# The Use of RNAi to Control Psyllids in Citrus

Bill Dawson, UF/IFAS CREC

**Using Citrus tristeza virus to produce products to manage HLB**

Why?

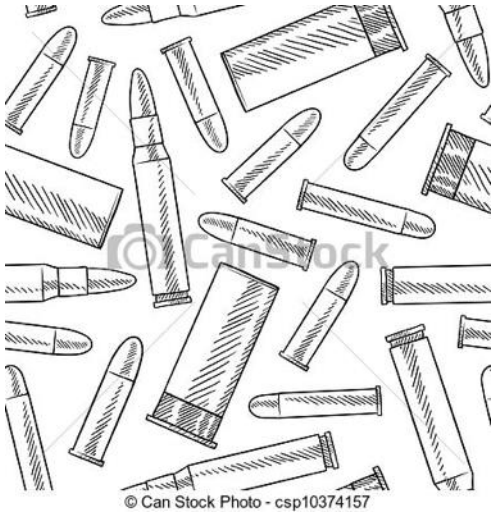
Can get to the field quicker than transgenics

No cost after putting the virus in the tree

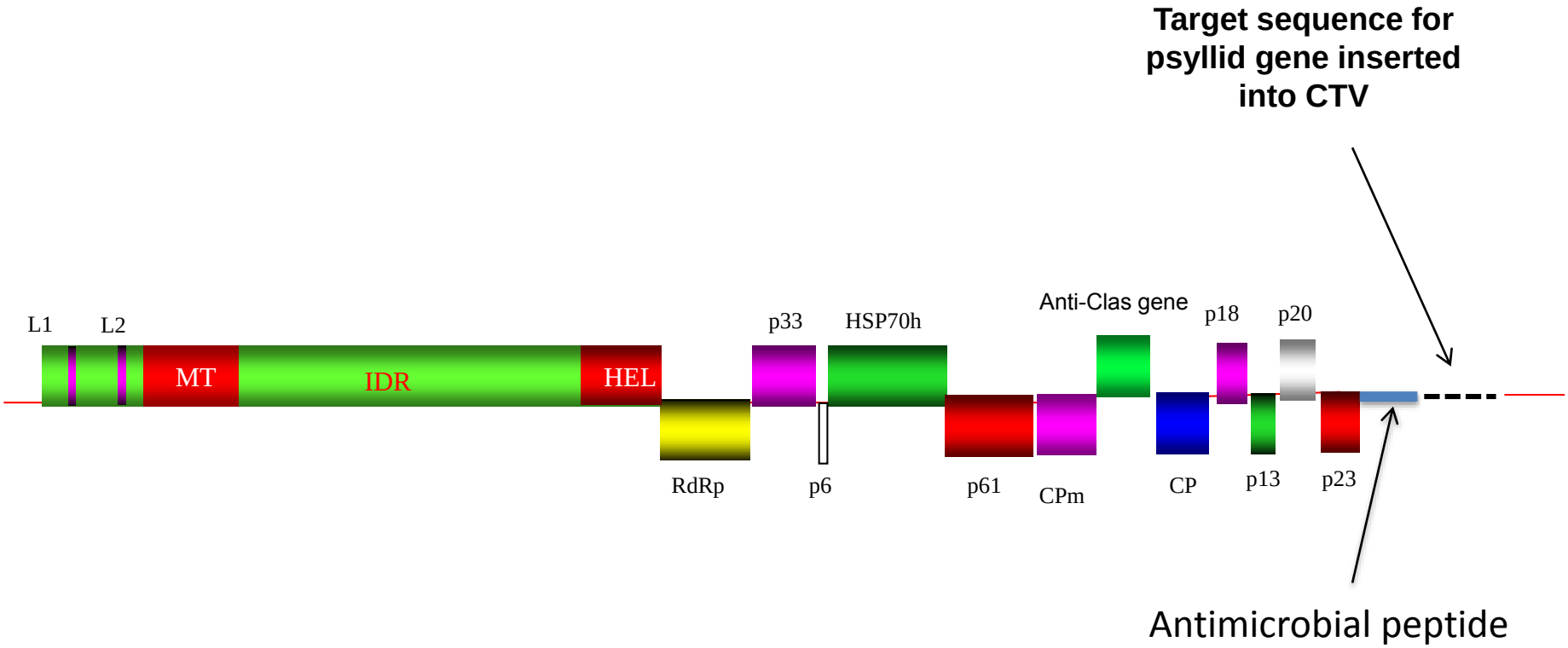
How?

Use antimicrobial peptide to control CLas

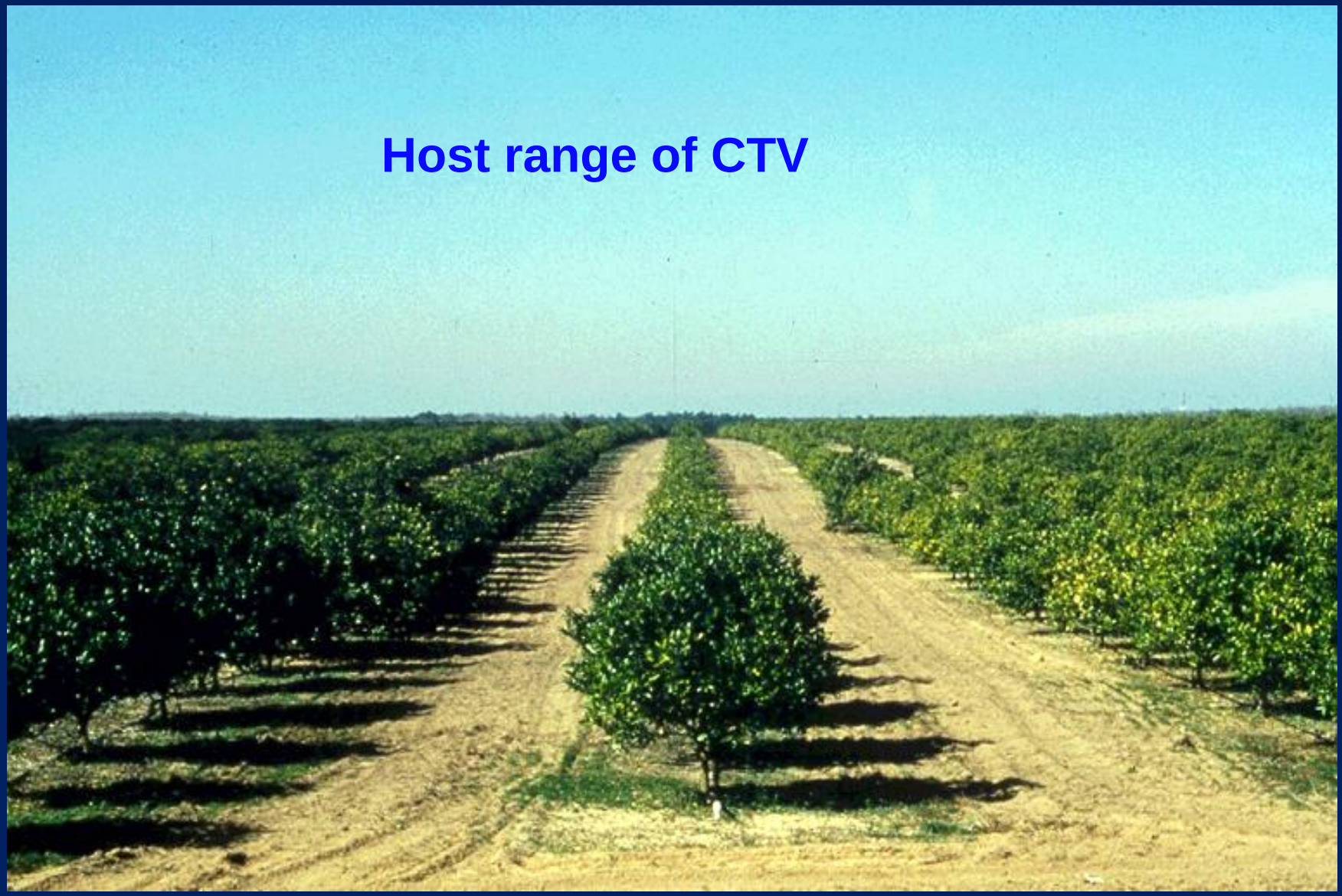
Use RNAi to control psyllid reproduction and spread of HLB

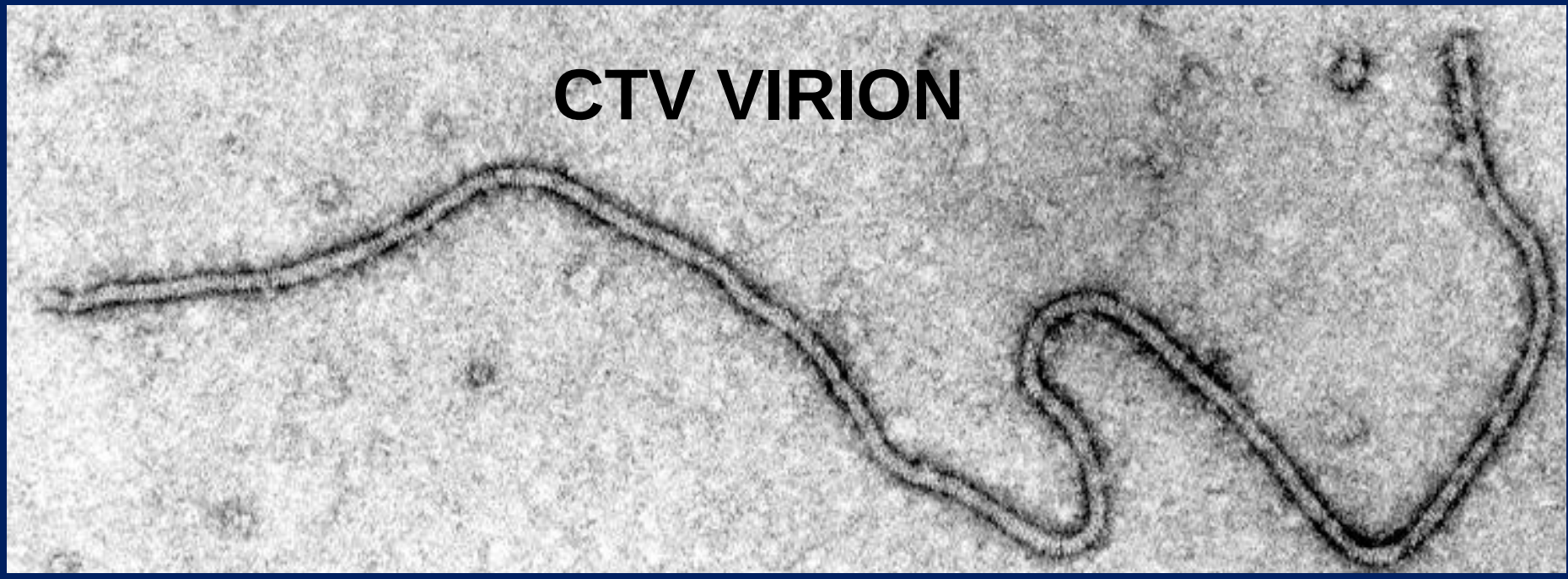


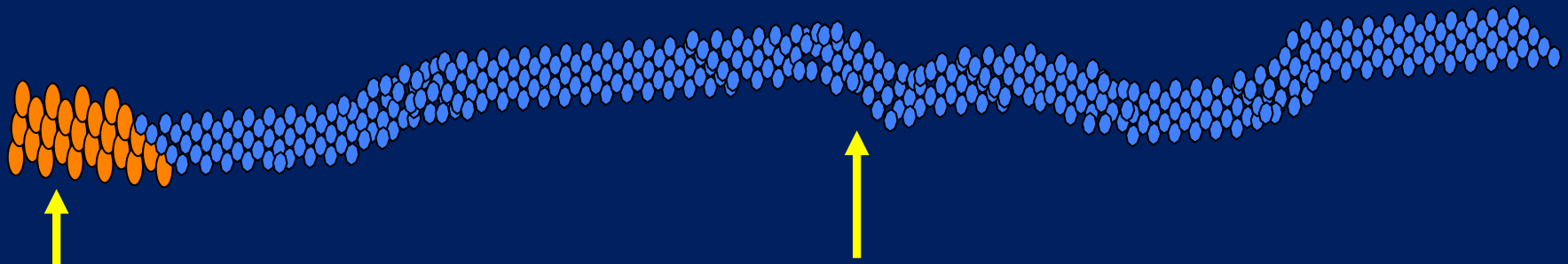
# Screening antimicrobial peptides from CTV against HLB


Target sequence for psyllid gene inserted into CTV
L1
L2
MT
IDR
HEL
RdRp
p33
p6
HSP70h
p61
CPm
Anti-Clas gene
CP
p18
p13
p20
p23
Antimicrobial peptide

# Host range of CTV



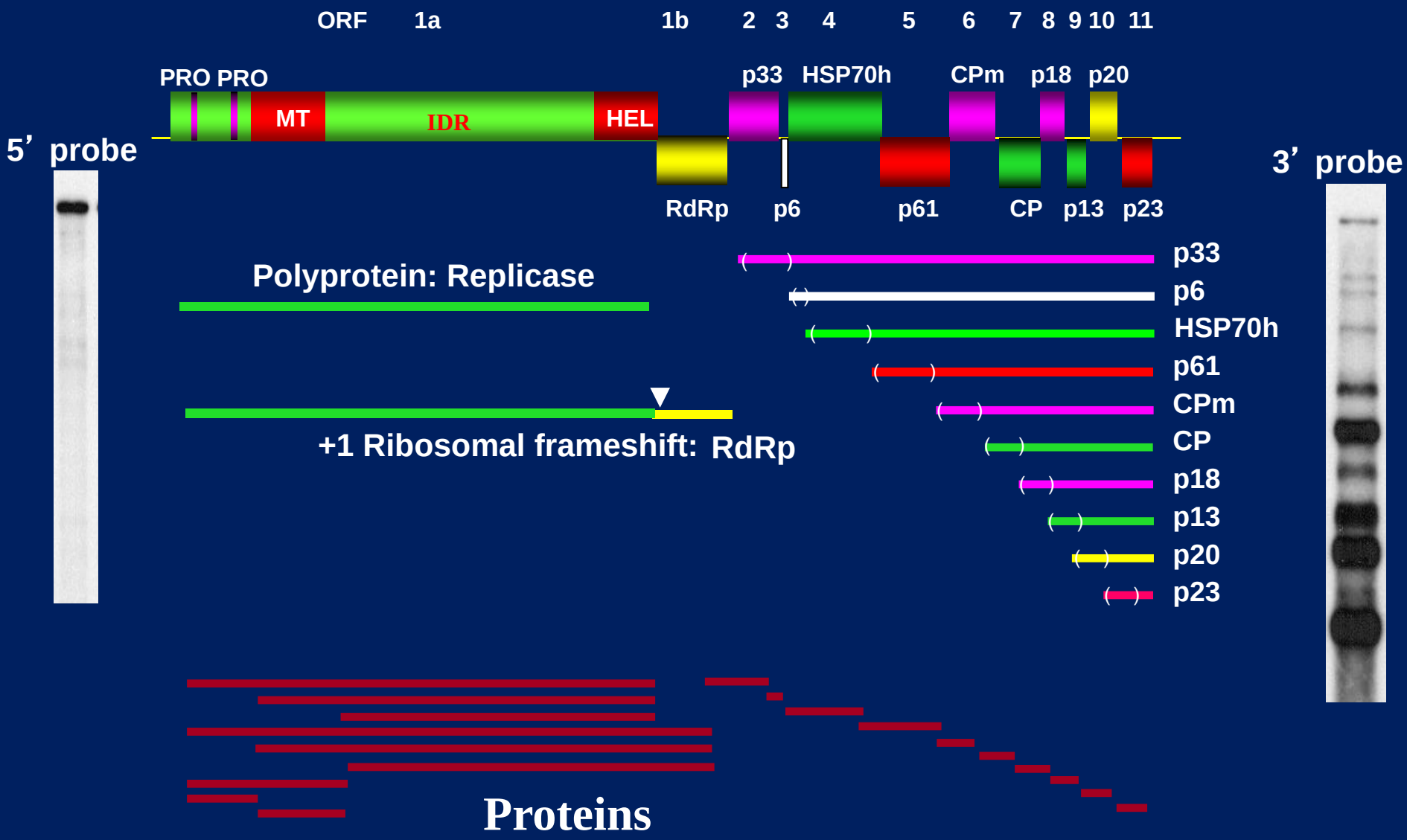


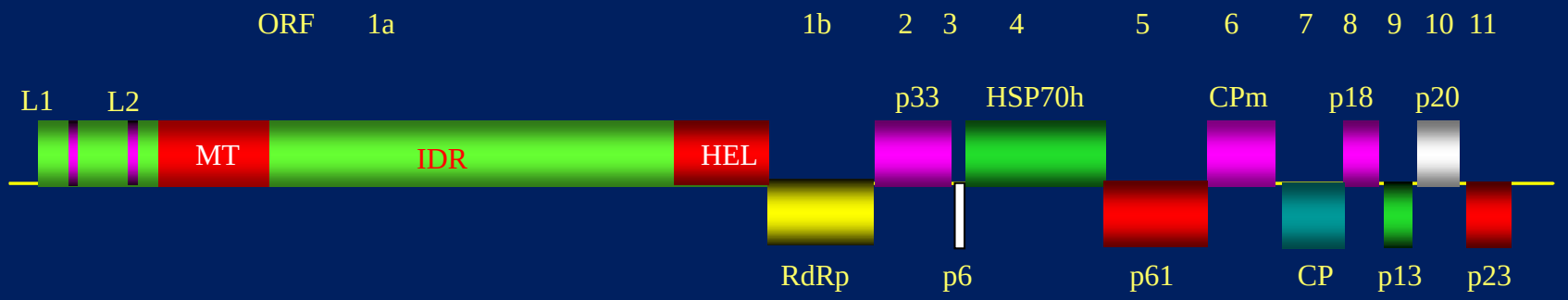
p27
(minor capsid protein, CPm)

p25 (major capsid protein, CP)

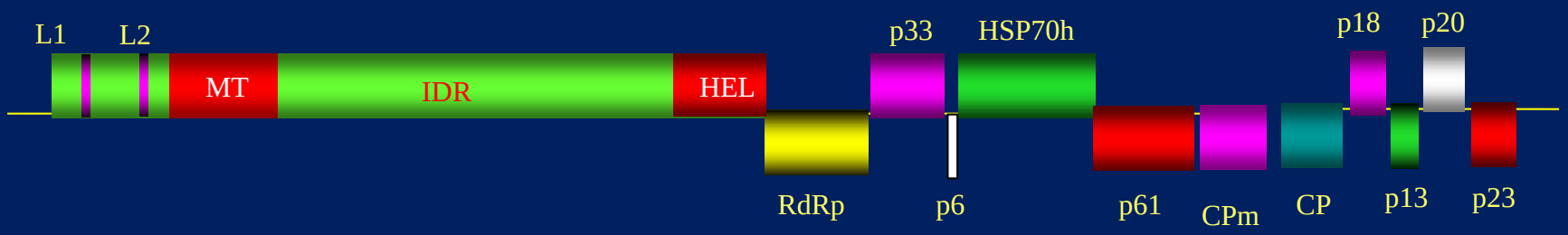
# *Citrus tristeza virus*

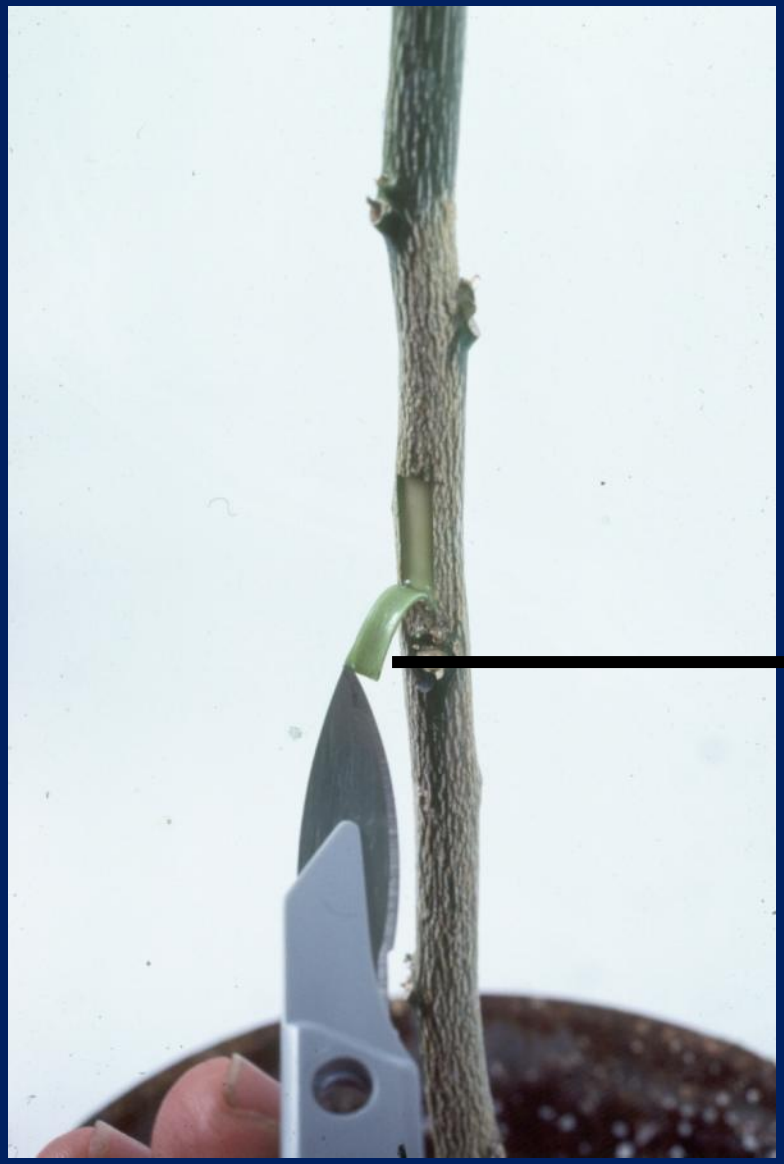


# CTV genome

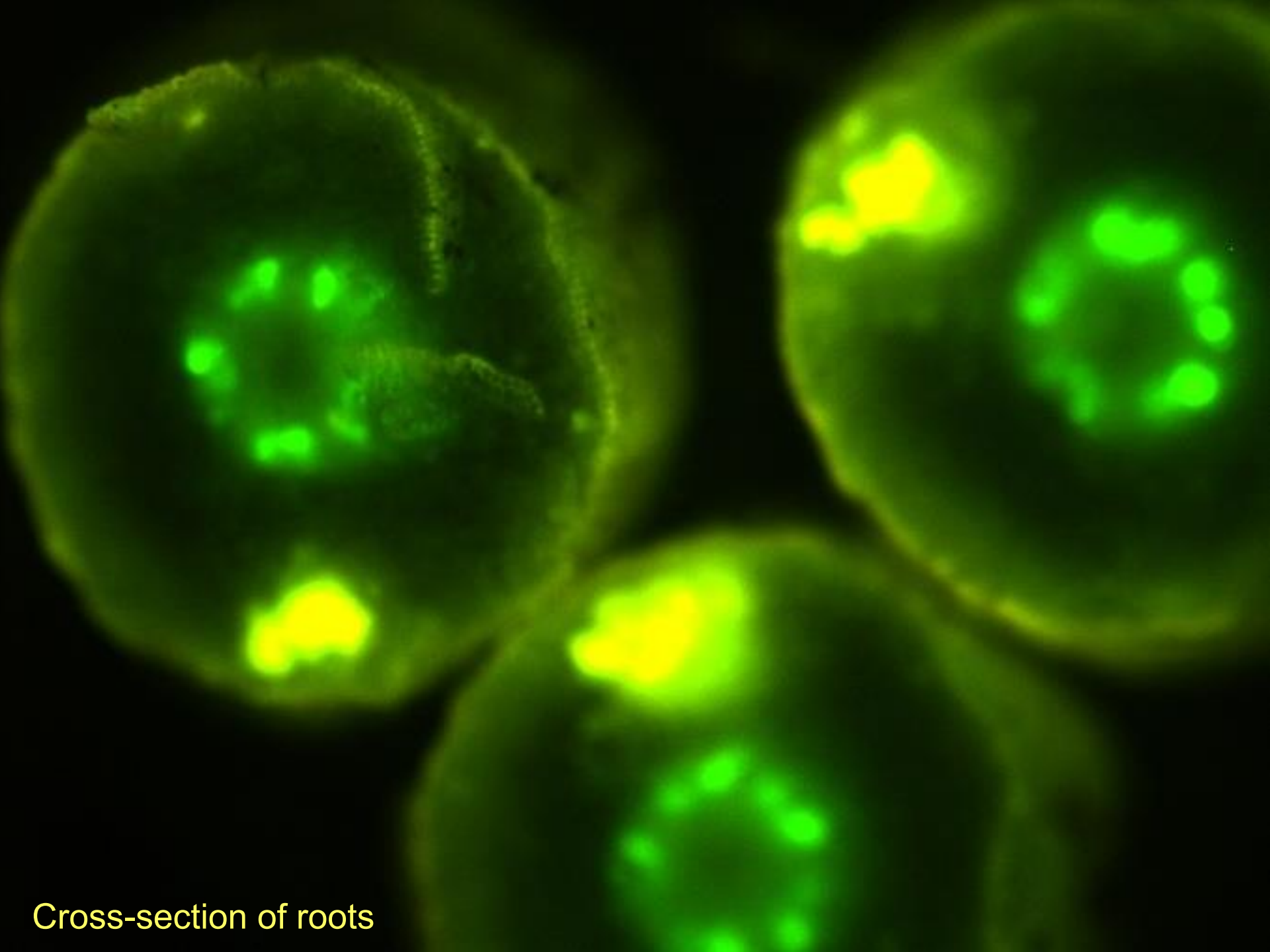



# CTV-based expression vector

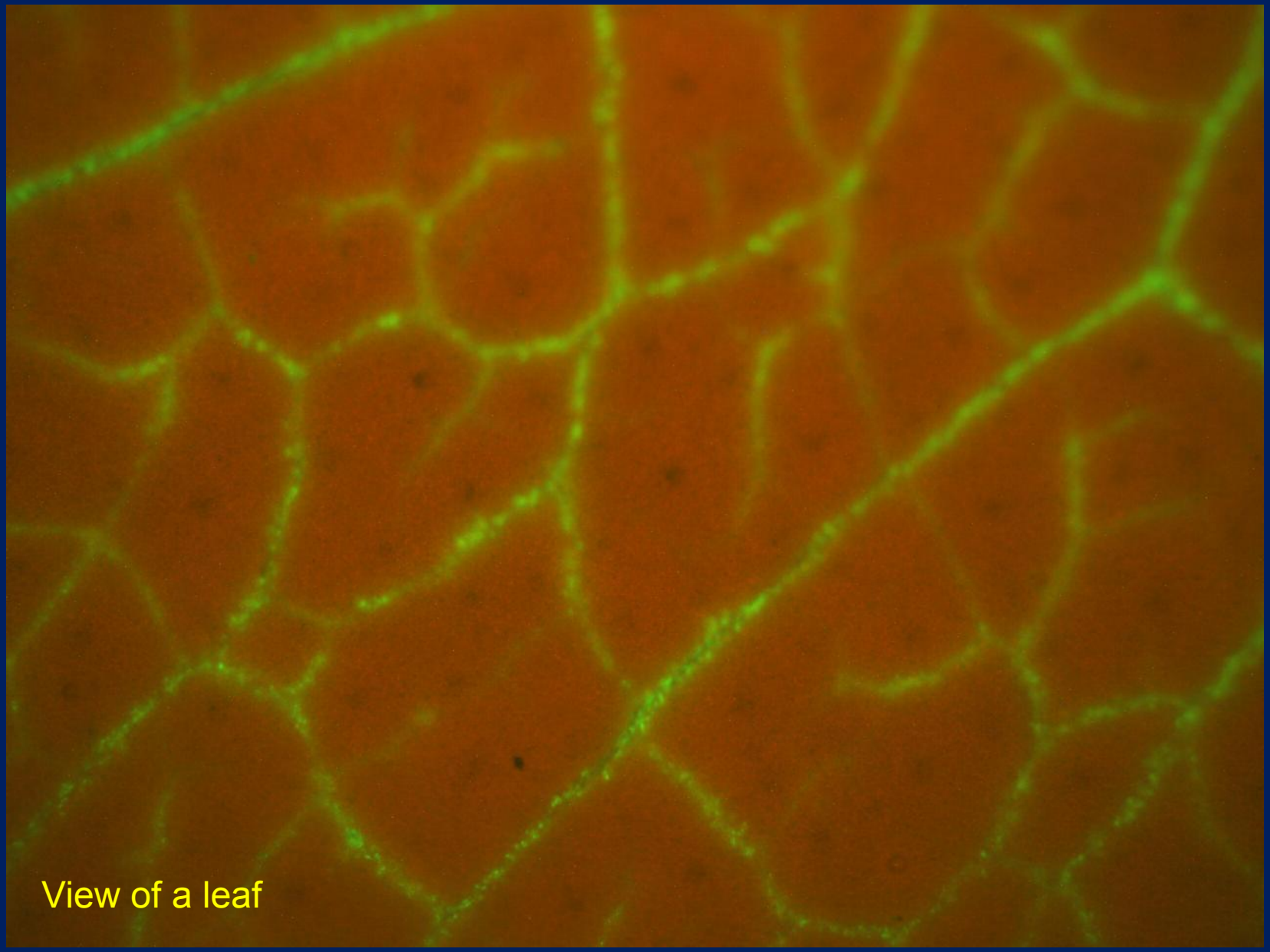




Cross section of a stem

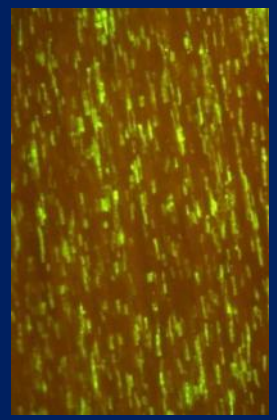


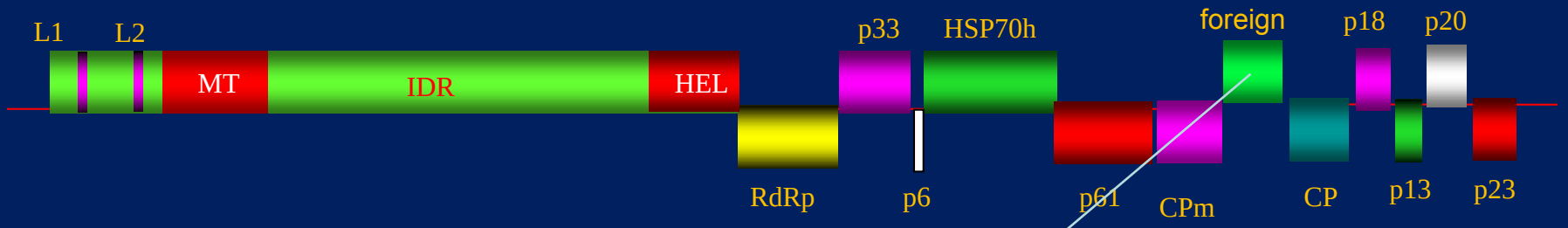
Cross-section of roots


View of a leaf

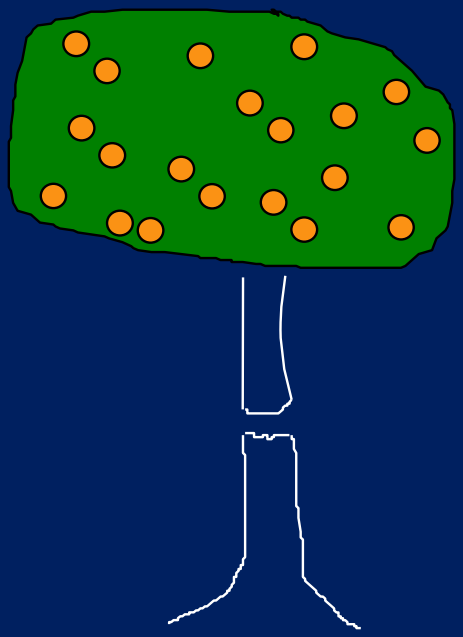
# Citrus tree infected for 14 years

Tree was inoculated in April 2003






Antibacterial gene or anti-psyllid gene or siRNA



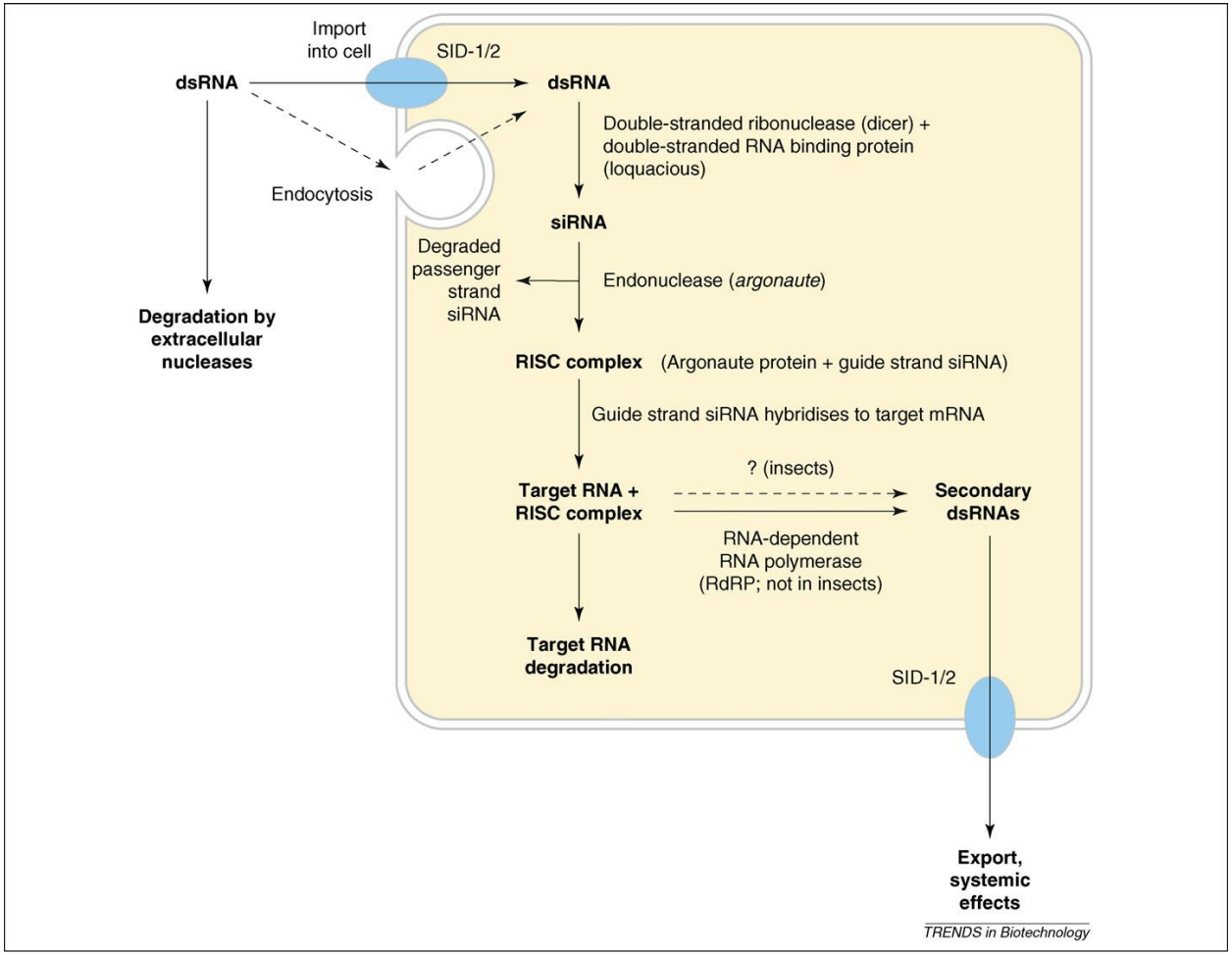

VICTORINOX
SWITZERLAND
STAINLESS

# What are the possibilities?

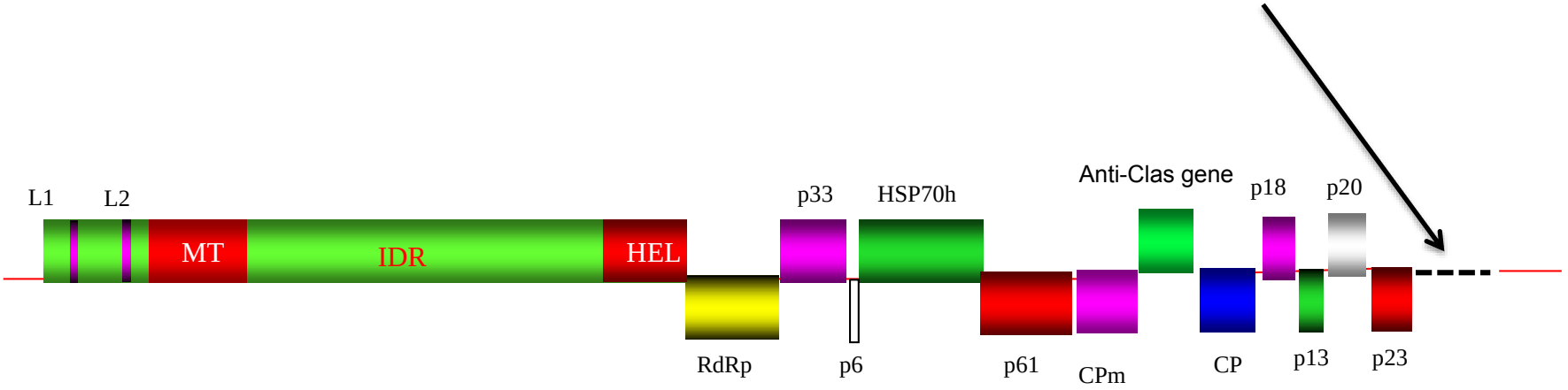
- Protect new plantings?
  Put CTV vector + gene in nursery trees

- Treat HLB infected trees already in the field?
  Graft-inoculate trees in the field

# RNAi mechanism – in plants it is a defense against viruses

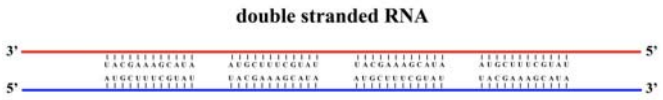


Sequences against a plant gene (mRNA)

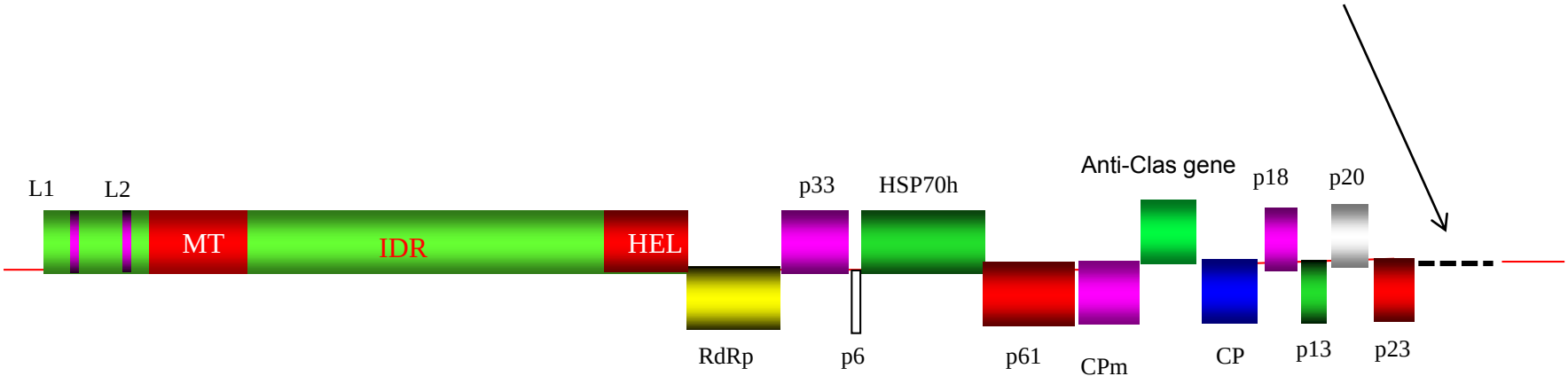


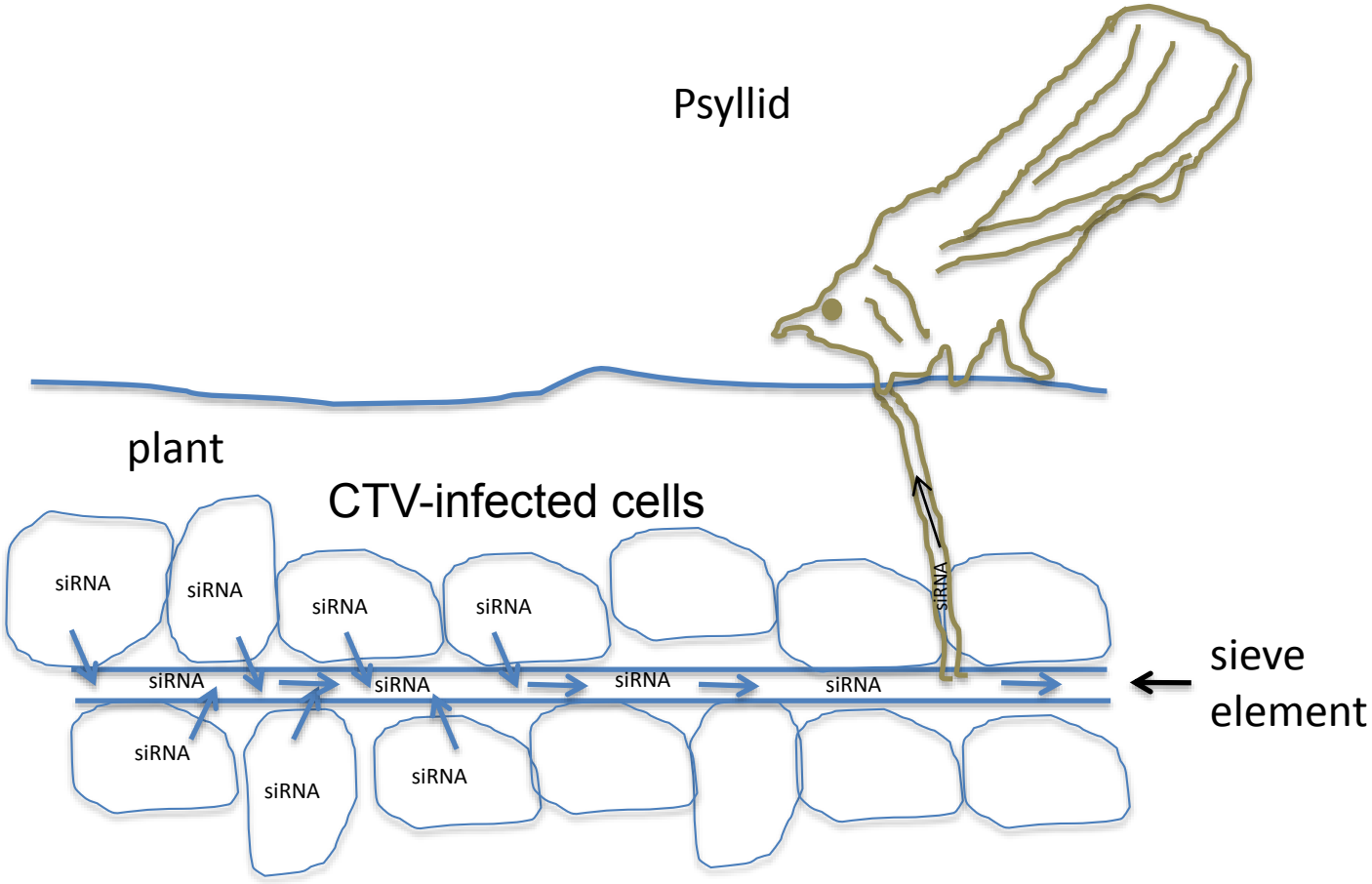

Results in white veins

Shatters Lab (USDA Fort Pierce), Killiny Lab (UF CREC), Falk Lab (UC Davis) first tested double stranded RNAs against psyllids and provided effective sequences to us.




**Target sequence for psyllid gene inserted into CTV**





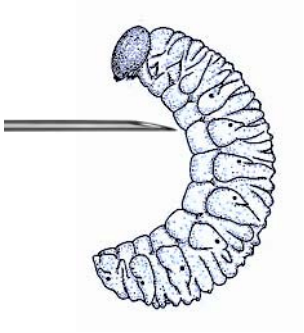

Peptides would be taken up the same as siRNAs

Nabil Killiny, CREC

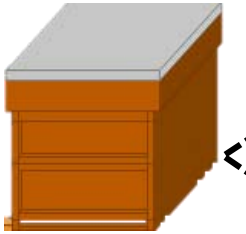
**RNA extraction**

For gene expression

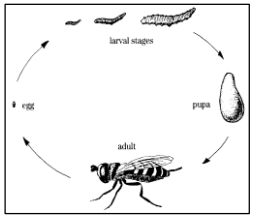
**Microinjection**



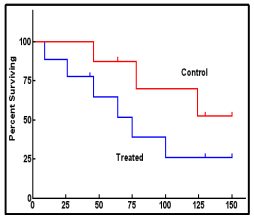
**Malformation**



**Back to hive**




**Metamorphosis**




**Survival**

**THIS RESEARCH HAS BEEN FUNDED**

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**BARD**

**(Binational Agricultural Research & Development Fund)**

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