TE: Think of Next Experiment

Generate Questions and Problems

1. How effective would phage therapy be in preventing disease incidence after multiple generations of tomato plant growth?

   Brainstorm: Perform the same experiment as was conducted in the greenhouse initially, but continue planting the tomato seeds for at least five generations. Since this study showed that phage resistance in *R. solanacearum* can occur quite rapidly after just the growth of a single tomato plant generation, it'd be interesting to see if this trend continues when adding more ancestral phages per generation. Could the pathogen evolve to be phage resistant and virulent as well in this shifting rhizosphere community?

2. Would the efficacy of the combination phage therapy improve if more genetically varied phages were used in the experiments (instead of >99% genetically similar phages used in this study)?

   Brainstorm: This would require the collection of soil samples from tomato fields, sequencing these soil samples for phages, and then performing a streak assay against *R. solanacearum* to see if the novel phage is able to properly infect, lyse, and inhibit pathogenic growth. If these phages exist or are sequenced and identified through this process, this experiment in the greenhouse can be repeated but with phages that are much more genetically different than over 99%. Perhaps this will reveal results that differ from those found in this study; can also pair this idea with the multigenerational study idea.

3. What is the mechanism behind how the phages used in this study infect and kill the pathogen?

   Could involve sequencing pathogens that are phage-resistant and comparing these sequences to wildtype pathogens. Study could include TEM images of wildtype and phage-resistant bacteria with the sequenced information to attempt to elucidate mechanisms of what is required for phage infection and what is selected in phage resistance.

4. Based on the discovery of certain bacteria that seem to be antagonistic toward the pathogen, how would disease incidence decrease if batches of these bacteria were added to the tomato plant rhizosphere instead of the phages?

   Instead of introducing the phages used in the rhizosphere, researchers could introduce the bacteria that was enriched by the phages. Even though the researchers claimed bacterial inoculants could be ineffective, it would be interesting to explore these bacteria since they appear to be native to the tomato plant rhizosphere and seemed to be somewhat responsible for limiting growth of the pathogen alongside phage treatment.

5. Why were only three phage combination therapies used in the greenhouse experiment rather than four-phage combinations when four different phages were available, and four phage therapy combinations were tested in the field experiment?

   This experiment would simply be addressing a gap that I found with the first greenhouse experiment. Since the researchers had four different phages at their disposal, and they used four phages in their field experiment, I would like to see the results of using these four phages in the greenhouse as well. Perhaps the results would follow the observed pattern that researchers showed in their figures, but it would still be informative to perform this experiment as extra exploration into more phage combination.

Design New Experiments

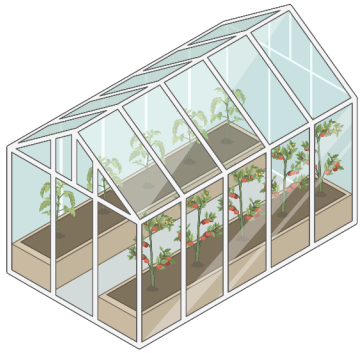
1. **Observing Disease Incidence Over Multiple Generations of Phage Therapy** (see methods flowchart)

   The experiment in the greenhouse researchers performed only involved one generation of tomato plant growth and interaction in the rhizosphere. As a future direction, one could perform the same initial experiment as the researchers, but continue this for at least five generations. In this future direction, researchers would continue raising tomato plant offspring in the same soil rhizosphere. For each offspring/generation, the ancestral phage combination could be added to the soil. After these generations, researchers could again analyze the soil samples and perform tests that look at pathogen phage resistance. It would be interesting to see if disease incidence is still lowered through this time when compared to the control with no phage treatment, and if the pathogen is able to evolve phage resistance in addition to increased virulence within the community during this time.

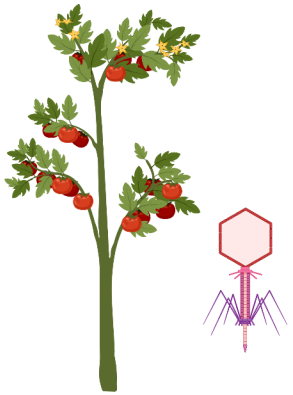
2. **Pairing Phage Therapy with *R. solanacearum* Antagonistic Bacteria Found in Study** (see methods flowchart)

   Instead of introducing only the phages used in the rhizosphere, researchers could introduce the bacteria that was enriched by the phages. These bacteria were found to inhibit the growth of the pathogen in competition assays. Even though the researchers claimed bacterial inoculants could be ineffective, it would be interesting to explore these bacteria since they appear to be native to the tomato plant rhizosphere and seemed to be somewhat responsible for limiting growth of the pathogen alongside phage treatment. By pairing the two in a greenhouse experiment from the start, disease incidence may be negatively affected even more. With that, this could be a way to reduce phage resistance as the pathogen will also have to compete for nutrients with the increased count of these antagonistic bacteria. For the experiment, instead of just adding the phage combinations, one would add these antagonistic bacteria. After a period of growth (~45 days), researchers could analyze pathogen resistance and the composition of the rhizosphere community.

Future Direction #1: Observing Disease Incidence Over Multiple Generations of Phage Therapy



Initially grow tomato seeds with exposure to *R. solanacearum* per study, and expose to the combination phage therapies with one control without phages (Same as paper's procedures)-- Done in greenhouse so conditions are consistent



Continue growing tomato plants to produce offspring: grow offspring in same soil and reintroduce ancestral phages according to what phage treatment was initially given

After 5+ generations...

Sample rhizosphere from each planter box: isolate bacteria and phages present in sample

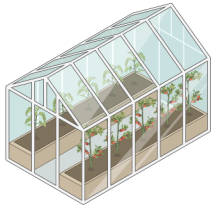
- Test for phage resistance of pathogen

- Test microbiome diversity

- Record disease incidence and see if pathogen is more virulent AND phage resistant after these generations

Research Question/Goal: Could the pathogen evolve to be phage resistant and virulent as well after generations of exposure to phage in the rhizosphere community? Explore effectiveness of phage therapy in a controlled envrionment after multiple tomato plant generations.

Pairing Phage Therapy with *R. solanacearum* Antagonistic Bacteria Found in Study



Plant tomato seeds in separate planter boxes in greenhouse for controlled environment

Experimental: Add pathogenic *R. solanacearum* to soil then add both phage combinations and amounts of the antagonistic bacteria

Controls:
1. no treatment
2. add only phages
3. add only antagonistic bacteria

after 45d

Obtain soil rhizosphere sample and isolate *R. solanacearum* while freezing more sample for subsequent analyses

Calculate disease incidence

Measure phage resistance in pathogen

Record composition of rhizosphere for all groups

Research Goal: Explore addition of antagonistic bacteria to phage therapy treatmentsince they appear to be native to the tomato plant rhizosphere and seemed to be somewhat responsible for limiting growth of the pathogen alongside phage treatment. Perhaps, see if this increases efficacy of treatment and control of disease incidence.