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Overall research question: How do phages that infect the pathogen *R. solanacearum* responsible for bacterial wilt disease in tomato crops affect the disease in hosts affected by the pathogen and by what evolutionary and ecological mechanisms do these effects take place.

Overall hypothesis: Since phages can affect bacterial proliferation through both killing the host bacterium or necessitating evolutionary trade offs that impact the ability to intake nutrients or perform metabolic functions, if the inoculation of tomato plant rhizospheres affected with *R. solanacearum* with phages targeting that host has a robust ecological effect that benefits the health of the plant, then there will be a decrease in incidence of disease and an increase in overall microbial diversity that correlates with increased unique phages used for inoculation.

Experiments:

- 1.) This experiment sought to determine the effects on disease incidence in tomato plants affected by bacterial wilt disease in response to several combinations of four phages that were determined to infect the host bacterium *R. solanacearum*. This was accomplished by applying these phages to the roots of affected plants in both a greenhouse and field setting to measure the effect in both a controlled and practical environment.

Type: experimental test

- 2.) This experiment sought to determine the development of phage resistance in coevolved bacteria across experimental groups inoculated with varying amounts of phage by culturing the evolved phages in and out of the presence of the ancestral phages they were inoculated with to determine what effect phage therapy had on resistance development.

Type: experimental test

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2.2) This experiment sought to determine the effect on carrying capacity of pathogenic bacteria by the number of phages used in inoculation by culturing these bacteria without the presence of phage and comparing them to their ancestral form.

Type: experimental test

3.) This experiment sought to determine the bacterial composition of the rhizosphere and to characterize its changes in response to phage therapy, specifically as a result of the decreased *R. solanacearum* that is a direct consequence of increased phages used. This was determined by purifying soil samples and using bacterial rRNA to determine the concentration of each taxon.

Type: descriptive study (?)

4.) This experiment sought to determine whether non-*R. solanacearum* taxa were affected by the presence of phage in order to better characterize their ecological interactions with phages and other bacteria. This was done by culturing the bacteria alone, in the presence of the phage, in the presence of the pathogen, and in the presence of the phage and the pathogen.

Type: experimental test

5.) This experiment sought to determine the statistical significance of each mechanism through which phage treatment acts on the environment and affects the ecology of the rhizosphere.

Type: descriptive study

Hypotheses:

1.) Since phages decrease the population of their host species by inhibiting their ability to proliferate at their normal rate and forcing them to divert energy and resources towards

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phage management rather than growth, if more phages are used in conjunction with each other as a treatment for *R. solanacearum* in tomato plants then there will be a decrease in disease incidence.

- 2.) Since bacteria develop resistance to phages and this development is the result of random genetic mutations, if bacteria are treated with multiple phages then they will be less effective in developing phage resistance. (note: this hypothesis was not entirely correct)
- 2.2) Since the development of phage resistance can require evolutionary trade-offs that sacrifice other beneficial traits for increased resistance, if bacteria are treated with more phages and develop a more general phage resistance then the carrying capacity of these bacteria will be reduced even in the absence of phage.
- 3.) Since the phages in question decrease the amount of *R. solanacearum* in competition with other bacteria, if phages cause an increase in the concentration of those taxa with the greatest direct competition with *R. solanacearum* then there will be an increase in bacterial diversity.
- 4.) Since the phages in question target the bacterium *R. solanacearum* as their host and have been observed to increase the success of many other taxa, if the phages do not have an effect on bacteria other than *R. solanacearum* then there will be no significant decrease in bacterial diversity when cultured in the presence of phage.
- 5.) Since phage therapy has been demonstrated to have several effects on the rhizosphere of the plant on which it is applied, if phage has an impact on disease incidence and non-pathogenic microbial diversity then a statistical analysis will reveal significance.