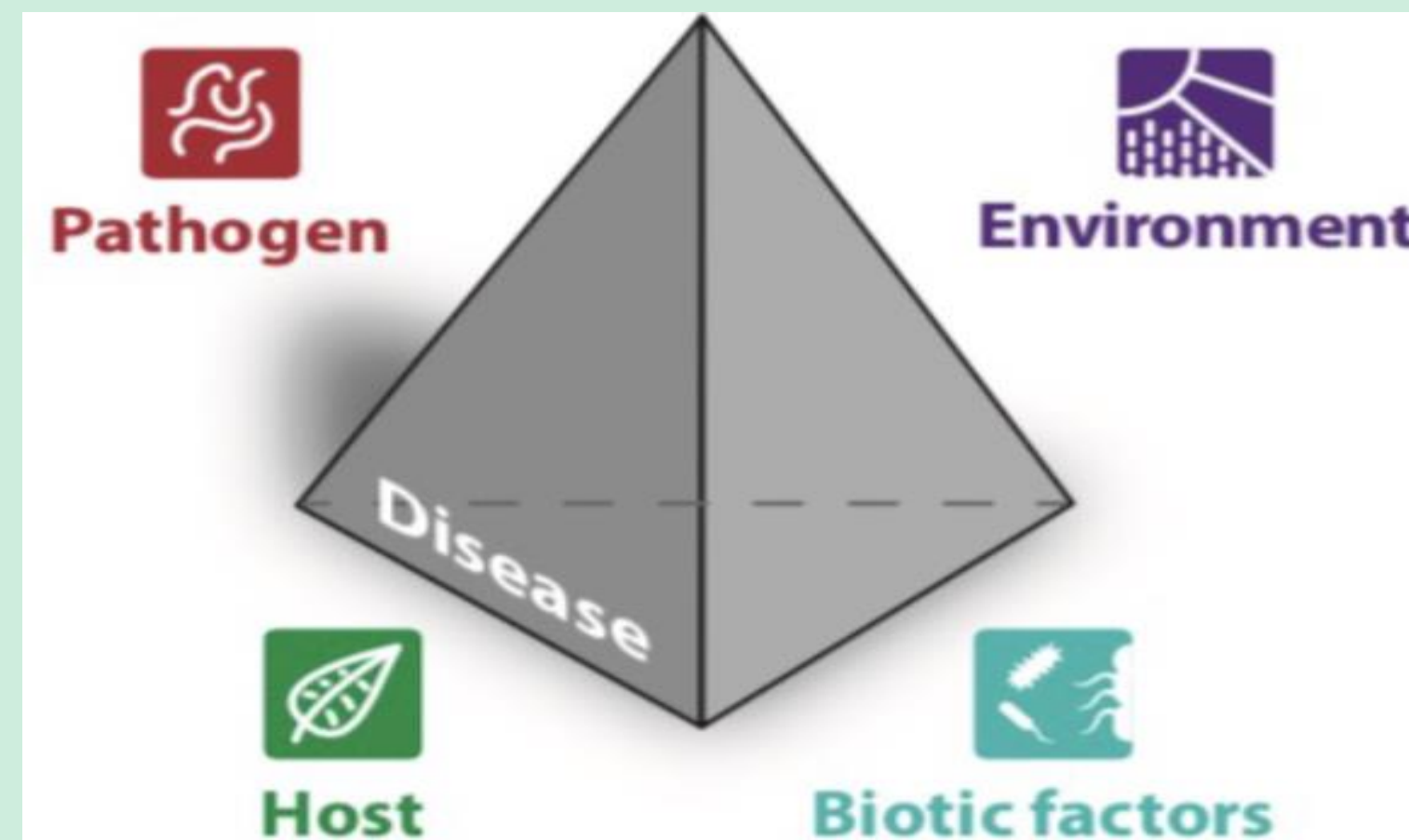


Introduction

A microbiome includes all of the microorganisms around an environment. All plants have evolved to form an intimate relationship with microorganisms, leading to their dependence on the microbes around them. In the disease triangle (pictured right) the pathogen, environment, host, and biotic factors all contribute to the disease. By studying these components separately, more knowledge is gained about the disease as well.

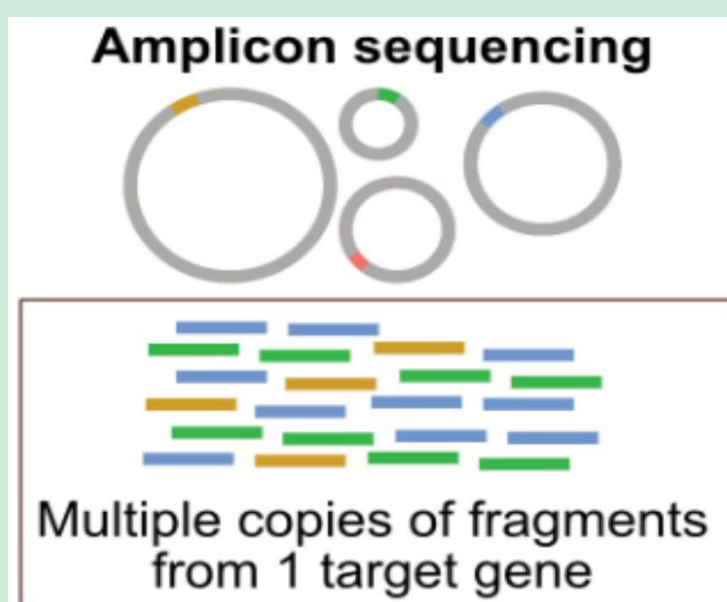


Objective

To gain a greater understanding of the different aspects of plant pathogens; how they function, where they arise, and how they evolve to infect multitudes of hosts. This knowledge will then be used to create different techniques and treatments to protect the crops and help them fight off future infections.

Abstract

Soybean plants are the 3rd highest crop worldwide, thus the study of their microbiome is useful in protecting this needed source. DNA amplicon sequencing was used on the Internal Transcribed Spacer region and 16s rRNA genes to study the fungal and prokaryotic communities around soybean plants. Soybean plants were sampled from conventional, non-tilled fields throughout the growing season. Primers were prepared for PCR, and the amplification of all the microbiome genome occurred together. Then, the results were sequenced to identify the individual microbes present in the soil.

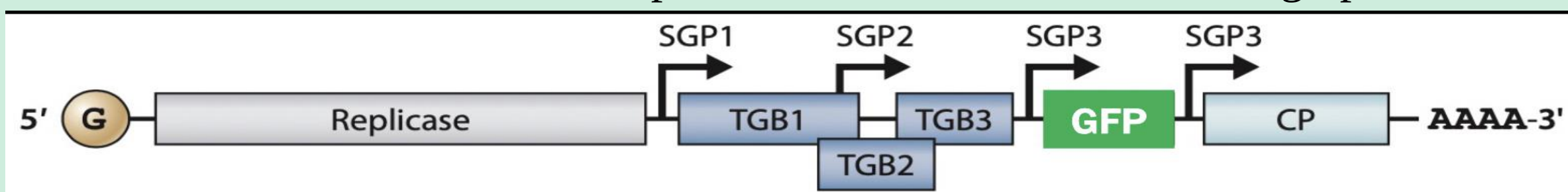


(Pictured: process of amplicon sequencing. This sequencing allows for mass production of one specific gene. This technique was used in this experiment to sequence the multitudes of fungi present in soybean soil.)



(Pictured: culturing of Sporobolomyces OTUs on agarose plates. The results showed that Sporobolomyces increased in abundance only soybeans.)

The RNA genome of the citrus tristeza virus is one of the most harmful diseases to citrus plants, quickly spreading and destroying orchards across Florida. In this work, the p33 protein, a nonconserved protein of this virus, was observed for its interactions within the host. The genes coding for the p33, CP, p20, p23, and other proteins were amplified by PCR and introduced into Stellar competent cells. These cells were cultured and screened for the presence of these cloned genes through restriction enzyme digestion. Once confirmed, the proteins were tagged with fluorescent markers to indicate location within the cells and to show interaction between the different proteins present in these cells. Coimmunoprecipitation assays were used to indicate where the fluorescent markers on these proteins were located, all showing up in the CTV replication factories.

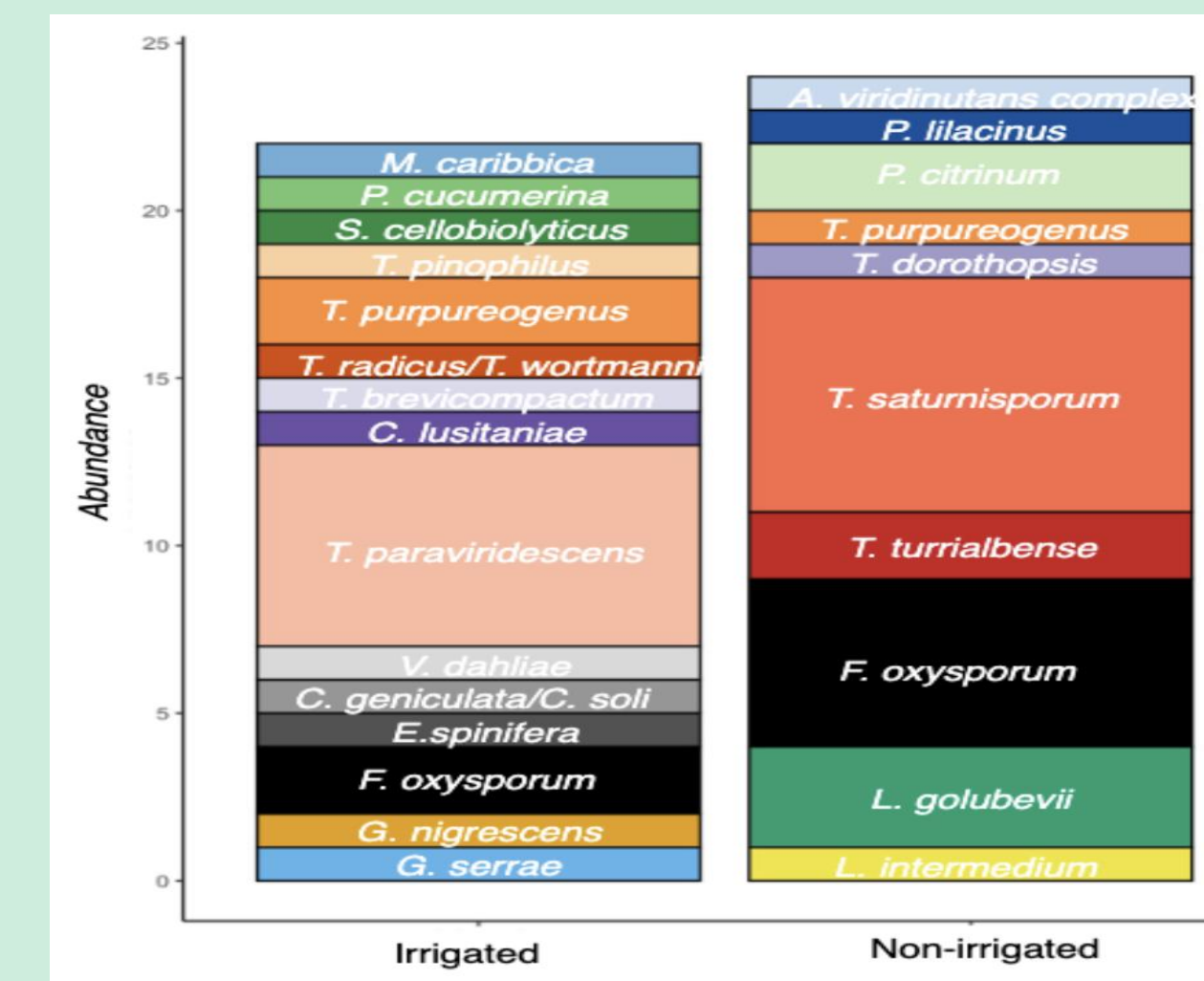


(Pictured: RNA sequence of CP protein with the fluorescent marker attached.)

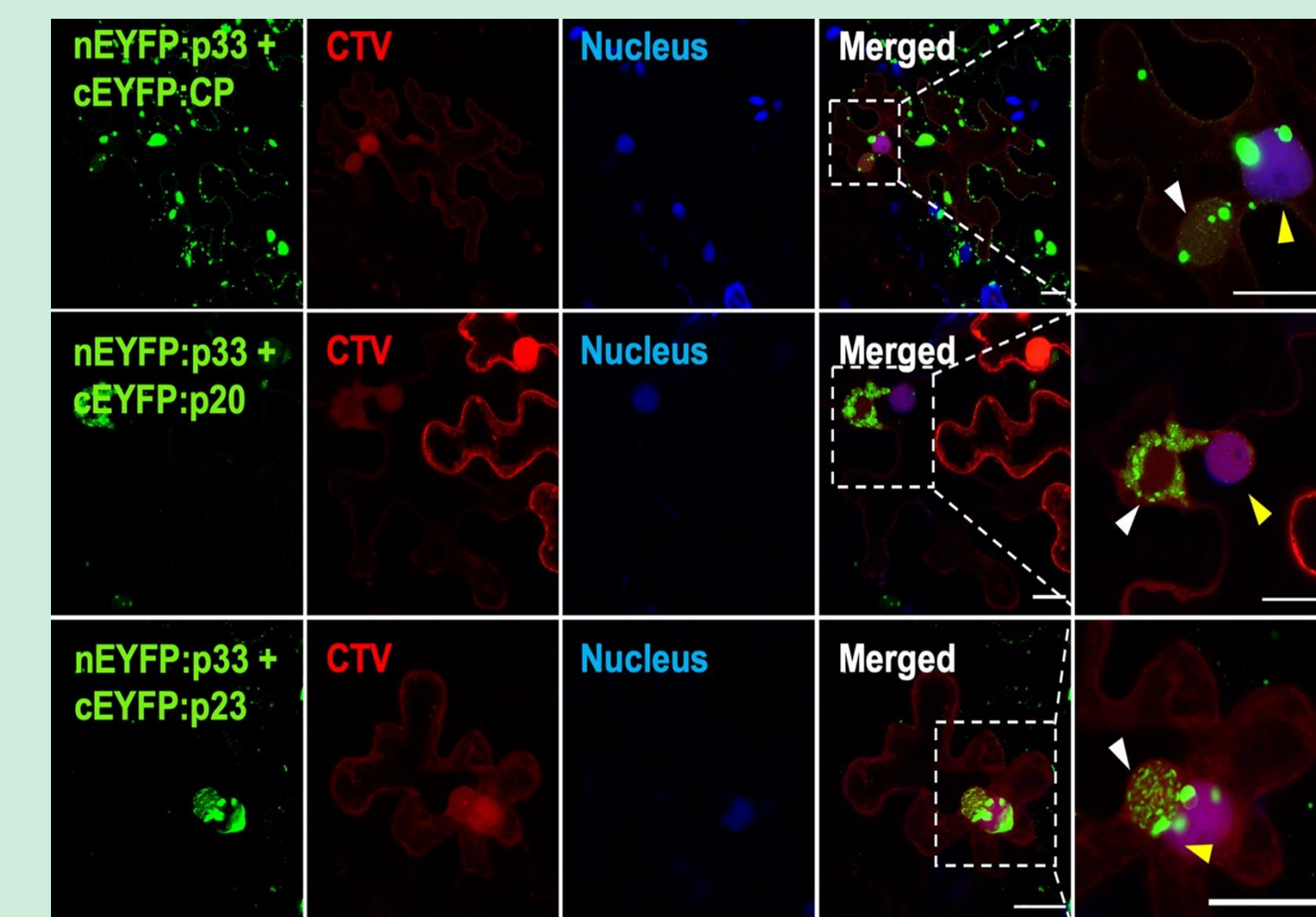
Results

The results showed that even though location created the greatest influence in microbial diversity, management of land and current plant stage contributed heavily as well. When the land was not tilled, beneficial organisms, including *Bradyrhizobium*, began to show up again. This research demonstrated that long term crop management techniques alter not just the plant's growth, but the microbiome around it.

The results also showed that p33 is an integral membrane protein, and this action allows it to extend its host range easily. It also is key for CTV infection, as it can control the host's immune response. P33 will interact with the viral proteins CP, p20, and p23, leading to faster infection and extension of host range. These interactions will also hinder the protection mechanism of RNA silencing within the host, thus making the infection worse.



(Pictured: results of testing for fungal presence in soybean fields, depicting the different fungi that are present depending on if the soil was irrigated for the crops (Noel et al., 2020))



(Pictured: fluorescent imaging depicting where p33 interacted with the CP, p20, and p23 proteins (Dao et al., 2020))

Future Direction

Understanding how viral proteins interact is one step towards understanding how viruses infect their hosts. This study revealed previously unknown interactions between non-related proteins and their effect on RNA silencing, creating a new focus on plant treatment to fight against this suppression. These results need further research to better understand how these proteins control the virus-host reactions in the CTV pathosystem. By targeting the mechanic used to suppress RNA silencing, a new treatment can be developed to stop the epidemic of CTV in citrus groves.

Through learning that irrigation can cause certain pathogens to arise, further research can be conducted in determining the appropriate amount or technique for irrigation should be used to keep the microbiome healthy for plants. Potentially, with the correct use, irrigation can be used to protect the plants by giving rise to healthy fungi that benefit the crop's growth.

This research could also lead to the study of the pathogens that arise from irrigation to understand what encourages their growth. Once this cause is found, the irrigation method could be modified to avoid this trigger for the harmful pathogens.

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