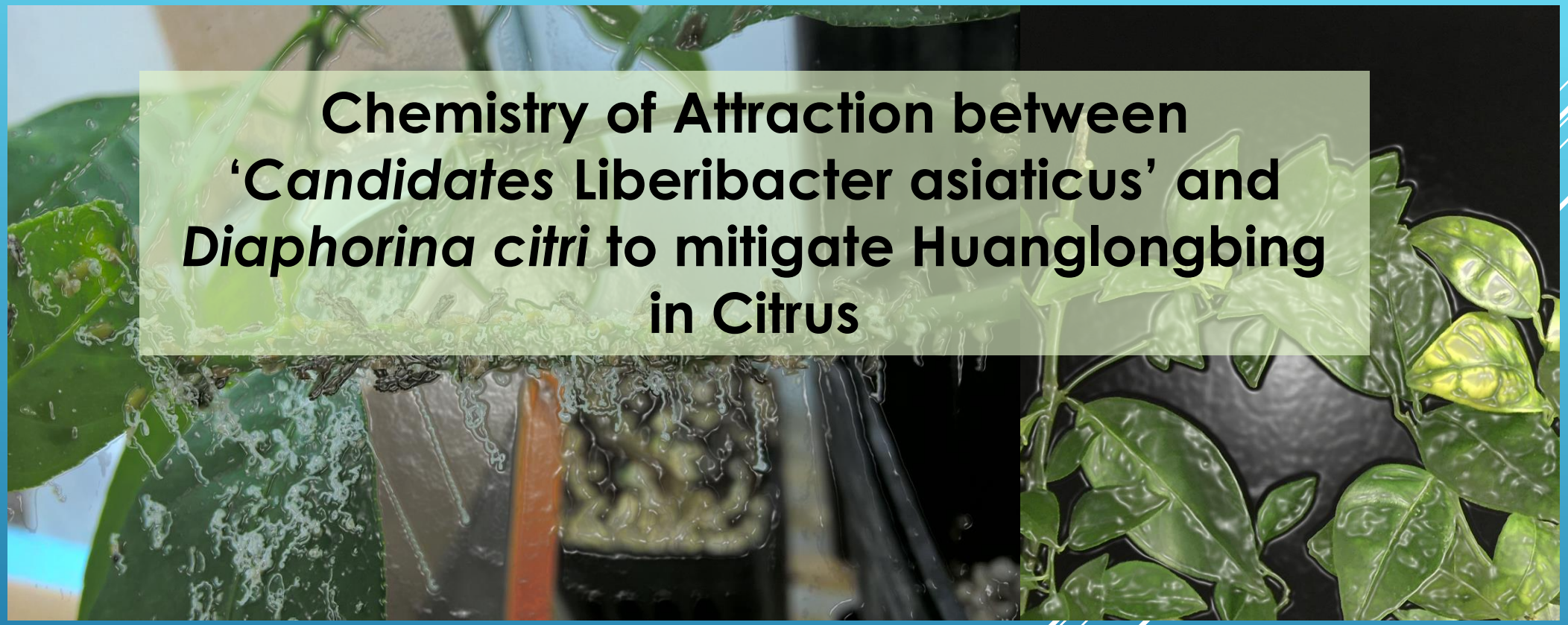


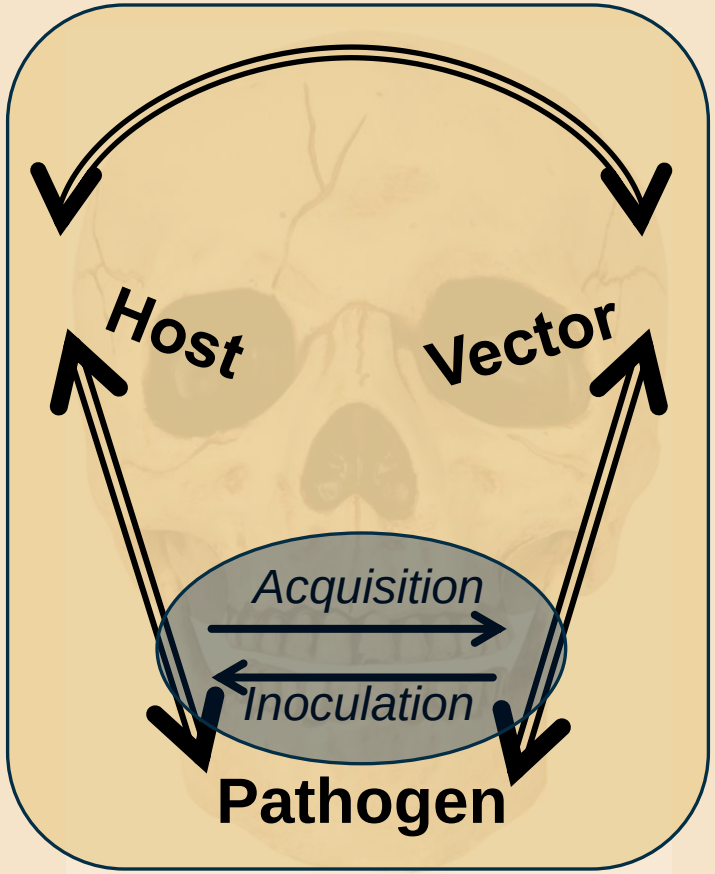
# Chemistry of Attraction between '*Candidates* Liberibacter asiaticus' and *Diaphorina citri* to mitigate Huanglongbing in Citrus

**Nabil Killiny**
Associate Professor
Plant Pathology
Citrus Research and Education Center, IFAS,
University of Florida

# Insect transmission of plant pathogens

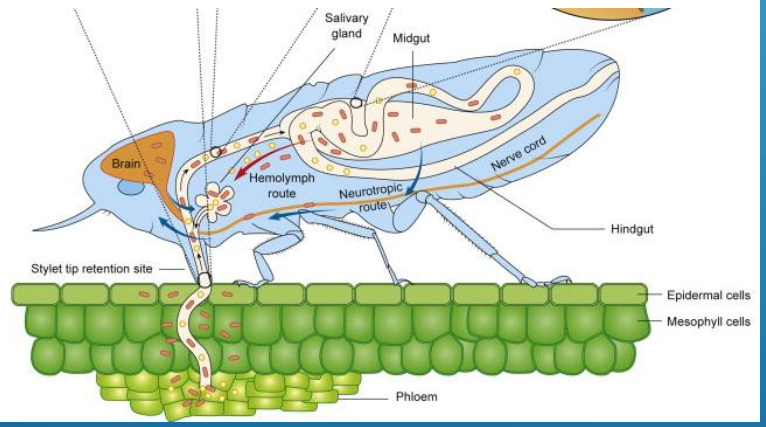
## Mechanisms of transmission

**Persistence** **Circulation** **Propagation**

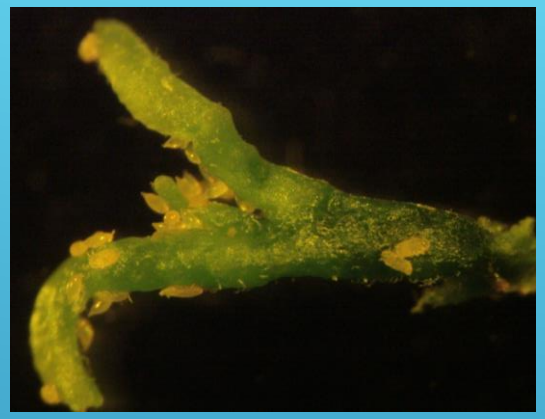



## Transmission parameters

- ***Acquired*** mostly by nymphs.
- Circulates within insect body(cross the gut to haemolymph where it
- multiplys and form biofilm on the gut surface then invade salivary glands)
- Persists in insect during its entire life.
- ***Inoculated*** into new plants by adults.

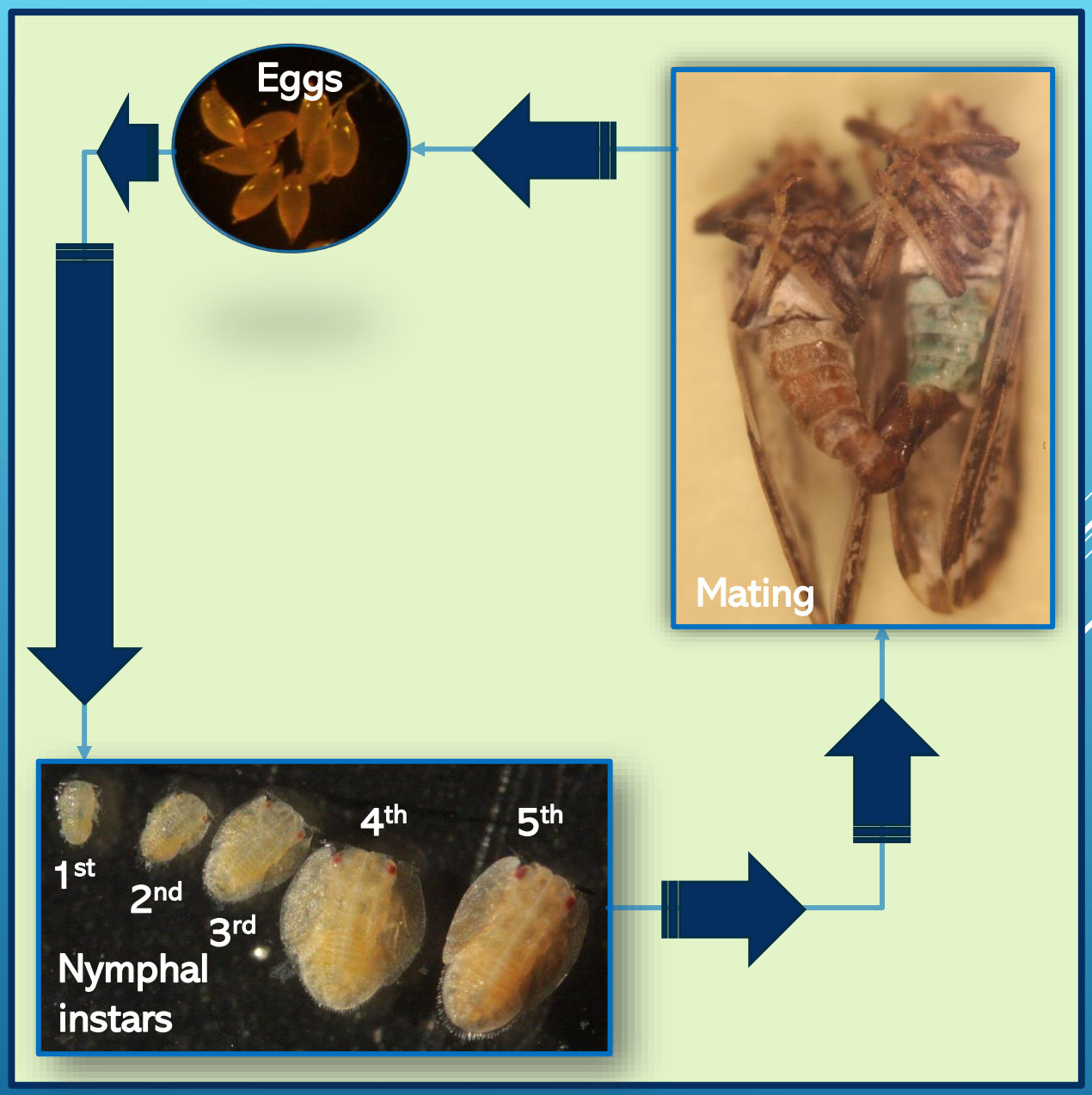



Laying eggs



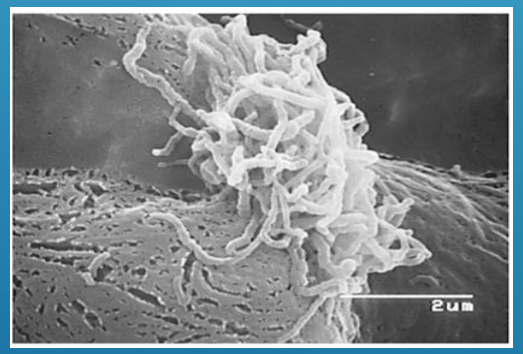

Hatching


Emerging


Eggs
Mating
1st
2nd
3rd
4th
5th
Nymphal instars

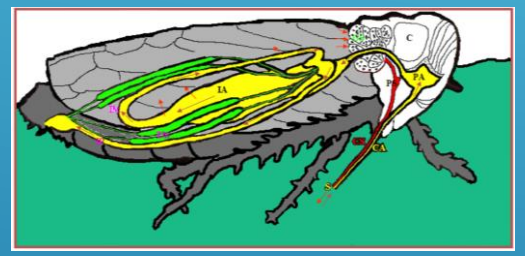
# ‘*Ca*. L. asiaticus’ transmission by *D. citri*

Forms Biofilm



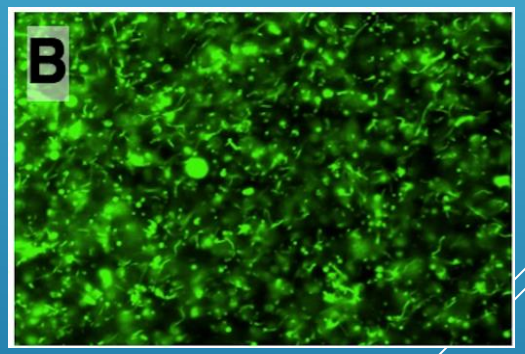

Quorum sensing!
**Two components system**

Circulative

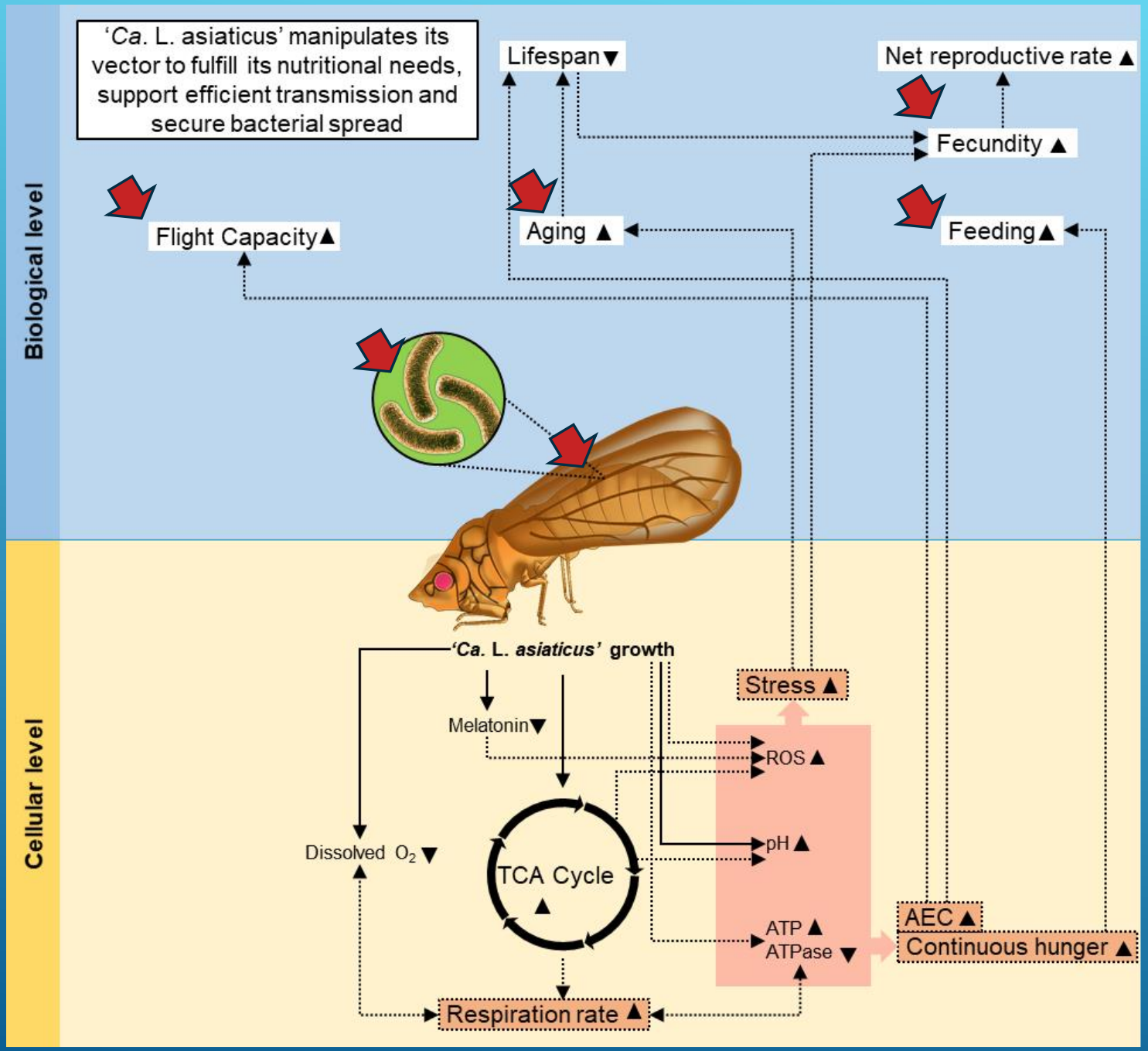


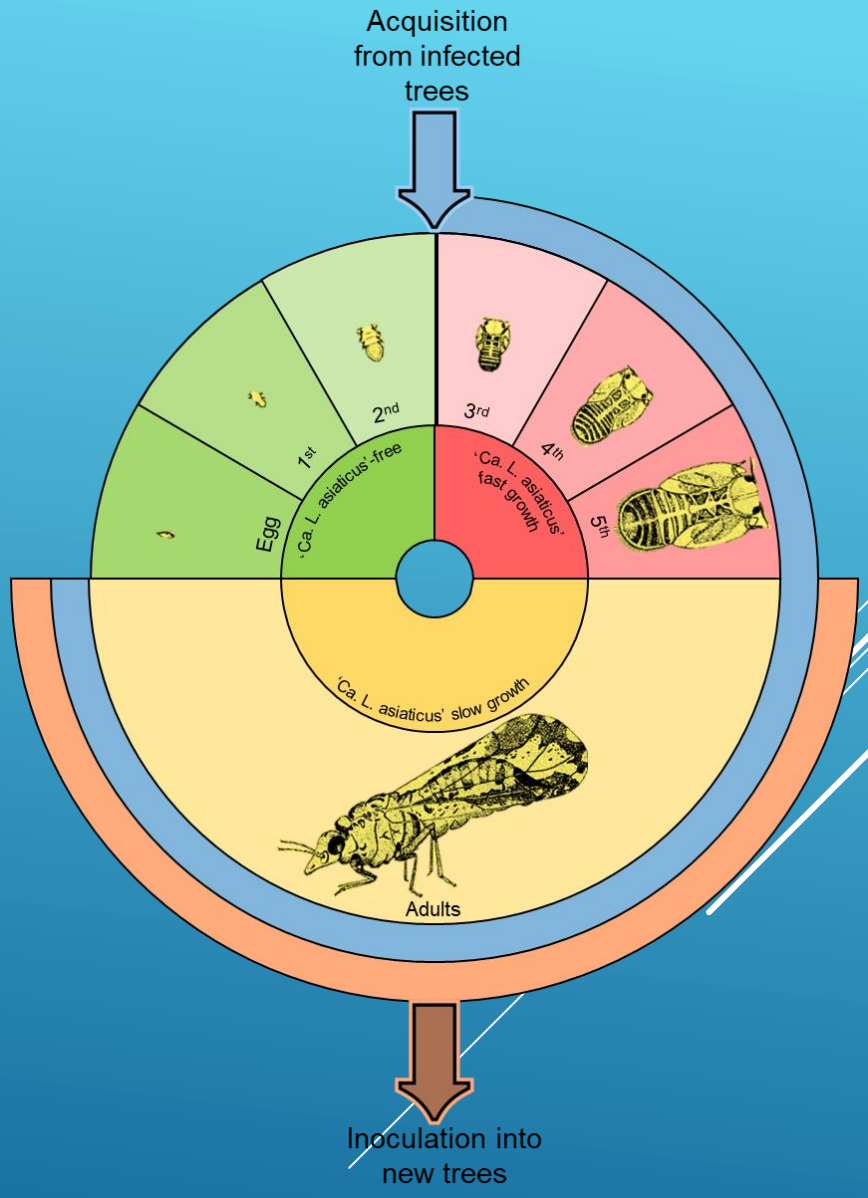
Bacteria pass through the biological barriers (Gut and salivary glands)!
**Specific interactions (receptor-ligand)**

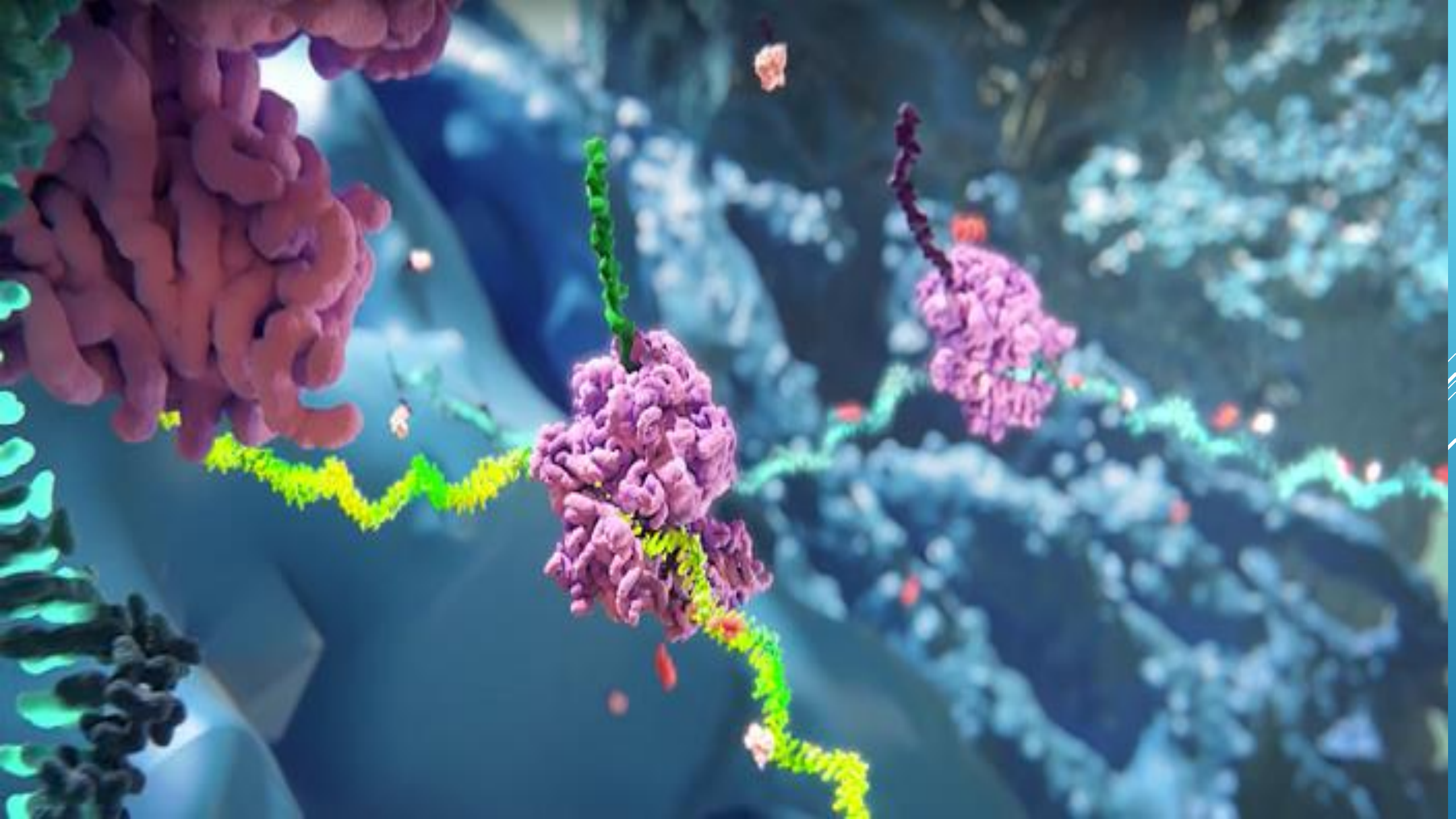
Propagative

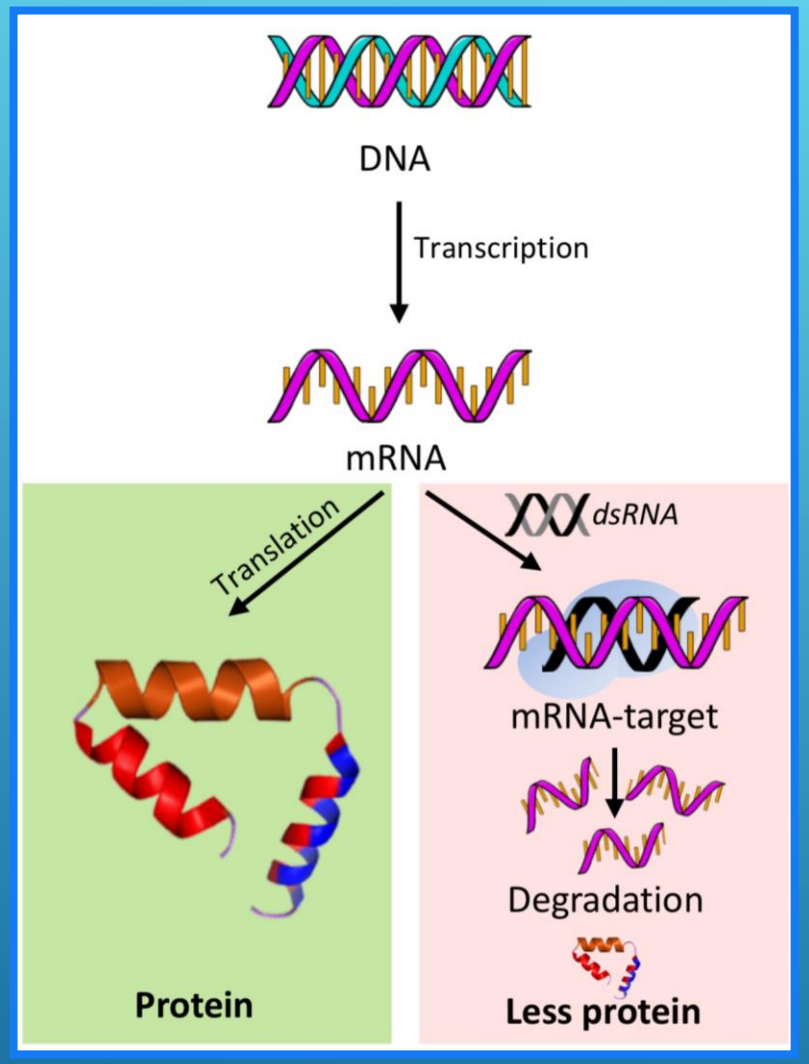



Bacterial multiplication in haemolymph!
**Nutrition (sugars, Amino acids, …. )**

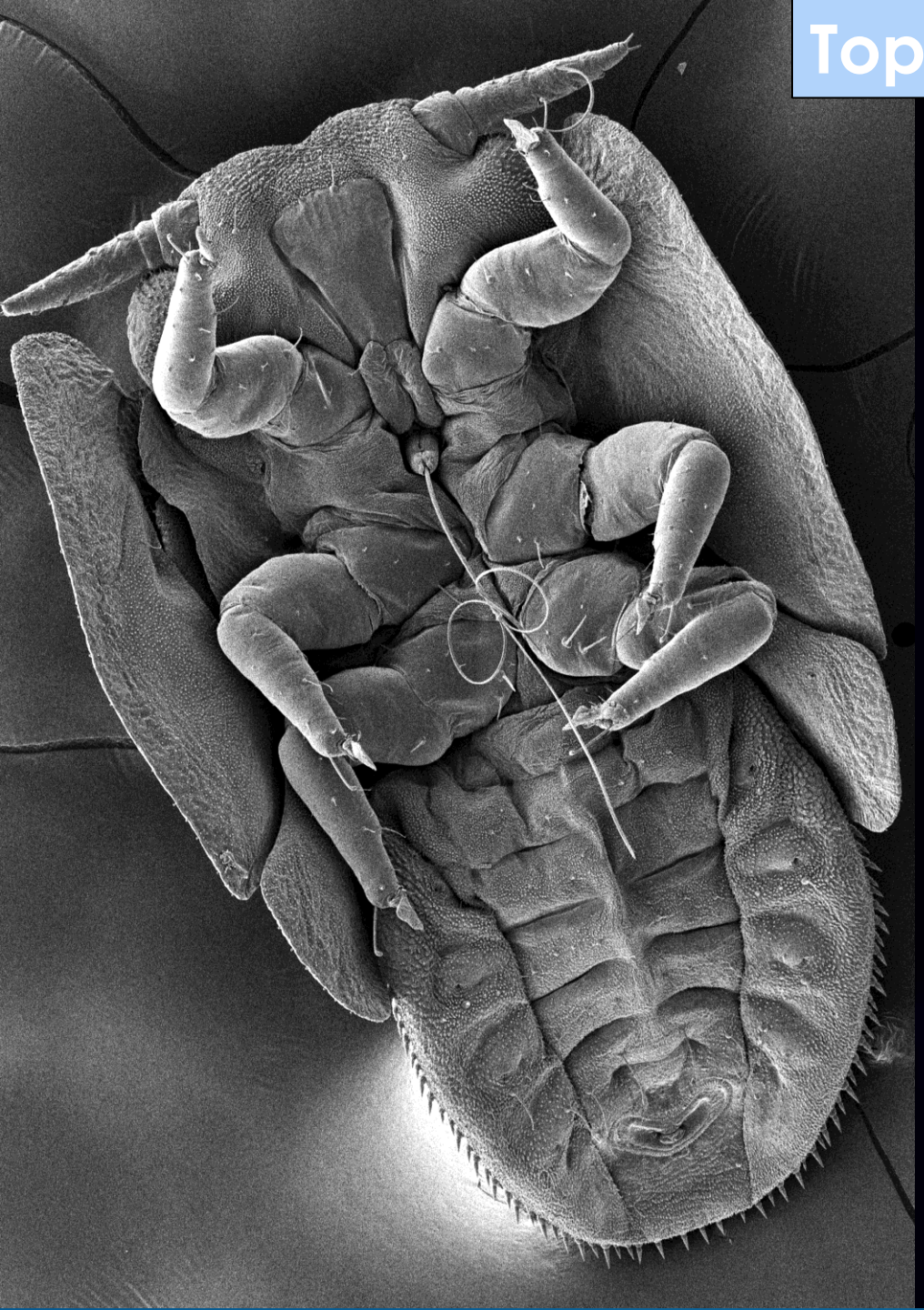

'Ca. L. asiaticus' manipulates its vector to fulfill its nutritional needs, support efficient transmission and secure bacterial spread
Biological level
Cellular level
Lifespan▼
Net reproductive rate ▲
Fecundity ▲
Flight Capacity▲
Aging ▲
Feeding▲
'Ca. L. asiaticus' growth
Stress ▲
Melatonin▼
ROS ▲
pH ▲
Dissolved $O_2$ ▼
TCA Cycle
ATP ▲
ATPase ▼
AEC ▲
Continuous hunger ▲
Respiration rate ▲


Acquisition from infected trees
Egg
1st
2nd
3rd
4th
5th
'Ca. L. asiaticus'-free
'Ca. L. asiaticus' fast growth
'Ca. L. asiaticus' slow growth
Adults
Inoculation into new trees



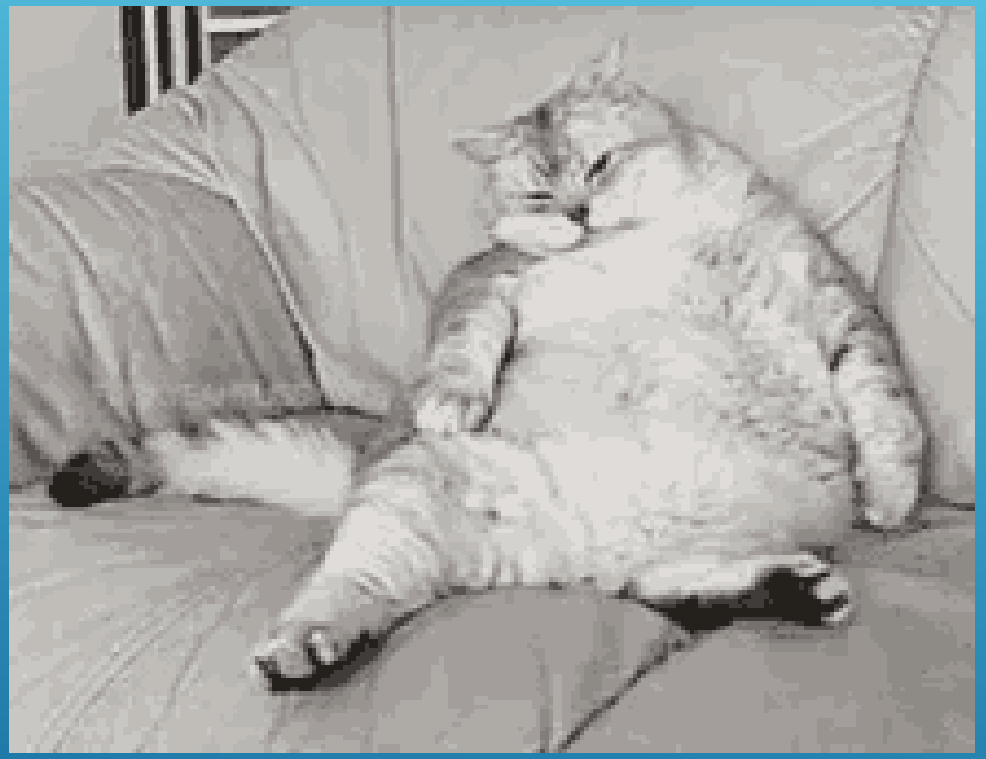

DNA
Transcription
mRNA
Translation
dsRNA
mRNA-target
Degradation
Protein
Less protein

# Topical feeding

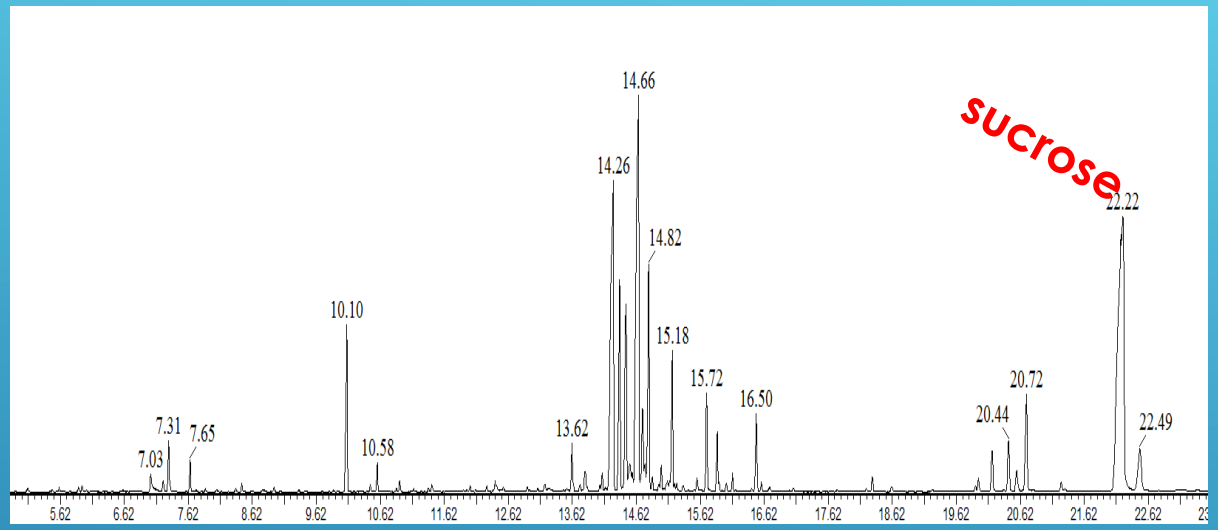


# Screening for target genes

## Food coma

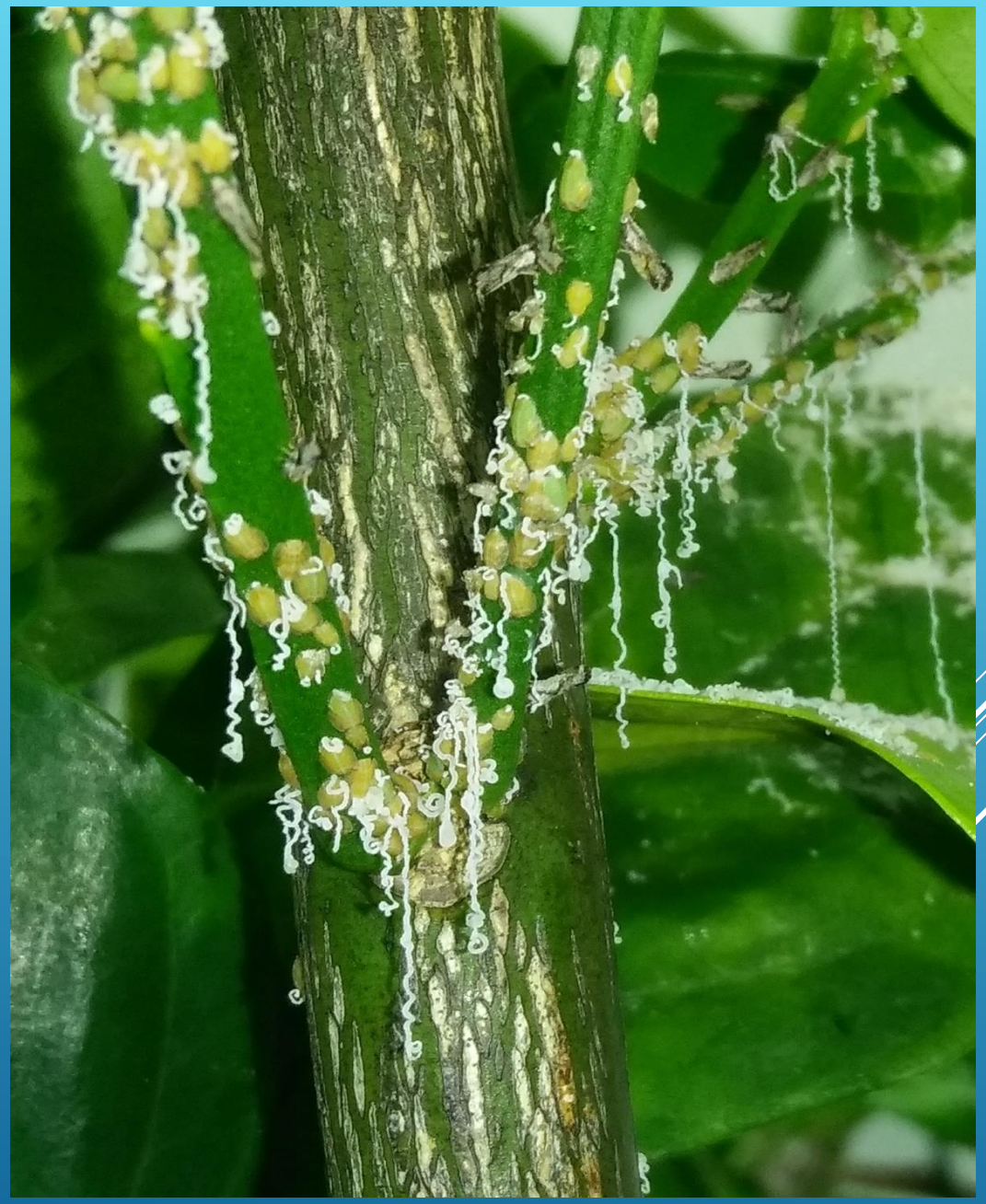


# Target: Sucrose hydrolase

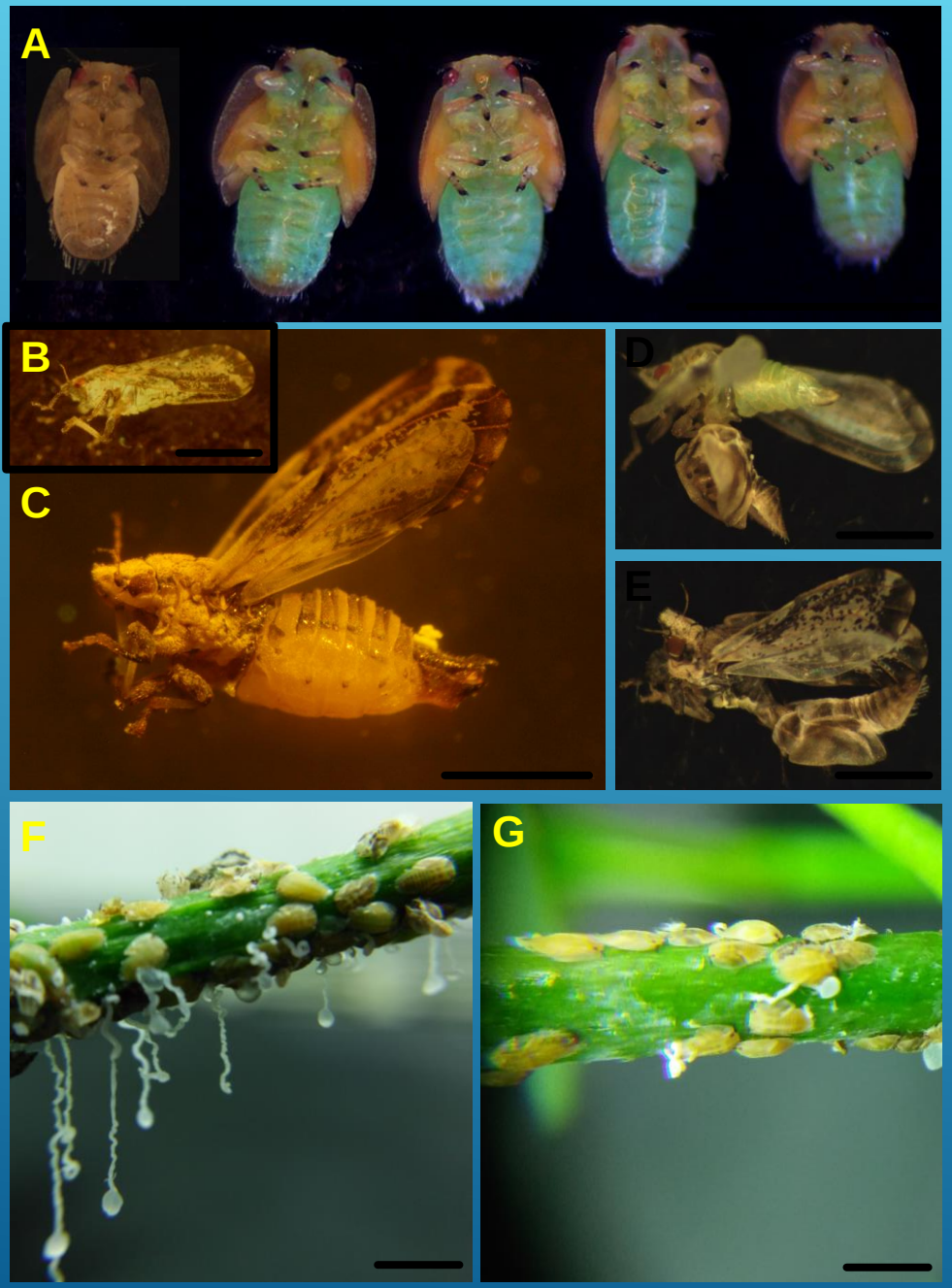


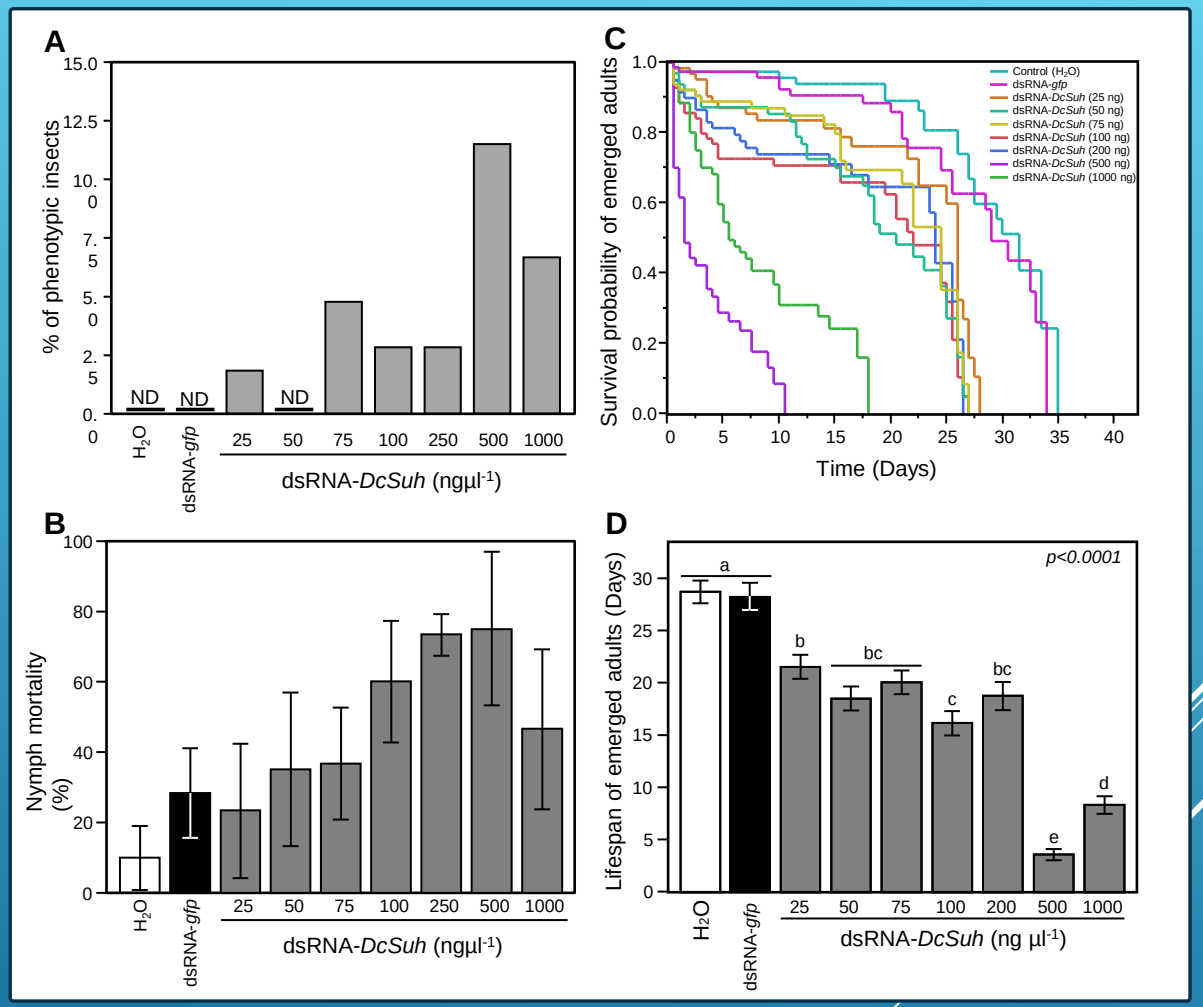

## Osmotic potential:

- Hydrolysis of sucrose
- Transglycosidation into oligosaccharides (honeydew)
- Dilution with water by feeding on xylem sap or transferring body fluids



# Target: Sucrose hydrolysis



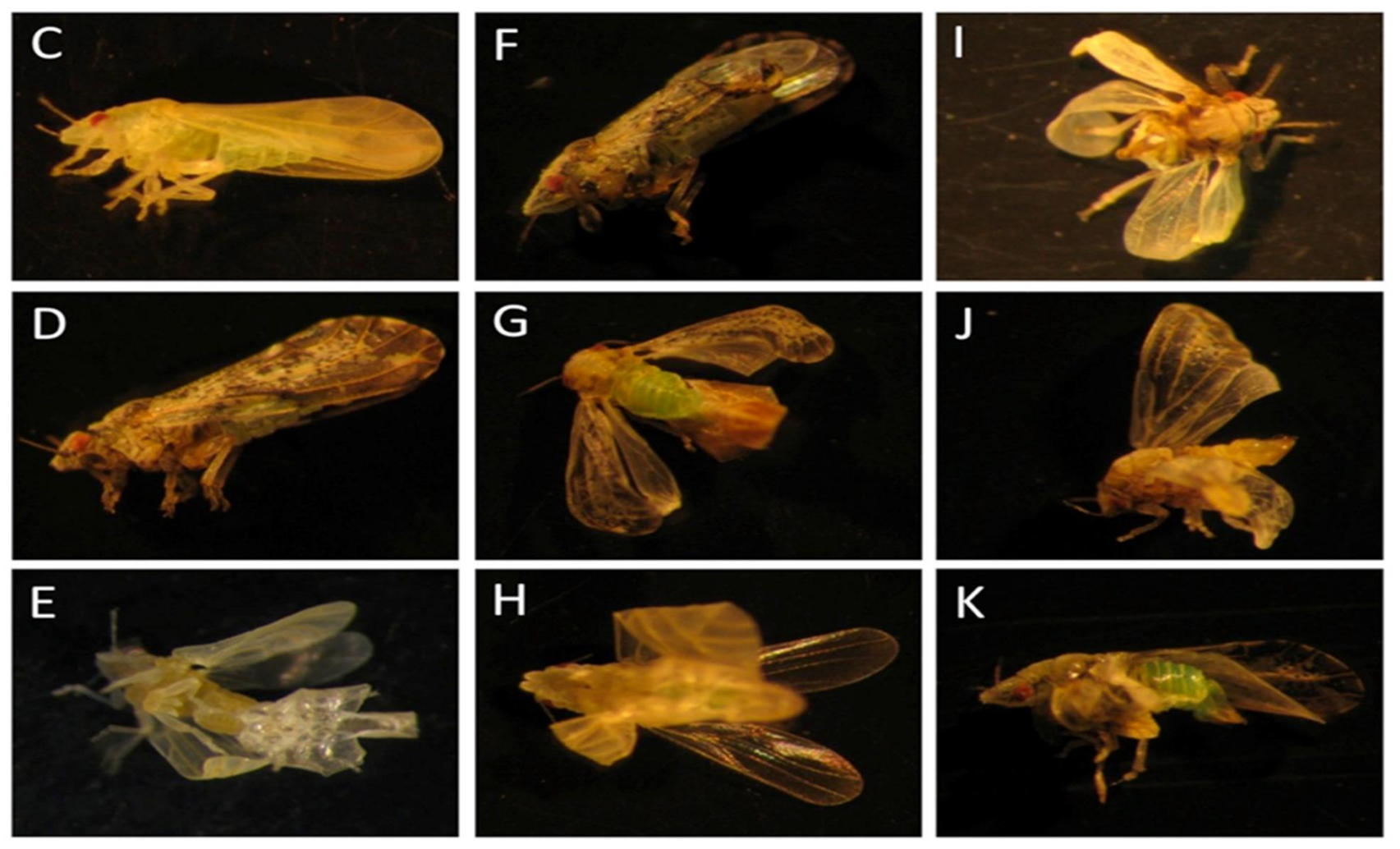




- Nymphal mortality
- Shorter adult lifespan
- Swollen abdomen phenotype

# Screening for target genes

## Fly the coop

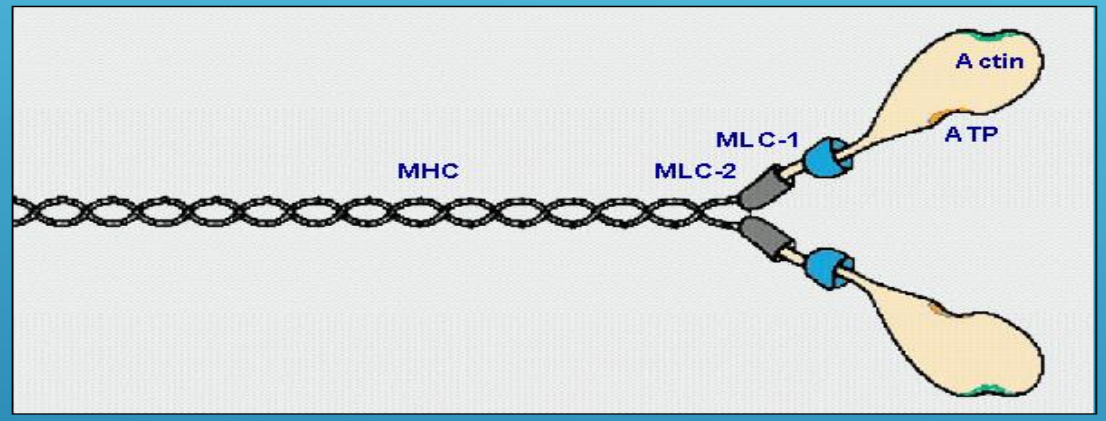
## Target: abnormal wing disc

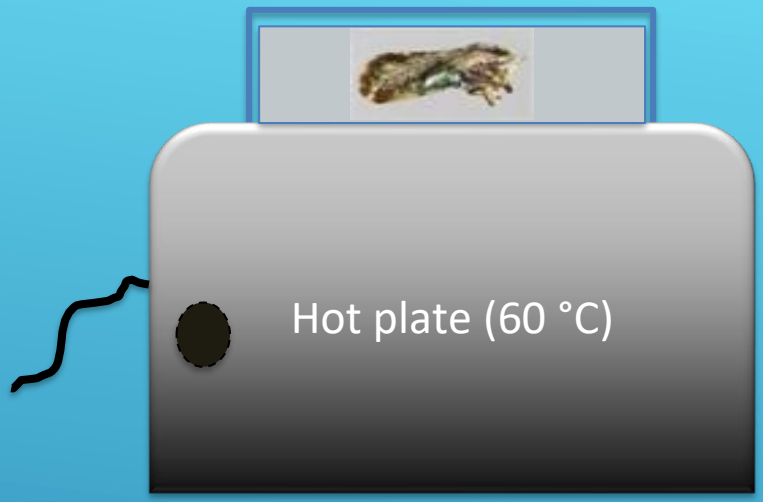


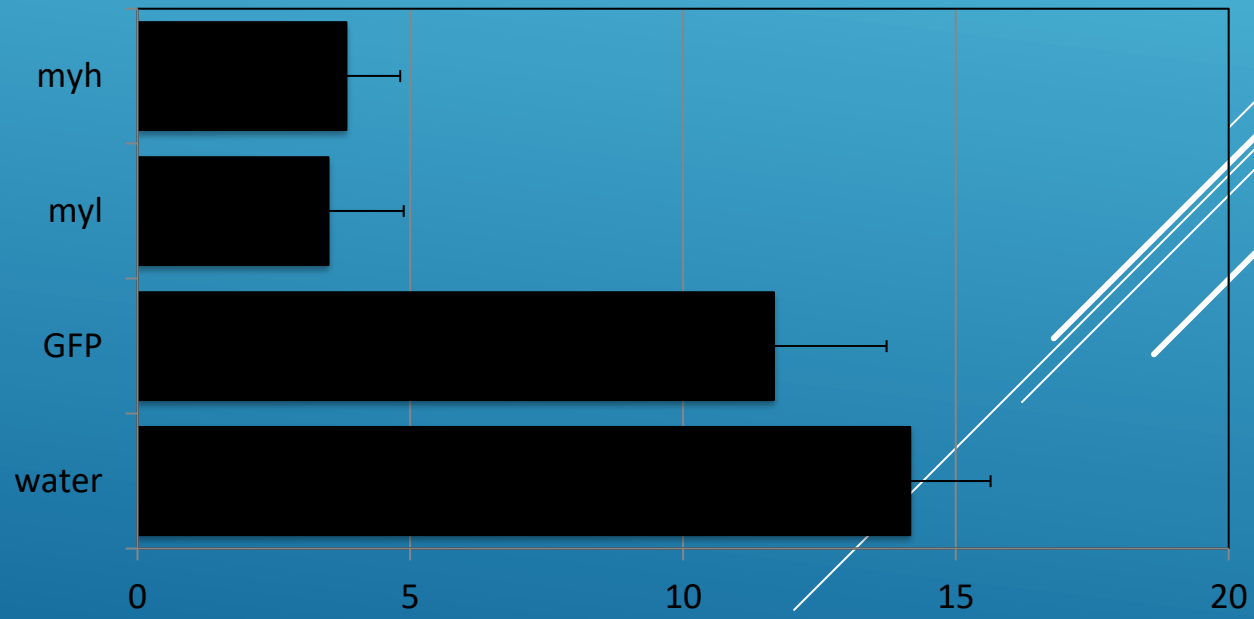
# Screening for target genes

## Lazy bone

### Target: myosin



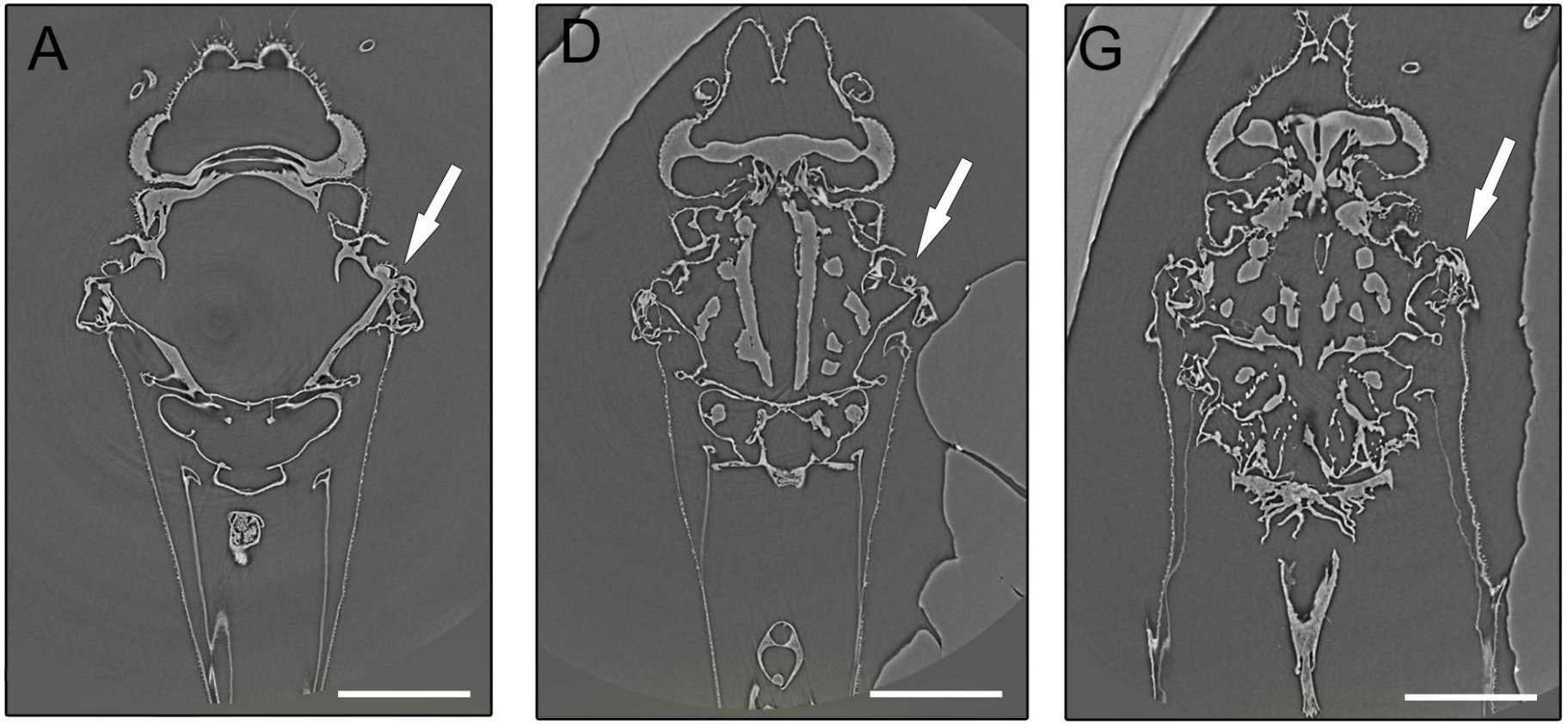




# of jumps/ 10 sec

Control

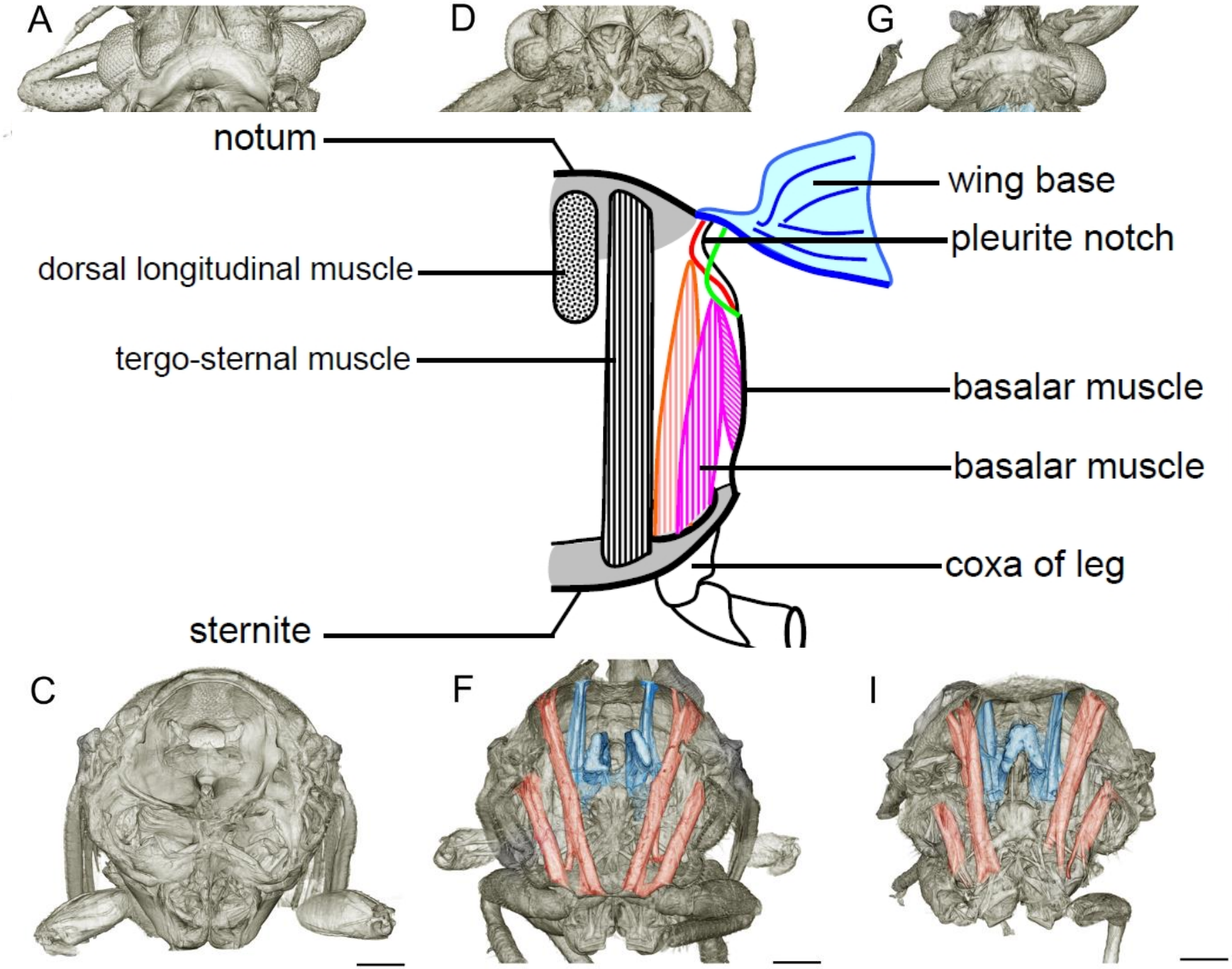
*myl*-dsRNA

*myh*-dsRNA



Normal ACP adults
Internal organs and tissues are invisible

ACP adults merged from treated nymph
Flight muscles are visible


A
D
G
notum
wing base
pleurite notch
dorsal longitudinal muscle
tergo-sternal muscle
basalar muscle
basalar muscle
coxa of leg
sternite
C
F
I

# Screening for target genes

## Breaking the insecticides resistance

## Target: Cytochrome P450

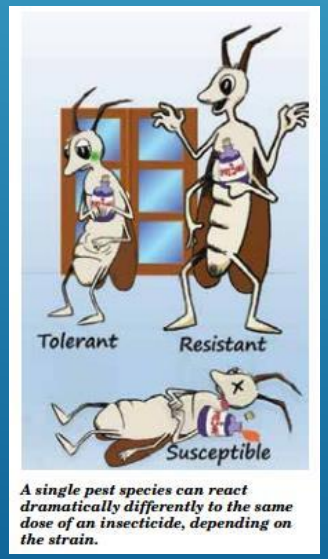
OPEN ACCESS Freely available online

PLOS ONE

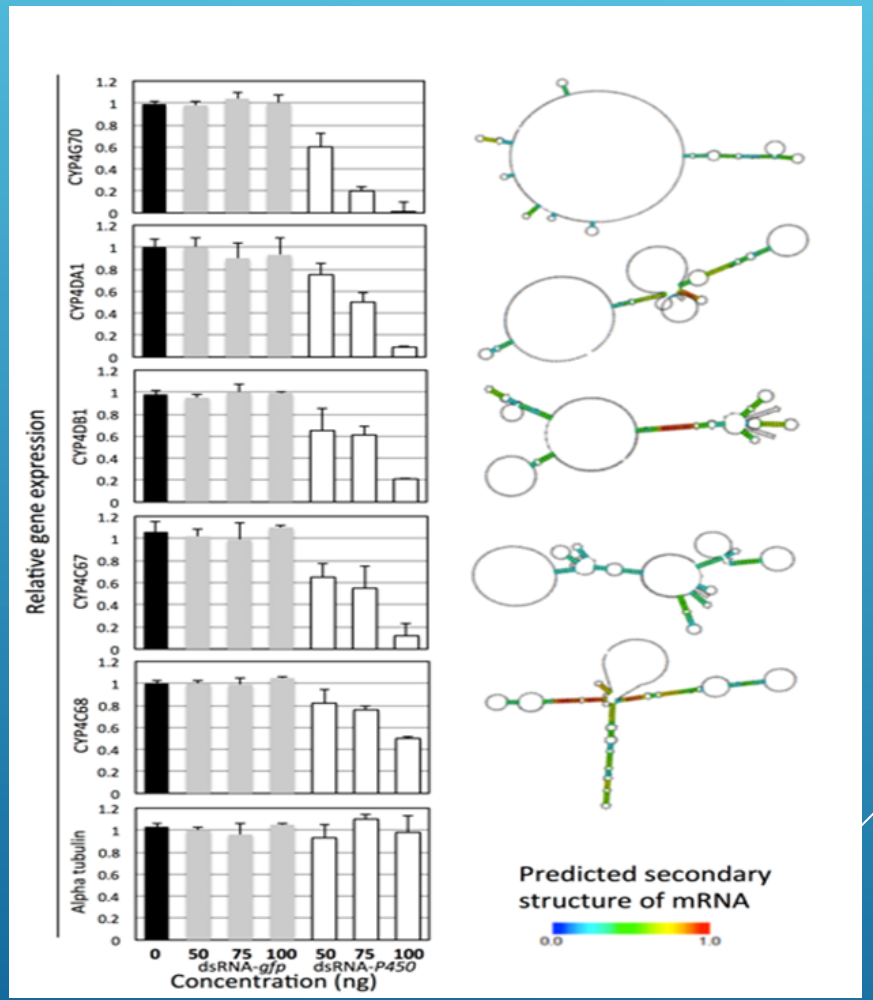
### Double-Stranded RNA Uptake through Topical Application, Mediates Silencing of Five CYP4 Genes and Suppresses Insecticide Resistance in *Diaphorina citri*

**Nabil Killiny[1]*, Subhas Hajeri[2], Siddharth Tiwari[1], Siddarame Gowda[2], Lukasz L. Stelinski[1]**

1 Department of Entomology and Nematology, Citrus Research and Education Center, IFAS, University of Florida, Lake Alfred, Florida, United States of America,
2 Department of Plant Pathology, Citrus Research and Education Center, IFAS, University of Florida, Lake Alfred, Florida, United States of America




*A single pest species can react dramatically differently to the same dose of an insecticide, depending on the strain.*




URGENT


Download from
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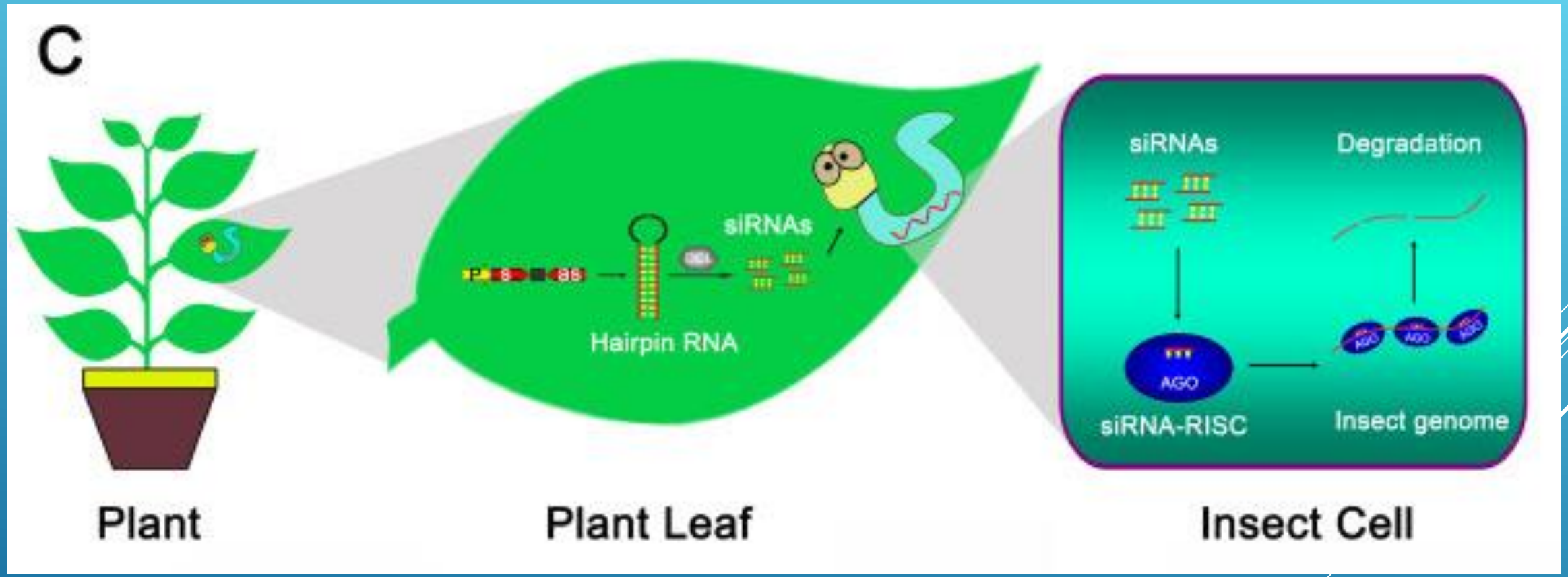
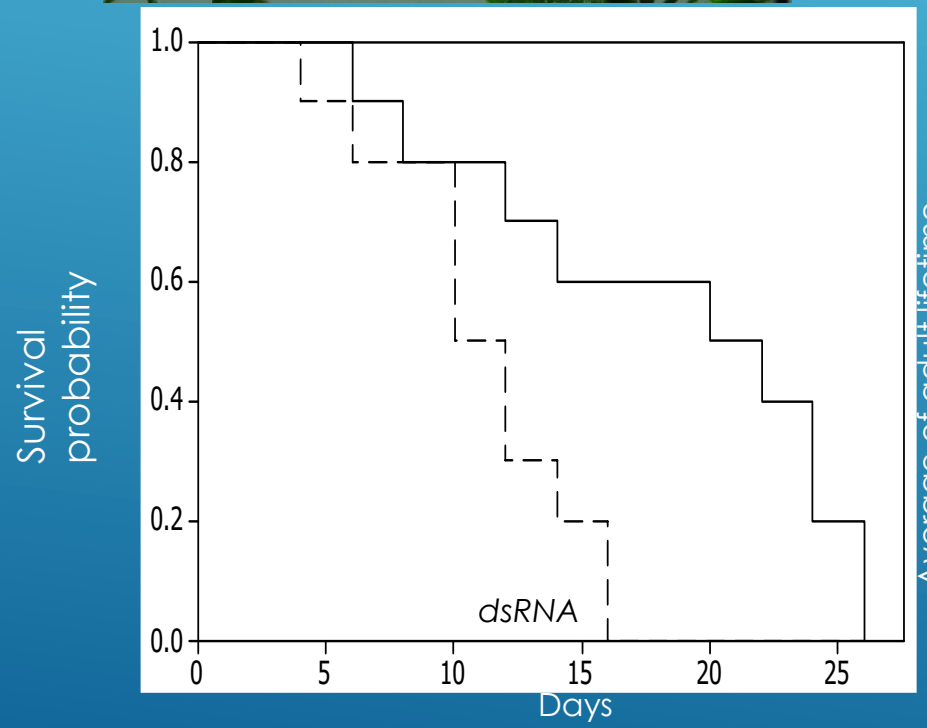
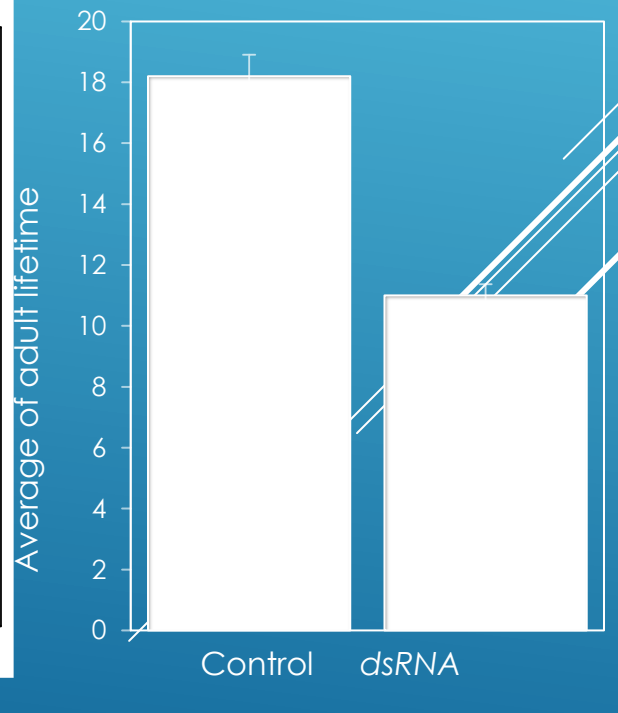
# 1-Virus induced gene silencing using CTV



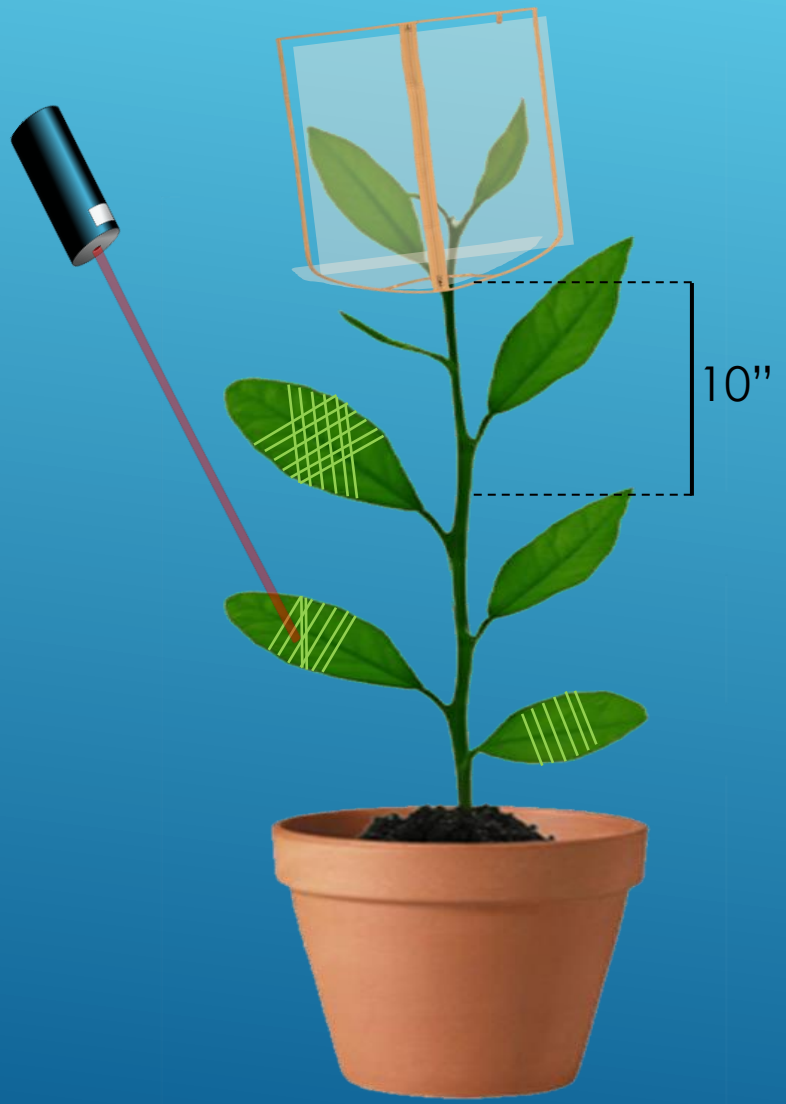
# Virus induced gene silencing

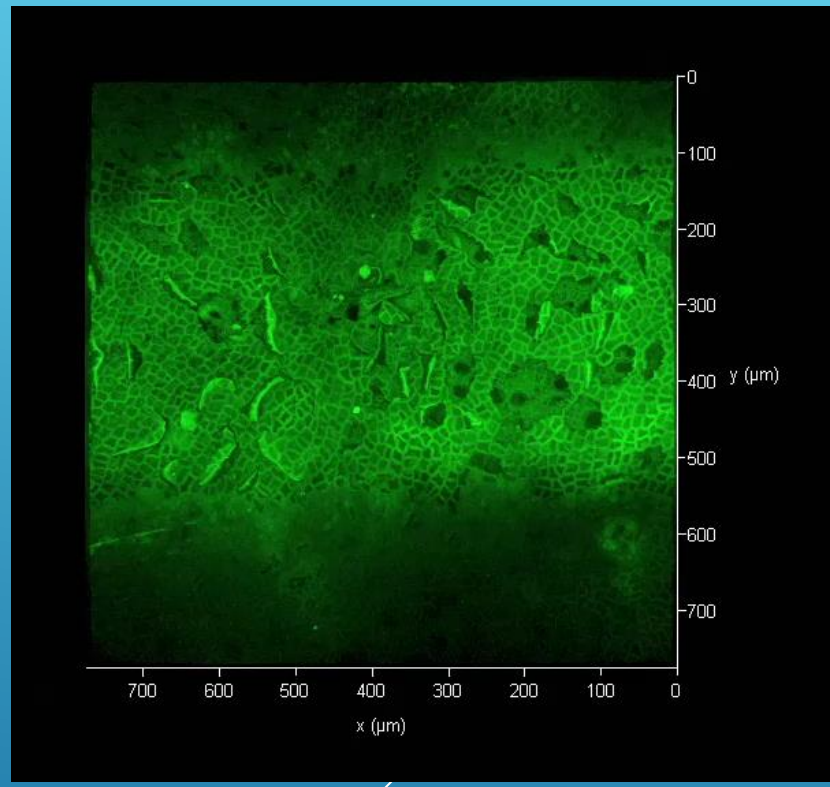




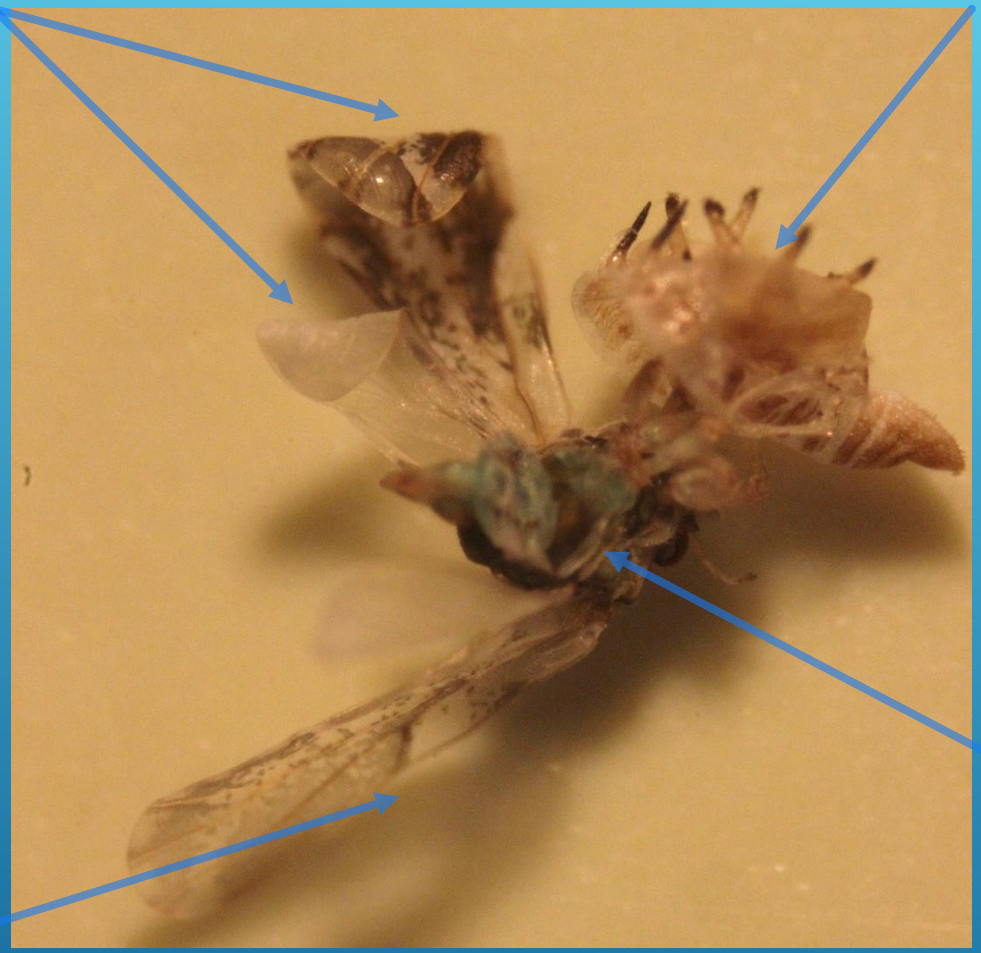
# 2- Laser delivery of dsRNA



Curled wings
(cup shaped)

Incomplete
molting



Stretched wing

Stunted
malformed body

# 3- Gene-editing using CRISPR-Cas9

# Establishment of an artificial medium to support the entire life (from egg to adult) cycle of *D.citri*

# Uses of the medium

- Deliver dsRNA at earlier stages (eggs) for RNAi to study functional genomics such as transformer gene.
- Deliver plasmid for CRISPR/Cas9 for gene editing



# ACKNOWLEDGE

THANK YOU

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Anders Omsland

Haluk Beyenal