A Flexible Self-amplifying RNA System for Silencing Plant and Insect Genes to Control Huanglongbing and Other Emerging Threats

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Summary: The purpose of this project is to continue staged experimental field trials in commercially grown citrus that was heavily infected with huanglongbing (HLB), using incrementally improved combinations of dsRNAs and delivery systems to silence several citrus and psyllid target genes that affect transmission and propagation of HLB. Both 'Hamlins' and grapefruits were used in 2020 in a large scale field trial to compare application rates and also application methods (spray/etching vs. injection). Commercially prohibitively high application rates of plain dsRNA applied by spray/etching were required to produce a significant, but transient effect on the host target gene. Although disease indices and fruit yield trended in the right direction, the effects on HLB were not significant for any treatment evaluated. A further large-scale trial in 2021 revealed that the dsRNA formulated into a nanoemulsion demonstrated that five times less dsRNA could be used to achieve the same effects. Some of the dsRNA became phloem mobile, likely through the formation of small interfering RNAs (siRNAs), detected seven days after application, and declining by day fourteen. The host target gene was also suppressed. In an effort to enhance efficacy, a self-amplifying (SAM) messenger RNA (mRNA) was engineered to form siRNAs to silence the original host target gene and injected into sweet orange citrus, where the siRNAs appeared to become phloem mobile and were readily detected in non-inoculated leaves within two days. In preliminary experiments, much lower levels of the SAM mRNA (approximately 50 times) appeared to be needed to suppress the host target gene.

Take Home Message:

- dsRNA applied by foliar spray can achieve temporary, systemic silencing of one or more citrus genes, allowing rapid screening of candidate genes to control HLB.
- Formulations of RNA into nanoemulsions allowed use of five times less dsRNA to achieve the same effect.
- Self-amplifying RNA may allow use of 50 times less RNA to achieve the same effect and much more flexibility in screening new gene targets.

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