



**CALIFORNIA STATE SCIENCE FAIR  
2016 PROJECT SUMMARY**

<b>Name(s)</b> <b>Krystal R. Horton</b>	<b>Project Number</b> <b>S0514</b>
<b>Project Title</b> <b>A Simple Field Detection of Citrus HLB Associated Bacteria in Insect Vectors</b>	
<p style="text-align: center;"><b>Abstract</b></p> <p><b>Objectives/Goals</b> Asian Citrus Psyllids infected with the Candidatus Liberibacter bacteria have caused more than \$4 billion damage to the citrus industry in Florida by infecting trees with Huanlongbing or Citrus Greening Disease. It is incredibly important to keep this infestation out of California with its \$2 billion citrus industry. Last year, I developed a test for the bacteria using the LAMP process and the SMART-Dart device. But that process is complicated and expensive. This year, my goal is to develop a test for the bacteria that citrus growers can perform themselves at low cost and high reliability.</p> <p><b>Methods/Materials</b> I studied the process of cutting, amplifying, and replicating DNA sequences from this bacteria to determine if there were any chemical by-products that could be detected with a chemical reaction, preferably with a product visible to the naked eye or possibly under a blacklight. I added primers, enzymes, and nuclease-free water to a sample of pulverized psyllids to extract segments of DNA. After preparation, the extract is heated at 65 degrees Celsius for 20 minutes to amplify the DNA. During this process, a hydrogen ion is released (greatly simplified). When an indicator is added at this step, a color change indicates a positive result. I tested numerous preparations and indicators to get a reliable, accurate result.</p> <p><b>Results</b> Through the use of specifically selected primers for the amplification of the bacteria, I was able to detect a pH change in one step of the process. Using pH indicators, I was able to detect this pH change reliably as well as show that there is no pH change when the bacteria is absent. This process can be conducted with nothing more than a few chemicals, a micro-centrifuge, and a small heater.</p> <p><b>Conclusions/Discussion</b> My goal was to develop a simple, effective test for the bacteria that causes Huanglongbing. Through testing combinations of enzymes, primers, and indicators, I was able to create such a test. This test will help prevent the spread of this disease outside of Florida and protect growers in other states.</p>	
<b>Summary Statement</b> I developed a simple test that a citrus grower could perform to determine whether a psyllid is infected with the bacteria that causes Huanglongbing.	
<b>Help Received</b> Dr. Manjunath Keremane allowed me to use his lab, taught me the LAMP process, and gave me access to the chemicals, psyllid samples, and equipment I needed.	