Investigating Dynamics of Tomato Fruit Sugars, in Response to Changing Ambient Temperature and Fruit Load, by Means of Mechanistic Modelling

Pannaree Boonyuen,

BA Natural Sciences, University of Cambridge

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Hosting affiliation: Biological Modelling Laboratory, Systems Biology and Bioinformatics research group (SBI), King Mongkut's University of Technology Thonburi (KMUTT), Thailand

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Introduction

- Sugars are important carbon compounds in tomato fruits. Apart from providing sweetness, they are precursors of aromatics, vitamins, antioxidants, and lycopene, all of which determine the quality of fruits. Additionally, sugars mediate developmental signalling; thus, sugar levels control also fruit yield (Kanayama, 2017; Ruiz-Nieves et al., 2021). The main tomato fruit sugars are glucose and fructose, although assimilates are transported as sucrose throughout the plant.
- Many environmental factors can significantly change the level of tomato fruit sugars, including light (Ji et al., 2020; Wang et al., 2022) and soil water (Abdel-Razzak et al., 2016). However, reported sugar responses to high temperatures are largely varied, depending on tomato variation and experimental design (table 1), which include how much fruit load is imposed per truss (a branch with flowers). Fruit pruning is a common agricultural practice used with tomato cultivation with the main aim to increase the yield (a larger size of the intact fruit) and quality of fruit (a greater sweetness of the intact).
- However, the mechanisms behind the increase in sugars are not well studied; therefore, we would like to use computation model to explain the likely dynamics of fruit sugars in response to different growing temperature and fruit load. This will require the model to be able to 1) account for the effect of temperature and number of fruit per truss on fruit sugar and 2) simulate the carbon in different forms because the level of sugars are related to the amount of carbon supply and carbon use by different cellular pathways. We selected SUGAR model as the based model and built on it.
- Our main hypothesis was that at a low fruit load, a higher temperature will increase the fruit sugar, while the opposite would happen under a high fruit load, according to Gautier et al. (2005).

Method

Referenced SUGAR model - mathematical and computational flow

- SUGAR model was originally developed for explaining the carbon metabolism of a peach fruit in 1996 (Génard & Souty, 1996), but further applied to other fruits e.g. grapes (Dai et al., 2009).
- It has been adapted and parameteried for tomato carbon metabolism by Luo et al., 2020 and Chen et al., 2020. The latter contains the model pseudo-code which was used as the starting model for this project.
- Chen et al., 2020 (Fig. 1 and Eqn 1-7) considered a scope of model to be one fruit. A carbon supply increases the total carbon of the fruit occurs at a rate dCsup/dt. All of the imported carbon is considered to be in soluble sugar forms (Csol) which is the centre of carbon metabolism. Csol is used in respiration (Csol transitioned to Cresp) and forming starch (Csol transitioned to Csta) and non-starch compounds (Csol transitioned to Coth). Equation (1) to (4) are the mass-balance equations of Csol, Csta, Coth, Csup.
- The rate of respiration (dCresp/dt) depends in dry weight (DW), dry weight change rate (growth rate) (dDW/dt) and temperature (T). The rate constant k1 (1/h) catalyse the sugar-to-non starch compound transition, while the model does not consider the reverse reaction. k3 catalyse the sugar-to- starch compound transition, and k2 catalyse the reverse transition. Only k2 that is assumed constant, and the other constants (k1 and k3) are explained by equation (6) and (7), based on the correlation plot between them and relative growth rate (dDW/dt / DW) or temperature sum (DD).
- Temperature (DD) is calculated using 5.7 °C as a base temperature (after which point, there is no growth of fruit (no DD increase))
- Water content (WC) is calculated by subtracting DW out of fresh weight (FW) (WC = FW – DW) and WCmax is fitted as a function of fruit age (hr after anthesis) from WC-age plot

- Therefore, in order to simulate the output from SUGAR model, there are five input variables: DW, FW, dDW/dt, Temp, age (Fig. 2)

$$\frac{dC_{sol}}{dt} = \frac{dC_{sup}}{dt} + k_2(t)C_{sta} - [k_1(t) + k_3(t)]C_{sol} - \frac{dC_{resp}}{dt} \tag{1}$$

$$\frac{dC_{sta}}{dt} = k_3(t)C_{sol} - k_2(t)C_{sta} \tag{2}$$

$$\frac{dC_{oth}}{dt} = k_1(t)C_{sol} \tag{3}$$

$$\frac{dC_{sup}}{dt} = c_{DW}\frac{dDW}{dt} + \frac{dC_{resp}}{dt} \tag{4}$$

$$\frac{dC_{resp}}{dt} = q_g\frac{dDW}{dt} + q_m DW Q_{10}^{(T-20)/10} \tag{5}$$

$$k_1(t) = \lambda \exp[\alpha(WC_{max} - WC)]\left(\frac{1}{DW}\frac{dDW}{dt}\right)^{\exp[\beta(WC_{max} - WC)]} \tag{6}$$

$$k_3(t) = \frac{k_{30}\exp[\gamma(WC_{max} - WC)]}{1 + \exp[(DD - u)/\tau]} \tag{7}$$

Input data

- We have access to the data of *Cervil* tomato growth (age, DW and FW) under different fruit loads (5 and 20 fruits per truss) collected from the year 2007, given in the paper Chen et al., 2020 (Table 2).
- The growth rate (dDW/dt) used in this report was calculated from 1) fitting the DW curve to Gompertz growth function and 2) calculating the derivative of the fitted curve. Consequently, we had age, DW, FW, and dDW/dt as the inputs for our model.
- However, they did not provide the exact temperature for each DW and FW records, despite recording it every minute (we could only estimate from their other plots that temperature range was likely to be between 13-17 °C)

- We then created the input temperature, according to our regimes – 1) lower temperature (CC): Day 17.4/Night 16.6 °C and 2) higher temperature (HH): Day 24.4/Night 20.3 °C. We selected this temperature according to the paper Gautier et al. (2005), on which we based the hypothesis.
- We used these artificially-created temperature values as model inputs, along with the other experimentally-collected variables (mentioned eariler)
- However, we were not totally confident that with changing temperature, DW and FW of fruits would be unchanged. Therefore, we examined both cases of model inputs – 1) assumed that DW and FW under CC and HH were not significantly different (non-adjusted DW and FW) and 2) assumed that DW and FW under HH would be reduced by a certain percentage (extracted from Gautier et al. (2005)), compared to CC (adjusted DW and FW). The pipeline in creating two types of model input shown in Figure 3.
- In conclusion, we have 4 treatments (5FCC, 5FHH, 20FCC, 20FHH), each run under 2 different model assumptions (heat-adjusted and non heat-adjusted input) (Table 2). However, they were added up to 6 runs in total because non heat-adjusted input data were the same between the same loads (5FCC = 5FHH and 20FCC = 20FHH)
- The common assumptions for all six runs were that
    - Regardless of the fruit load level, all processes related to carbon metabolism behave similarly (the values of explanatory variables and parameters were the same for 5F and 20F run)

Result and Discussion

Result 1: Examination of model inputs

Result 1.1: Fitted lines represent well the DW and FW raw data

- The distribution of the DW (Fig 4a) and FW (Fig 4b) roughly followed a sigmoid curve, explained by Gompertz function (Chen et al., 2020).
- Primary observation: The DW and FW per fruit of high-loaded plants (20 fruits per truss, 20F) were lower than those of low-loaded plants (5 fruits per truss, 5F)

Fig 4a) Dry weight raw data (points) and Gompertz fitted lines (bold line)

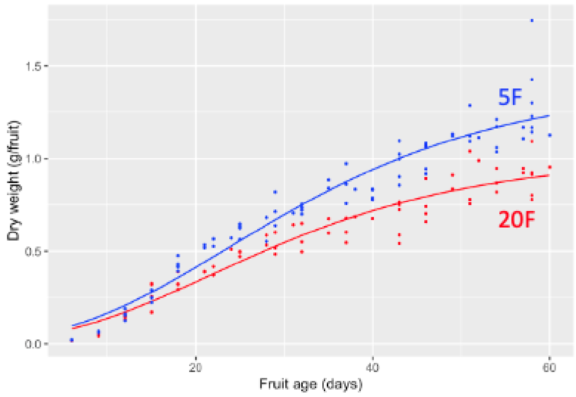
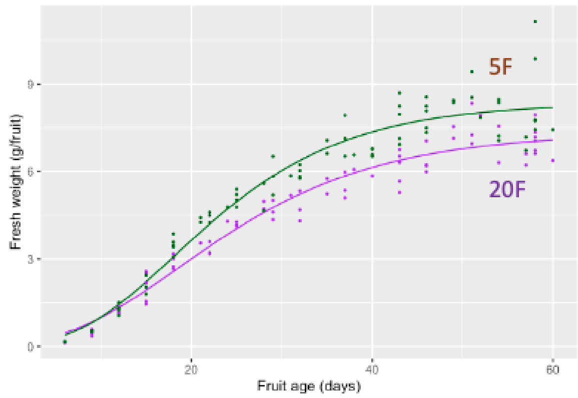


Fig 4b) Fresh weight raw data (points) and Gompertz fitted lines (bold line)



Result 1.2: Fitted lines of heat-adjusted version (reduced DW and FW)

- Under heat-adjusted version of data, the distribution of the DW (Fig 4c) and FW (Fig 4d) also tracked a sigmoid curve.
- Similarly, the DW and FW per fruit of high-loaded plants also remained lower than those of low-loaded plants

Fig 4c) Comparison between fitted lines from non-adjusted and adjusted dry weight data
Dash line is the heat-adjusted version of corresponding bold line

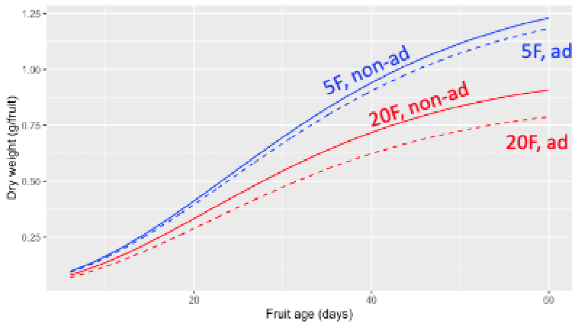
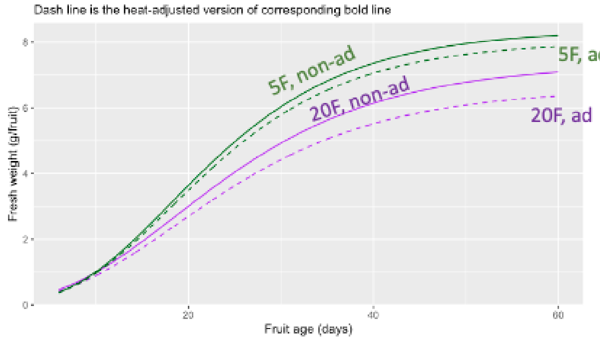


Fig 4d) Comparison between fitted lines from non-adjusted and adjusted fresh weight data
Dash line is the heat-adjusted version of corresponding bold line



- Why under more fruits per truss condition, each fruit grows less?
  - Possible explanation: the same carbon supply divided over a higher number of sinks

Result 2: Output simulations

Output 1: Amounts of sugars per FW (SSC, Fig 5a) and DW (SS, Fig 5b)

- On **both** a FW (Fig 5a) or DW (Fig 5b) basis, HH has a lower %sugar per weight than CC (blue vs pink) for the same fruit load. HL(20F) has a lower %sugar per weight than LL(5F) (bold vs dashed line) for the same growing temperature
- Therefore, either considering or not considering water content (FW or DW basis, respectively), the conclusion was not change. This means the dilution effect may not be the significant cause of the sugar content change due to the change in temperature and fruit loading. Therefore, the next question is whether the change in sugars was because the change in relative rate of sugar-related processes.

Fig 5a)

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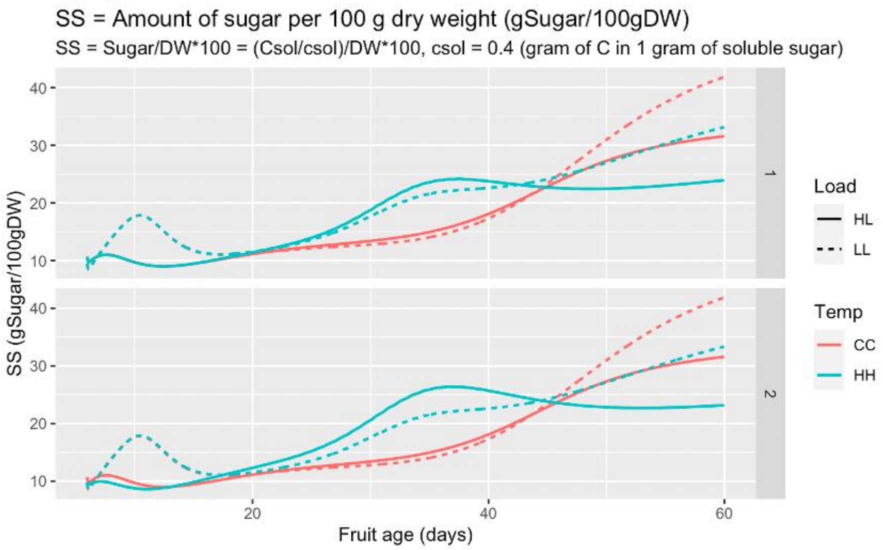
Fig 5b)



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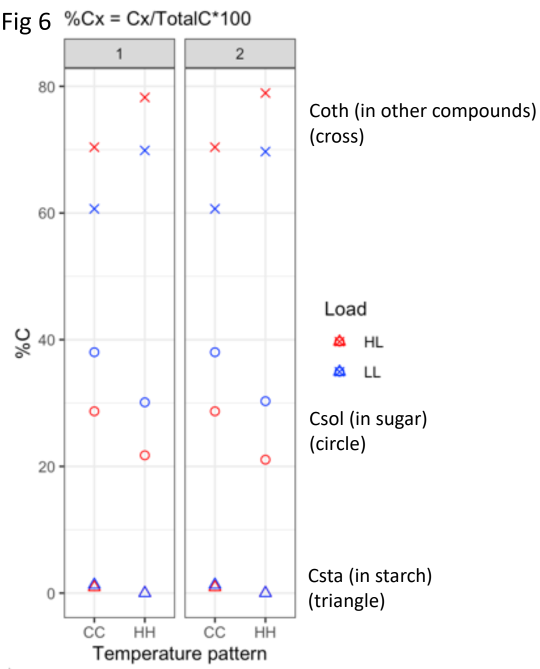
Output 2: Ratio of carbon in sugars or other forms per fruit at maturity

- Output at the last sampling date was used as amounts at maturity
- According to Fig 6, the ratio of any carbon form changes for CC vs HH (left vs right) and HL vs LL (red vs blue) e.g. for carbon in sugar form (Csol; circle)
- Therefore, it is possible that there is a metabolic shift between these processes as the condition changes.
- The candidate processes were 1) fruit carbon/sugar supply, 2) fruit respiration, 3-4) sugar conversion to starch and non-starch (other compounds e.g. acids, structural compounds), referred to fig 1.

Fig 6

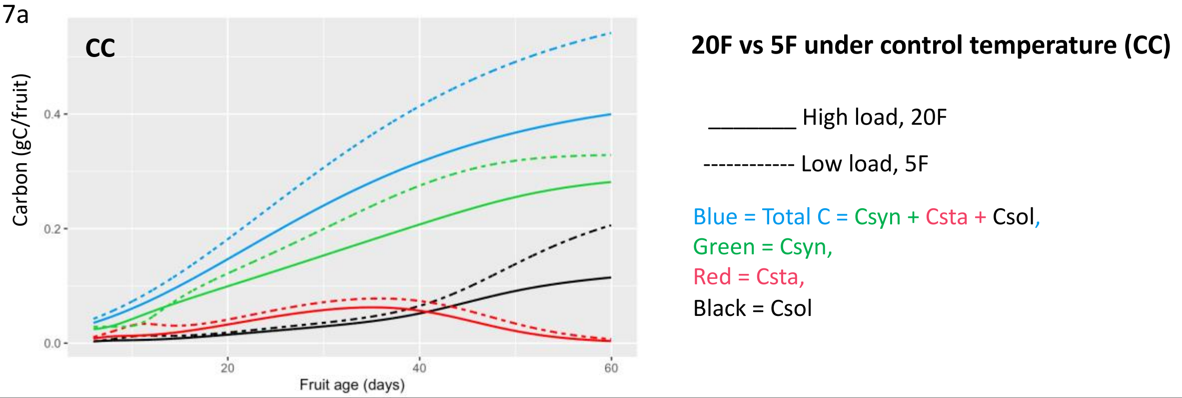


%Csol	CC	HH (1 and 2)
LL5F	38%	30%
HL20F	29%	21%

Output 3: Absolute amount of total carbon and in each form per fruit under CC

- Total C in HL20F is always lower than LL5F (Fig 7a, blue line)
  - The same trend with DW and FW were seen.
  - Physiologically, it made sense since the same carbon supply divided over a higher number of sinks.
- What make the most contribution to the change in total carbon?
  - According to Fig 7a, it is Csol. Csol in HL20F is always lower than that in LL5F (Fig 7a, black line)

Fig 7a



- Mechanistically, why Csol decreases under HL condition?
    - Hypothesis 1a: Is it because more sugars get respired under HL20F
    - Hypothesis 2a: sugars are formed less
    - Hypothesis 3a: more sugars get converted under HL20F to starch
    - Hypothesis 4a: more sugars get converted under HL20F to other compounds

Investigation of why Csol decreases under HL20F condition

Fig 8a: respiration rate (g/h)

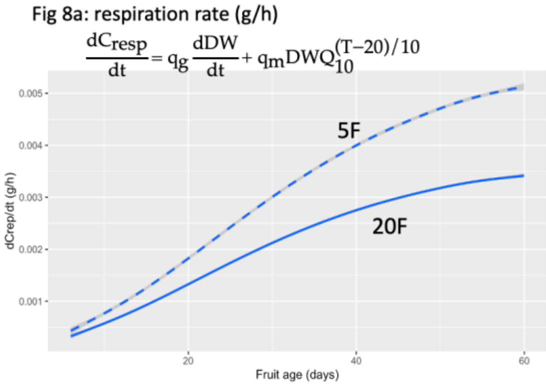
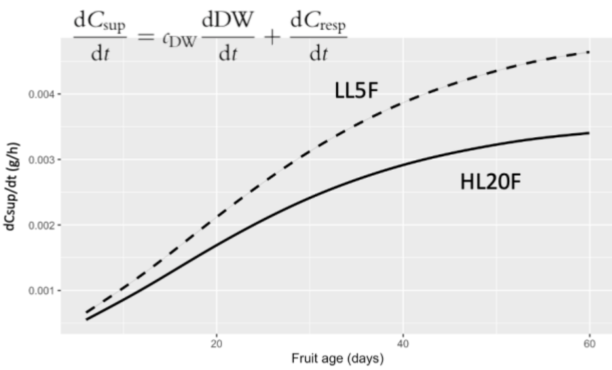
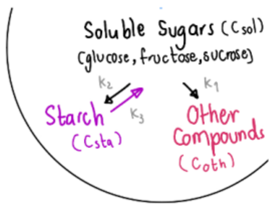


Fig 8b: carbon supply rate (g/h)



- H1a: higher respiration rate under HL20F
    - Looking at dCresp/dt (Fig 8a), HL20F actually has a lower respiration rate. Therefore, it was not the case.
- H2a: sugars are formed more in LL5F
    - Look at dCsup/dt (Fig 8b), HL20F indeed has a lower carbon supply rate. Therefore, it could be the reason behind the lower Csol under high load.



$$\frac{dC_{sta}}{dt} = k_3(t)C_{sol} - k_2(t)C_{sta}$$

Fig 9a: starch-to-sugar conversion coeff (k3)

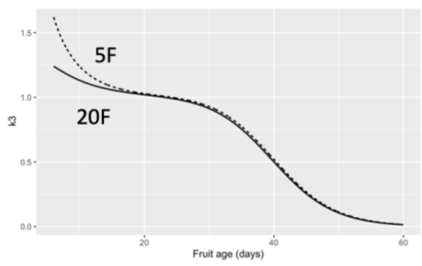
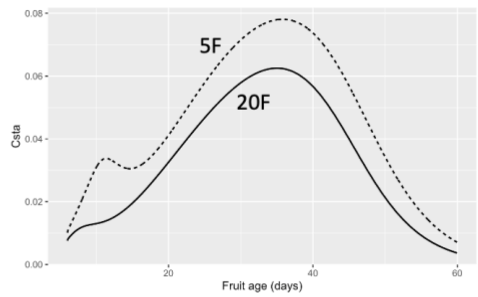
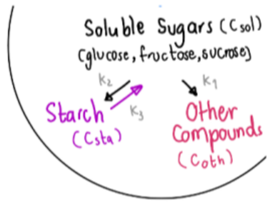


Fig 9b: carbon in starch form (Csta)



- H3a: higher net sugars-to-starch conversion under HL20F
    - Looking at k3, the lower k_3, the higher net sugar-to-starch conversion.
    - According to Fig 9a, only during early period, the k3 was lower under HL20F.
    - More importantly, Csta in HL20F was actually lower.



$$\frac{dC_{oth}}{dt} = k_1(t)C_{sol}$$

Fig 9c: sugar-to-other conversion coeff (k1)

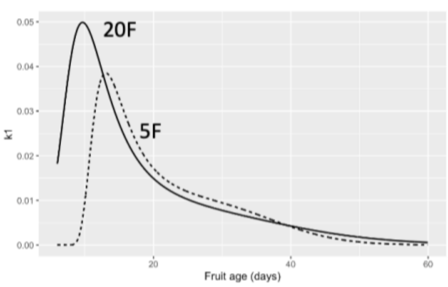
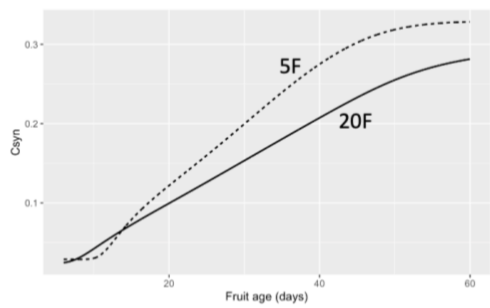


Fig 9d: carbon in other forms (Coth)



- H4a: higher net sugars-to-others conversion under HL20F
    - Looking at k1, the higher k_1, the higher net sugar-to-other conversion
    - Similarly, it was only support for early period and Coth in HL20F is actually lower
- **In summary, the likely reason behind the reduced sugar under high load, according to model output was that the sugars were supplied less under high load.**
    - Since a lower dCsup/dt (Csupply rate = Sugar supply rate) found in HL20F (Assuming they begin with the same level of Csup)
    - Similar to what suggested by Gautier et al. (2005) – although for the difference comparison.
- Not because more sugars get respired under HL20F since a lower respiration rate found in HL20F

- Nor more sugars get converted under HL20F
    - to starch since starch-to-sugars rate constant (k3) only differ at the beginning, and overall, does not cause a higher Csta in HL20F
    - Or to other compounds sugars-to-others rate constant (k1) only differ at the beginning, and overall, does not cause a higher Coth in HL20F

Output 4: What about when temperature effect considered?

Fig 10

- The SSC output for HL20F was reduced under heating (Fig 10, Bold blue vs Bold pink), which agreed with the finding by Gautier et al. (2005)
- The SSC output for LL5F was also reduced (Dash blue vs dash pink), and this was not in agreement with Gautier et al. (2005) that found increased sugars under low competition.
- Note that Case 1 and 2 (non-ad or adjusted DW and FW) did not change the conclusion.
- The question was then whether the reasons for the sugar drop in HH vs CC (under both fruit load) the same as in fruit load case (lower Csupply rate)?
- Therefore, we could carry out the same investigation to test all 4 hypotheses.

Investigation of why Csol decreases under HH condition for both fruit loads

Fig 11a)

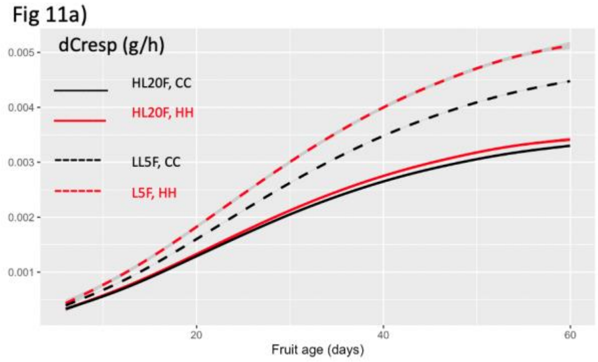
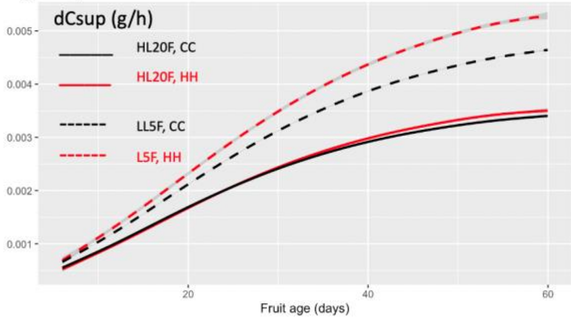


Fig 11b)



- H1b: higher respiration rate under HH
  - Looking at dCresp/dt (Fig 11a), HH indeed has a lower respiration rate. Therefore, it could be the reason behind the lower Csol under higher temperature.
- H2b: sugars are supplied more in CC
  - Look at dCsup/dt (Fig 11b), CC actually has a lower carbon supply rate. Therefore, it was not the case.

Fig 12a)

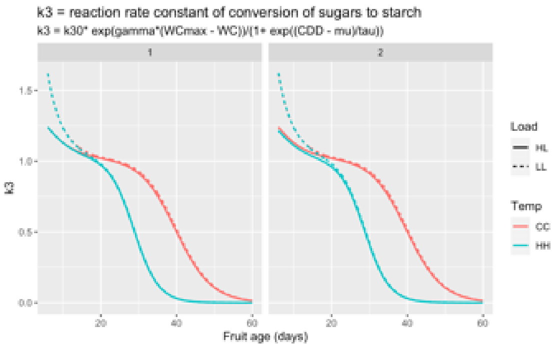
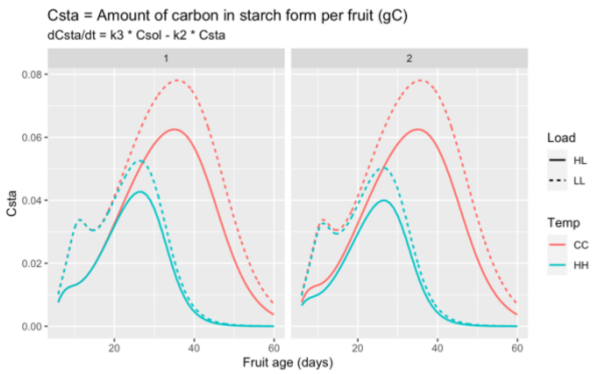


Fig 12b)



- H3a: higher net sugars-to-starch conversion under HH
  - Looking at k3, the lower k_3, the higher net sugar-to-starch conversion.
  - According to Fig 11a, the k3 was indeed lower under HH.
  - However, Csta in HH was actually lower.
  - Case 1 (non-adjusted DW and FW) and Case 2 (heat-adjusted DW and FW) did not make the difference in the output.

Fig 12c)

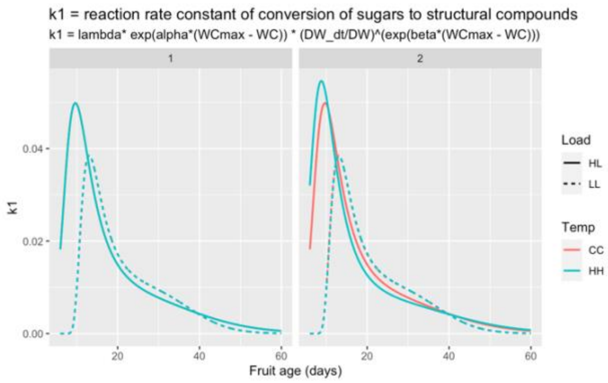
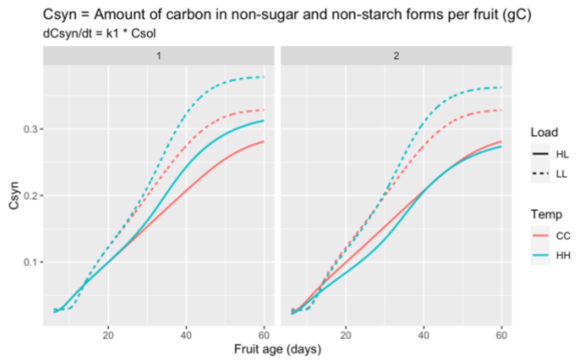


Fig 12d)



- H4a: higher net sugars-to-others conversion under HH
  - Looking at k1, the higher k_1, the higher net sugar-to-other conversion
  - For both temperature (CC and HH), k1 seemed to be similar.
  - However, Coth in HH was indeed higher, but only in case 1 (non-adjusted DW and FW) that this was true for both loads. In case 2, Coth in HL did not differ between two temperatures.
  - Note: Coth of HL under CC vs HH was the only simulation comparison that the adjustment of DW and FW (case 1 and case 2) made the difference in the conclusion.
- **In summary, the most likely reason that Csol decreases under HH conditions was that sugars were more respired under HH**
  - Since a higher respiration rate found in HH
- The second likely answer was that more sugars got converted non-sugar/starch compounds under HH
  - This was always true for low load (both case 1 and 2)
  - For high load, it was true only when we assumed the non-adjusted weight (case 1)
- Not because a higher Csup rate in CC since Csup rate was higher in HH
- Nor more sugars get converted under H to starch since even though k3 was lower, the Csta of HH appeared lower

Result summary and Limitations:

- In general,
  - Even though the fruit responses were the same, the mechanisms behind the changes can be different.
  - Modelling can help track change in metabolism (metabolic shift) over time, which may overcome some experimental limitations in collecting these data at a high-resolution time scale.
- Specifically,
  - Heat and fruit load can alter the level of carbon in several forms. Specifically, the increase in fruit load (for any temperature regimes) and in temperature (for any fruit loads) both reduced the carbon in sugar forms in the fruit. This partially contradicted to what were reported by Gautier et al. (2005) that found increased sugars when grown under heating under low competition.
  - However, in this paper, no statistical tests have been performed to consider the significance in these effects or the interactions between the two factors.
  - Another limitation of the model was the assumption that under different fruit loads, all parameters and explanatory variables were identical. We also assumed that every fruit behaved the same way, regardless of the fruit load on the plant and fruit age. However, this may not be true. The examples are that the ages of each fruit on a truss were different, so as their size and their respiration rate, and that there could be saturation in the ability to extract carbon from source as fruits grow.
  - Experimental work needs to be performed for the in-depth investigation of the differences in carbon compounds, firstly to refine the assumption and secondly to confirm the conclusion from this report.

References

- Abdel-Razzak, H., Wahb-Allah, M., Ibrahim, A., Alenazi, M., & Alsadon, A. (2016). Response of Cherry Tomato to Irrigation Levels and Fruit Pruning under Greenhouse Conditions. *J. Agr. Sci. Tech.*, *18*, 1091–1103.
- Chen, J., Vercambre, G., Kang, S., Bertin, N., Gautier, H., & Génard, M. (2020). Fruit water content as an indication of sugar metabolism improves simulation of carbohydrate accumulation in tomato fruit. *Journal of Experimental Botany*, *71*(16), 5010–5026. https://doi.org/10.1093/jxb/eraa225
- Gautier, H., Rocci, A., Buret, M., Grasselly, D., & Causse, M. (2005). Fruit load or fruit position alters response to temperature and subsequently cherry tomato quality. *Journal of the Science of Food and Agriculture*, *85*(6), 1009–1016. https://doi.org/10.1002/jsfa.2060
- Ji, Y., Nuñez Ocaña, D., Choe, D., Larsen, D. H., Marcelis, L. F. M., & Heuvelink, E. (2020). Far-red radiation stimulates dry mass partitioning to fruits by increasing fruit sink strength in tomato. *New Phytologist*, *228*(6), 1914–1925. https://doi.org/10.1111/nph.16805
- Kanayama, Y. (2017). Sugar Metabolism and Fruit Development in the Tomato. *The Horticulture Journal*, *86*(4), 417–425. https://doi.org/10.2503/hortj.OKD-IR01
- Ruiz-Nieves, J. M., Ayala-Garay, O. J., Serra, V., Dumont, D., Vercambre, G., Génard, M., & Gautier, H. (2021). The effects of diurnal temperature rise on tomato fruit quality. Can the management of the greenhouse climate mitigate such effects? *Scientia Horticulturae*, *278*, 109836. https://doi.org/10.1016/j.scienta.2020.109836
- Wang, S., Jin, N., Jin, L., Xiao, X., Hu, L., Liu, Z., Wu, Y., Xie, Y., Zhu, W., Lyu, J., & Yu, J. (2022). Response of Tomato Fruit Quality Depends on Period of LED Supplementary Light. *Frontiers in Nutrition*, *9*, 833723. https://doi.org/10.3389/fnut.2022.833723

Tables

Table 1: Effect of growing temperature on tomato fruit sugar levels from.

Genotypes	Sugar at higher temperature	Temperature regime	Additional factors	Ref.
Cherry tomato *S.lycopersicum L.* cv. Cervil	Low load - Higher High load - Lower	Heated by water pipe at with water of 45°C and ambient temp recorded	Fruit load	Gautier et al., 2005
Cherry tomato *S.lycopersicum* cv. Naomi	Sucrose – Lower Hexose – Higher	As measured (not manipulated)	Crop age	Rosales et al., 2007
Cherry tomato *S. lycopersicum L.* cv. Cervil	unchanged	21 or 26°C over 7 days During off-vine ripening	-	Gautier et al., 2008
Medium-fruit varieties (1)	Lower	33.4 (near cooling pad) vs 35.4°C since one week after transplanting	-	Shivashankara et al., 2015
Medium-fruit varieties (2)	Lower	control (~25°C) vs temperatures stress (40±2°C) since 30 Days after transplanting	(Stress)	Lokesha et al., 2019
Dwarf tomato cultivars "Ponchi Re" and "Tarzan"	At 22°C is the lowest level	16, 22, and 28°C after flowering	-	Affandi et al., 2022

(1) RF4A, Abhinava, Arka Saurabh, IIHR 2195 and Arka Vikas

(2) Arka Abha, IIHR-2627, IIHR-291, IIHR-2202, IIHR-2745 and IIHR-2841

Table 2: First three columns show the conditions (load and temperature) of all four treatments. The next two columns showed that functions used for the simulations. *Inputfun* is for retrieving data from raw data by Chen et al (2020) and *sugarmod* is the main function for the simulation. The codes can be found in the files in appendix.

Treatment	Load	Temperature	Inputfun	sugarmod		Inputfun	sugarmod
1	20F	CC	InputfunH	sugarmodH	=	InputfunH	sugarmodH
2	20F	HH	InputfunH	sugarmodH		InputfunH_H	sugarmodH_Ad
3	5F	CC	InputfunL	sugarmodH	=	InputfunL	sugarmodH
4	5F	HH	InputfunL	sugarmodH		InputfunL_H	sugarmodH_Ad

(total of six runs)

Figures*

Figure 1: Diagram of SUGAR model components (adapted from Chen et al., 2020)

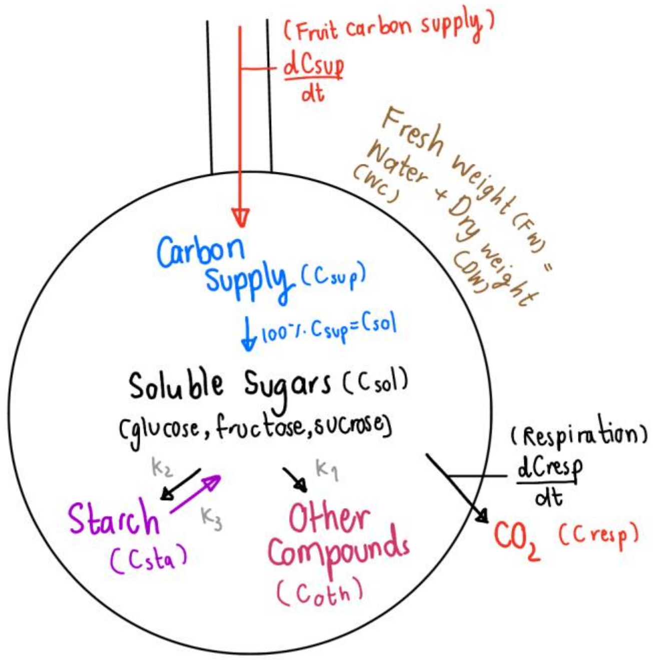
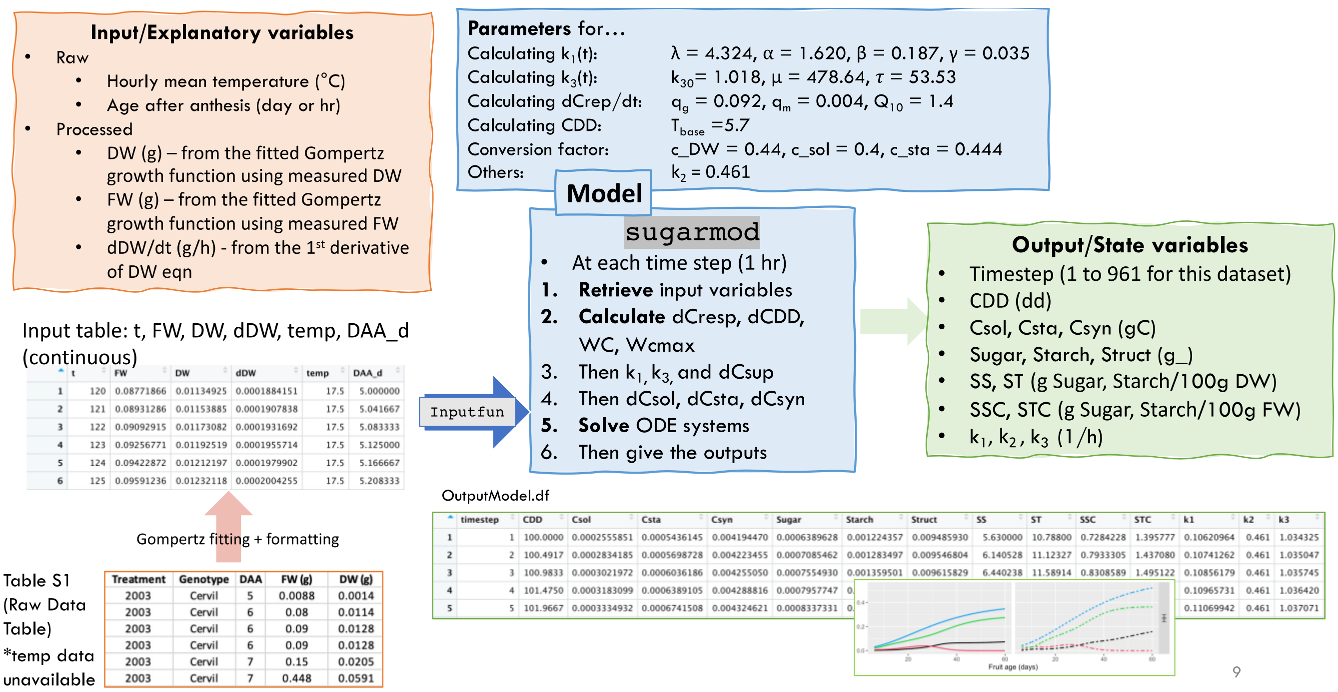


Figure 2: SUGAR model computational flow (Input, model and output)



	t	FW	DW	dDW	temp	DAA_d
1	120	0.08771866	0.01134925	0.0001884151	17.5	5.000000
2	121	0.08931286	0.01153885	0.0001907838	17.5	5.041667
3	122	0.09092915	0.01173082	0.0001931692	17.5	5.083333
4	123	0.09256771	0.01192519	0.0001955714	17.5	5.125000
5	124	0.09422872	0.01212197	0.0001979902	17.5	5.166667
6	125	0.09591236	0.01232118	0.0002004255	17.5	5.208333

Treatment	Genotype	DAA	FW (g)	DW (g)
2003	Cervil	5	0.0088	0.0014
2003	Cervil	6	0.08	0.0114
2003	Cervil	6	0.09	0.0128
2003	Cervil	6	0.09	0.0128
2003	Cervil	7	0.15	0.0205
2003	Cervil	7	0.448	0.0591

	timestep	CDD	Csol	Csta	Csyn	Sugar	Starch	Struct	SS	ST	SSC	STC	k1	k2	k3
1	1	100.0000	0.0002555851	0.0005436145	0.004194470	0.0006389628	0.001224357	0.009485930	5.630000	10.78800	0.7284228	1.395777	0.10620964	0.461	1.034325
2	2	100.4917	0.0002834185	0.0005698728	0.004223455	0.0007085462	0.001283497	0.009546804	6.140528	11.12327	0.7933305	1.437080	0.10741262	0.461	1.035047
3	3	100.9833	0.0003021972	0.0006036186	0.004255050	0.0007554930	0.001359501	0.009615829	6.440238	11.58914	0.8308589	1.495122	0.10856179	0.461	1.035745
4	4	101.4750	0.0003183099	0.0006389105	0.004288816	0.0007957747	0.						0.10965731	0.461	1.036420
5	5	101.9667	0.0003334932	0.0006741508	0.004324621	0.0008337331	0.						0.11069942	0.461	1.037071

Figure 3: Pipeline of creating the model inputs for DW and FW, which divided into 2 paths depending on the assumption of the difference between DW or FW under CC and HH. Note that dDW/dt not shown here but gained from finding the first derivative of Gompertz equation.

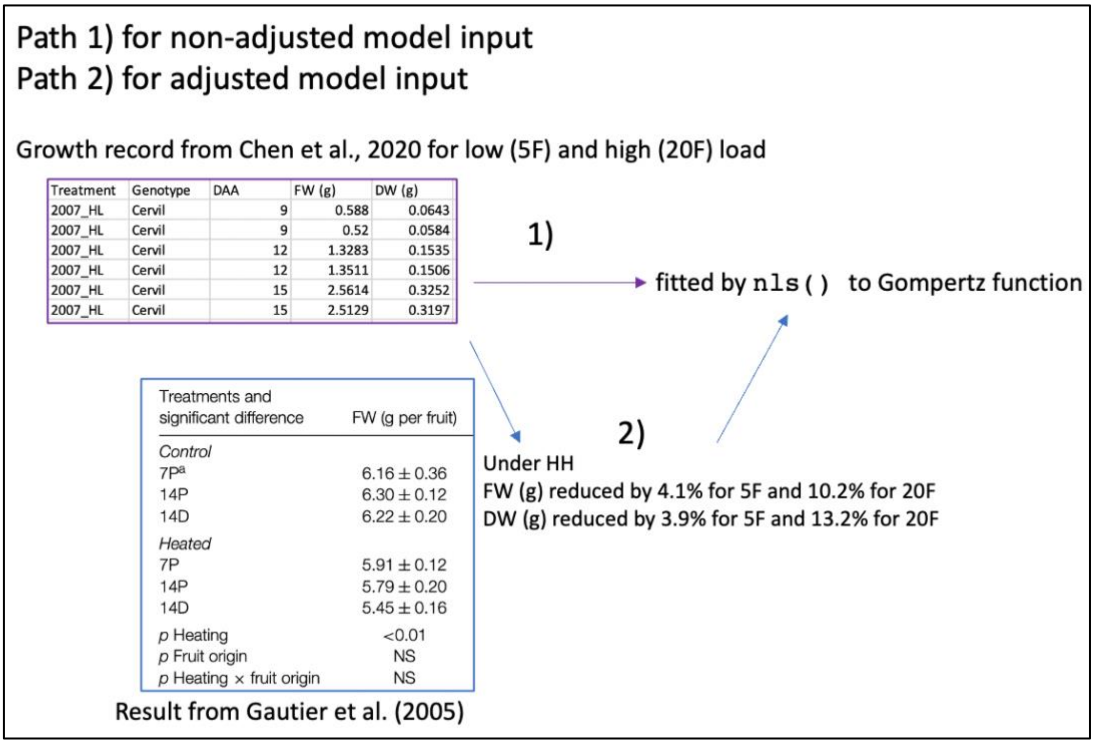


*Figure 4 to 12 were inserted within the text for the coherence.

Appendix

The codes, raw and processed data, and presentation slides are collected in to https://drive.google.com/drive/folders/1g62dvNs0LrC-lE9Pb4puF9QgqYHfXMUU?usp=sharing