Accelerating Growth and Treating Disease Using Metabolic Modeling of Citrus

This Research Involves Using Metabolic Models in New Ways to Address Citrus Needs:

1. Accelerating citrus breeding & engineering
2. Creating new strategies to make more drought tolerant citrus
3. Creating HLB-tolerant citrus

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Presentation Outline

What are Metabolic Models

What Can Metabolic Models be Used For

Accelerating Growth for Citrus Breeding and Engineering

Understanding Biological Processes for Drought Tolerance in Citrus and HLB Tolerance

What are Metabolic Models

Metabolic models are computer simulations of all the chemical reactions in an organism

What are some common chemical reactions?

Digesting food provides nutrients and fuel for the body

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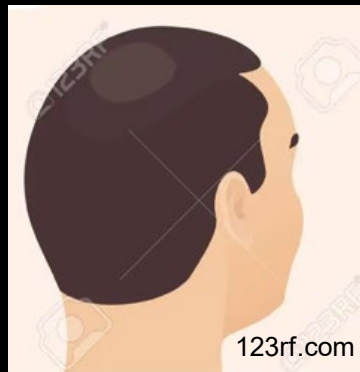
Digestive Enzymes →

- Carbohydrates
- Fats
- Protein

Nutrients can be used to create body components such as hair

- Carbohydrates
- Fats
- Protein

Hair Production Enzymes →



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What are Metabolic Models

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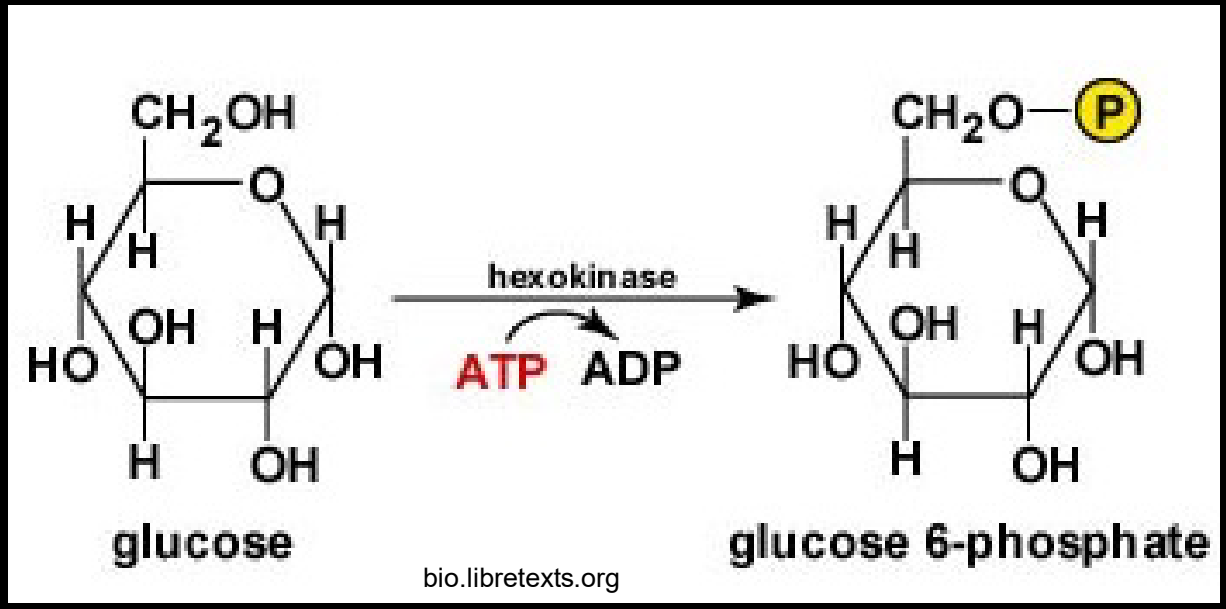
Digestive Enzymes

Carbohydrates
Fats
Protein

Chemical reactions use enzymes to transform one chemical into another



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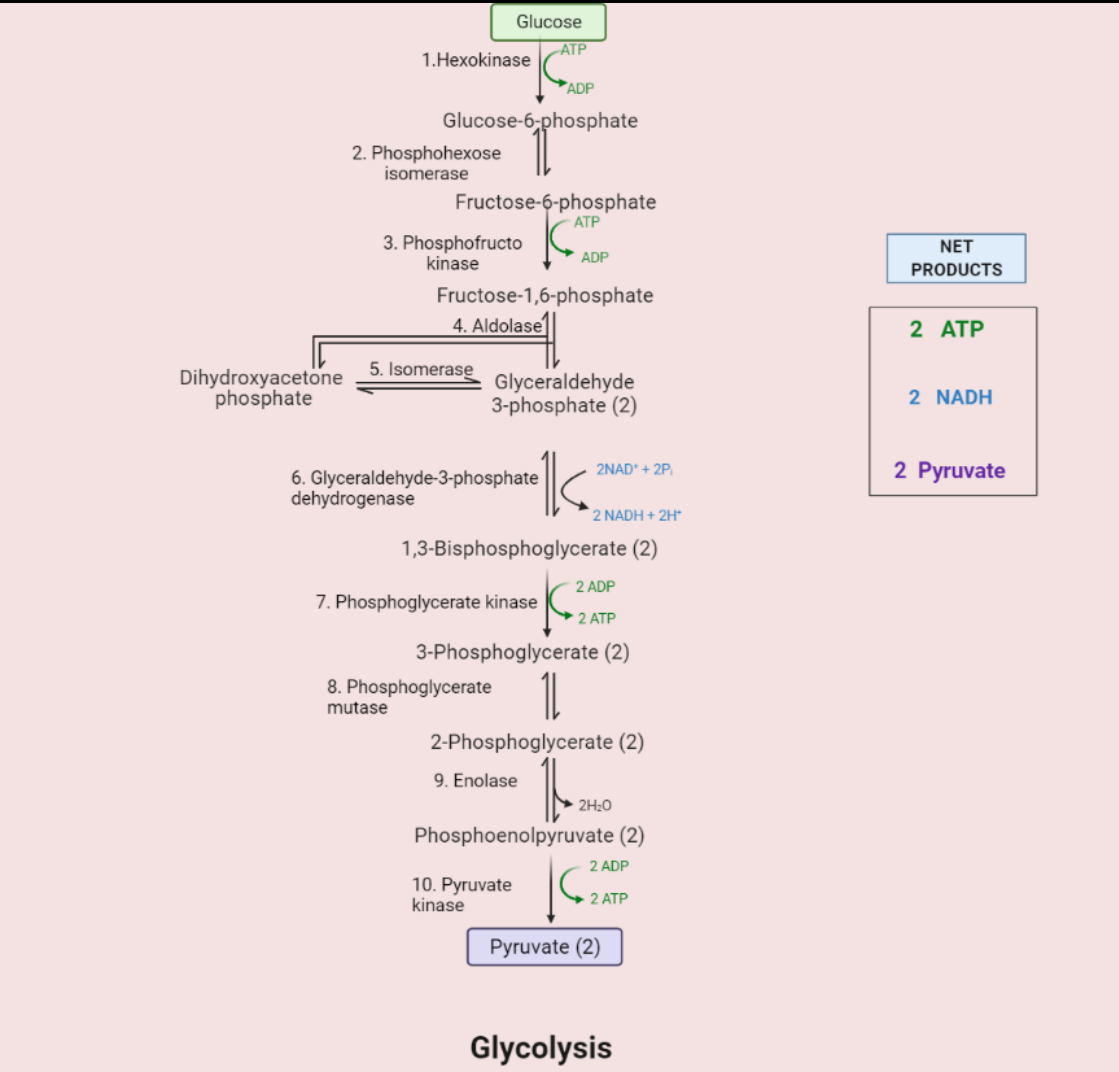
What are Metabolic Models

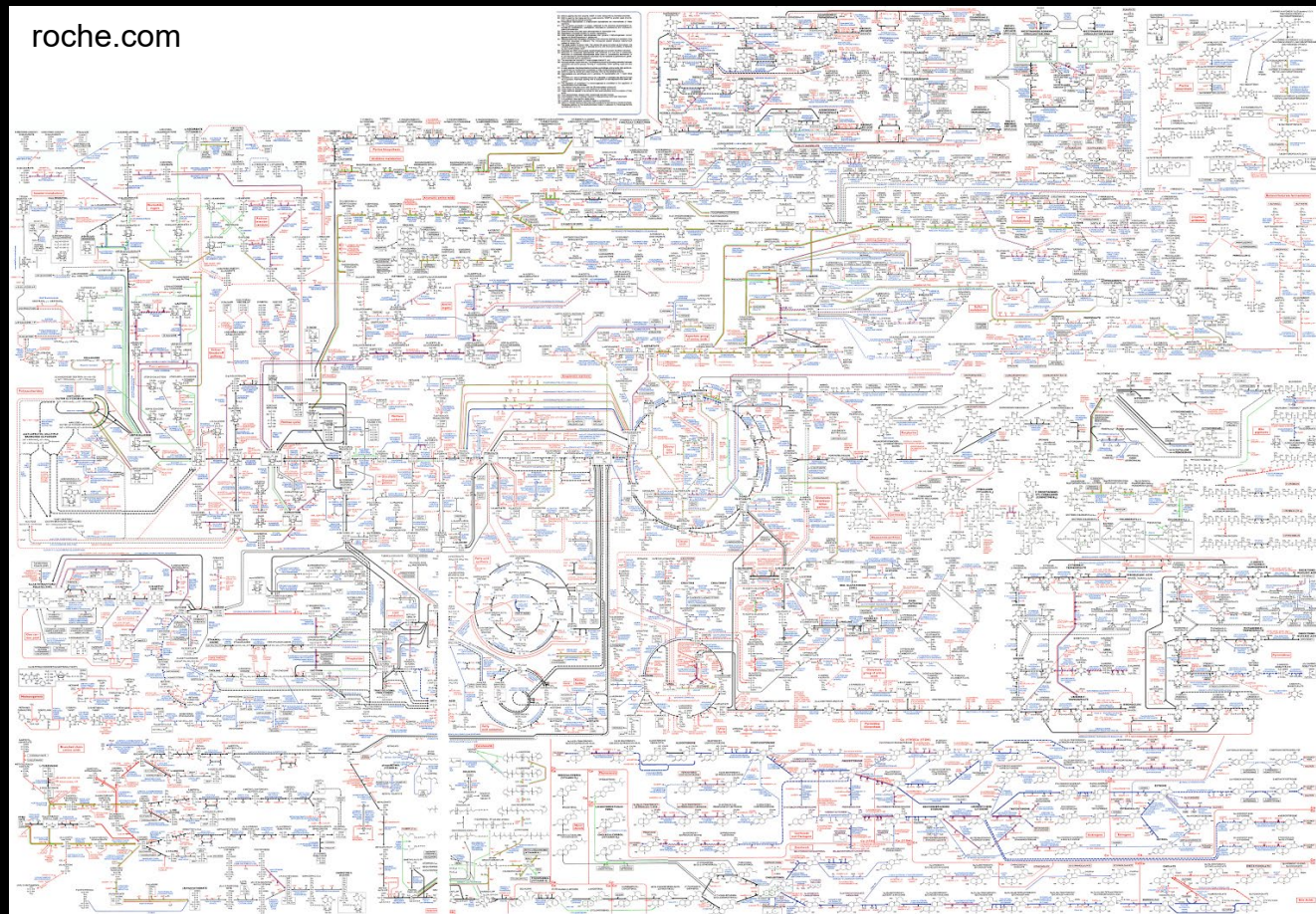
A series of chemical reactions forms a pathway that is used for a specific purpose – such as transforming nutrients into energy



What are Metabolic Models

Complex organisms like plants or animals have thousands of interconnected chemical reactions and pathways

Metabolic models are simulations of all of those interconnected reactions and pathways



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What Can Metabolic Models be Used For

Metabolic Models are not a commonly used method – because it takes a diverse skill set to both create and use them – but they are very powerful and they can be used for many purposes:

Identifying nutrients that accelerate growth rates of organisms

Identifying genetic modifications that accelerate growth rates

Obtaining a much greater understanding of biological processes such as drought tolerance

Identifying new strategies to kill organisms – which is being used to create new drugs for antibiotic resistant microbes

Many others

Presentation Outline

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Metabolic Models & Growth Rates

In the following – I will first provide a general example of how metabolic models can be used to examine growth

I will then describe how our team is using them for this purpose

To date – the most common use of metabolic models has been to understand growth & how to increase growth rates of organisms

For example – we all know people that eat very little and still gain weight – and people that eat an enormous amount and gain no weight – life is certainly not fair

But why does this occur?

Metabolic Models & Growth Rates

One reason could be how effectively a person digests food

Digestive Enzymes →

- Carbohydrates
- Fats
- Protein

The heavier person might more effectively digest food – leading to more nutrients entering the body

The skinnier person might digest food less effectively – leading to less nutrients entering the body

Metabolic Models & Growth Rates

Another reason could be how efficiently a person stores nutrients in the body

Carbohydrates
Fats
Protein

Nutrient Storage Enzymes →

The heavier person might more efficiently store nutrients in body

The skinnier person might store nutrients less efficiently in body

In sum – the variables that affect growth include all the chemical reactions in the body and all of the other processes that are simulated by a metabolic model

By collecting gene expression data from the two conditions above – heavier and skinnier – and analyzing it within the model – scientists can obtain a global understanding what is causing these differences and what can be done to change the outcomes

Our Work – Metabolic Models & Growth Rates

The goal of this project is to demonstrate that metabolic models can be used to accelerate plant breeding and engineering

Given that these activities can take many years – anything that can reduce the amount of time will be beneficial

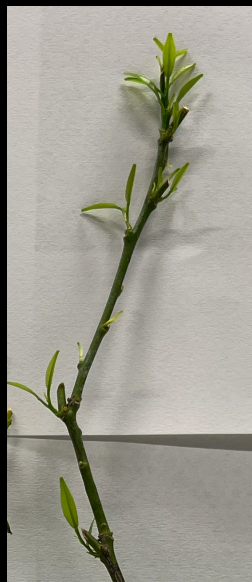
Our first project in this area is with citrus

We have recently completed the construction of the first citrus metabolic model – which contains 10,493 chemical reactions

We are now performing experiments to validate the model – comparing standard cultivation media to model enhanced media

Standard Media

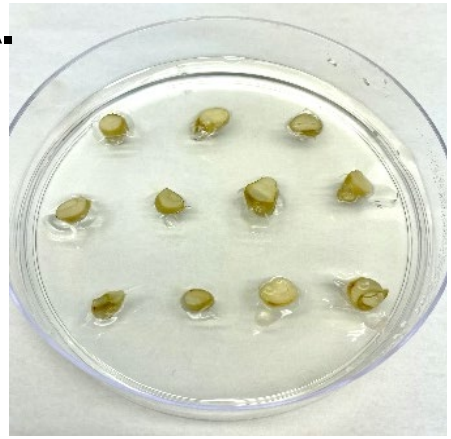
Model Media



Our Work – Metabolic Models & Growth Rates

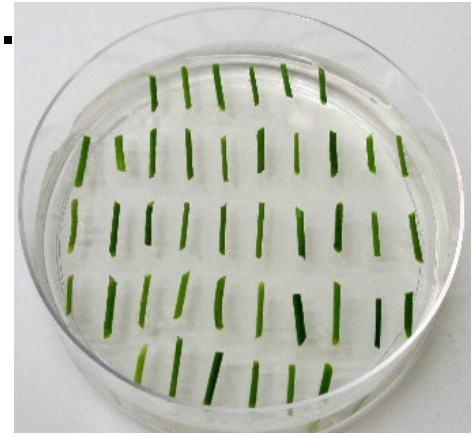
Our **Proof of Concept Project** is to accelerate two steps in the citrus engineering process using Washington Navel and Carrizo

A.



B.

C.



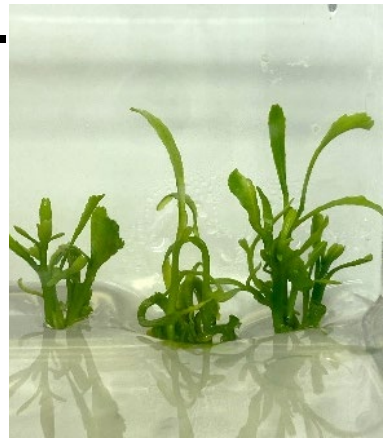
D.

~6 Weeks **~4 Weeks**

E.

~1 Day

F.



~6 Weeks

G.

~8 Weeks

H.

+12 Weeks

Our Work – Metabolic Models & Growth Rates

If we are successful in this project – we expect that we will have provided a blueprint for our team and others to use metabolic models to accelerate:

1. Breeding of different citrus varieties and rootstocks

2. Engineering of different citrus varieties and rootstocks

In addition – a recent study showed that the accuracy of molecular markers used in breeding – was improved by over 50% when a metabolic model was incorporated into the breeding process – doi.org/10.1038/s41467-020-16279-5

Presentation Outline

What are Metabolic Models

What Can Metabolic Models be Used For

Accelerating Growth for Citrus Breeding and Engineering

Understanding Biological Processes for Drought Tolerance in Citrus and HLB Tolerance

Metabolic Models & Understanding Biological Processes

The second most common use of metabolic models is to obtain a greater understanding of biological processes

One general example is some people lose their hair and others don't

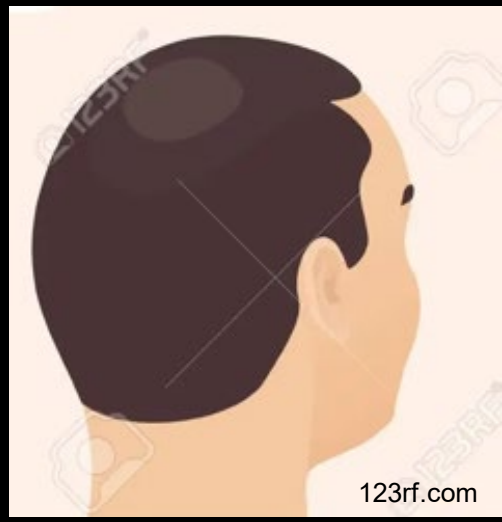
But why does this occur?

Metabolic Models & Understanding Biological Processes

One reason could be how effectively one is producing hair

Carbohydrates
Fats
Protein

→ **Normal Amounts** of Hair Production Enzymes



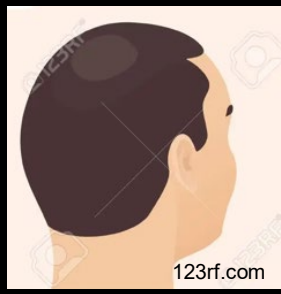
Carbohydrates
Fats
Protein

→ **Low Amounts** of Hair Production Enzymes

Metabolic Models & Understanding Biological Processes

Carbohydrates
Fats
Protein

Normal Amounts of Hair Production Enzymes →



Carbohydrates
Fats
Protein

Low Amounts of Hair Production Enzymes →

In the example above – we want to find the genes or pathways that make the hair production enzymes

The problem is – **current state-of-the-art methods often cannot detect these genes and pathways**

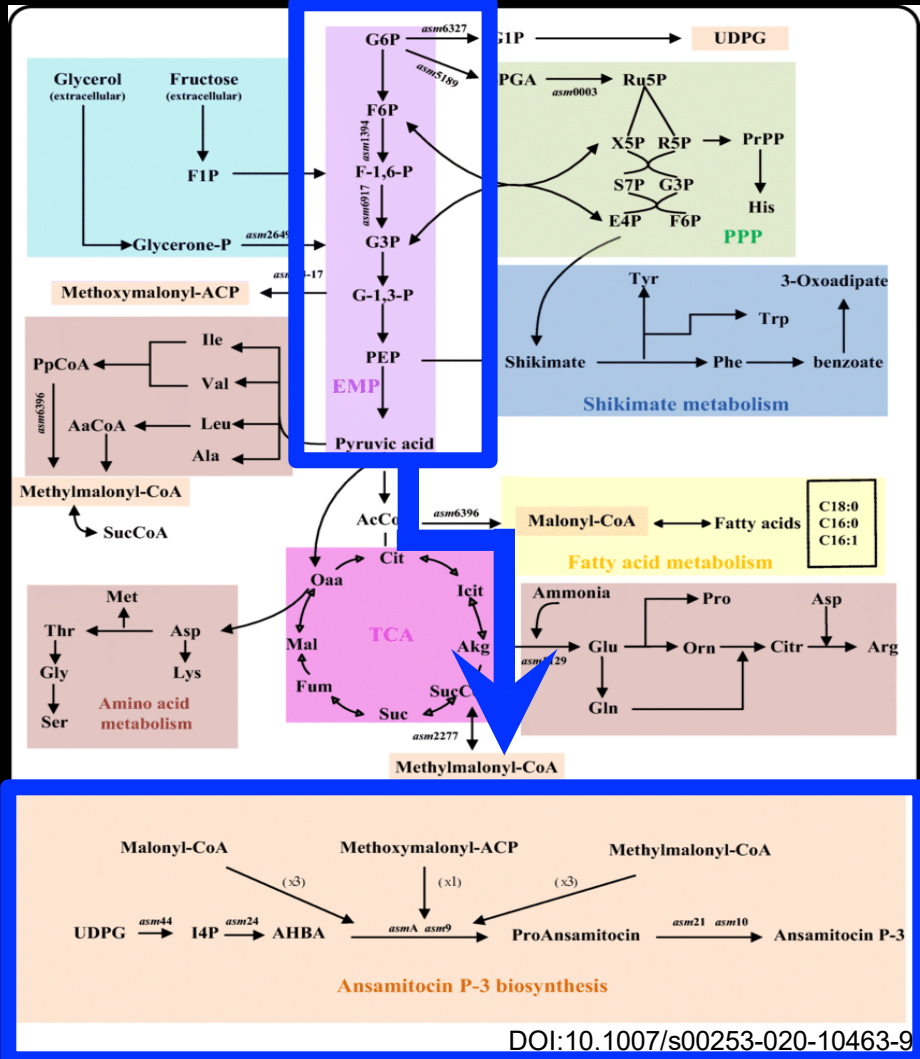
The reasons for this are complicated and involve different ways genes are regulated – a good explanation is in the following – doi:10.1371/journal.pgen.1002887 – and/or we could talk off line

Metabolic Models & Understanding Biological Processes

The good news is that metabolic models can be used to find such genes – and the feature that allows metabolic models to do this is the interconnected pathways

For example – lets pretend the reactions at bottom of this figure make the key hair production enzymes – and they are not detectable by current methods

Because the reactions at the top are detectable – and they are linked to the hair production enzymes at the bottom – we can use the amount of the top reactions to predict the amount of reactions at bottom



DOI:10.1007/s00253-020-10463-9

Our Work – Metabolic Models & Understanding Biological Processes

We are using this approach in two projects that are examining

1. Drought Tolerance in Citrus

2. HLB Tolerance in Citrus

Our Work – Metabolic Models & Understanding Biological Processes

The Goal of the Drought Tolerance Project is to – first obtain a more thorough understanding of drought tolerance in citrus – and then use that knowledge to create drought tolerance in commercially relevant varieties in California

Drought tolerance is likely to be important because

This current drought in California is predicted to last until at least 2030 – https://doi.org/10.1038/s41558-022-01290-z

Water shortages caused by climate change are predicted to cause considerable agricultural losses in California including yield reductions of 20% to 40% in avocados, oranges, walnuts, almonds, and table grapes

– https://calclimateag.org/climatethreatstoag/

Our Work – Metabolic Models & Understanding Biological Processes

This project is currently in the planning phase

Our plan is to leverage a prior study that measured the drought tolerance of 12 different citrus rootstocks

– doi:10.1016/j.scienta.2011.03.039

We will then use metabolic models to identify the genes and pathways that enable certain rootstocks to have more drought tolerance than others

We expect that our team and others will be able to use that knowledge to increase the drought tolerance in commercially relevant citrus varieties using at least two different strategies

Our Work – Metabolic Models & Understanding Biological Processes

Those Strategies will likely include

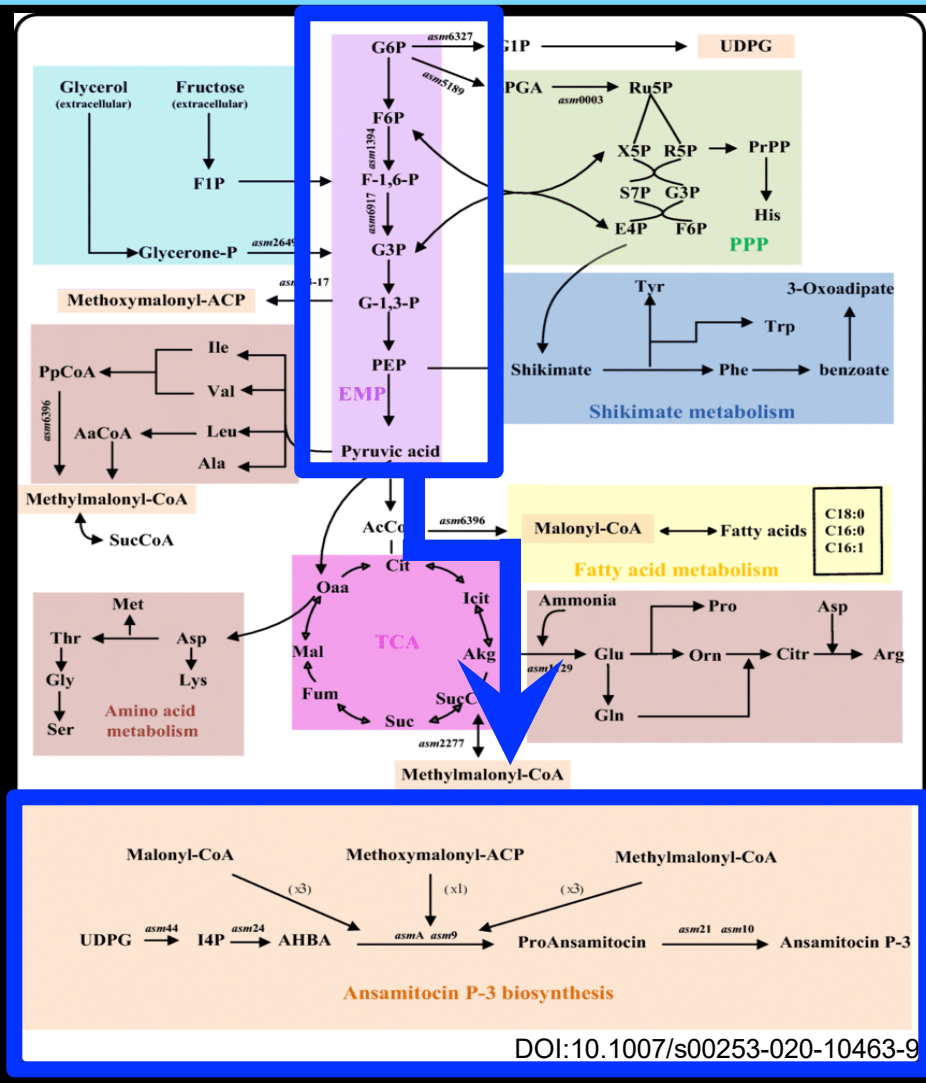
Longer-Term Work that will use our newly obtained knowledge to engineer citrus that have increased drought tolerance – for example – by using CRISPR to edit genes

Shorter-Term Work that could involve manipulating pathways to increase drought tolerance – for example – by adding nutrients that redirect existing pathways involved in drought tolerance

Our Work – Understanding Biological Processes

Shorter-Term Work

Using the example we discussed earlier – because pathways are interconnected – it may be possible to add nutrients that have downstream effects on pathways involved in drought tolerance



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DOI:10.1007/s00253-020-10463-9

If we are successful in this project – we expect that we will have provided a blueprint for our team and others to use metabolic models to create additional drought tolerant varieties of citrus

Our Work – Metabolic Models & Understanding Biological Processes

In our second project using this approach – the Goal is to obtain a thorough understanding of the Survivor Tree Phenotype – and then use that knowledge to create HLB tolerance in commercially relevant citrus varieties

Huanglongbing – or HLB – is a disease that is causing large losses in Florida – and it has the potential to cause considerable damage to the Californian citrus industry

Our Work – Metabolic Models & Understanding Biological Processes

The origins of the Survivor Tree Phenotype involved observations by growers – where most trees quickly succumb to HLB – but a very some number of trees remain relatively healthy

– Wang and Gmitter, Citrus Industry, July 2014, pages 16-17

Our team – led by Georgios Vidalakis – wanted to determine whether these observations were valid

We made annual trips to Florida – where we rated the same trees in several orchards to determine if some of them did not exhibit the normal HLB-associated decline

Our Work – Metabolic Models & Understanding Biological Processes

After seven years of this study – we have identified a small number of trees in one orchard that not only did not decline – but they have actually become healthier – moving from 3 to 1.5

Our Work – Metabolic Models & Understanding Biological Processes

The question then became – what is causing this phenotype?

Genetics is not likely involved in this phenotype – because citrus trees in orchards contain the same scion & rootstock

We hypothesized that the most likely cause of this phenotype is – differences in the tree-associated microbes and the products that they are making

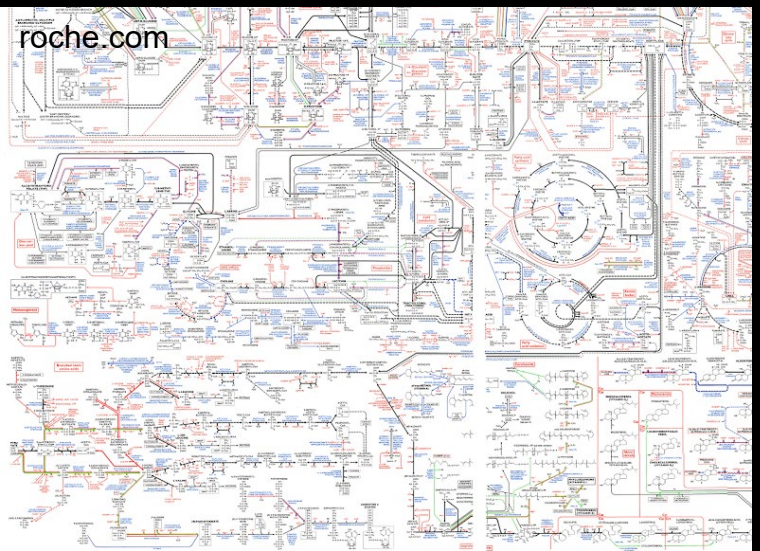
We are using a model-based approach for this project – but it is slightly different than what we have discussed so far

Our Work – Metabolic Models & Understanding Biological Processes

In this work – we are constructing metabolic models of the microbes most likely involved in the Survivor Tree Phenotype

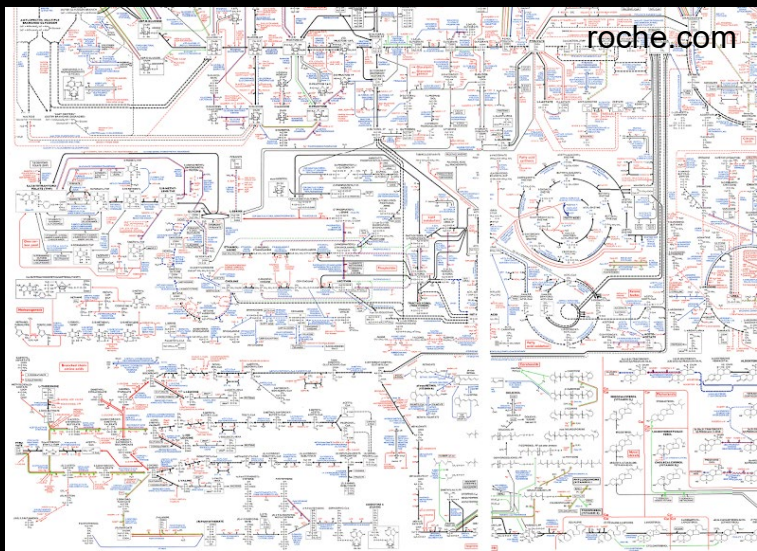
We will then create an "interaction model" with citrus – which will enable us to identify the molecules that are being transferred between these two organisms – which we posit will include molecules from the microbes that are causing phenotype

Microbe Metabolic Model



Exchange of Molecules

Citrus Metabolic Model



Our Work – Metabolic Models & Understanding Biological Processes

Thus far – we have identified several microbial molecules that could be responsible for the Survivor Tree Phenotype

We are currently working on testing these findings by expressing these molecules in citrus exposed to the HLB-associated pathogen

Summary

Metabolic models are powerful tools – and we are attempting to use them in Several New Ways:

We are using them to accelerate citrus breeding and engineering

We are using them to create new strategies to make more drought tolerant citrus

We are using them to create HLB tolerant citrus

Thank You for Your Time -- Questions?