct each equation are listed in Table 1 and are listed alongside each equation as appropriate. This first model is depicted in solid lines in Figure 1.

(1.1) $$FS_s(x) = \frac{2 \times RMS2_{gs}}{1 + BIP_s(x-1)}$$ *hyp2, hyp7, hyp17*

(1.2) $$RMS1_{ts}(x) = FS_s(x-1) \times RMS1_{gs}$$ *hyp1, hyp14*

(1.3) $$RMS5_{ts}(x) = FS_s(x-1) \times RMS5_{gs}$$ *hyp5, hyp14*

(1.4) $$SMS_s(x) = RMS1_{ts}(x) \times RMS5_{ts}(x)$$ *hyp6, hyp10, hyp11*

(1.5) $$FS_c(x) = Ygraft \times \left(\frac{2 \times RMS2_{gc}}{1 + BIP_c(x-1)} \right)$$ *hyp2, hyp7, hyp17*

(1.6) $$RMS1_{tc}(x) = Ygraft \times \left(FS_c(x-1) \times RMS1_{gc} \right)$$ *hyp1, hyp14*

(1.7) $RMS5_{tc}(x) = Ygraft \times (FS_c(x-1) \times RMS5_{gc})$ *hyp5, hyp14*

(1.8) $SMS_c(x) = Ygraft \times (RMS1_{tc}(x) \times RMS5_{tc}(x))$ *hyp6, hyp10, hyp11*

(1.9) $FS_{r1}(x) = RMS2_{gr1}$ *hyp2, hyp7, hyp17*

(1.10) $FS_{r1t}(x) = FS_{r1}(x) + splitD \times DOWN \times FS_s(x-2) + Ygraft \times splitD \times DOWN \times FS_c(x-2)$

hyp13, hyp19, hyp20, hyp22, hyp24, hyp26

(1.11) $RMS1_{tr1}(x) = FS_{r1t}(x-1) \times RMS1_{gr1}$ *hyp1, hyp14*

(1.12) $RMS5_{tr1}(x) = FS_{r1t}(x-1) \times RMS5_{gr1}$ *hyp5, hyp14*

(1.13) $SMS_{r1}(x) = RMS1_{tr1}(x) \times RMS5_{tr1}(x)$ *hyp6, hyp10, hyp11*

(1.14) $FS_{r2}(x) = Tworoot \times RMS2_{gr2}$ *hyp2, hyp7, hyp17*

(1.15) $FS_{r2t}(x) = Tworoot \times (FS_{r2}(x) + splitD \times DOWN \times FS_s(x-2))$

hyp13, hyp19, hyp20, hyp24, hyp26

(1.16) $RMS1_{tr2}(x) = Tworoot \times (FS_{r2t}(x-1) \times RMS1_{gr2})$ *hyp1, hyp14*

(1.17) $RMS5_{tr2}(x) = Tworoot \times (FS_{r2t}(x-1) \times RMS5_{gr2})$ *hyp5, hyp14*

(1.18) $SMS_{r2}(x) = Tworoot \times (RMS1_{tr2}(x) \times RMS5_{tr2}(x))$ *hyp6, hyp10, hyp11*

(1.19) $SMS_{st}(x) = SMS_s(x) + splitU \times UP \times SMS_{r1}(x-2) + Tworoot \times splitU \times UP \times SMS_{r2}(x-2)$

hyp12, hyp18, hyp21, hyp23, hyp24, hyp26

(1.20) $BI_s(x) = RMS3_{gs} \times RMS4_{gs} \times SMS_{st}(x-1)$

hyp3A, hyp4A, hyp8, hyp9, hyp15, hyp16

(1.21) $SMS_{ct}(x) = Ygraft \times (SMS_c(x) + splitU \times UP \times SMS_{r1}(x-2))$

hyp12, hyp18, hyp21, hyp24, hyp26

(1.22) $BI_c(x) = Ygraft \times (RMS3_{gc} \times RMS4_{gc} \times SMS_{ct}(x-1))$

hyp3A, hyp4A, hyp8, hyp9, hyp15, hyp16

(1.23) $BIP_s(x) = BI_s(x)$ *hyp16*

(1.24) $BIP_c(x) = Ygraft \times BI_c(x)$ *hyp16*

(1.25) $CK_{r1}(x) = \frac{2}{1 + FS_{r1t}(x-1)}$ *hyp25*

(1.26) $CK_{r2}(x) = Tworoot \times \frac{2}{1 + FS_{r2t}(x-1)}$ *hyp25*

Algebraic rules: final model

The hypotheses (*hyp#*) that were utilised to construct each equation are listed in Table 1. The final model is depicted in solid and dashed lines in Figure 1. In this model, if *RMS2* is mutant, then $RMS2_{gs} = LEAKY$, $RMS2_{gc} = LEAKY$, $RMS2_{gr1} = LEAKY$, and/or $RMS2_{gr2} = LEAKY$, as appropriate (Table 1: *hyp27*).

Equations 2.1 through 2.10 are the same as equations 1.1 through 1.10.

(2.11) $RMS1_{tr1}(x) = RMS1_{gr1} \times \left(1 + FS_{r1t}(x-1) - RMS3_{gr1} \times RMS4_{gr1}\right)$

hyp1, hyp3B, hyp4B, hyp14, hyp28

(2.12) $RMS5_{tr1}(x) = RMS5_{gr1} \times \left(1 + FS_{r1t}(x-1) - RMS3_{gr1} \times RMS4_{gr1}\right)$

hyp5, hyp3B, hyp4B, hyp14, hyp28

Equations 2.13 through 2.15 are the same as equations 1.13 through 1.15.

(2.16) $RMS1_{tr2}(x) = Tworoot \times RMS1_{gr2} \times \left(1 + FS_{r2t}(x-1) - RMS3_{gr2} \times RMS4_{gr2}\right)$

hyp1, hyp14, hyp28

(2.17) $RMS5_{tr2}(x) = Tworoot \times RMS5_{gr2} \times \left(1 + FS_{r2t}(x-1) - RMS3_{gr2} \times RMS4_{gr2}\right)$

hyp5, hyp14, hyp28

Equations 2.18 through 2.26 are the same as equations 1.18 through 1.26.

After the branching inhibition (*BI*) is determined by *X* iterations of equations 2.1 through 2.26, the following equations are executed to determine *RMS1* gene expression in the

rootstock and basal zone of the scion, and aerial branching inhibition as a result of the previously calculated branching phenotype.

(2.27) $FS_s(x) = \frac{2 \times RMS2_{gs}}{1 + BIP_s(X)}$ *hyp2, hyp7, hyp17*

(2.28) $BDFS_s(x) = \begin{cases} 1 - BIP_s(X) \text{ if } BIP_s(X) \le 1 \\ 0 \text{ if } BIP_s(X) > 1 \end{cases}$ *hyp29*

(2.29) $RMS1_{ts}(x) = (FS_s(x) + BDFS_s(x)) \times RMS1_{gs}$ *hyp1, hyp14, hyp31*

(2.30) $RMS5_{ts}(x) = (FS_s(x) + BDFS_s(x)) \times RMS5_{gs}$ *hyp5, hyp14, hyp31*

Equation 2.4

(2.31) $FS_s(x) = Ygraft \times \left(\frac{2 \times RMS2_{gs}}{1 + BIP_s(X)} \right)$ *hyp2, hyp7, hyp17*

(2.32) $BDFS_c(x) = \begin{cases} Ygraft \times (1 - BIP_c(X)) \text{ if } BIP_c(X) \le 1 \\ Ygraft \times 0 \text{ if } BIP_c(X) > 1 \end{cases}$ *hyp29*

(2.33) $RMS1_{tc}(x) = Ygraft \times (FS_c(x) + BDFS_c(x)) \times RMS1_{gc}$ *hyp1, hyp14, hyp31*

(2.34) $RMS5_{tc}(x) = Ygraft \times (FS_c(x) + BDFS_c(x)) \times RMS5_{gc}$ *hyp5, hyp14, hyp31*

Equation 2.8, Equation 2.9

(2.35) $FS_{r1t}(x) = FS_{r1}(x) + splitD \times DOWN \times FS_s(x) + Ygraft \times splitD \times DOWN \times FS_c(x)$

hyp13, hyp19, hyp20, hyp22, hyp26

(2.36) $BDFS_{r1}(x) = splitD \times DOWN \times BDFS_s(x) + Ygraft \times splitD \times DOWN \times BDFS_c(x)$

hyp19, hyp20, hyp22, hyp30

(2.37) $RMS1_{tr1}(x) = (1 + BDFS_{r1}(x) + FS_{r1t}(x) - RMS3_{gr1} \times RMS4_{gr1}) \times RMS1_{gr1}$

hyp1, hyp3B, hyp4B, hyp14, hyp28, hyp31

(2.38) $RMS5_{tr1}(x) = (1 + BDFS_{r1}(x) + FS_{r1t}(x) - RMS3_{gr1} \times RMS4_{gr1}) \times RMS5_{gr1}$

hyp5, hyp3B, hyp4B, hyp14, hyp28, hyp31

Equation 2.13, Equation 2.14

(2.39) $FS_{r2t}(x) = Tworoot \times (FS_{r2}(x) + splitD \times DOWN \times FS_s(x))$

hyp13, hyp19, hyp20, hyp26

(2.40) $BDFS_{r2}(x) = Tworoot \times (splitD \times DOWN \times BDFS_s(x))$ *hyp19, hyp20, hyp30*

(2.41) $RMS1_{tr2}(x) = Tworoot \times (1 + BDFS_{r2}(x) + FS_{r2t}(x) - RMS3_{gr2} \times RMS4_{gr2}) \times RMS1_{gr2}$

hyp1, hyp3B, hyp4B, hyp14, hyp28, hyp31

(2.42) $RMS1_{tr2}(x) = Tworoot \times (1 + BDFS_{r2}(x) + FS_{r2t}(x) - RMS3_{gr2} \times RMS4_{gr2}) \times RMS5_{gr2}$

hyp5, hyp3B, hyp4B, hyp14, hyp28, hyp31

Equation 2.18

(2.43) $SMS_{st}(x) = SMS_s(x) + splitU \times UP \times SMS_{r1}(x) + Tworoot \times splitU \times UP \times SMS_{r2}(x)$

hyp12, hyp18, hyp21, hyp23, hyp26

(2.44) $AerialBI_s(x) = RMS3_{gs} \times RMS4_{gs} \times SMS_{st}(x)$ *hyp3B, hyp4B, hyp8, hyp9, hyp15, hyp16*

(2.45) $SMS_{ct}(x) = Ygraft \times (SMS_s(x) + splitU \times UP \times SMS_{r1}(x))$

hyp12, hyp18, hyp21, hyp26

(2.46) $AerialBI_c(x) = Ygraft \times (RMS3_{gc} \times RMS4_{gc} \times SMS_{ct}(x))$

hyp3B, hyp4B, hyp8, hyp9, hyp15, hyp16

(2.47) $AerialBIP_s(x) = AerialBI_s(x)$ *hyp16*

(2.48) $AerialBIP_c(x) = Ygraft \times AerialBI_c(x)$ *hyp16*

Equation 2.49 is only implemented if $BIP_s(X)$ is less than the relevant WT grafted control.

(2.49) $Branchlength = \begin{cases} \text{short if X - CK} \le \text{WT control} \\ \text{long if X - CK} > \text{WT control} \end{cases}$ *hyp32*

Model implementation

All model components were randomly assigned a value between 0 and 1 as an initial condition, while the genotypic inputs were set by the graft type and genotype combination. The models were iterated until a steady state was reached for the branching inhibition output from each graft-genotype combination, which occurred within 1000 iterations. An example of the branching inhibition of a WT self-grafted plant reaching a steady-state value is depicted in Supplemental Figure 6 online. In the final model, once the branching inhibition, *RMS1* gene expression and rootstock X-CK outputs are determined, equations are re-evaluated to determine the resulting *RMS1* gene expression and aerial branching phenotype.

Biological data

It was not reasonable to aim for a model output that could be compared directly to the primary biological data, as the experiments reported over the years were not all conducted under the same conditions. Although always recorded, the node along the stem at which the plants of different treatments undergo branching was not always reported. Total lateral length (TLL; as the summative data value) has been used to determine the branching phenotype, rather than considering the location of each branch. Trends and relationships conserved between experiments, rather than the exact TLL values, were considered important as they not only capture the branching phenotype of different graft combinations, but also overcome the problem of variable experimental conditions (e.g., Beveridge et al., 1996 compared to Foo et al., 2005).

For ease of data comparison, the biological branching data were grouped into three classes of branching inhibition (Supplemental Tables 2, 3 and 4 online). This allowed us to capture branching phenotypes repeatedly the same as, or intermediate to, non-branching and branching control plants as described and schematically represented by Beveridge et al. (1997a). A WT plant on any genetic background was considered to have total branching inhibition, value 1, while any mutant self-graft (*rms1*/*rms1* for example) was considered to have no inhibition of branching, value 0. The phenotypes of all graft combinations were compared to the WT and mutant self-grafts, and grouped into their respective classes. A graft combination with an intermediate phenotype was included in an intermediate class of branching inhibition, value 0.5. Due to the wide range of results observed biologically for *RMS1* gene expression, these data were grouped into five classes using the same procedure (Supplemental Figure 2 online). Again using the same procedure, rootstock X-CK data for

different graft combinations were grouped into three phenotypic classes (Supplemental Figure 5 online).

Comparison of biological and model-generated data

Biological data was entered into a text file, allowing the model simulation to access it for automated comparison with the model output to verify that the hypotheses and mathematical translation described the biological network sufficiently and correctly. This involved evaluating if all grafts remained in the correct groupings for level of branching inhibition, *RMS1* gene expression and X-CK. All data were required to be captured by the model, as one result not captured may indicate that the hypotheses are inadequate to explain all emergent behaviours of the branching regulatory network.

The models were run with all possible combinations of *UP, DOWN* and *LEAKY* parameter values from 0 to 1 at 0.05 intervals (9261 combinations). For each parameter combination, the graft combinations were sorted based on the branching inhibition output of the model. The branching inhibition output was considered acceptable for any given parameter combination if the resulting order matched the order for biological branching inhibition classes. Parameter combinations that yielded acceptable branching inhibition outputs were recorded (Supplemental Table 1 online).

For rootstock X-CK, the outputs of the models were grouped into three classes, based on their level relative to WT self-grafts. Outputs that were much greater than that of WT self-grafts were grouped into a class of high X-CK content, output similar to that of WT self-grafts were grouped into a class of medium X-CK content, and output much less than that of WT self-grafts were grouped into a class of low X-CK content. Trends were then compared to biological classes (Supplemental Figure 1 online).

RMS1 gene expression output from the model was graphed, and trends were visually compared to the biological classes (Supplemental Figures 2 and 5 online).

SUPPLEMENTAL REFERENCES

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