



# BAPC-CRISPR/Cas9 System for Heritable Gene-Knock OUT:

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## Asian Citrus Psyllid

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In 2017, the Genome of *Diaphorina citri*, the Asian citrus psyllid, and the Official Gene Set, OGS\_v2 were used to design psyllid control strategies. [www.citrusgreening.org] We used the CRISPR/Cas9, Clustered regularly interspaced palindromic repeats (CRISPR) & CRISPR associated proteins(Cas).

System to disrupt gene expression in psyllids. The targeted gene was the Thioredoxin gene, TRX, in *D. citri*, deletions of 220bp and 505 bp in in the 3' region of the TRX gene.

The knockout psyllids had longer development time (~6 d) longer to eclose to adult, lower fecundity (3-5 eggs/wk/female) versus (2-3 eggs/day/female control); and shorter Adult lifespans post eclosion (8-9 d) versus (11-14 d) of mock injected controls.



Asian citrus psyllid, *Diaphorina citri*, Kuwayama.

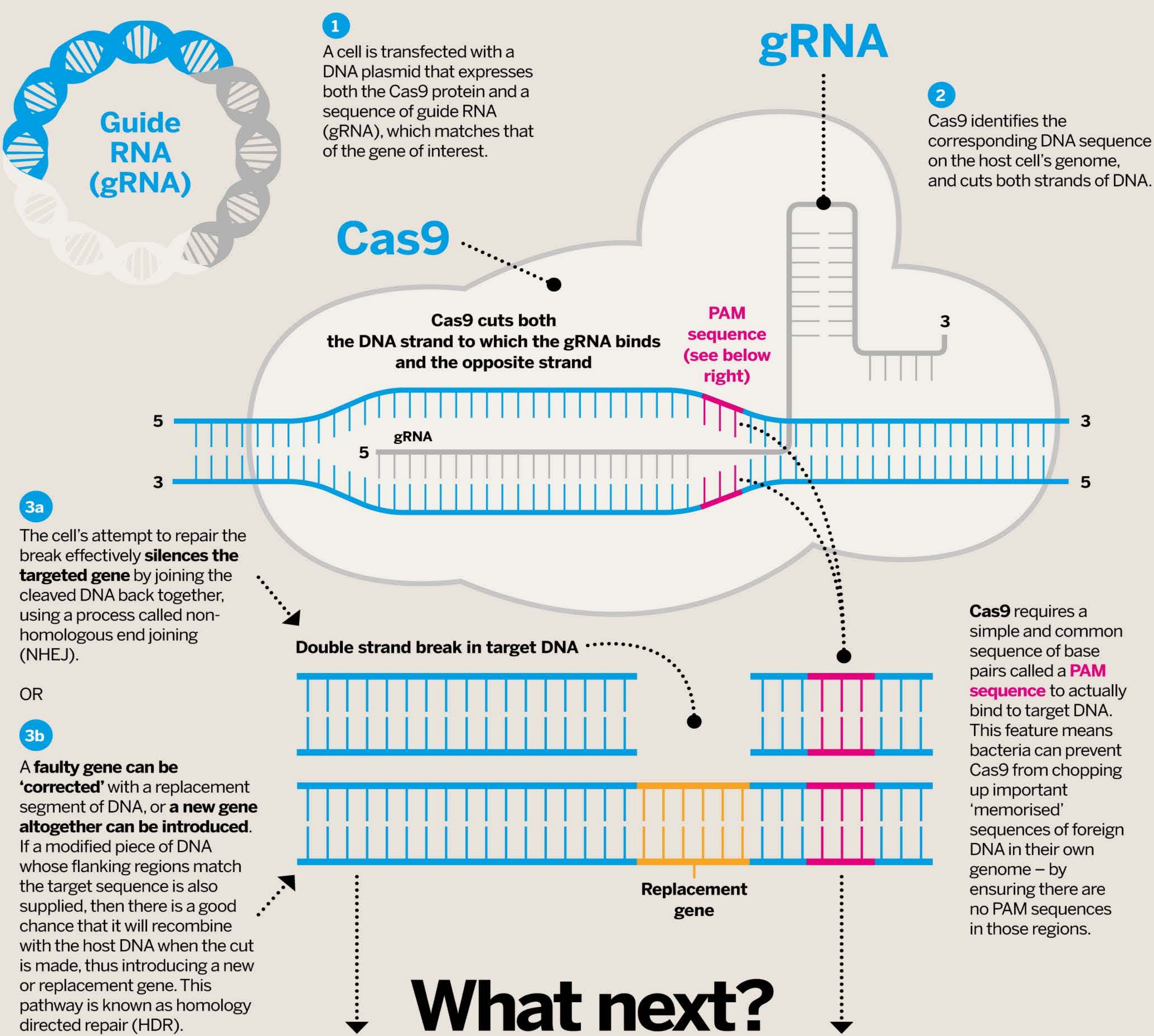
Conduct gene knockout, which Slowed development, reduce Fecundity, Shortened Adult Lifespan post eclosion.

### CRISPR/Cas9, designed to Thioredoxin

- Two guide RNA, gRNAs (Dharmacon).
- Cas9 Protein
- BAPC, ~ 4.2 fmol/nL (Tomich, KSU).
- Nanojet III, (Dummond)

## CRISPR-Cas9

How the genome editor works



**FOOD AND LIVESTOCK MODIFICATION**  
Researchers have already created plants and mammals with edited genomes. It is hoped such technology could help boost productivity and improve food security.

**GENE DRIVE**  
Some genes are more likely to be passed on than others. If an 'edit' is linked to these genes, it will quickly spread through a wild population. That sounds alarming, but could help eradicate malaria-carrying mosquitos.

**GENE THERAPY**  
Genetic disease could be treated by introducing gene editing systems into affected cells. Researchers in the USA are trialling this to treat HIV by knocking out the gene for the specific T-cell receptor that the virus targets.

**HUMAN GERM LINE**  
Modifying human embryos, sperm or eggs would introduce changes to the genome of future generations. Some argue that other techniques, such as embryo screening, can just as effectively prevent genetic disease.

**MPM**  
**MODIFIED-PEST MANAGEMENT**  
In the future, each Specific insect pest 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 not Vectors for CLas bacteria

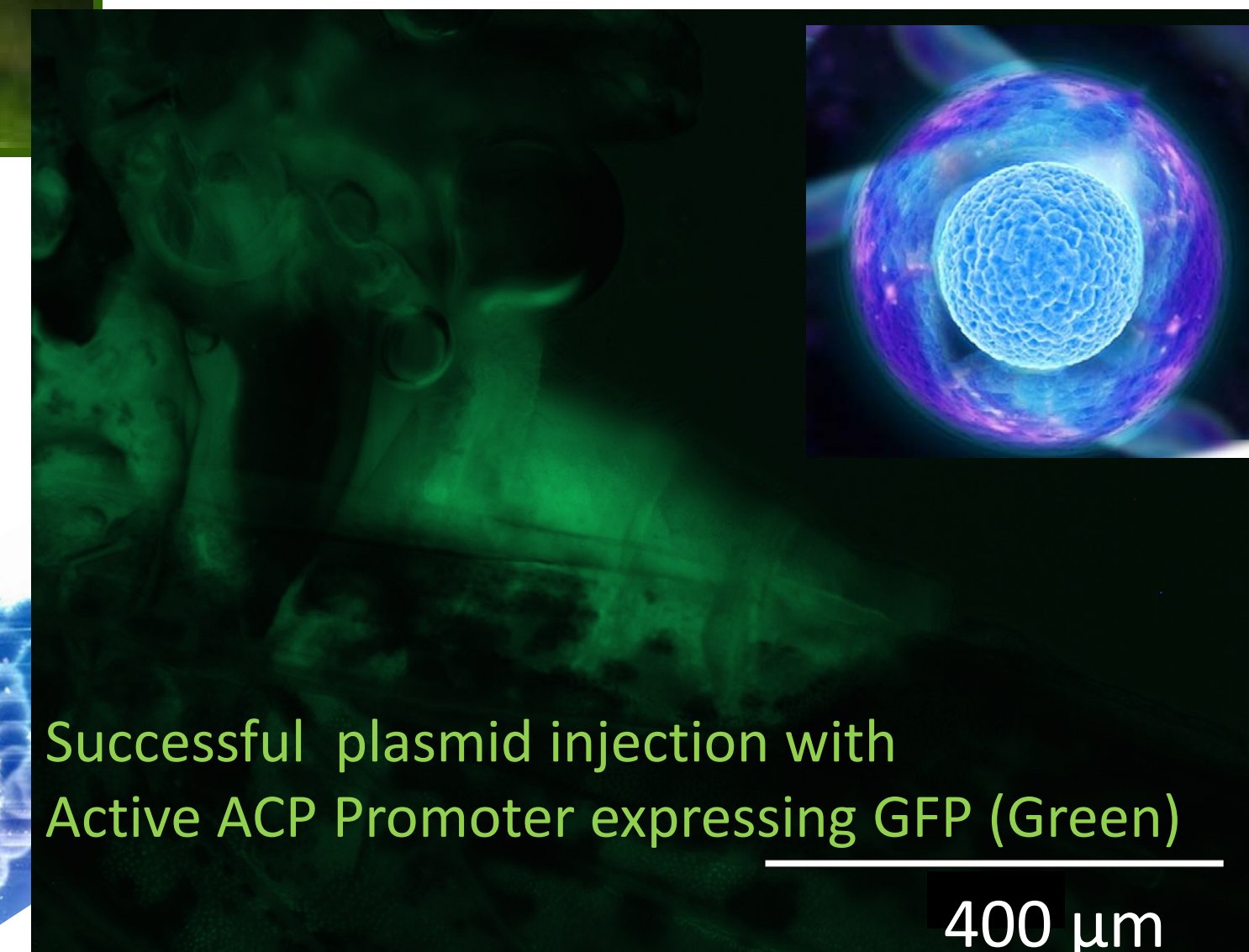
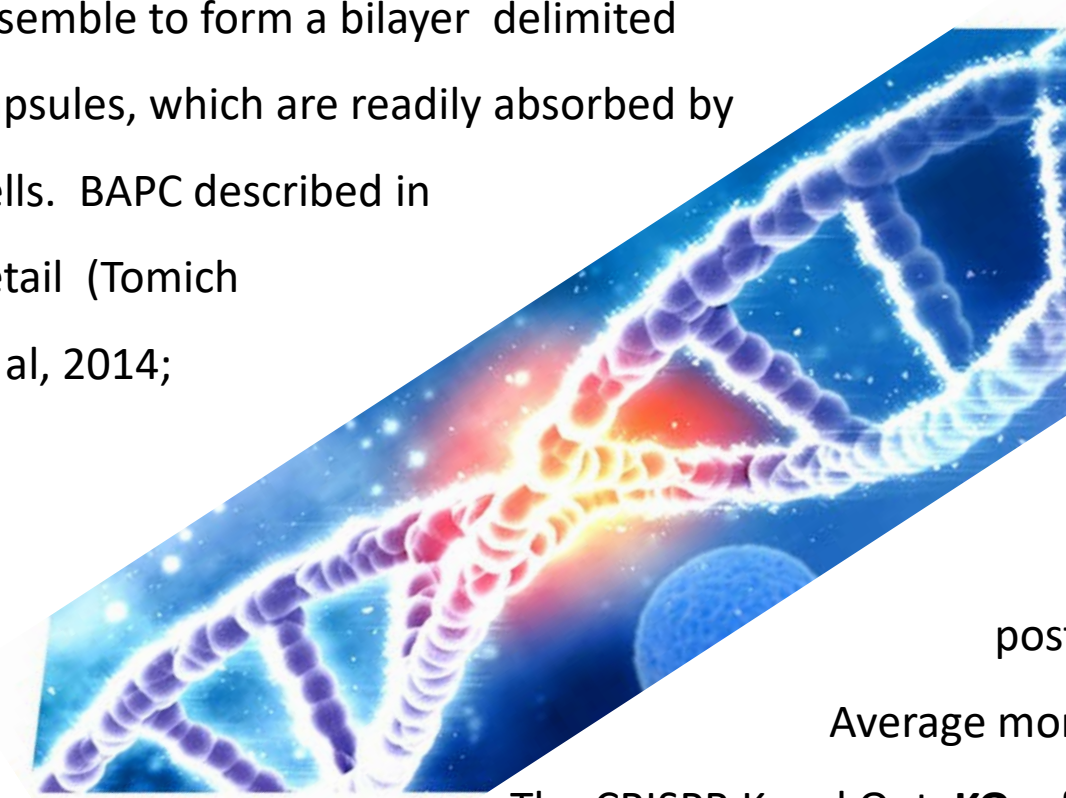
### Methods:

Cas9 protein was designed and then purchased as two gRNAs, (Dharmacon). Microinjections used Nanojet III (Dummond), purchased tips with filament. Solutions used as recommended on kits. Conc. BAPC, 1.9 nL x 10<sup>16</sup>, per vol. injected/psyllid. Psyllid gene TRX, data mined from DIACI\_1.01 genome: www.citrusgreening.org.

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### Branched amphiphilic peptide capsules (BAPC)

Branched amphiphilic peptides self-assemble into capsules or vesicles. The peptide nano-spheres are comprised of equimolar proportions of two branched peptide sequences bis(FLIVI)-K-KKKK that self-assemble to form a bilayer delimited Capsules, which are readily absorbed by Cells. BAPC described in detail (Tomich et al, 2014;



Successful plasmid injection with Active ACP Promoter expressing GFP (Green) 400 μm

The TRX treated psyllid adults lifespan averaged 8.5 d post eclosion compared to controls injected with buffer, which averaged 16 d. Average mortality averaged 58%-69% across treatments at 3 d post injections.

The CRISPR KnockOut, KO, of TRX resulted in psyllids with: Longer development times, Shorter adult lifespans, Reduced fecundity. There is room for a lot of improvement with this technique.

Interestingly of the few F<sub>1</sub> eggs oviposited, post TRX injection near ovaries of an F<sub>0</sub> adult female which also were KO positive, when collected as nymphs, one 4<sup>th</sup> instar out of six analyzed, was TRX KO positive. Surprisingly the development of a second generation psyllid from eggs, NOT injected, but oviposited from a TRX-injected F<sub>0</sub> adult female, had the missing TRX-KO sequence. **Thus, it appears that 1)** Purposefully co-inject Cas9 protein and two sgRNAs, with 0.1 ng BAPC, into the region of the adult female psyllid ovaries may produce stable KO offspring. **2)** A **CRISPR – BAPC combination** method would provide a much easier gene-editing strategy for insects where it has not been feasible to conduct egg injections. Previous egg injections trials in psyllids over a one year period, and thousands of eggs, did not produce any positive KO results, and few psyllids. Very expensive.

### CONCLUSIONS

- **CRISPR/Cas9 works in psyllid nymphs, and adults, microinjections.**
- **BAPC-CRISPR/Cas9 system works for Adult Ovary Injection modifies- next generation, resulting in Heritable, gene editing.**
- **TRX knockout, produces psyllids with slower development, reduced adult lifespan, and reduced fecundity.**
- **CRISPR/Cas9 provides a system for population modification management, MPM (2018). Future prospects: to produce Non-Vector Psyllids, HLB.**

### References:

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