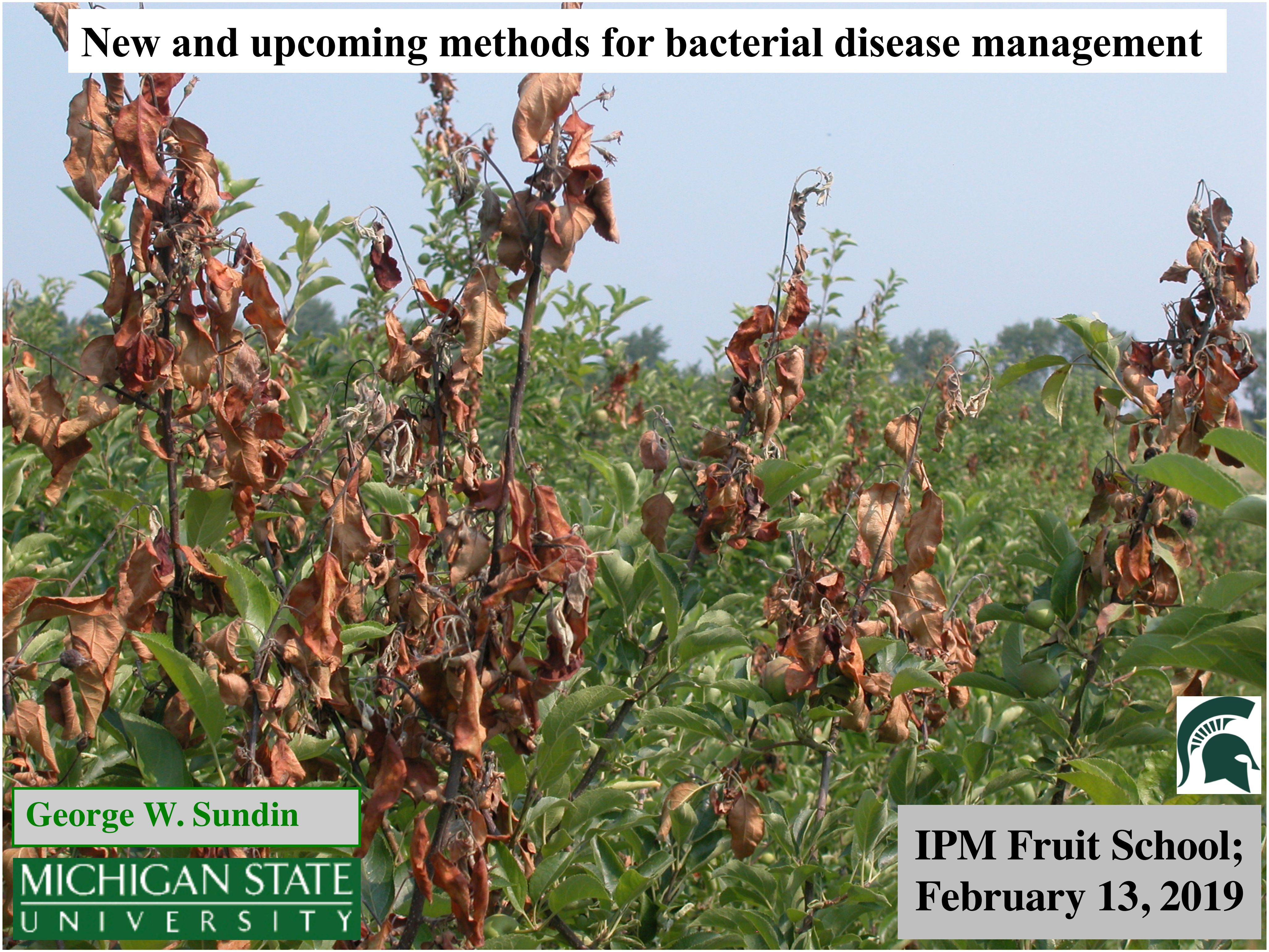
New and upcoming methods for bacterial disease management



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MICHIGAN STATE UNIVERSITY

IPM Fruit School;
February 13, 2019

Newly available methods for bacterial disease management

- **Materials that induce host disease resistance**
  - **Actigard**
  - **LifeGard**

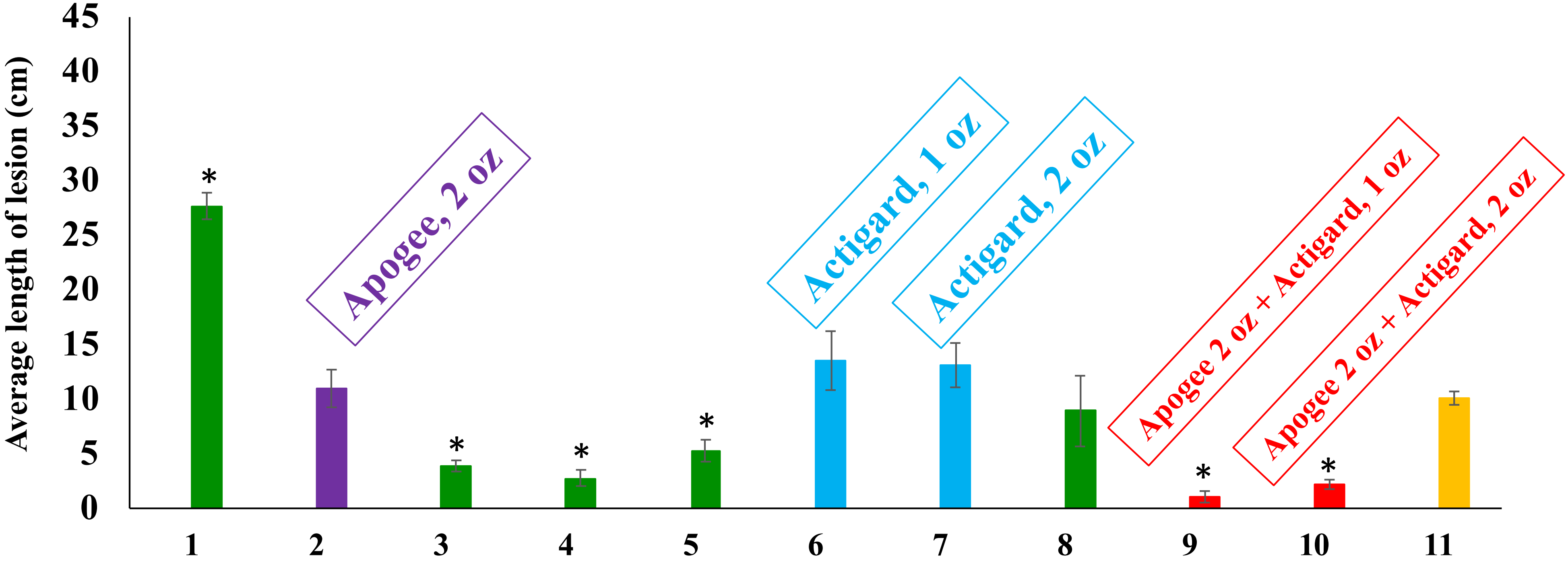
Disease resistance inducers

- **Actigard – chemical [acibenzolar-*S*-methyl (ASM)]**
- **LifeGard – *Bacillus mycoides* bacterium**

- **Application to plants elicits a resistance response which is generally active against plant pathogens**

- **Questions:**
  - **how fast is the response triggered?**
  - **how does the intensity of the response compare?**
  - **how long does the response last?**
  - **how effective is the response in flowers vs. shoot tips?**

Average shoot length and lesion length (26 June 2018)



2 = 2 oz / A Apogee

4 = 4 oz / A Apogee

5 = 8 oz / A Apogee

9 = 2 oz Apogee + 1 oz Actigard

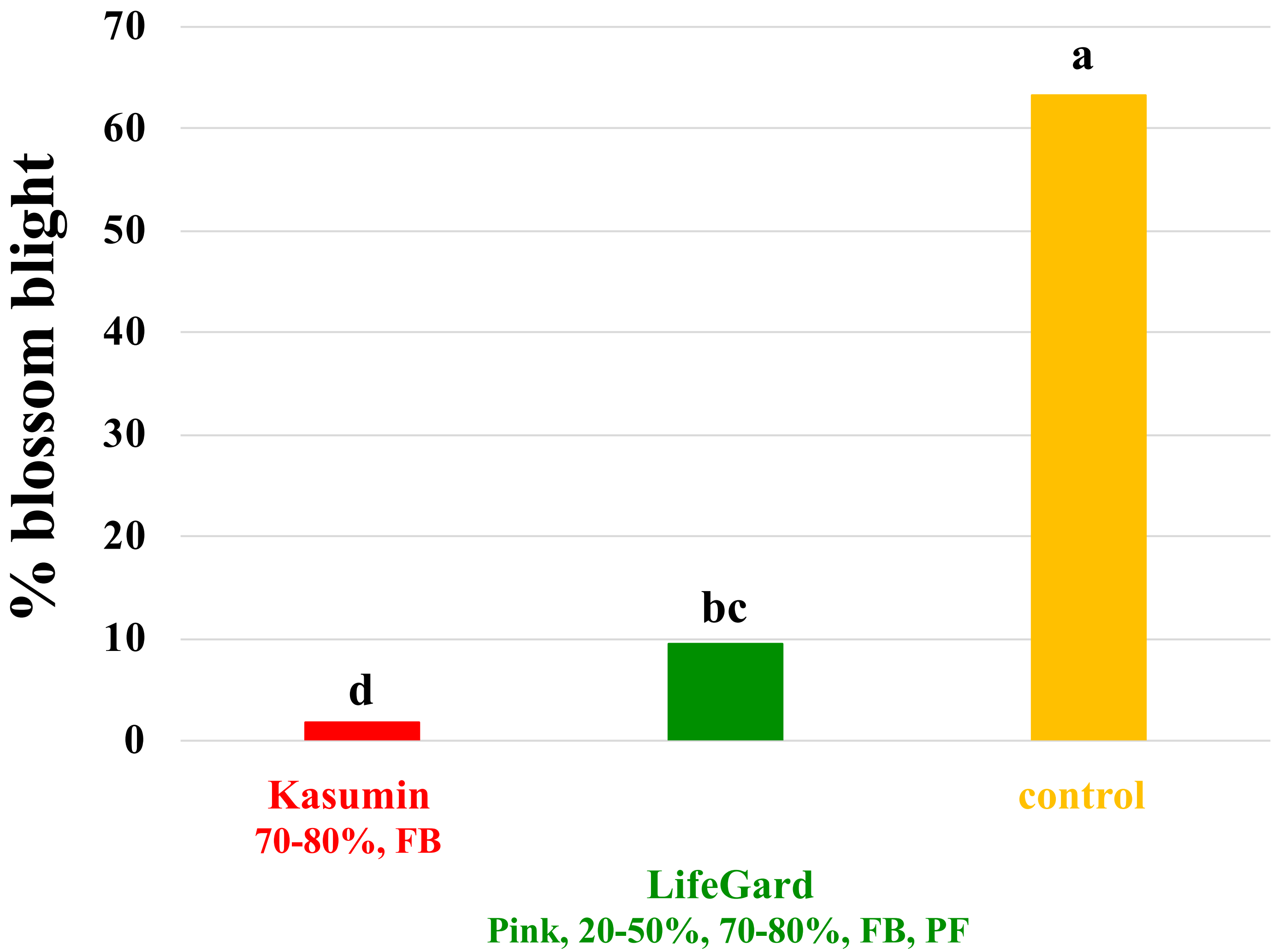
10 = 2 oz Apogee + 2 oz Actigard

11 = control

6 = 1 oz Actigard

7 = 2 oz Actigard

LifeGard – field test targeting blossom blight



2017, MSU Plant Pathology farm

Newly available methods for bacterial disease management -- SUMMARY

- **Materials that induce host disease resistance**
  - **Actigard**
  - **LifeGard**

- **Actigard and LifeGard have both been variable for blossom blight control**

- **Actigard is promising for shoot blight control, partnering with Apogee**
- **Further testing needed with both to optimize usage**

Pipeline methods for bacterial disease management

- **Nanotechnology**
  - **Zinkicide**
- **Bacteriophage**
  - **Agriphage (Certis)**
  - **Experimental phage**
- **Chemical inhibitors of bacterial virulence**
  - **Experimental compounds**

Zinkicide

- **Invented by a research at the University of Central Florida in 2013**
- **Contains zinc + undisclosed nanoparticles**
- **Small particle size and surface structure allow it to be absorbed by plant and mobile**
- **Stated to be translaminar**
- **Huge ongoing effort in developing this as a bactericide for citrus greening disease and citrus canker**

Zinkicide

Table 1. Minimal inhibitory concentration (MIC) of antimicrobial compounds against selected gram negative bacteria[z]

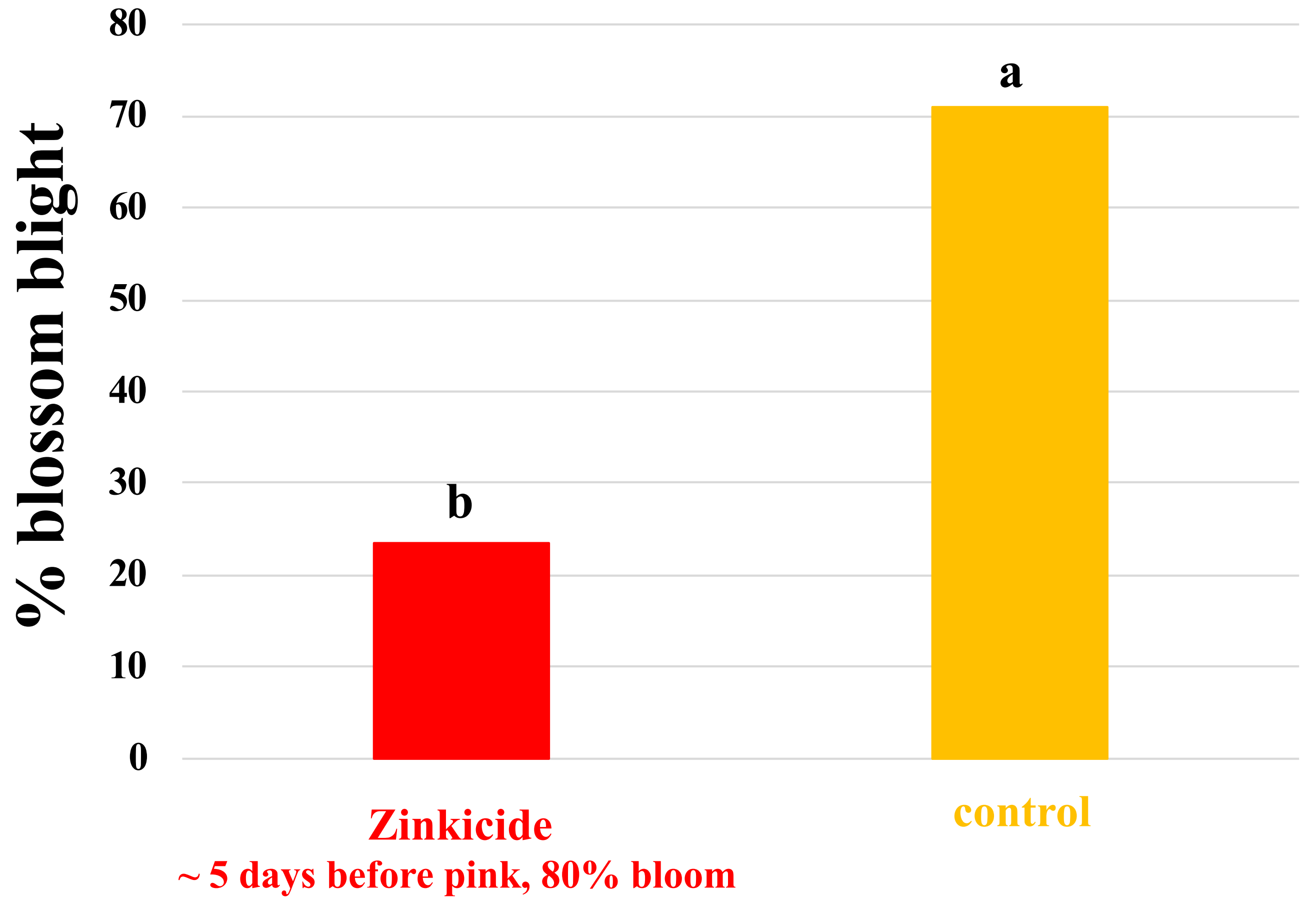
Antimicrobial compound	MIC (µg/ml)	
	Xanthomonas alfalfa subsp. *citrumelonis*	*Escherichia coli*
Zinkicide SG6	62.5	31
Zinkicide SG4	62.5–125	125–250
Copper sulfate	250	250
Copper hydroxide	250–500	250–500
Cuprous oxide/zinc oxide	125–250	125–250

Zinkicide – field test targeting citrus canker

Table 4. Effect of foliar applications at 21-day intervals for cuprous (Cu) oxide, cuprous oxide/zinc (Zn) oxide, and Zinkicide formulations on incidence of citrus canker on fruit of 6- and 7-year-old 'Ray Ruby' grapefruit trees at Vero Beach, FL in October 2014 and 2015[z]

Trial year Treatment – rate	Incidence of old lesions (%)	Incidence of young lesions (%)	Total incidence (%)
2014			
Untreated check	45 a	18 a	63 a
Cu oxide – 1.12	17 b	4.4 b	21 b
Cu oxide/Zn oxide – 0.56	16 b	8.8 b	25 b
Zinkicide SG4 – 0.56	3.0 c	6.2 b	9.2 c
Zinkicide SG6 – 0.56	4.6 c	2.4 b	7.0 c
2015			
Untreated check	23 a	37 a	60 a
Cu oxide – 1.12	10 bc	20 b	29 b
Cu oxide/Zn oxide – 0.56	8.2 bcd	13 cd	21 cd
Cu oxide/Zn oxide – 0.28	9.0 bd	14 c	23 c
Zinkicide SG4 – 0.56	6.2 bd	11 cd	17 de
Zinkicide SG6 – 0.56	5.6 d	10 cd	16 e
Zinkicide SG4 – 0.28	5.2 d	8.4 c	14 e
Zinkicide SG6 – 0.28	11 b	13 cd	24 c

Zinkicide – field test targeting blossom blight

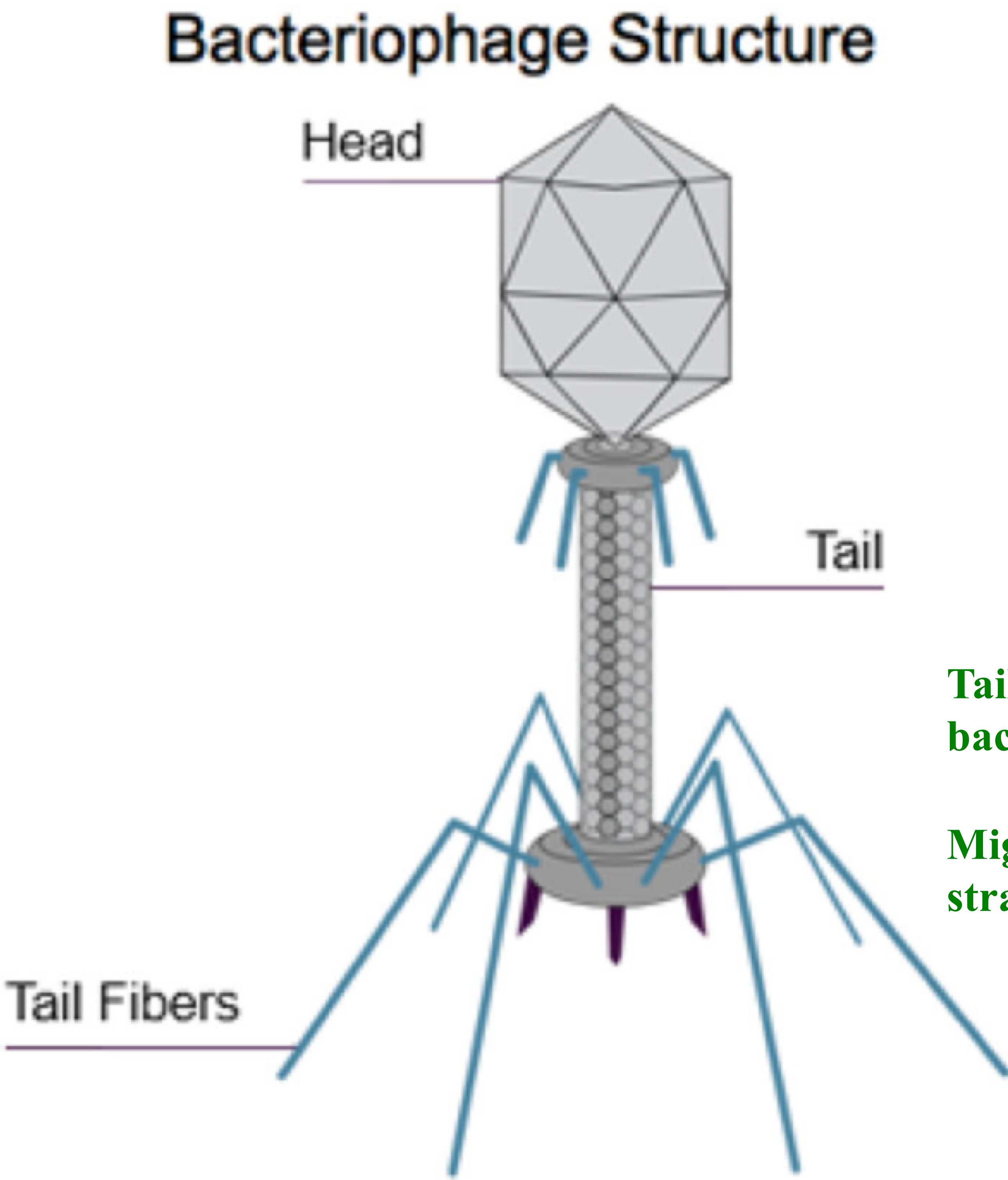


2018, MSU Plant Pathology farm

Can Zinkicide protect shoot tips from infection?

Bacteriophage

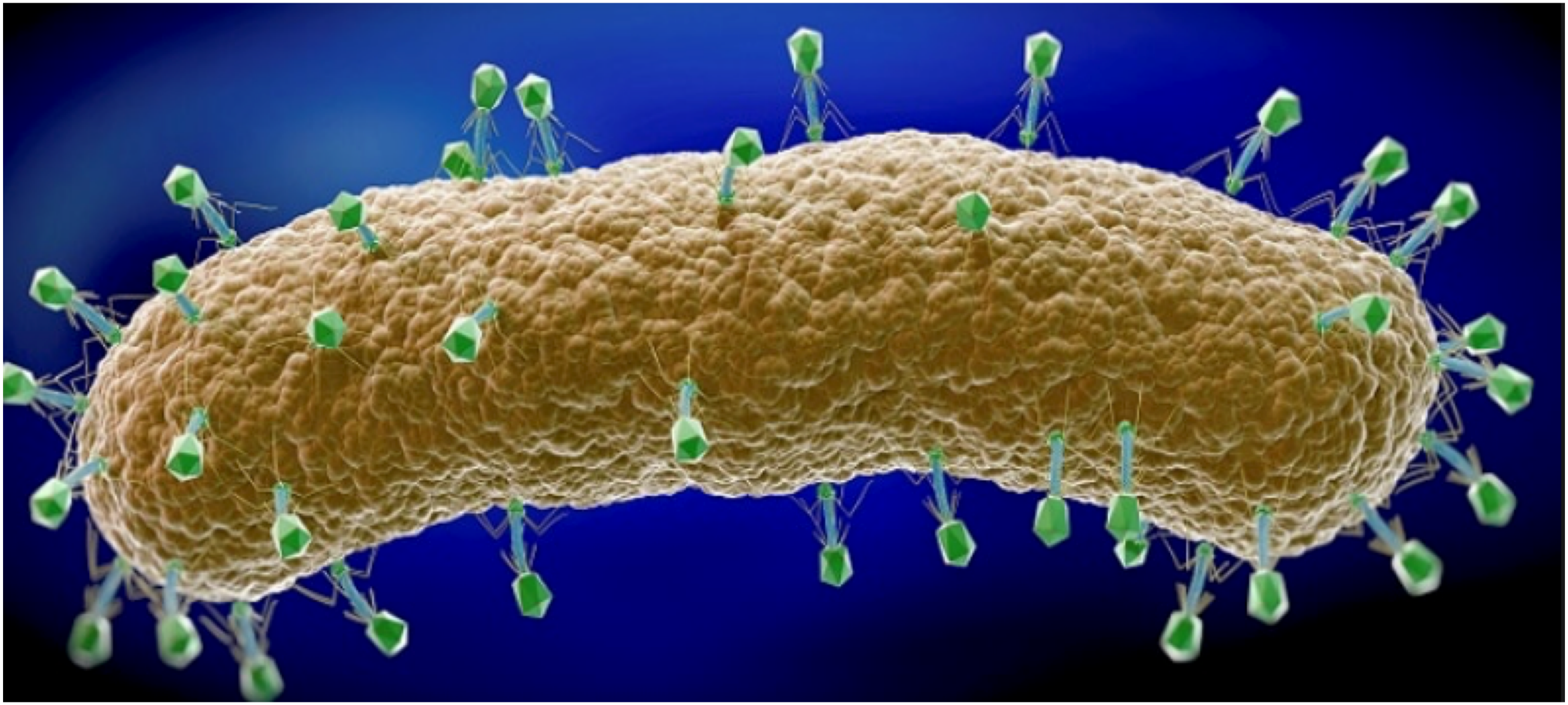
- Viruses that specifically infect bacteria
- Most phage also are very specific in terms of the bacteria they can infect
  - One or a few related species
  - Individuals within a species
- Infection:
  - Phage particle attaches to host
  - Infection, propagation of new phage inside bacterial cell
  - Host is killed, phage released

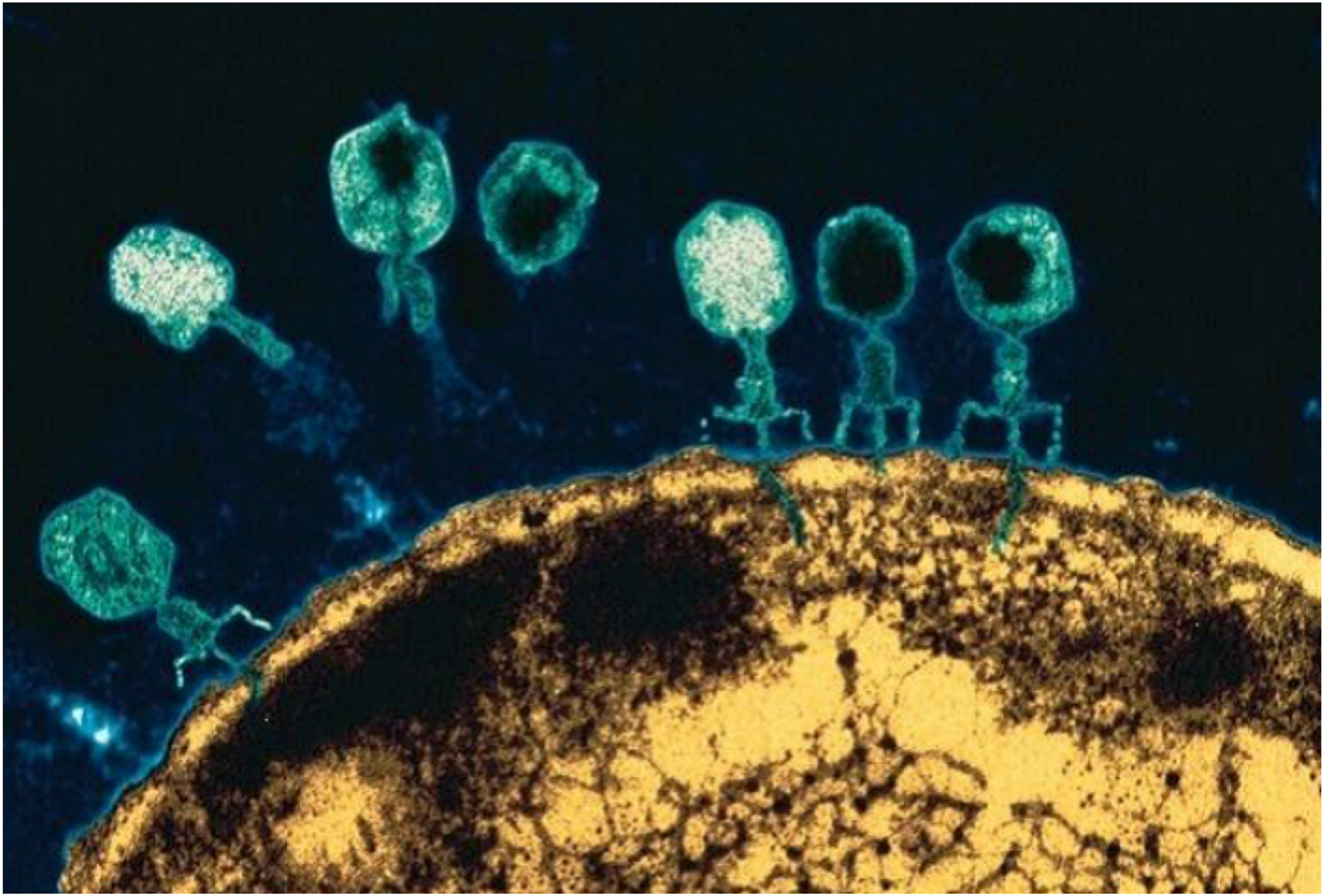


Tail interacts specifically with bacterial host

Might be one species or even strains within a species

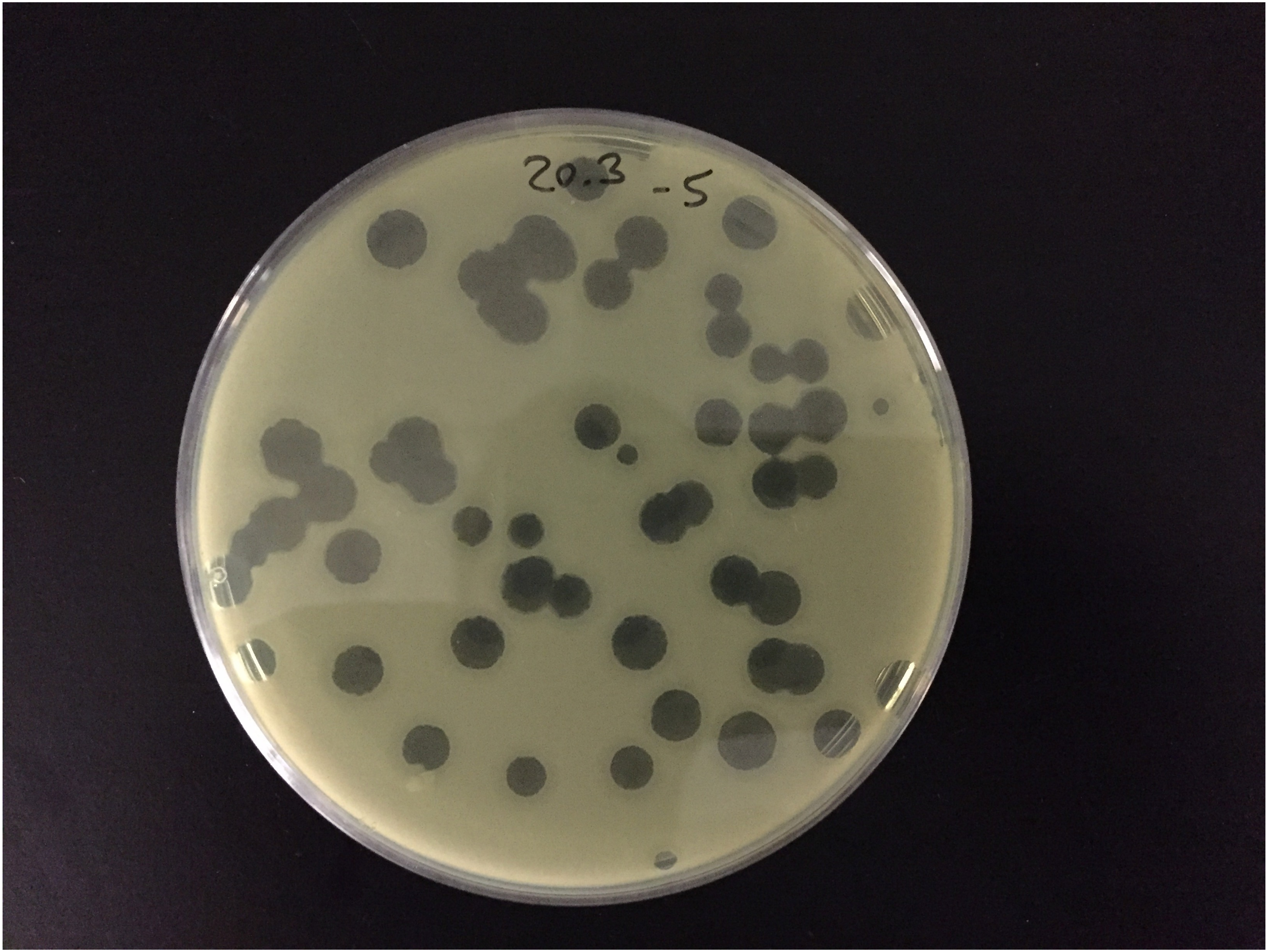
Bacteriophage

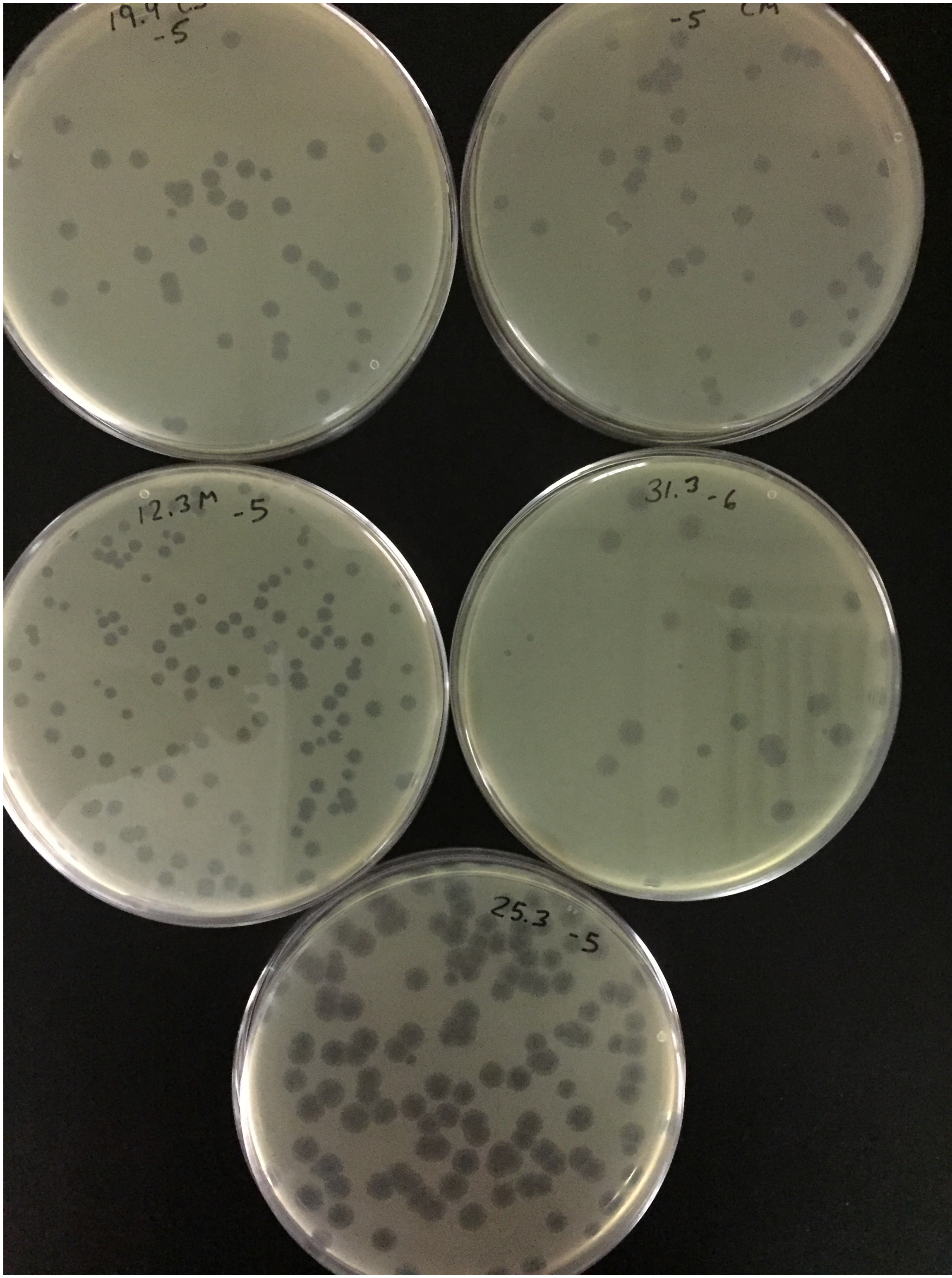


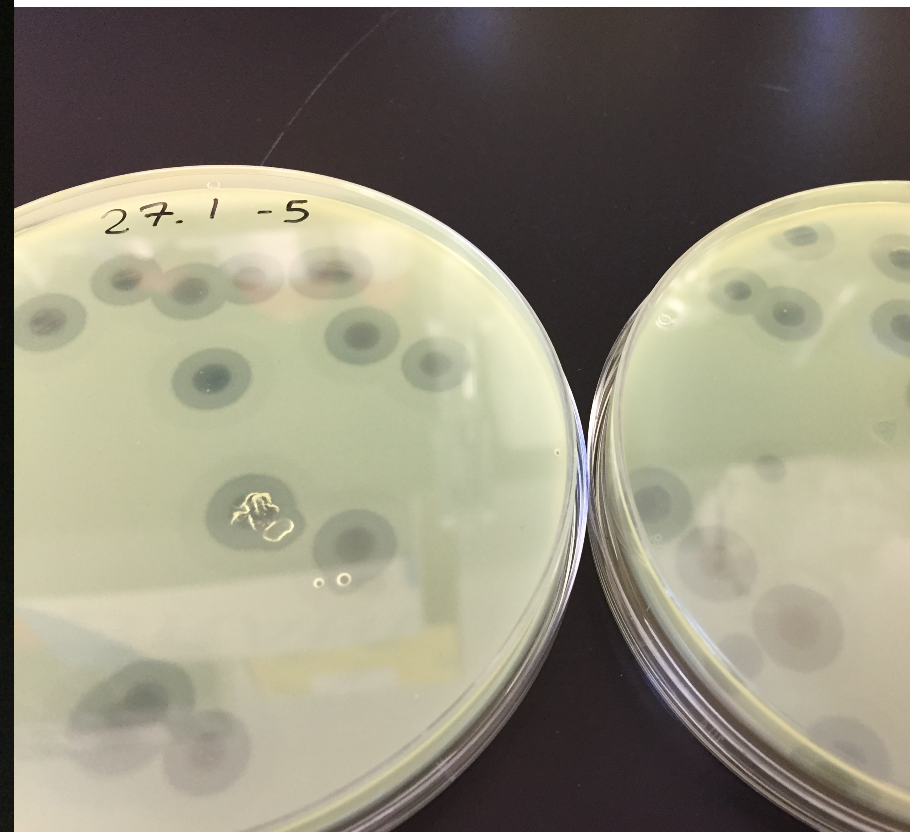


Bacteriophage

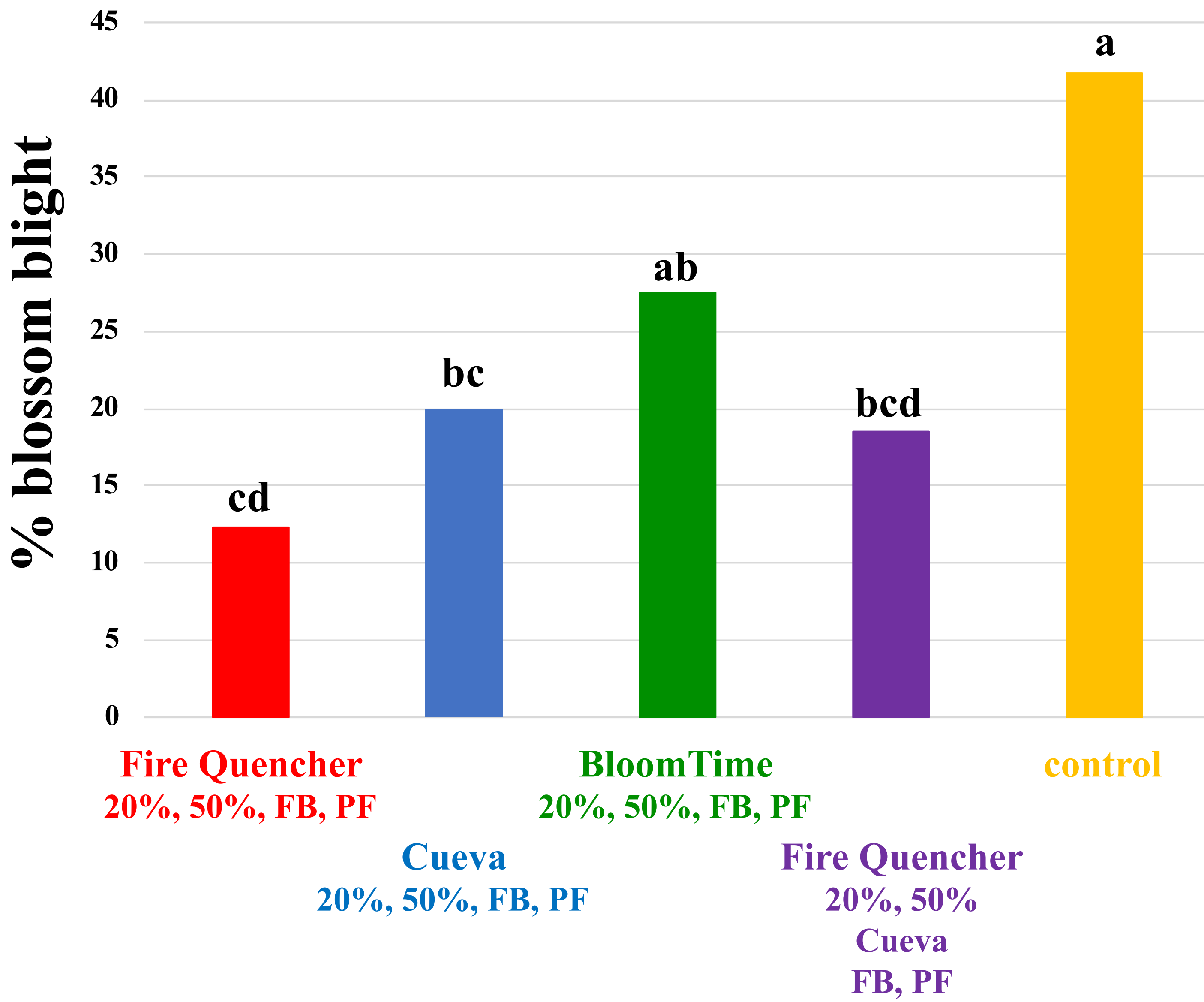
- **Phage occur in the same environments as their host cells**
- **Phage can be isolated in the lab and increased to very high numbers**
- **Interest in many different systems for using phage to control bacteria**
    - **Human and animal diseases**
    - **Food safety**
    - **Plant diseases?**


20.3 -5


19.4
-5
-5 CM
12.3M -5
31.3 -6
25.3 -5


27.1 -5

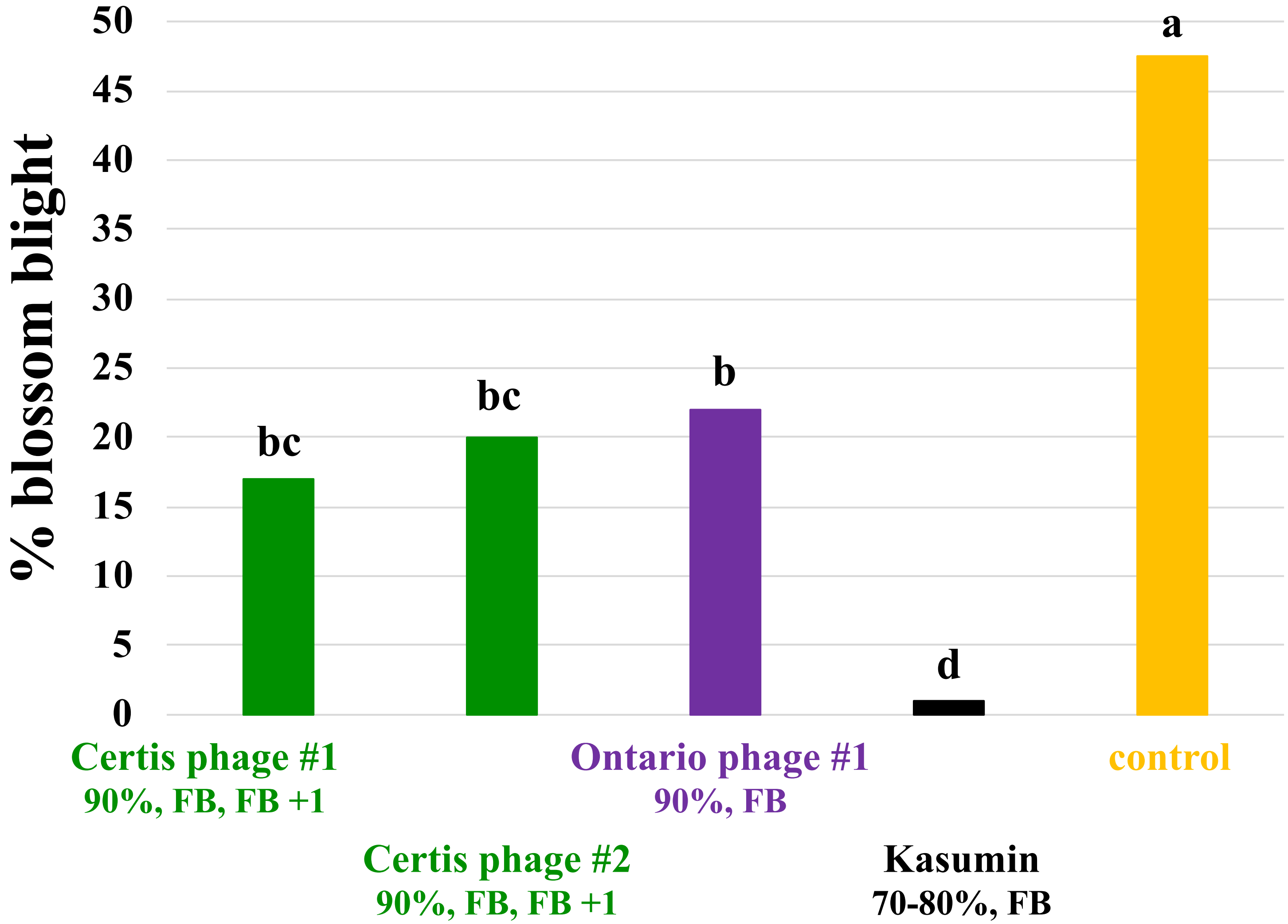
Blossom blight efficacy – Fire Quencher phage



2015, MSU Plant Pathology farm

FQ – developed by researcher at Brigham Young Univ.

Blossom blight efficacy – bacteriophage test



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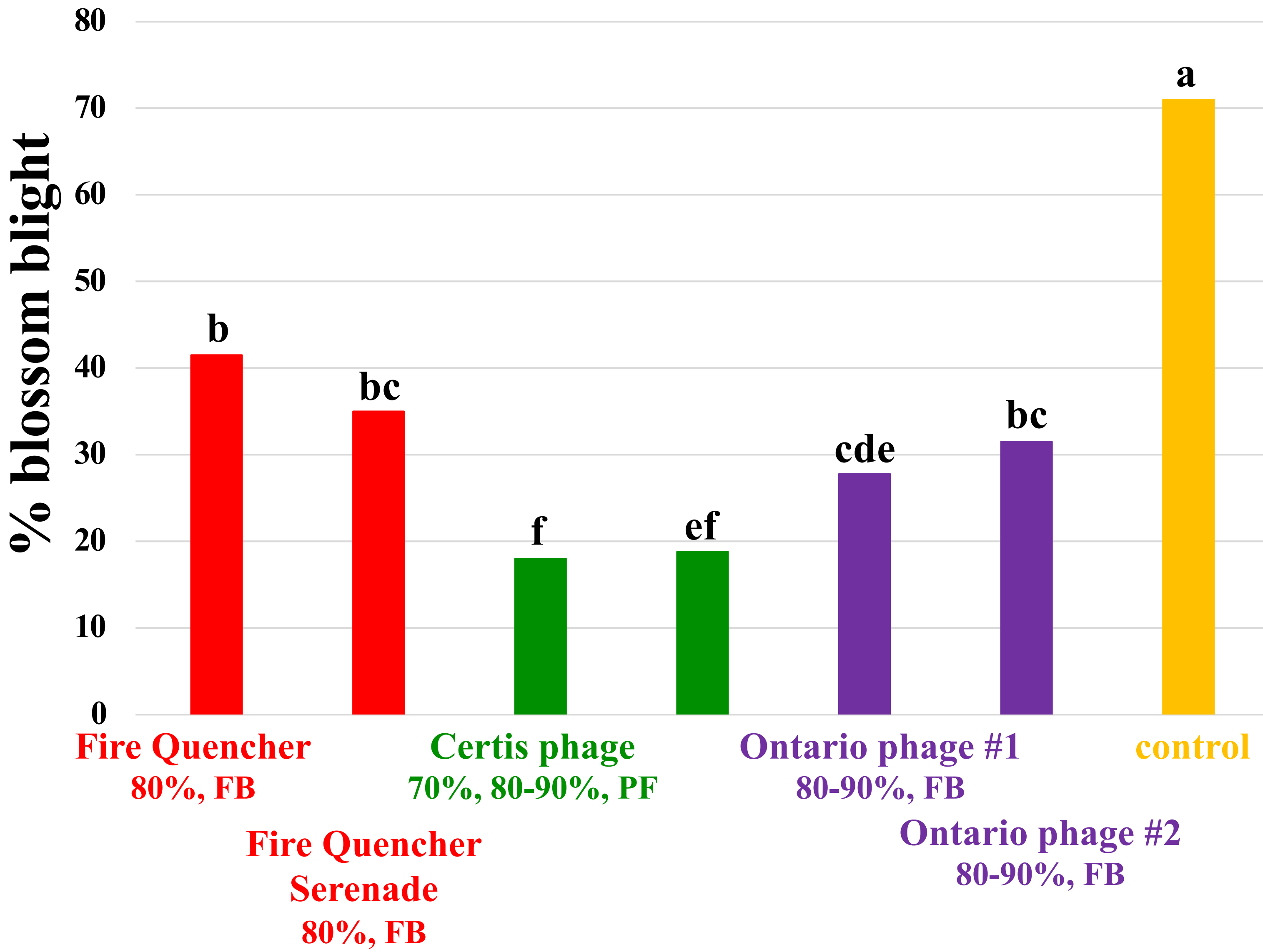
Blossom blight efficacy – bacteriophage test



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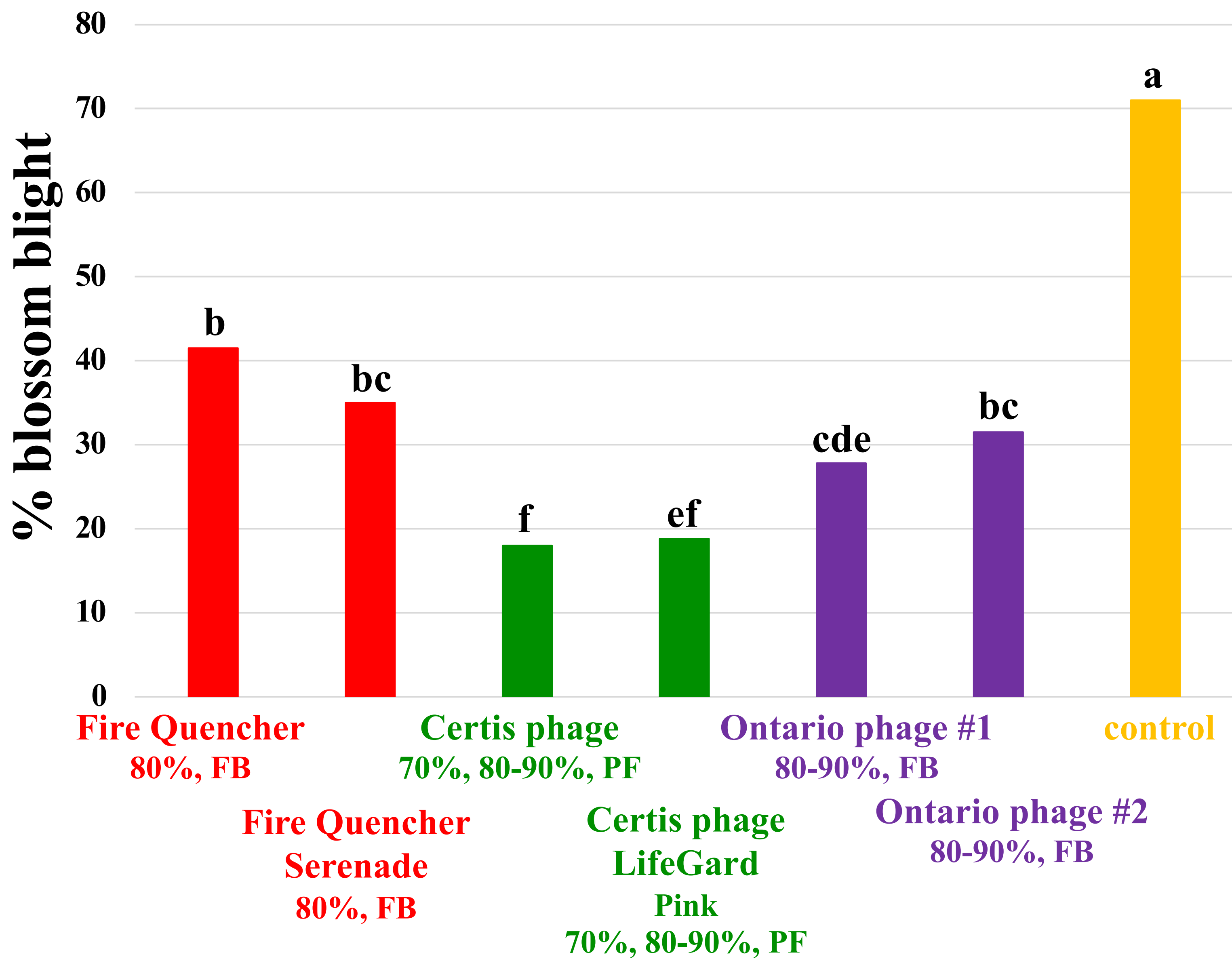
Blossom blight efficacy – bacteriophage test



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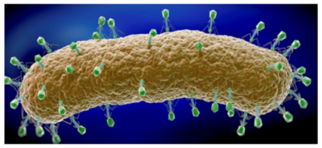
Issues with bacteriophage deployment for bacterial disease control

- **Survival – phage are fairly UV sensitive**
- **Phage "die" off quickly in the absence of their host**
- **When tested for efficacy where control is needed on leaf surfaces, phage don't work too well**
  - **Heat also a factor affecting survival**

Sundin lab – NIFA phage grant for fire blight

- **Collaborating with Dr. Sara Villani, NC State Univ., Dr. Antonet Svircev, AgCanada (Ontario)**
- **Main objective is to improve phage efficacy**
  - **Studying different phage "cocktails"**
  - **UV protectants**
  - **Adjuvants**
  - **Understanding phage dynamics on flowers with and without host present**
  - **Can phage be applied with a *Pantoea* (biol. control strain) that they can also infect?**

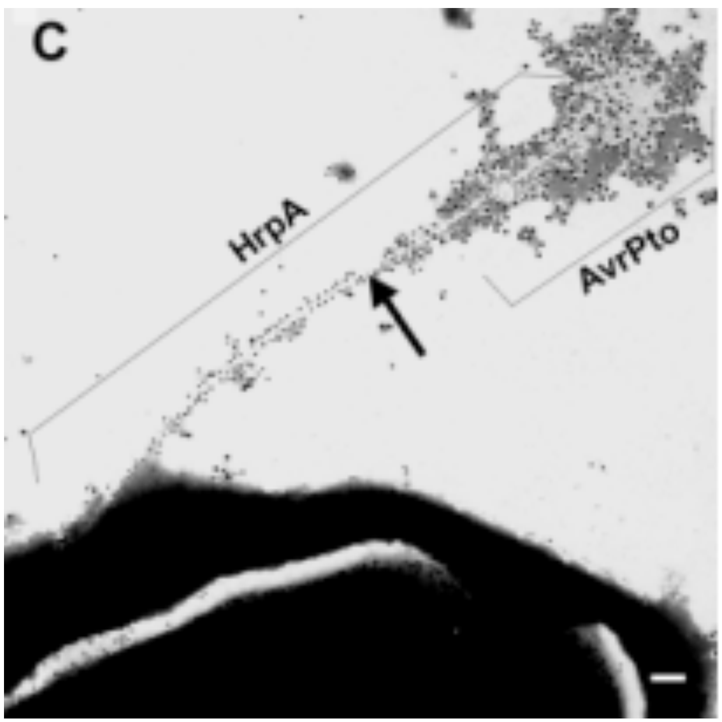


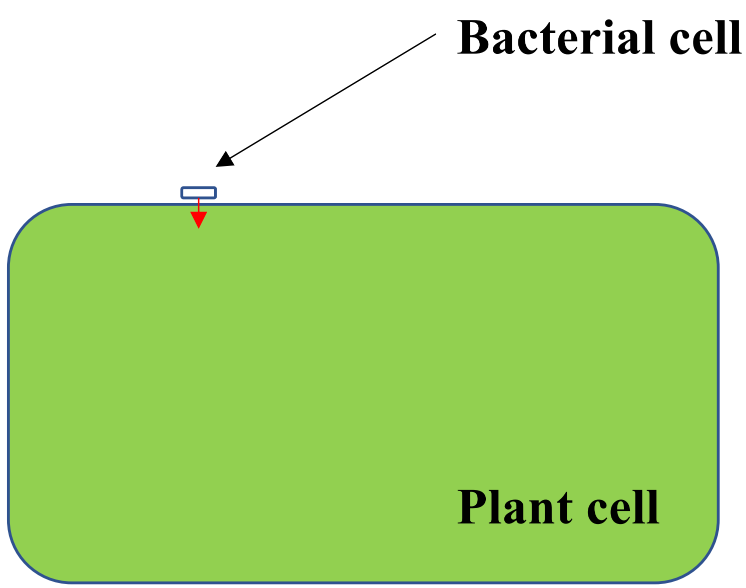
Bacteriophage – potential for other tree fruit diseases?

- **Targets – bacterial canker pathogen *Pseudomonas syringae* (PS), bacterial spot pathogen *Xanthomonas arboricola* (XA)**
- **Sundin lab collaborating with Rothwell, Shane, and Rob Jackson (UK)**
  - **Isolate phage that attack PS or XA – how many different phage can we find?**
  - **Assess host range**
  - **Assess infectivity: activity at low temperatures, best dose for infection etc.**
  - **Incorporate knowledge from fire blight phage grant into improving phage efficacy for these other diseases**

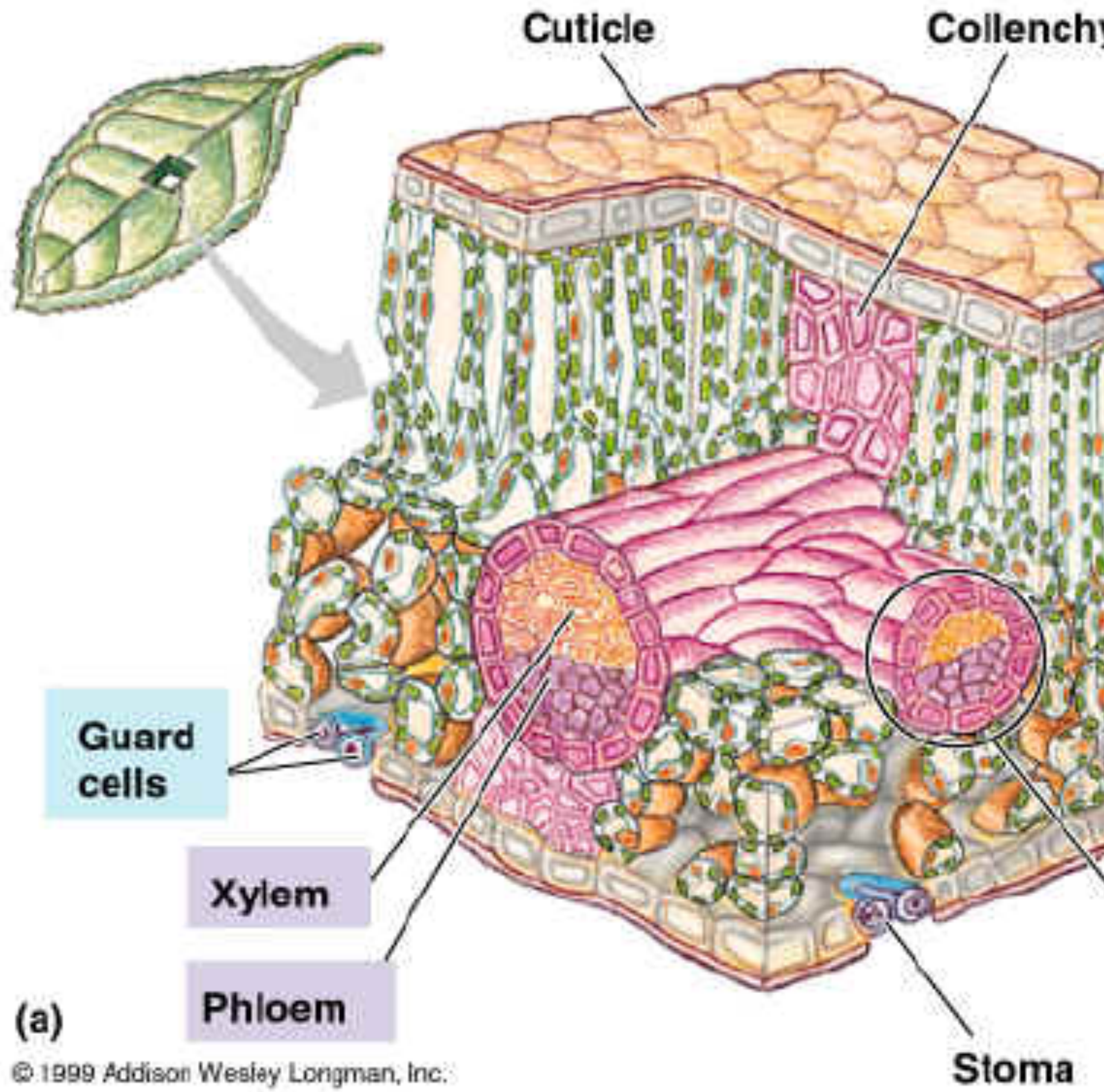
Chemical inhibitors of bacterial virulence

- **Studies of bacterial infection at genetic level**
- **For fire blight, we've identified two pathogen traits that are required for infection**





Type III secretion


Cuticle
Collenchyma
Upper epidermis
Palisade parenchyma
Spongy parenchyma
Guard cells
Xylem
Phloem
Stoma
(a)
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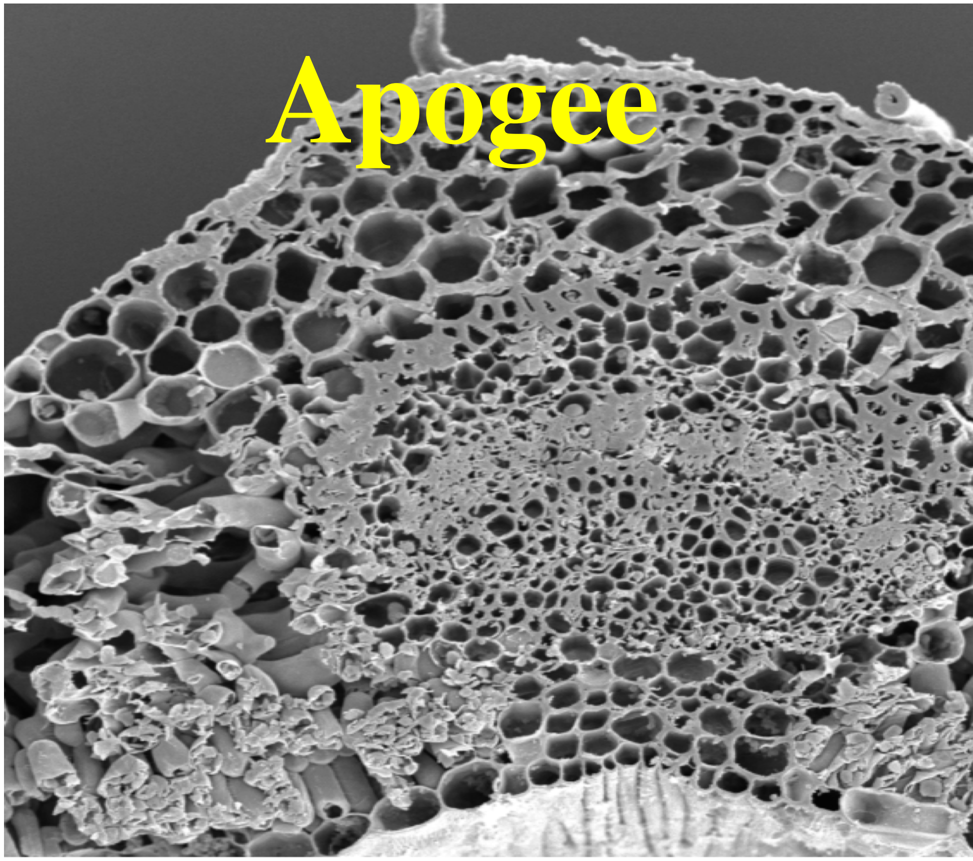
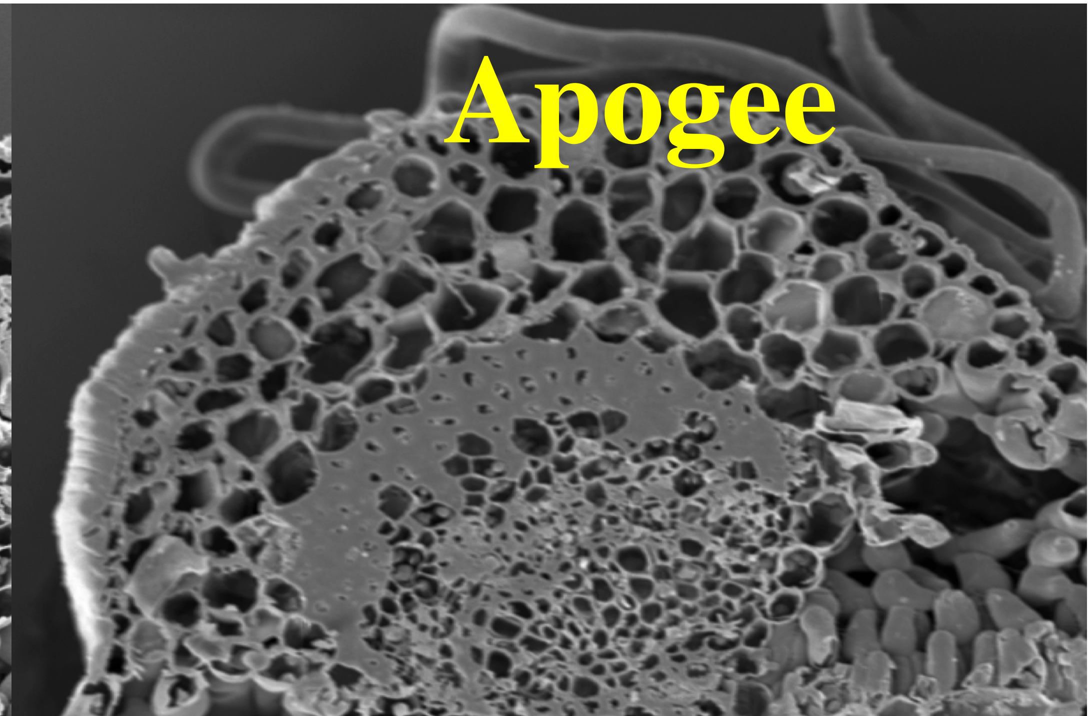
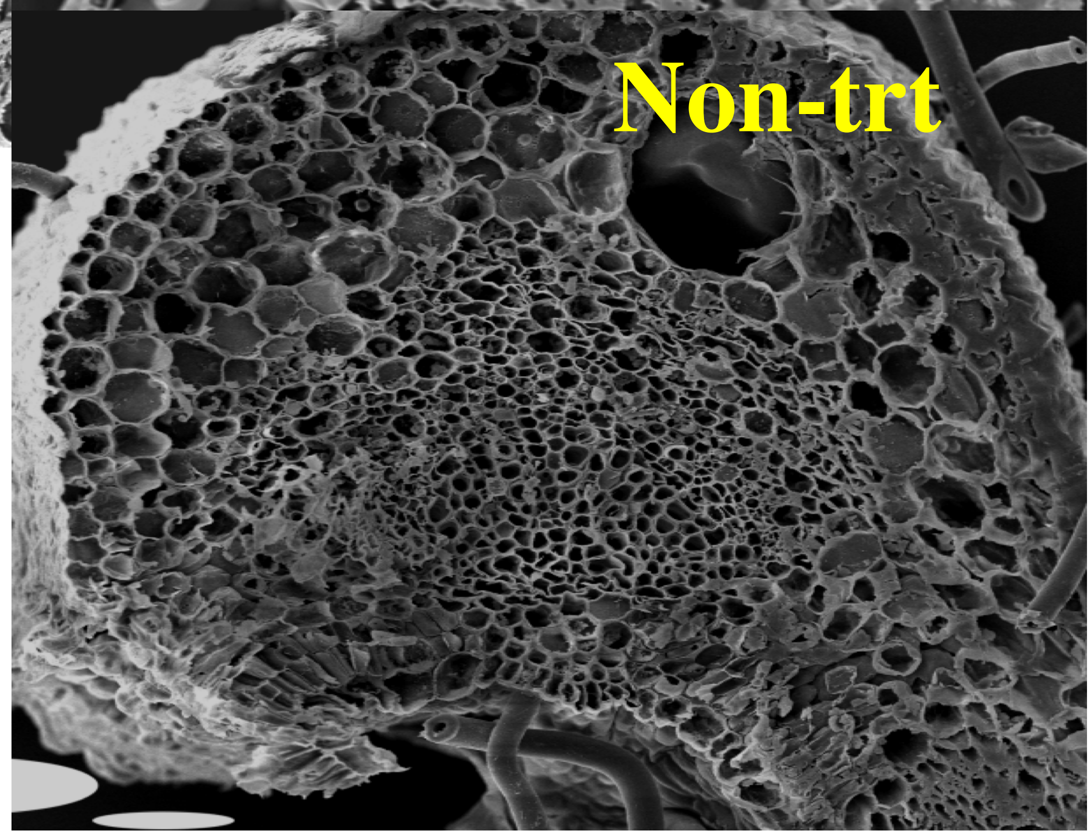
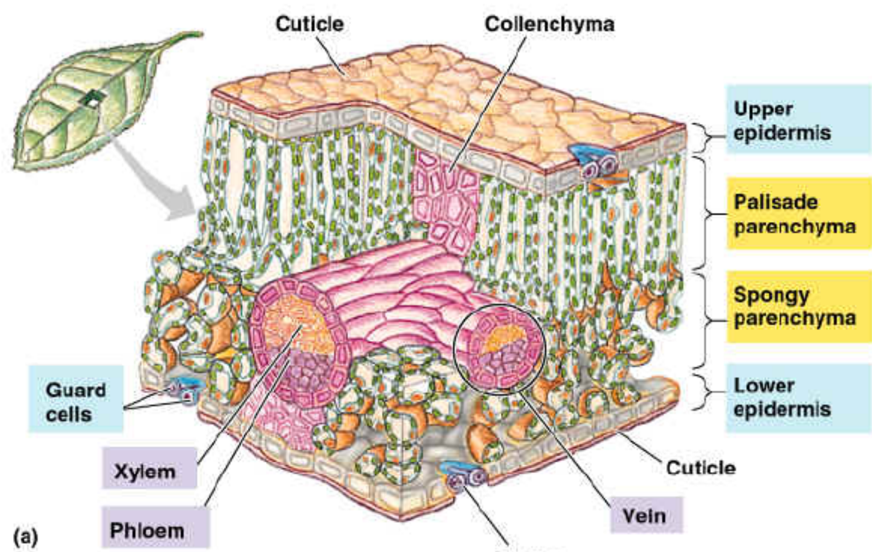

Apogee

Apogee

Non-trt

Cuticle
Collenchyma
Upper epidermis
Palisade parenchyma
Spongy parenchyma
Lower epidermis
Guard cells
Xylem
Phloem
Cuticle
Vein
Stoma
(a)

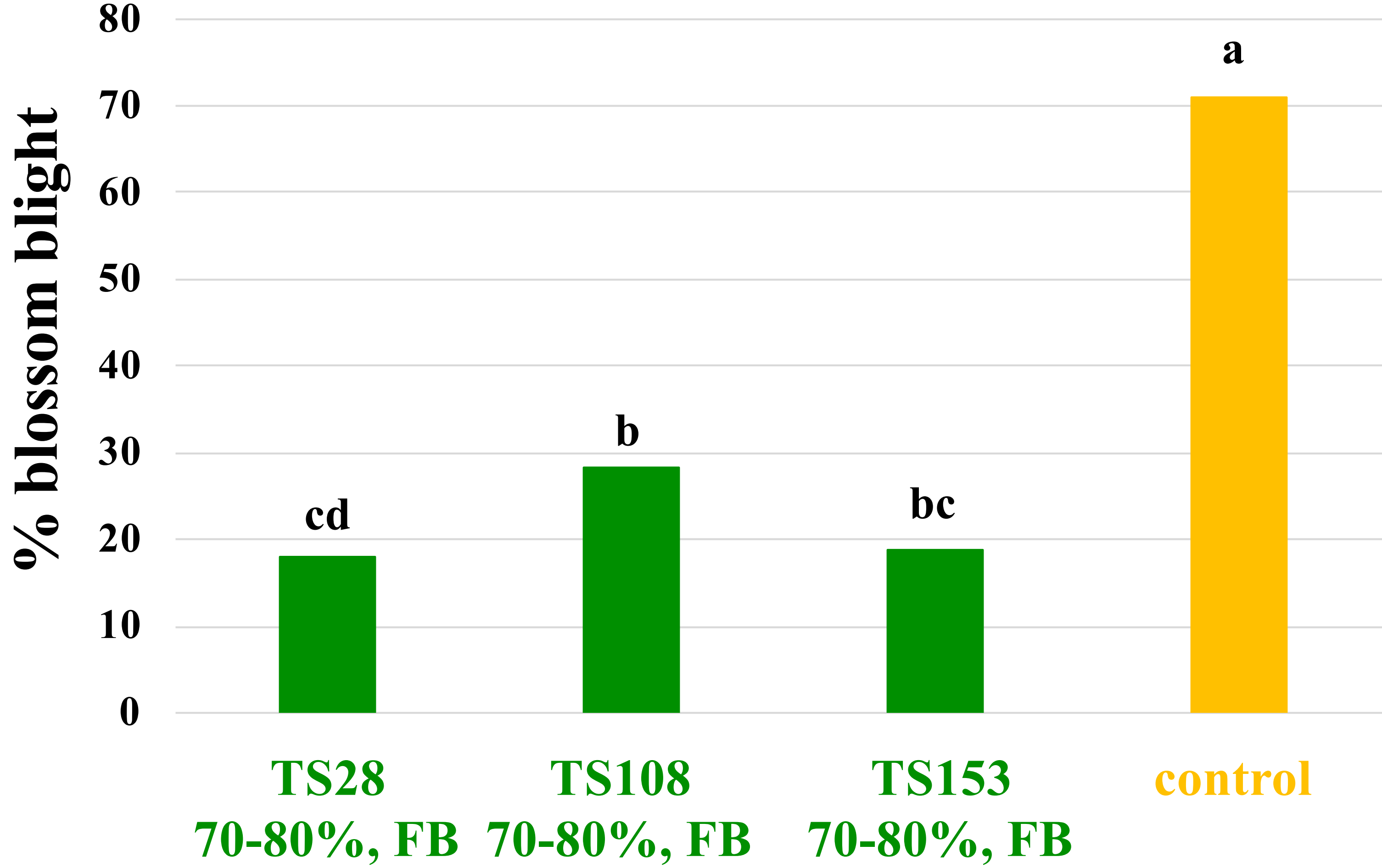
Chemical inhibitors of bacterial virulence

- Collaborator Dr. Ching Hong Yang (University of Wisconsin, Milwaukee)
- Yang group screened a library of phenolic compounds to identify inhibitors of type III secretion in the fire blight pathogen
  - Requirement: inhibition at low concentration
- Several promising compounds identified
- We determined that the inhibitory effect lasted 12 hr but was gone at 24 hr

Chemical inhibitors of bacterial virulence
Small-scale field testing

	2014 % INFECTION	2015 % INFECTION
• TS28	27.8	
• TS108	23.5	20.8
• TS152	46.2	
• TS153	25.0	28.1
• TS160	38.5	
• Control	48.5	49.0

Blossom blight efficacy – virulence inhibitors



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SUMMARY

- **Zinkicide – broad spectrum, partially systemic, very promising**
- **Bacteriophage – definite promise as a biological control**
  - **Fire blight – research to improve phage survival and efficacy**
  - **Bacterial canker, bacterial spot – phage discovery, then efficacy research**
- **Virulence inhibitors – also show promise for disease control**
  - **Improve efficacy – higher concentrations? adjuvants?**
  - **Partner with other materials**

Acknowledgements

Thanks to:

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Michigan Apple Committee
Michigan Cherry Committee
Michigan State Horticultural Society
Michigan Tree Fruit Commision
USDA, NIFA
Michigan State University
AgBioResearch, Project GREEEN

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