Does *Phyllocoptes fructiphilus* harbor the Rose Rosette Virus?

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ABSTRACT

The Rose Rosette Disease has been a growing virus for the past 72 years. Even though it has been around for a few decades, there is very little known about this disease. The main question asked is how is this disease spread? In an attempt to answer that question, we first have to overcome the hurdle of if enough RNA can be extracted to yield a positive or negative result. For the past 10 weeks, symptomatic rose tissue was collected and tested for Rose Rosette Disease. In addition, mites were searched for on the tissue in hopes of discovering a vector.

Introduction and Objectives

Since 1940, the species of the wild rose, Multiflora Rose, has been highly susceptible to the Rose Rosette Virus (6). Within the past two years, there has been an increased incidence of this disease seen in the Dallas-Fort Worth area. While many factors could contribute to the spread and infectivity of this virus, the rose leaf curl mite *Phyllocoptes fructiphilus* has raised suspicion as a vector for this virus. Because of this, I want to ask the question: do the mites harbor this deadly virus? The results could aid in steps towards the right direction of further research of the disease. Research might someday conclude that toxicogenic reactions from mite feeding cause the Rose Rosette Virus in rose species.

Figure 1: Distribution of known Rose Rosette disease

Methods

A sample survey was collected of different symptomatic varieties of Rose species. Roses were searched for mites, and plant tissue was collected for RNA extraction using Norgen Biotek Viroid RNA kit and liquid nitrogen. The extracted RNA underwent PCR to detect if Rose Rosette Virus was present, and the PCR products were run on a 2% gel by electrophoresis. The first mite extraction trial was unsuccessful. It was conducted using black construction paper to better see the mites. Once the vials were poured onto the paper, the color bled through and the mites were lost. In the second approach, the mites were contained in vials of 95% ethanol (figure 1.2) and placed in a shredder column from a Quiagen blood and tissue extraction kit. After being spun down, the mites were crushed down onto the column and extracted using lysis buffer and the Norgen Biotek Viroid RNA kit.

Figure 1.2 Molly Giesbrecht photo of eriophyid mites under the sepal of a rose. Size less than 0.3 mm long

Results

After collecting thirty rose samples, ten of those samples were positive for Rose Rosette Disease, showing positive bands (figure 1.3). All of the roses positive for Rose Rosette Virus were symptomatic to the disease. Only a few mites could be collected at a time, but later samples revealed numerous numbers of eriophyid mites. The mites, being tiny in size only produced 6.4 ng/mL of RNA, which is miniscule compared to the amount of RNA collected from plant tissue ranging from 120's-500's ng/mL. Due to this factor, the mites were unable to show any bands for RNA to make the test positive or negative for Rose Rosette Virus within the mites themselves (figure 1.4). Refer to Figure 2 and 2.2 for Rose Rosette Variety Results

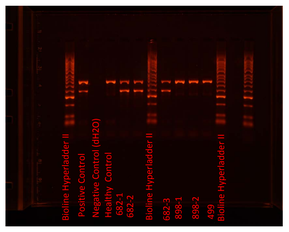

Figure 1.3 First positive reading for Rose Rosette using 2% gel and electrophoresis. 5/2/13

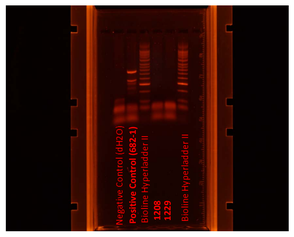

Figure 1.4 Mite extraction on a 2% Agarose gel and electrophoresis. 7/30/13

Conclusion

- The size of the mites made it extremely difficult to extract enough RNA capable of giving a positive reading for Rose Rosette Virus and it is still unknown if the mites are the vector to this virus.
- Using an Arthropod specific primer could help expose the PCR band for the mites.
- Also, there is a need for an abundance of mites (100+) to be able to obtain enough RNA.
- We are still working on a consistent protocol that will be useful for extracting RNA from the mites
- This experience has helped me understand more about viral pathogens that will aid me in my future goals of medicine.

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	Variety	Number of Symptomatic Roses	Positive for RRV
Knockout	12	6	6
Rose	16	8	3
Lupo	1	1	1
Belinda Dream	1	1	0
Koverlandus	1	1	0

Figure 2:Table of Rose Varieties and Rose Rosette Results

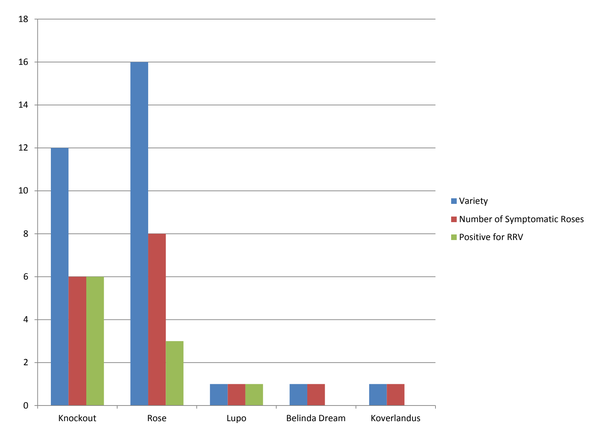

Figure 2.2: Graph of how the different varieties stacked up with the Rose Rosette Results