MPM

MODIFIED-PEST MANAGEMENT

In the future, each Specific insect could be modified Directly, or the bacteria Inside, to alter the 'pest traits'. So that the insect is ***No longer*** a pest. E.g. Psyllids that are non-Vectors for CLas bacteria

Product Design, Applications for RNA-suppression-Pest Management

RNA interference, Antisense Oligos, Gene editing

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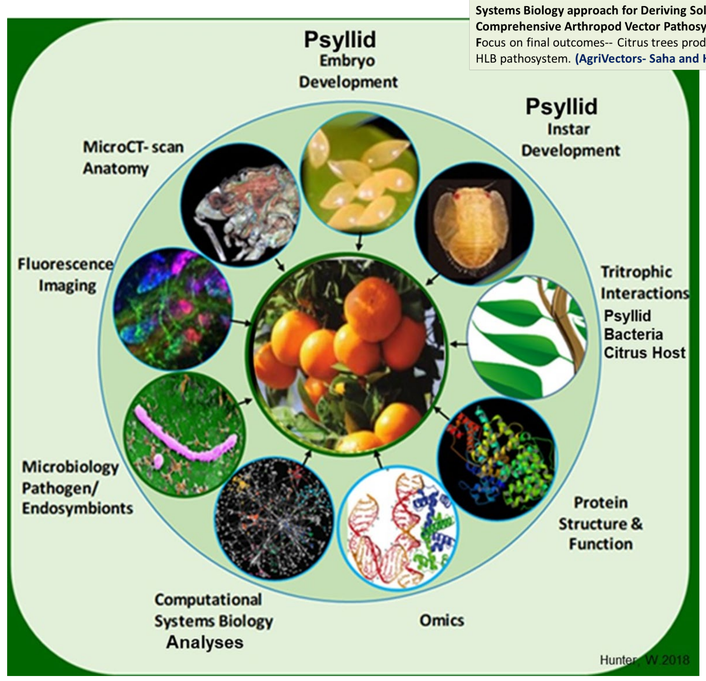
Introduction

- **Multiple technologies for RNA suppression and gene editing have been shown to provide new methods and strategies to reduce arthropod pests and pathogens (*Sinisterra-Hunter and Hunter, 2018*). These technologies have been applied to develop control strategies towards the Asian citrus psyllid (ACP), *Diaphorina citri (Hemiptera: Liviidae)* (*Andrade & Hunter 2017; Hunter &Sinisterra-Hunter 2018*). Population suppression, and preventing bacteria transmission are just two of the objectives researchers have examined (*Hunter et al, 2012; Hunter, Gonzalez, Tomich 2018*). These efforts have advanced significantly with the completion of the psyllid genome (*Hunter et al, 2009, 2014; Arp et al, 2016; Surya et al, 2017, 2018*; www.citrusgreening.org open source database).**
- **Other strategies using antisense oligonucleotides have successfully been developed to target the bacteria (*Candidatus* Liberibacter asiaticus, CLas), in infected citrus trees and psyllids, as well as targeting the endosymbiotic bacteria within the psyllids (*Hunter et al 2017; Pelz-Stelinski et al, 2017; Hunter and Sinisterra-Hunter 2018; Hunter et al, 2019*).**



Systems Biology approach for Deriving Solutions from a Comprehensive Arthropod Vector Pathosystem Model. Focus on final outcomes-- Citrus trees productive under a HLB pathosystem. **(AgriVectors- Saha and Hunter 2019)**

RNA modification, altered gene expression--

RNA interference, RNAi, uses a natural mechanism that recognizes double-stranded RNA, dsRNA, which is the TRIGGER that starts dicing up any complimentary mRNA sequences. This prevents or greatly reduces the protein titer associated with the mRNA target. This disrupts normal biological needs within the insect.

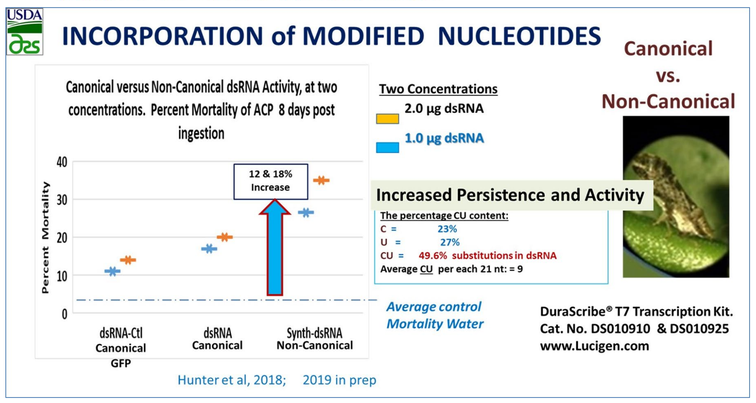
Because this is built upon sequence recognition, RNAi is more specific than broad spectrum insecticides, providing a measure of safety to Beneficial's and other non-target insects.

Example of dsRNA with the C & T replaced with modified, noncanonical nucleotides, Shown in RED. This increases resistance to nuclease degradation, improving activity.

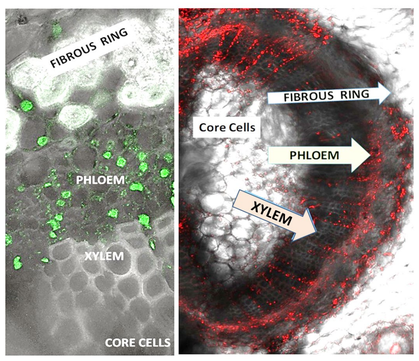
CCAAUCCAUCAAAAUGUGUGACGAUGAUGUAGCGCGUUUGGUCGUGGACAAUGGUUCCGUAUGUGCAAG

GCCGGAUUCGCCGGAGAUGACGCUCCCCGAGCUGUCUUCCCCUCAAUCGUCGUAGACCCCGUCAUCAGG

GUGUCAUGGUGGGUAUGGGUCAAAAAGACUCCUACGUCGGUGAUGAGGCUCAAUCCAAGAGAGGUAUCCU



Hunter et al, 2018; 2019 in prep

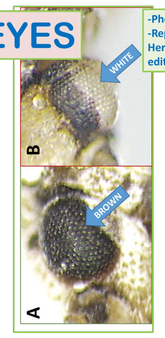


Delivery of molecules into citrus trees and other plants can be tracked by Fluorescently labeling the RNA treatment. *Figure shows -L*eaf midrib systemic movement of the tagged dsRNA in citrus tree and leaves, sweet orange, *Madam vinous* and *Citrus sinensis.* & *Carrizo rootstock. V*isualization of G-tagged dsRNA in the xylem and phloem of treated citrus leaf at 3 days post soil applied treatments. (ULYSIS® Nucleic Acid Labeling Kit [*Thermo* Fisher Scientific]: Molecular Probes cat#U21650].

Methods can detect down to 0.4 picograms Tagged-dsRNA / 1 g citrus leaf tissue, using the BioTek H-1, fluorescent plate reader (Hunter & Sinisterra-Hunter 2018)

CRISPR injection into adult psyllids produced Eye color phenotypes, as well as gene knock outs In the subsequent generations of psyllids.

The method provides a fast method to identify gene functions, And their effect if suppressed on psyllid survival, reproduction, and Pathogen transmission.



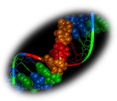
-Phenotype injected Adults
-Repeating to Determine if Heritable Embryonic Gene edit, KO, *Vermilion*

***Peptide assisted* CRISPR Delivery Systems:**

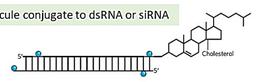
To turn ACP into a non-vector Hunter (USDA) is using a direct Gene-editing CRISPR-Cas9 strategy (*Hunter, Gonzalez, Tomich 2018)* produced 2nd generation mutants, by injecting adult females and nymphs. The gene knock outs greatly reduced survival, and fecundity (A), or alter eye color (B). This is only the 2nd demonstrated method in insects of using adult insects for embryonic gene editing. The other study used yolk peptide, in mosquito (2018).

Other delivery systems being developed: using virus, yeast, bacteria, and a variety of carriers, peptides, lipids, chitin, cholesterols, synthetic and organic nanoparticles, and many others.

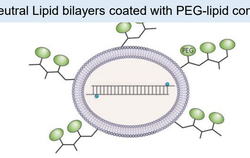
BAPC (Branched Amphiphilic Peptide Capsules)
self-assembling peptide nano-capsular spheres



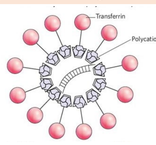
Molecule conjugate to dsRNA or siRNA



Neutral Lipid bilayers coated with PEG-lipid conjugates



Transferrin-cyclodextrin polycation nanoparticle surrounding dsRNA or siRNA



ROUND-Larger Double-hairpins each end- DogBone

FLAT-Double-hairpins each end Covers dsRNA

Two nucleotide overhang, usually GC

Single-hairpin 19 nucleotides Away from dsRNA

Antisense Oligos are single-stranded Duplexes of RNA/DNA and can be from 10 to 27 nt, But are usually around 21nt in length.

Often referred to as Gapmers, Oligos are made in many different forms, With modified linkage groups, and sugars to build a connective backbone that resists nuclease degradation. Some of these need carriers like peptides to enter cells, others Can autodeliver into cells of organisms or into bacteria. These types of molecules provide Treatments that can reduce and kill bacteria inside of citrus trees and psyllids.

Wild Type Psyllid

4 days to hatch | Ave. 12 days to pass thru 1st to 4th nymphal stages | 3.5 days Pupae | Ave. 18-20 days as Adult

TXT-KO Type Psyllid

4 to 6 days to hatch | 15 to 22 days to pass thru 1st to 4th nymphal stage | 4-5 days Pupae | Ave. 8 days as Adult

Longer Development Time, with Increased Mortality — Shortened Lifespan

Reduced Fecundity

16 days X 8 eggs/day = **128 eggs in Lifetime**

Wild Type Psyllid

8 days X (1 egg *every 3 days*) = **~3 eggs over Lifetime**

TXT-KO Psyllid

Kit available- BAPtofect-25™, (Phoreus Biotechnology, Inc., Olathe, KS. https://phoreusbiotech.com

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