Bt toxins for suppression of Asian citrus psyllid

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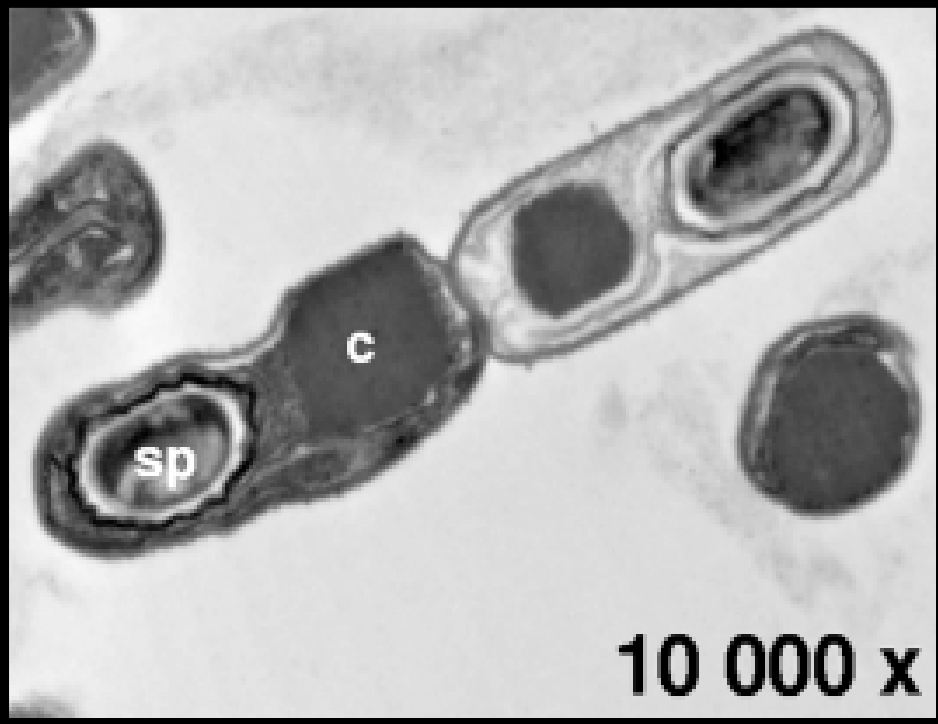


8-15-19

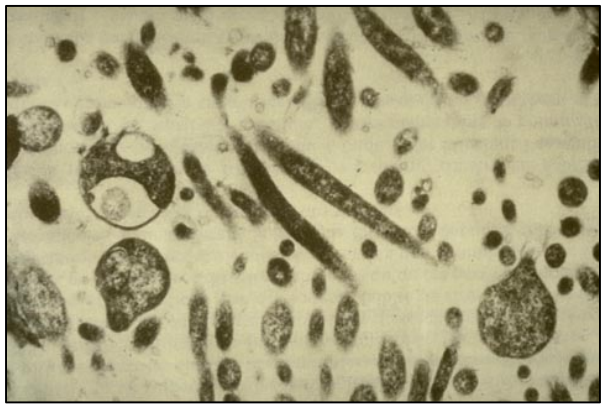
Plant host

Citrus spp.

Insect vector

Asian citrus psyllid (ACP),
Diaphorina citri Kuwayama (Hemiptera: Psyllidae)

Pathogenic bacterium



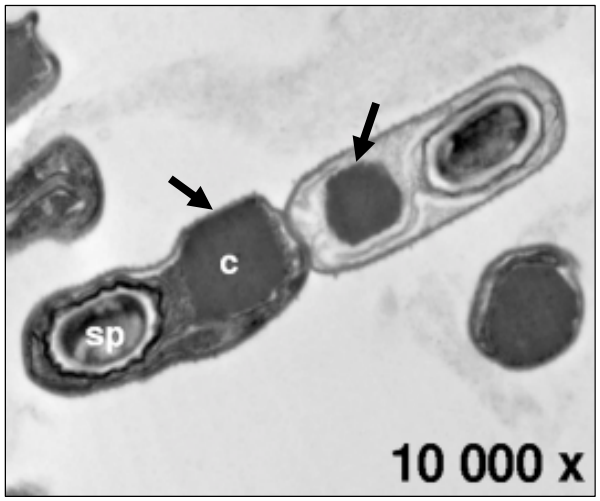
Candidatus Liberibacter asiaticus (CLas)

Citrus Greening Huanglongbing (HLB)

Bacillus thuringiensis (Bt)

Bacterium

- Gram +ve, spore forming soil bacterium
- Crystal (Cry) proteins produced during sporulation
  - Different Bt strains produce different toxin combinations
- Widely used in sprays for organic agriculture and for control of mosquitoes and other disease vectors



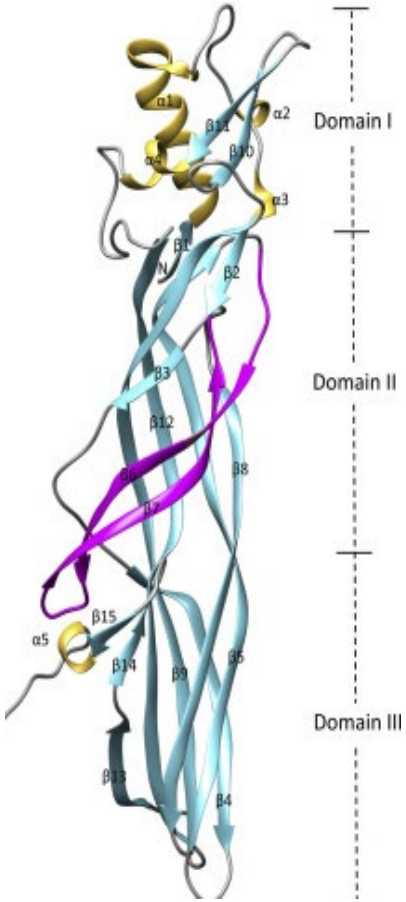
Sporulated cells of *B. thuringiensis* with Cry1Ab crystals (B.A. Federici).

Bt toxins

- Successfully used for insect pest control
- Used in transgenic crops for management of agricultural pests:
  - 79% of corn, 84% of cotton in U.S. in 2016
  - not toxic to humans or non-target organisms

How do Bt toxins work?

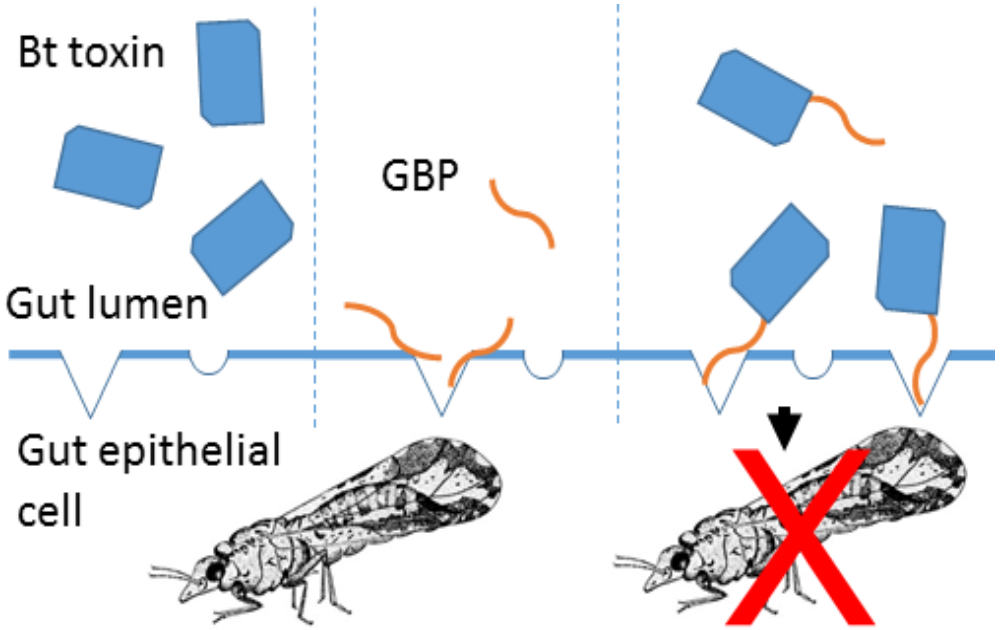
- Ingested by the target insect
  - toxin may require proteolytic activation
- Bind to and damage the insect gut epithelium
  - stop insect from feeding
- Bt toxins widely used to reduce caterpillar damage but don't bind well to the guts of sap-feeding insects



Cry51Aa1

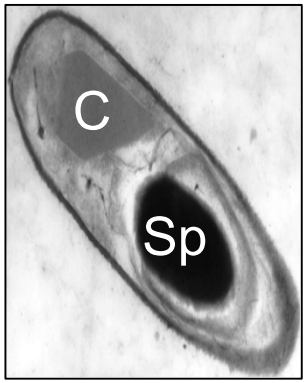
How can we increase the effectiveness of Bt toxins against psyllids?

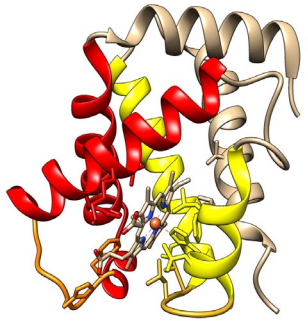
- Attach an artificial anchor to a toxin that has basal activity
  - anchor (gut binding peptide, GBP) binds well to the gut making the toxin more effective



Project Goals

1. Screen toxin mixtures derived from selected Bt strains and identify individual toxins that are toxic to ACP

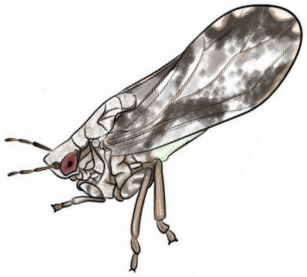




2. Modify the toxin using gut binding peptide (GBP)

Optimize toxin by addition of peptide anchor
(as reported by *N.P. Chougule et al, PNAS 2013)*

3. Assess methods to deliver toxin to ACP via plants

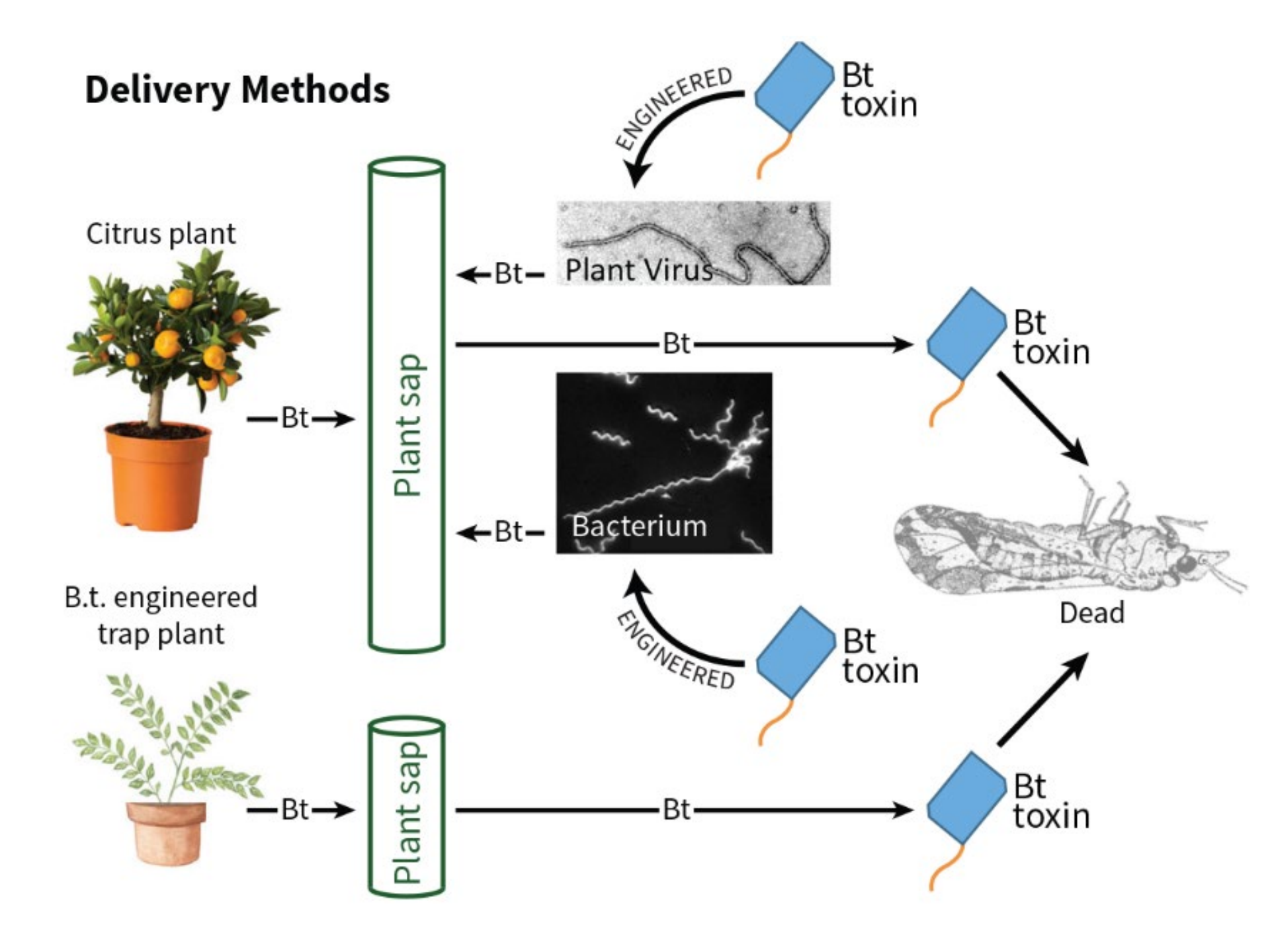


How do we apply this to the field?

Bt toxin must be located in plant sap (phloem) to affect sap feeding insects such as ACP

Approaches:

- Modify plants to express toxin in phloem
- Modify naturally occurring viruses or microbes that reside in the phloem to deliver the toxin


Delivery Methods
ENGINEERED
Bt toxin
Citrus plant
Bt
Plant Virus
Plant sap
Bt
Bt toxin
Bt
Bacterium
Bt
Dead
B.t. engineered trap plant
ENGINEERED
Bt toxin
Bt
Plant sap
Bt
Bt toxin

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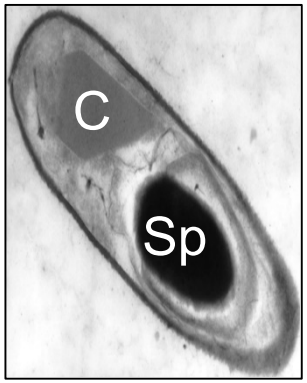

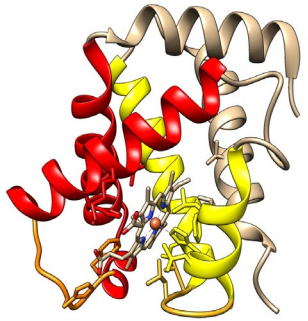

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3. Assess methods to deliver toxin to ACP via plants

- Transgenic plants (Dr. Vladimir Orbovic)
  - Citrus
  - Trap plant, Indian curry
- Citrus tristeza virus (CTV; Dr. Bill Dawson)
- Phloem-inhabiting bacteria (Drs. Caroline Roper and James Borneman, UC Riverside)

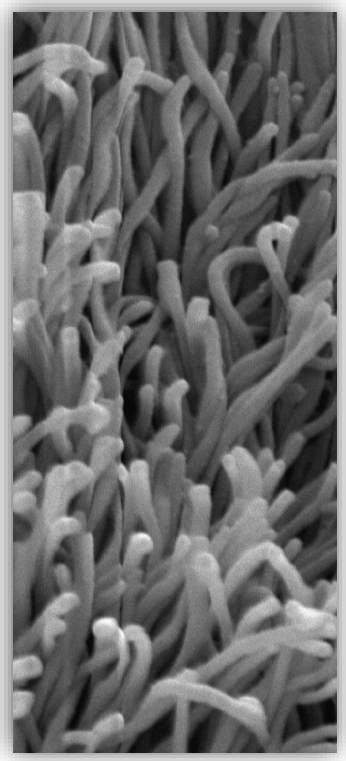
Bt toxins active against ACP

- ~4,000 Bt strains available for testing
  - Dr. Michael Blackburn, USDA ARS, Beltsville, MD
- Toxins derived from different strains were tested for toxicity to ACP
  - Five strains were toxic at 500 µg/ml
- Three individual toxins showed comparable toxicity against ACP
  - Cry1Ab, Cry1Ba, and Cry51Aa1
  - LC50 100-200 ppm: *Further optimization needed*

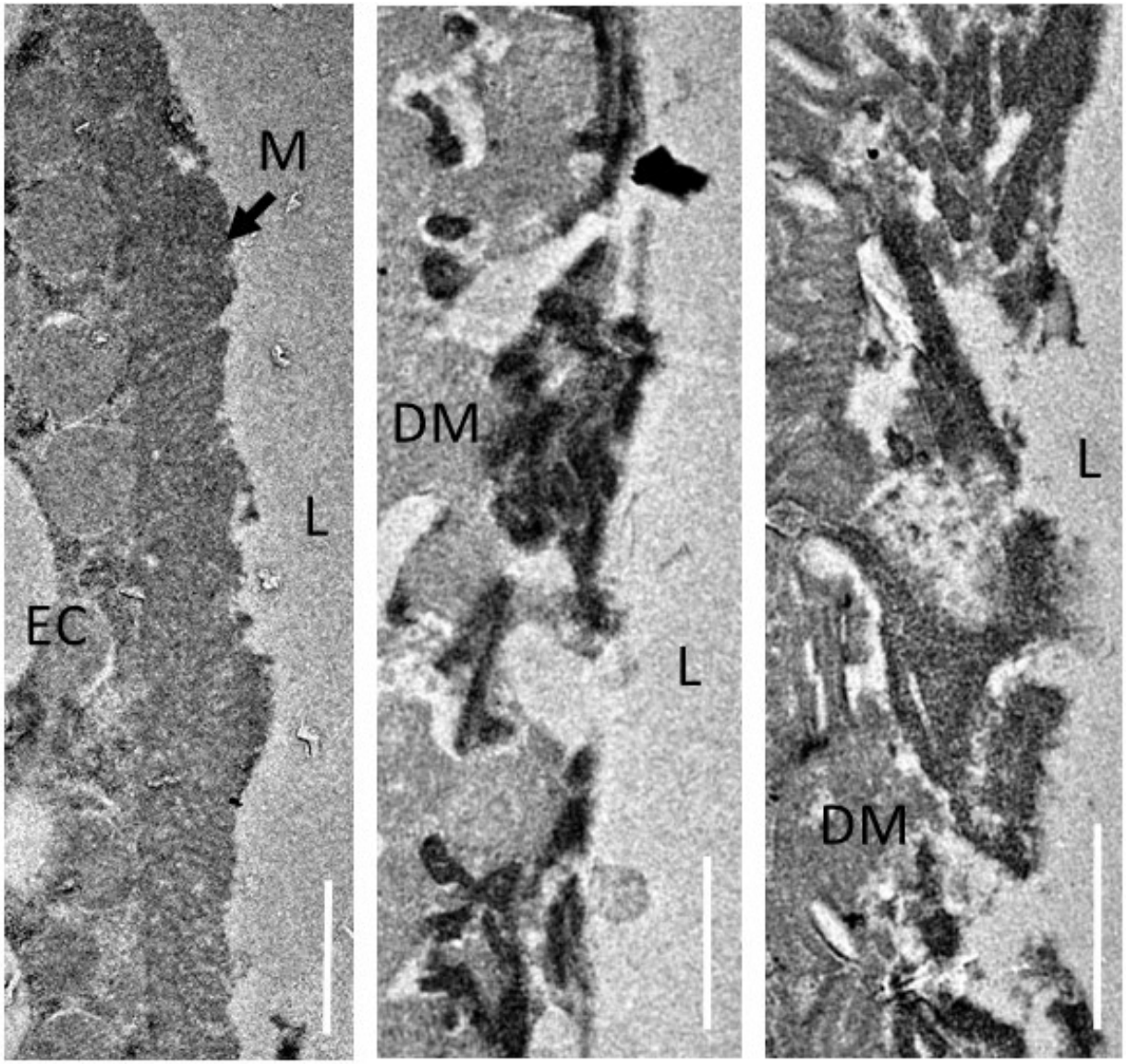
CRDF Citrus Research and Development Foundation, Inc.

Fernandez-Luna MT et al., 2019. Toxins pii: E173.
doi: 10.3390/toxins11030173

Cry1Ba disrupts the ACP midgut epithelium



Insect gut microvilli



Buffer control IBP-00200 Cry1Ba

Cry1Ba may be phytotoxic

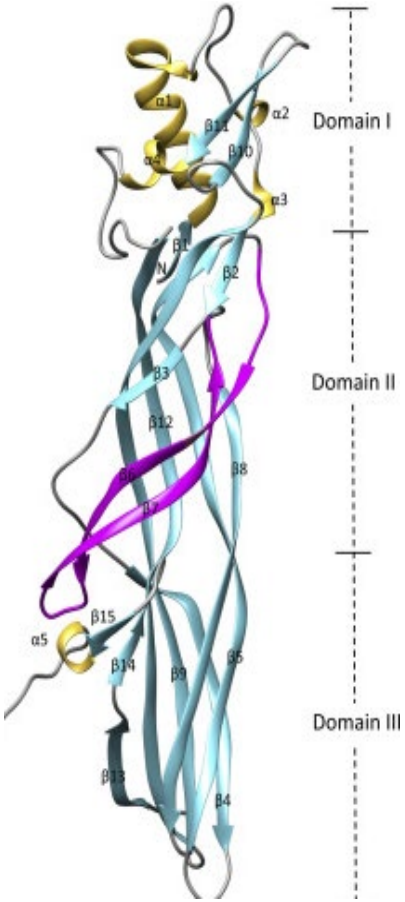
Transformation of Indian curry (trap plant), Duncan grapefruit and Valencia orange :

- Low rate of recovery of transformed lines
- Transcription (production of RNA) confirmed
- Translation (production of protein) not confirmed

CTV constructs with Cry1Ba mutated so no expression

Focus is now on Cry51Aa1

- Cry51Aa1 currently being expressed in plants
  - Transgenic plants
  - CTV delivery
- Testing of toxins modified with gut binding peptide underway
  - CTV delivery



Cry51Aa1

Four ACP gut binding peptides identified

Peptide 12 ( )

Peptide 15 ( )

Peptide 18 ( )

Peptide 22 ( )

Peptide-mCherry fusion proteins used to confirm binding

Summary

1. Toxin mixtures from 5 Bt strains showed toxicity against ACP at 500 µg/ml
2. Individual toxins Cry1Ab, Cry1Ba and Cry51Aa1 have comparable toxicity against adult ACP
3. Cry1Ba expressed in CTV (Dawson) and transgenic citrus and Indian curry leaf plant (Orbovic), but Cry1Ba1 appeared to be phytotoxic
4. Peptide 15 binds specifically to ACP gut proteins
5. Transgenic plant- and CTV-mediated delivery of Cry51Aa1 is underway

Challenges

- Identify the best method to deliver sufficient toxin for psyllid control
- Address regulatory requirements to allow growers to use the technology

Bt Toxin-based Strategies for Management of *Diaphorina citri* and Citrus Greening

DCBT
D CITRI – BT TOXIN

NIFA

Bt toxin isolation and optimization:

Dr. Pavan Kumar

Mariah Kemmerer

Transgenic plant delivery:

Dr. Seyed Ali Ravanfar

Dr. Vladimir Orbovic

CTV delivery:

Dr. Choaa El Mohtar

Dr. Bill Dawson

In planta bioassays

Dr. Freddy Ibanez-Carrascoa

Dr. Lukasz Stelinski

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