Targeting the Asian Citrus Psyllid

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Feb 19, 2021

Insect vector

Asian citrus psyllid (ACP),
Diaphorina citri Kuwayama (Hemiptera: Psyllidae)
Detected in Florida 1998

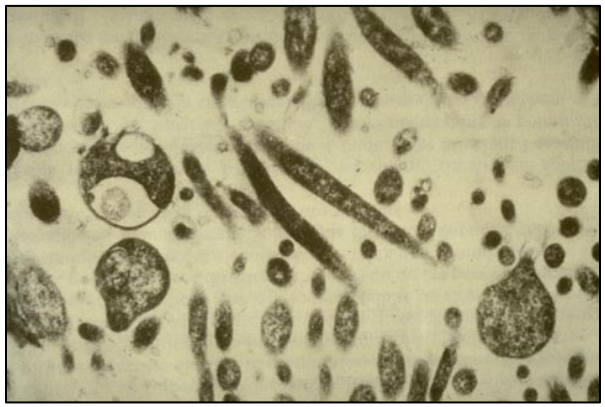
1 hr

Plant host

Citrus spp.

15-30 min feeding

Pathogenic bacterium



Candidatus Liberibacter asiaticus (CLas)

Citrus Greening

Detected in Florida 2005

Targeting the Asian Citrus Psyllid

- Primary target to interrupt transmission of CLas

- Widespread application of classical chemical insecticides
  → Insecticide resistance

What alternatives are there for suppression of ACP?

Alternative approaches include –

1. Pesticidal proteins that target the insect gut (e.g. from Bt)
2. Silencing of genes essential for insect survival (RNA interference)
3. Biological control: Insect pathogens (e.g. viruses), parasitoids
4. Attractants (pheromones), repellents, trapping

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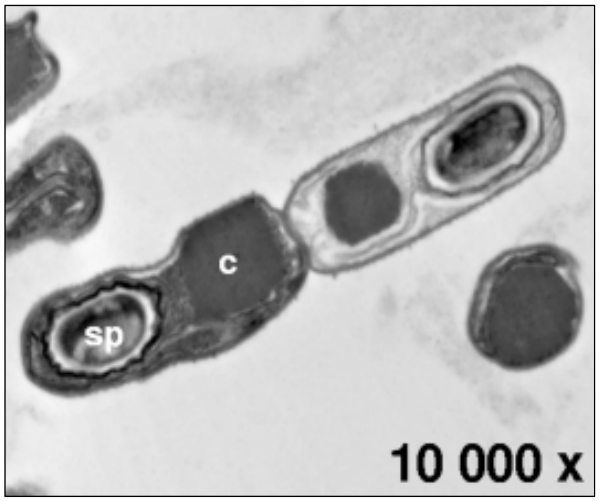
- Deliver both to the insect gut
- Combination works better than individual strategies

Pesticidal Proteins that Target the Insect Gut

Bacillus thuringiensis (Bt)

- Spore forming soil bacterium
- Pesticidal proteins produced during sporulation
  - Different Bt strains produce different protein 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).

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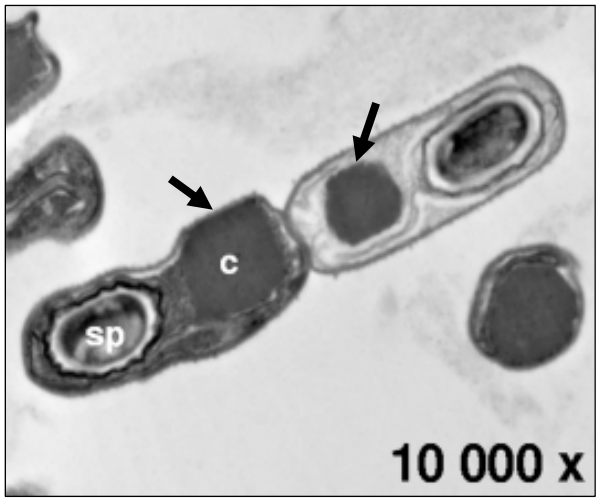


Sporulated cells of *B. thuringiensis* with Cry1Ab crystals (B.A. Federici).

Bt pesticidal proteins

- Successfully used for insect pest control
- Used in transgenic crops for management of agricultural pests:
  - majority of corn and cotton in U.S. expresses Bt proteins
  - not toxic to humans or non-target organisms

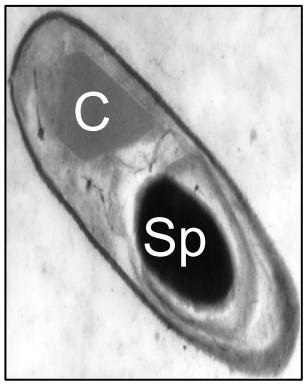
Asian citrus psyllid (ACP),
Diaphorina citri Kuwayama
(Hemiptera: Psyllidae)

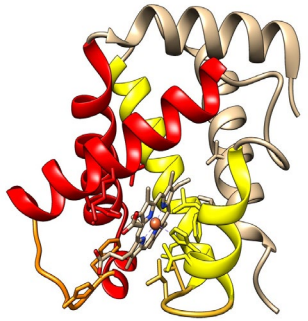
Identify ACP-active pesticidal proteins

1. Screen pesticidal protein mixtures derived from Bt strains and identify proteins that are toxic to ACP

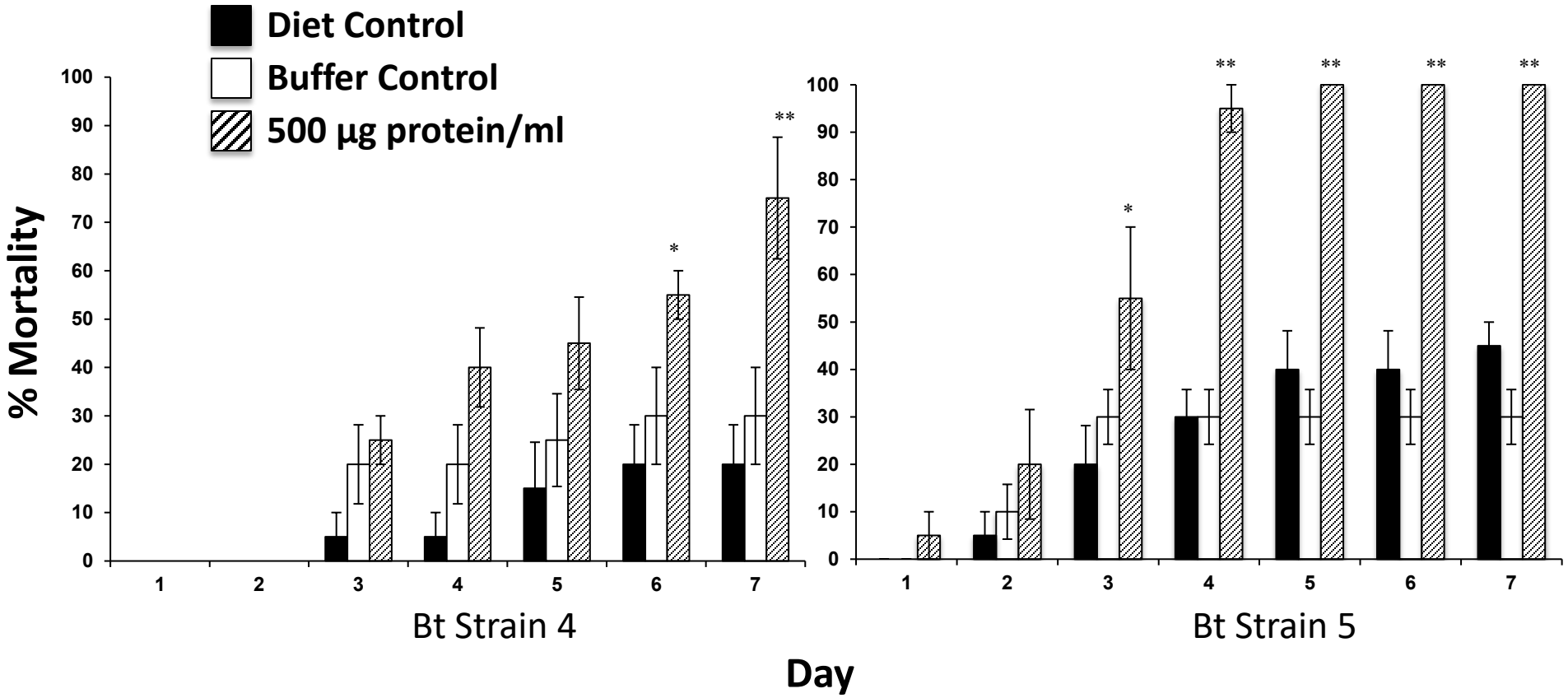
~4,000 Bt strains available for testing

Dr. Michael Blackburn, USDA ARS, Beltsville, MD





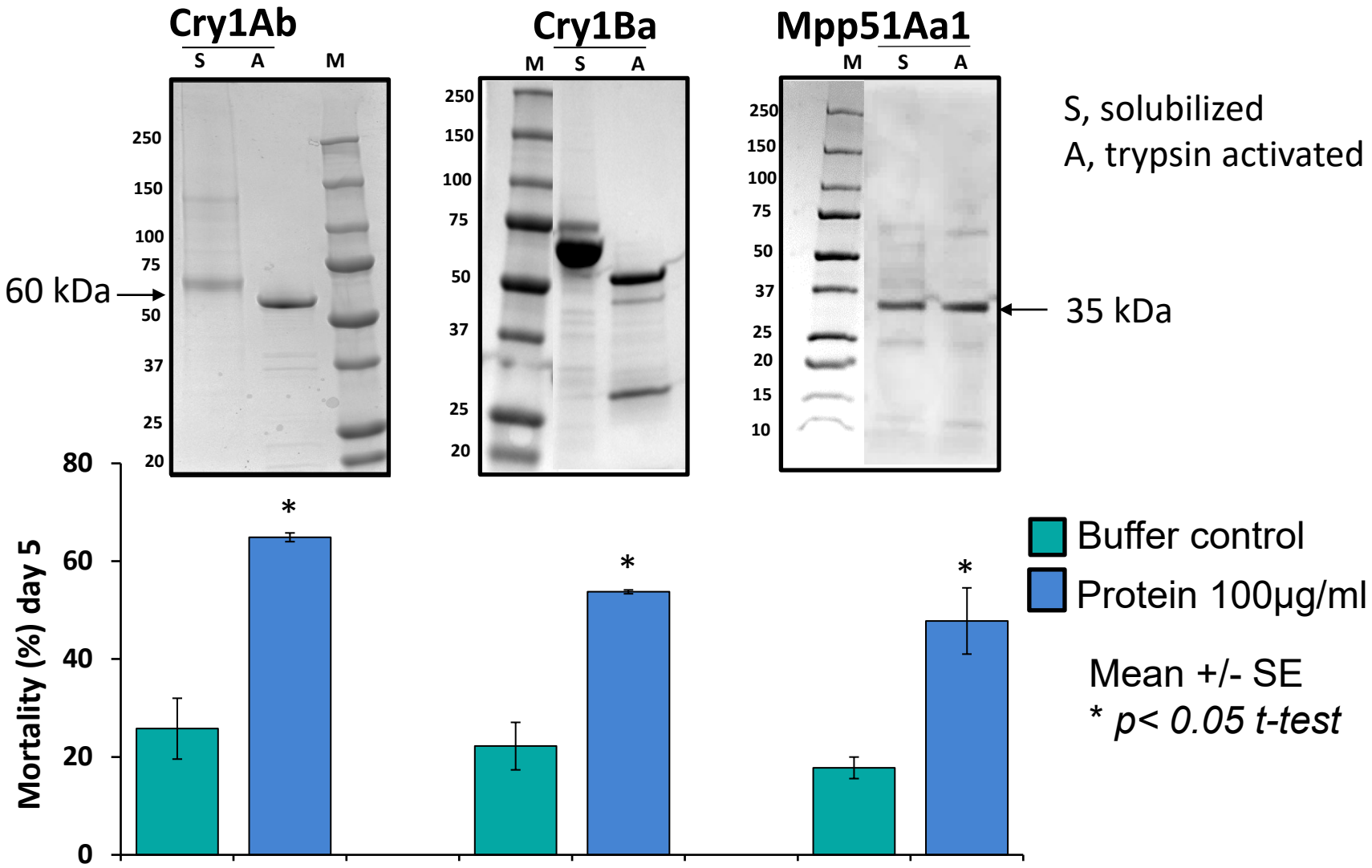
Pesticidal protein mixtures derived from strains 4 and 5 are toxic to ACP



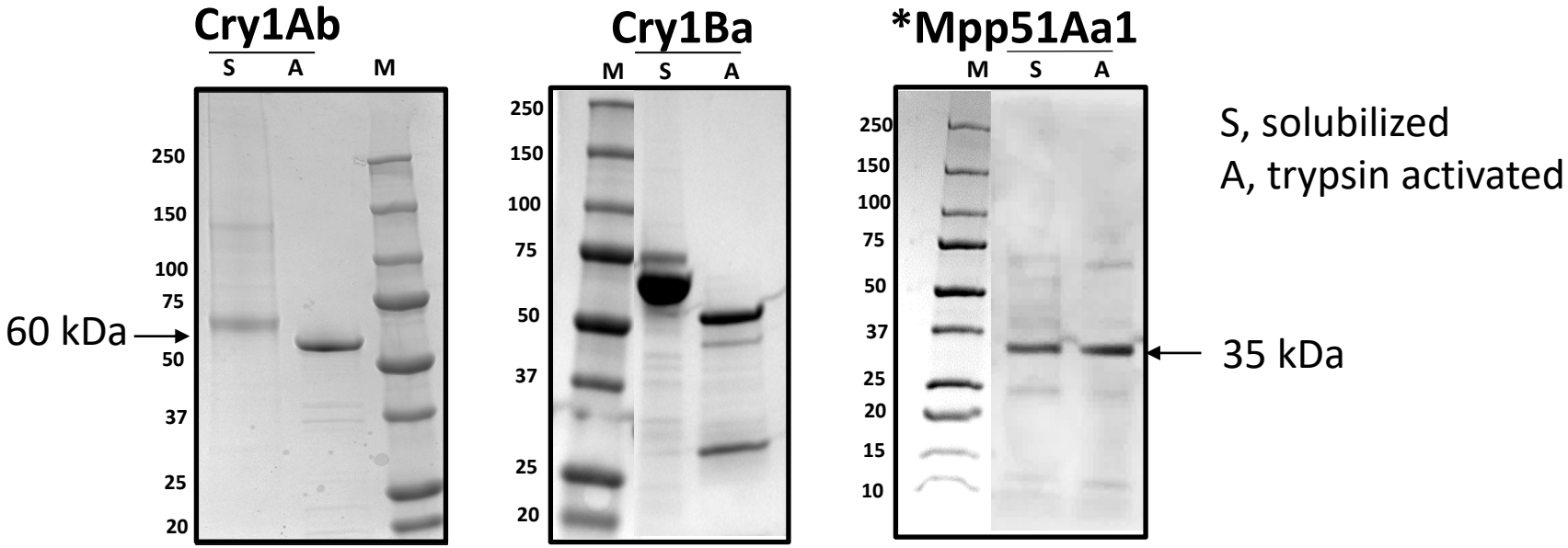
Mean ± SEM (n=5). Significant differences with reference to control treatments indicated as * p<0.05, ** p<0.01 (One-way ANOVA, Tukey's test).

M.T. Fernandez-Luna et al., 2019

Bt pesticidal proteins active against ACP



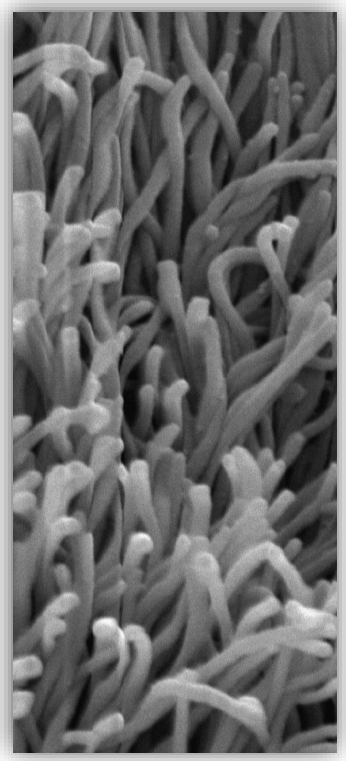
Bt pesticidal proteins active against ACP



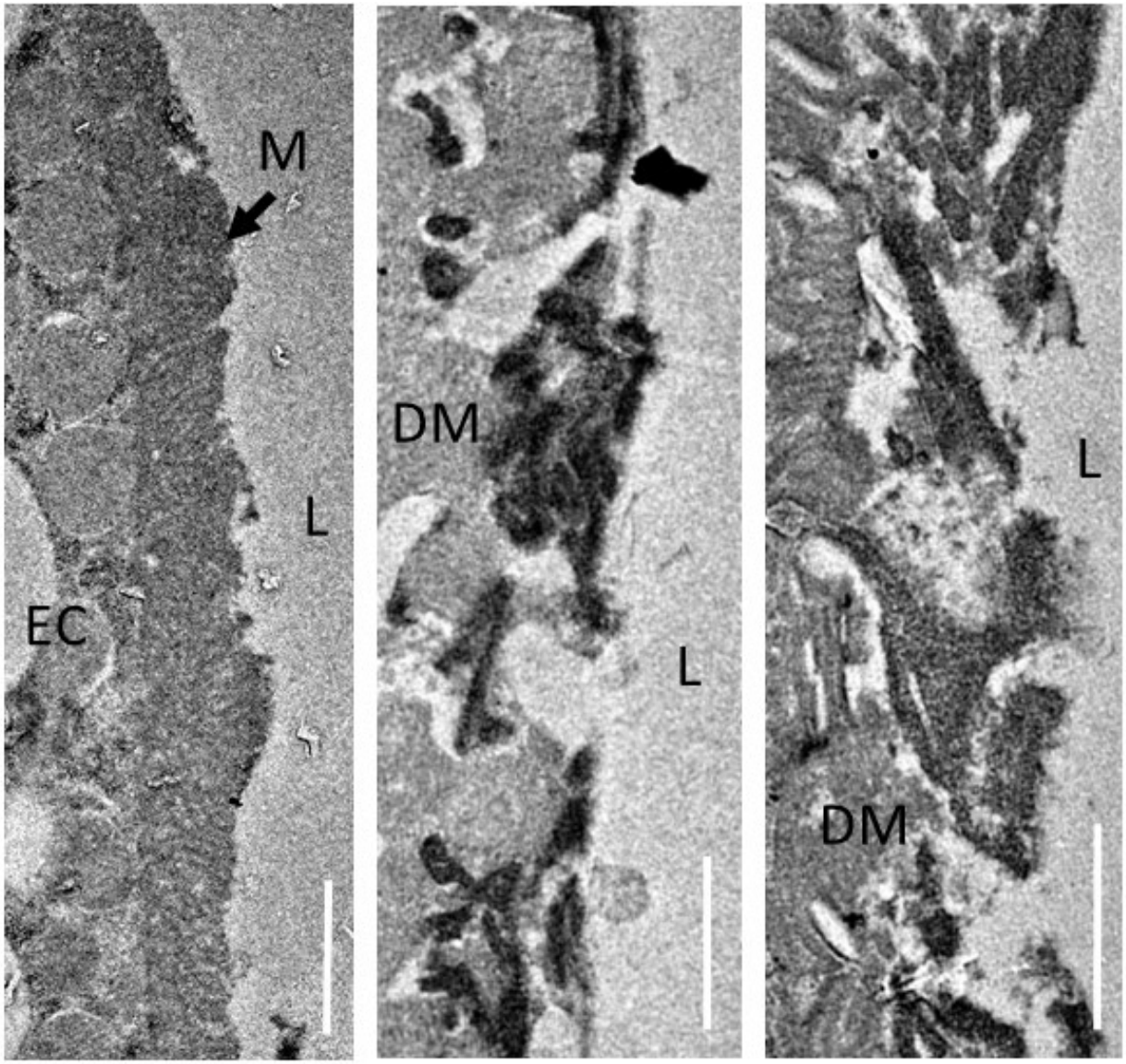
Bacterial Pesticidal Protein Resource Center, BPPRC.org

*N. Crickmore et al., 2020. A structure-based nomenclature for *Bacillus thuringiensis* and other bacteria-derived pesticidal proteins. J. Invertebr. Pathol.

Cry1Ba disrupts the ACP midgut epithelium



Insect gut microvilli



Buffer control IPB-00200 Cry1Ba

Toxicity of ACP-active pesticidal proteins

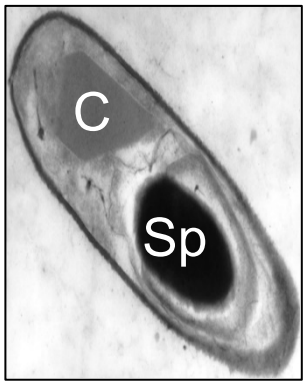
Pesticidal protein	LC_{50} on day 5 against adult ACP (µg/ml: ppm)	Standard error (+/-)
Cry1Ab	**116.5**	31.9
Cry1Ba1	**123.7**	12.9

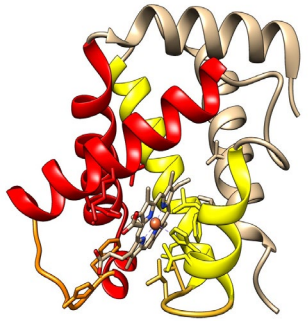
Further optimization is needed!

M.T. Fernandez-Luna et al., 2019

Enhance efficacy against ACP

1. Screen pesticidal protein mixtures derived from Bt strains and identify proteins that are toxic to ACP



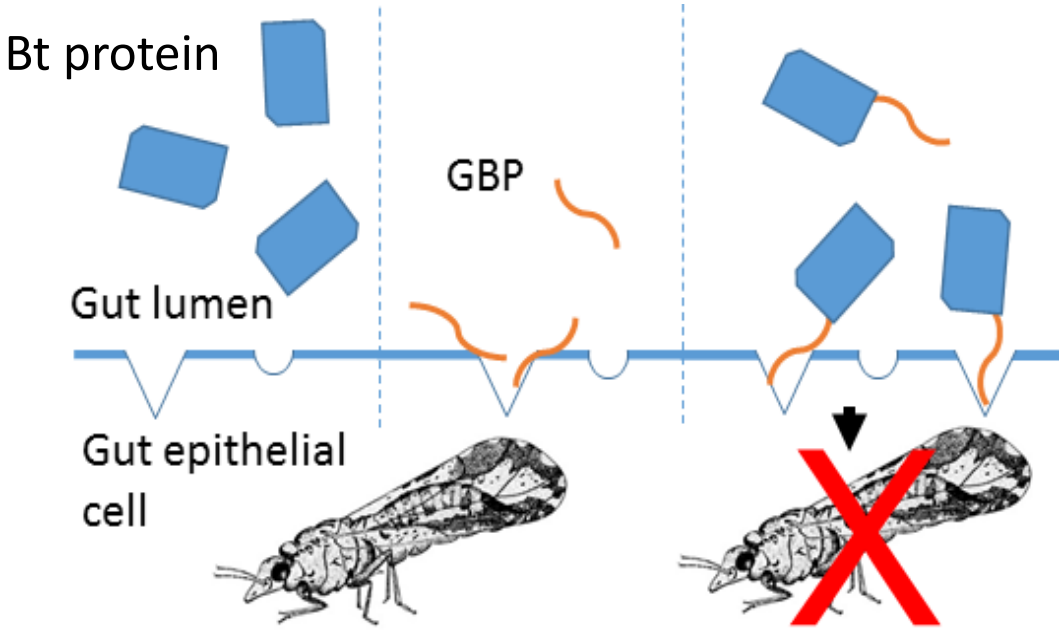


2. Modify the pesticidal protein using gut binding peptide (GBP)

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

How can we increase the effectiveness of Bt pesticidal proteins against psyllids?

- Attach an artificial anchor (gut binding peptide, GBP) to the pesticidal protein
  - anchor binds well to the gut making the toxin more effective



N.P. Chougule et al, PNAS 2013

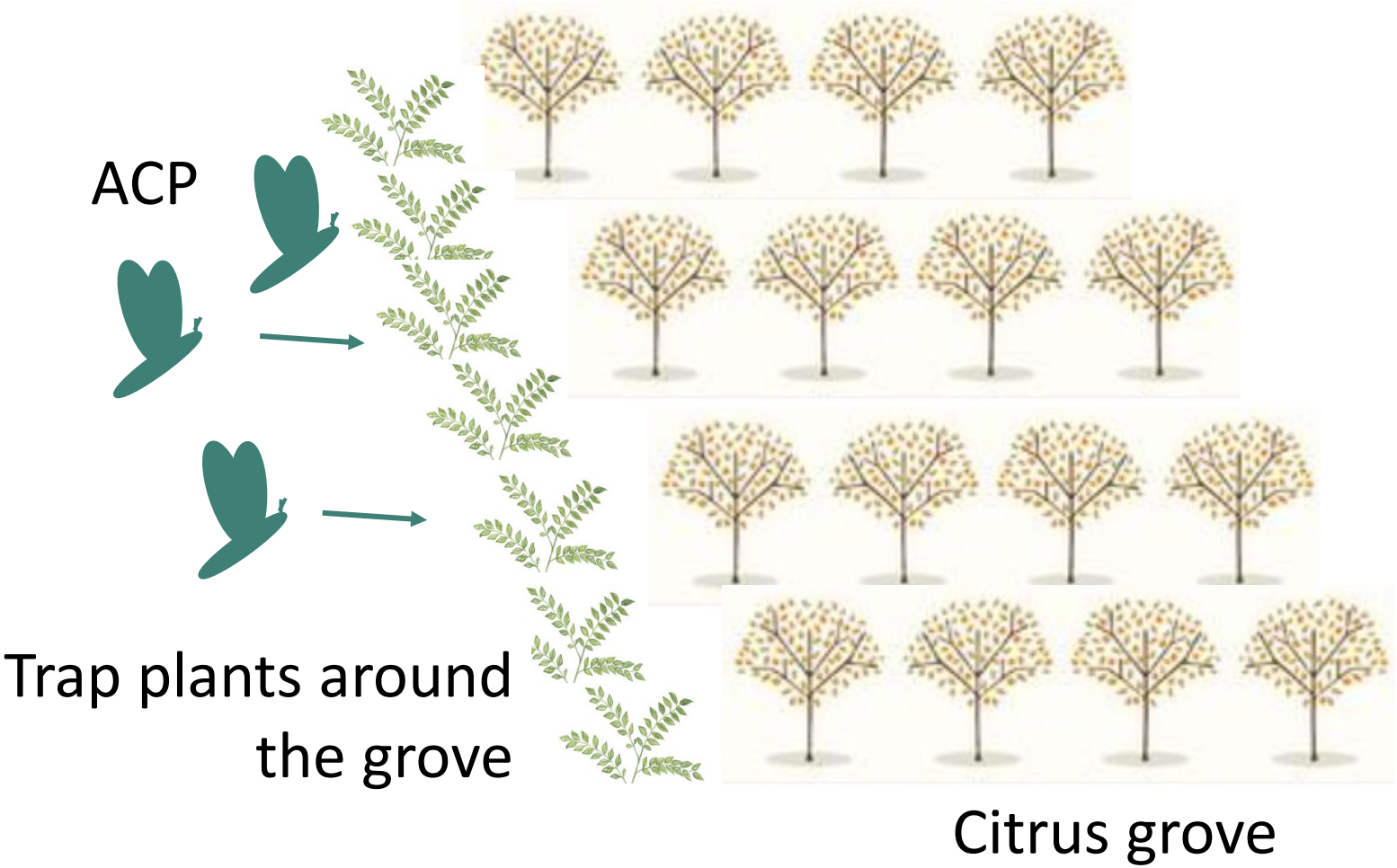
How do we apply this to the field?

Need to get Bt-derived pesticidal proteins into the plant sap (phloem) for ingestion by ACP

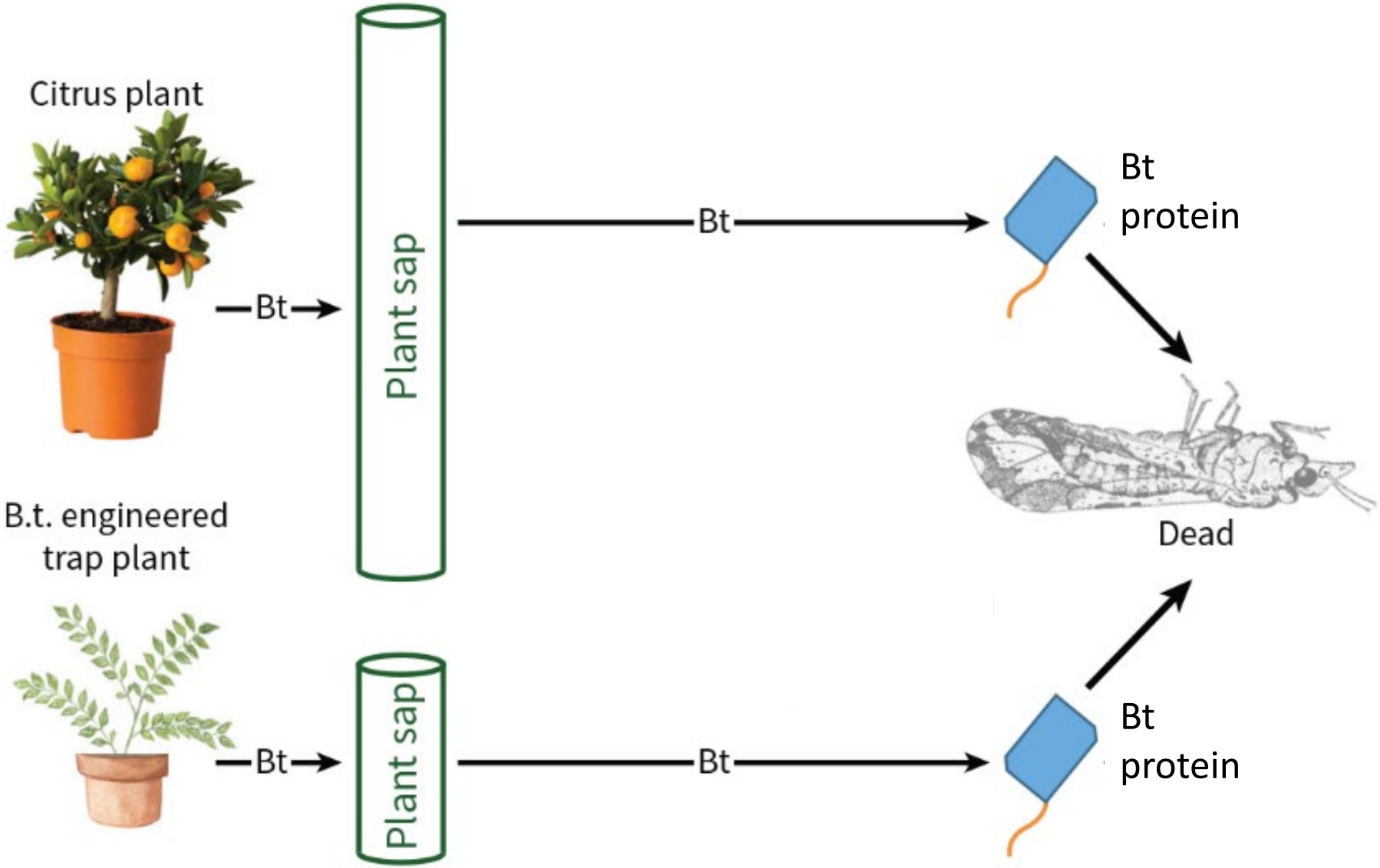
Approaches:

- Modify plants to express proteins in phloem

Trap Plants to Deflect ACP from Citrus Grove



Delivery Methods

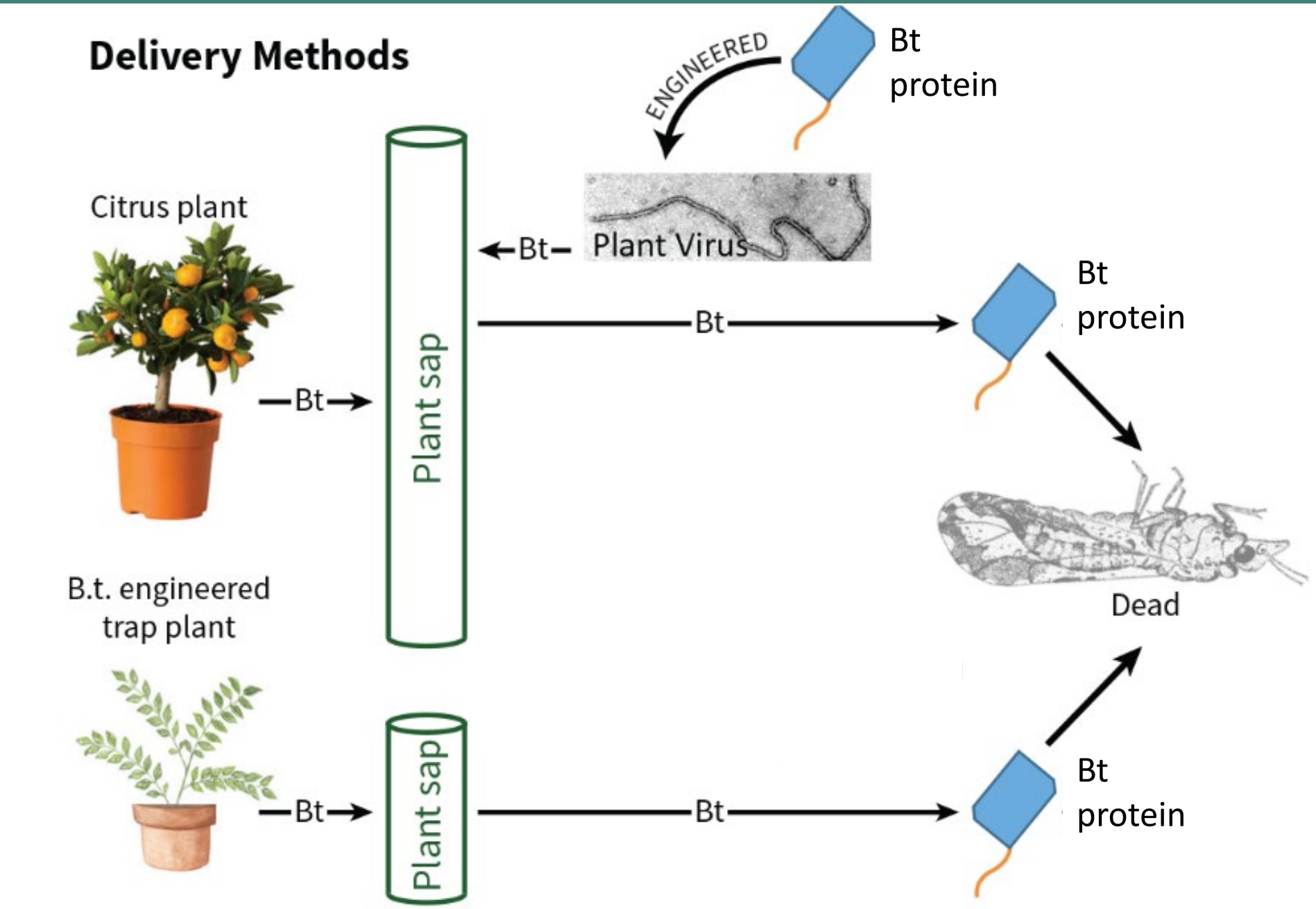


How do we apply this to the field?

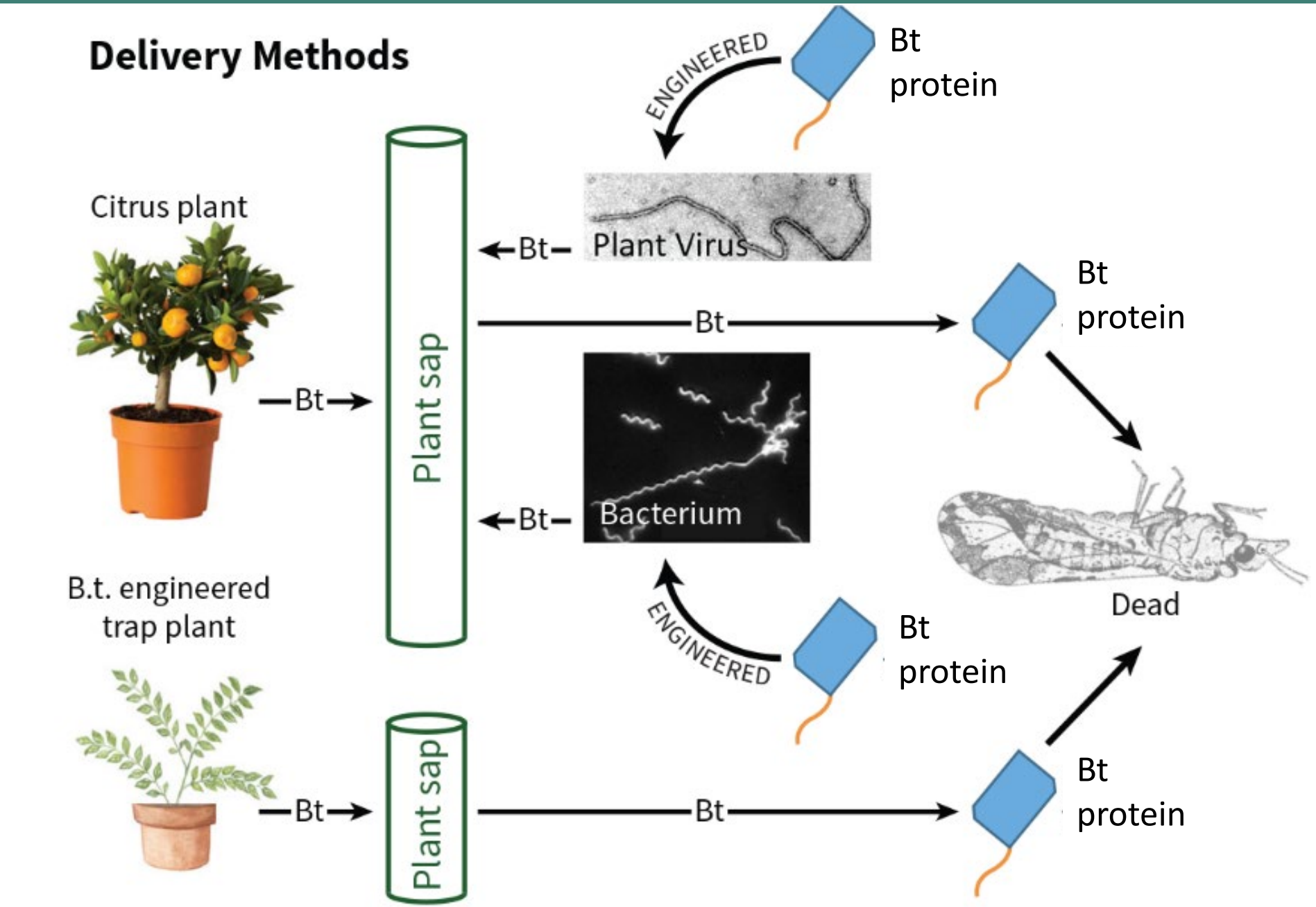
Need to get Bt-derived pesticidal proteins into the plant sap (phloem) for ingestion by ACP

Approaches:

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

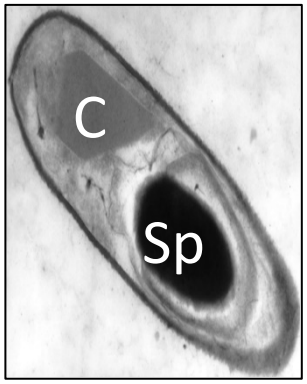

Delivery Methods
ENGINEERED
Bt protein
Citrus plant
Bt
Plant Virus
Bt
Plant sap
Bt
Bt protein
Dead
B.t. engineered trap plant
Bt
Plant sap
Bt
Bt protein

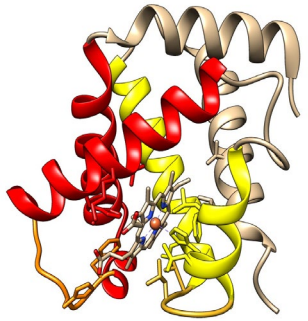
Bt Proteins for Suppression of Asian Citrus Psyllid



Assess Delivery Options

1. Screen pesticidal protein mixtures derived from Bt strains and identify proteins that are toxic to ACP





2. Modify the pesticidal protein using gut binding peptide (GBP)

3. Assess methods to deliver pesticidal proteins 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)

Summary

Multiple ACP-active pesticidal proteins have been identified

- Protein optimization for increased efficacy by addition of gut binding peptides

Transgenic citrus and trap plants expressing pesticidal proteins are effective for suppression of ACP under greenhouse conditions

- CTV also shows promise
- Bacterial delivery in early stages

IPM for management of ACP and citrus greening

Bt-derived pesticidal proteins provide additional tools against ACP for use with other strategies in an *integrated pest management* approach.

Bt-derived pesticidal proteins now being tested in combination with gene silencing RNAs to target ACP

Bt proteins + RNAi more effective

USDA NIFA ECDRE 2020-70029-33177

- Antimicrobial peptides to target CLas

Future

- Identify effective methods to deliver bacterial pesticidal proteins for psyllid control
  - Assess efficacy
  - Familiarize the public with this new approach

- Address regulatory requirements to allow growers to use the technology

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

DCBT
D CITRI – BT TOXIN

NIFA

Bt protein isolation and optimization:

Dr. Pavan Kumar

Ms. 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

USDA NIFA #2017-70016-26755