Antimicrobial strategies for controlling *C*Las and the Asian citrus psyllid

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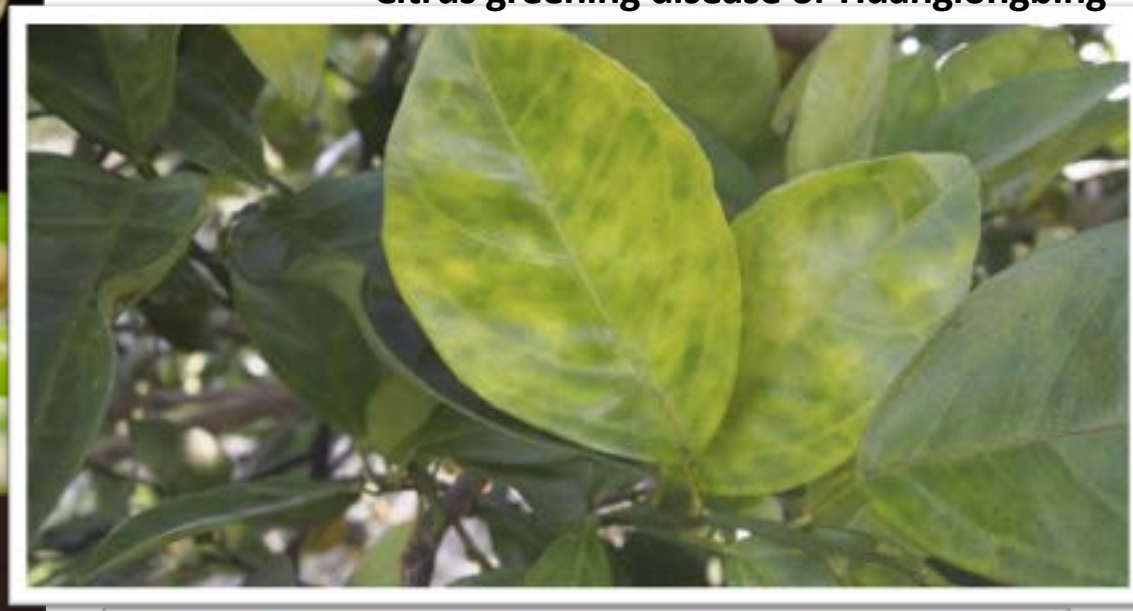
Citrus greening disease or Huanglongbing



Current psyllid management requires significant input of insecticides

- Use of broad-spectrum insecticides targeting adult psyllids with possible rotation with insect growth regulators

- Applications made prior to new flush are most effective in reducing psyllid populations

  - 10-12+ annual sprays annually

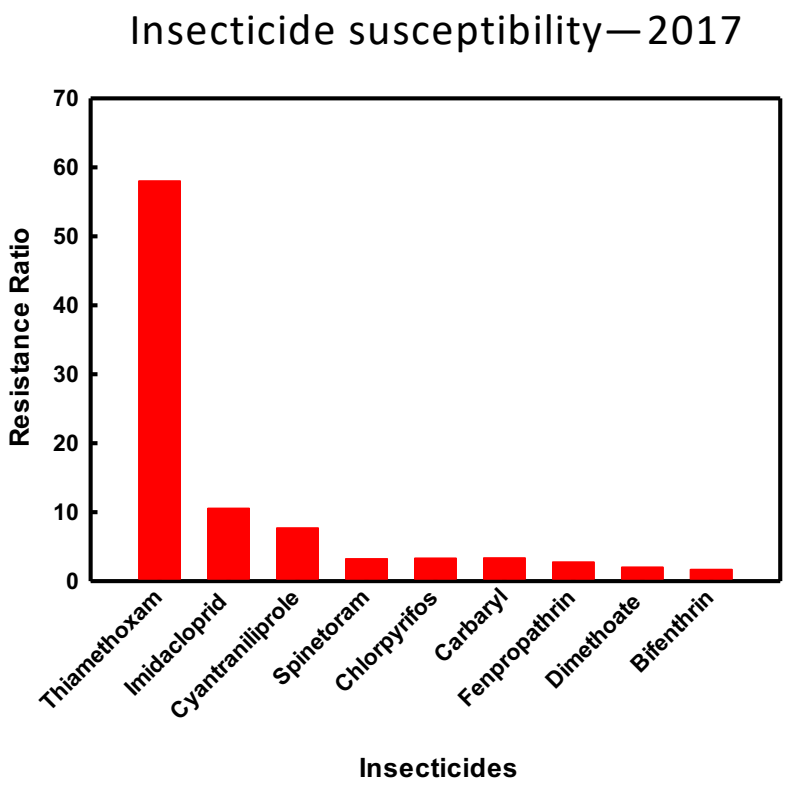
Antimicrobials for reducing pathogen transmission

Current management of psyllids with broad spectrum insecticides is unsustainable

- High cost
- Physiological resistance

Challenge: Targeted manipulation of symbionts provides a unique opportunity for vector/pathogen management

- Symbiont elimination/suppression
  - Antibiotics
  - Bactericides: oligonucleotide suppression of Las and endosymbionts



Antimicrobials for reducing pathogen transmission

Current management of psyllids with broad spectrum insecticides is unsustainable

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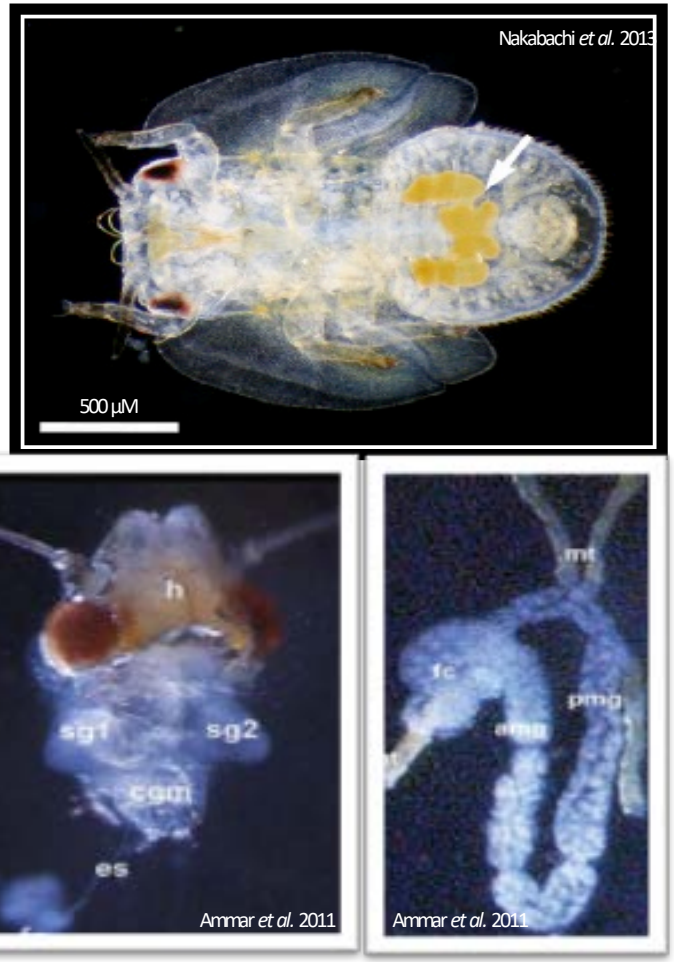
Can we manipulate microorganisms present in the vector and host plant to reduce pathogen transmission?

- Disruption of symbionts for ACP/CLas management

ACP bacterial endosymbionts



- *Candidatus* Carsonella ruddii
- *Candidatus* Profftella armature
- *Wolbachia* (*wDi*)

- *Candidatus* Liberibacter asiaticus
- *Wolbachia* (*wDi*)

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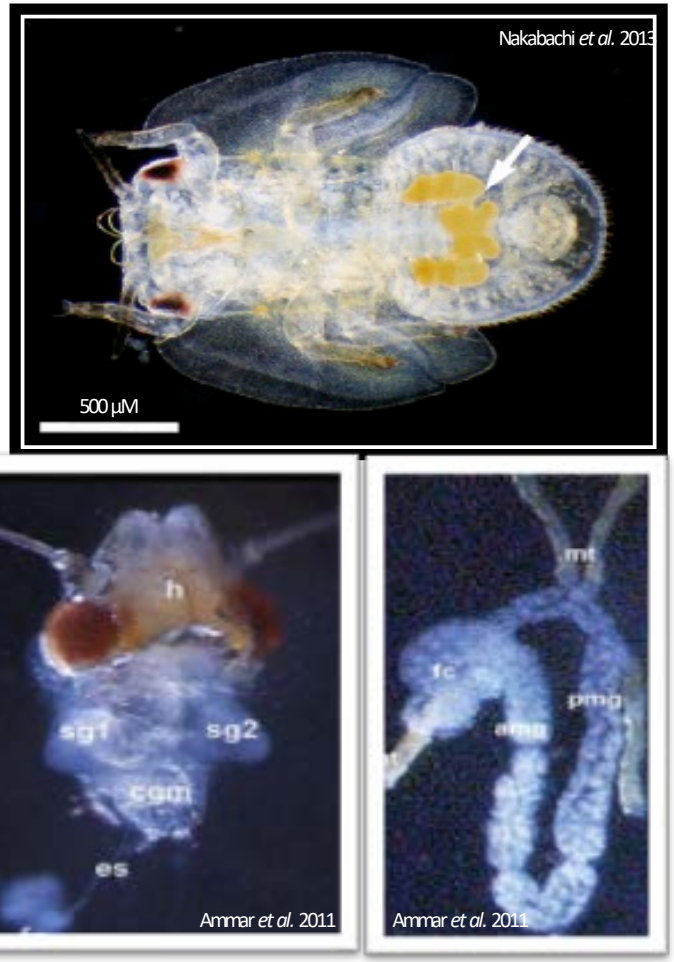

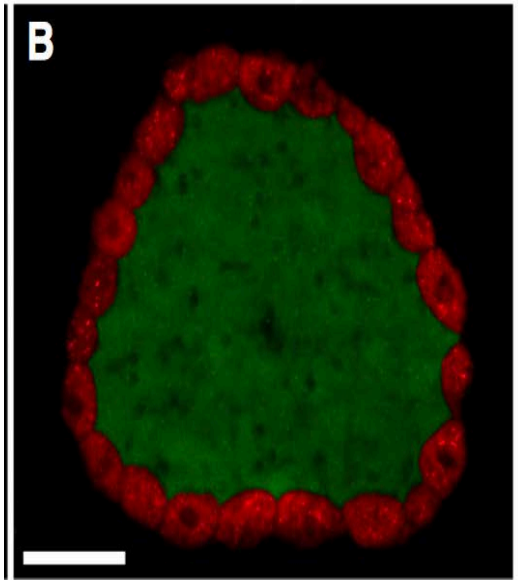

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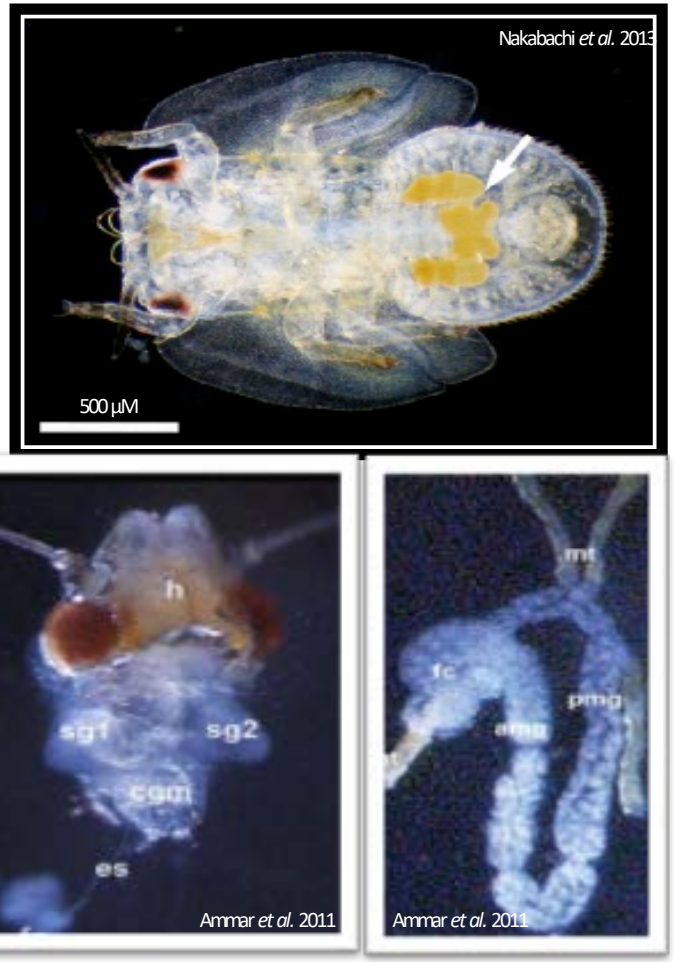

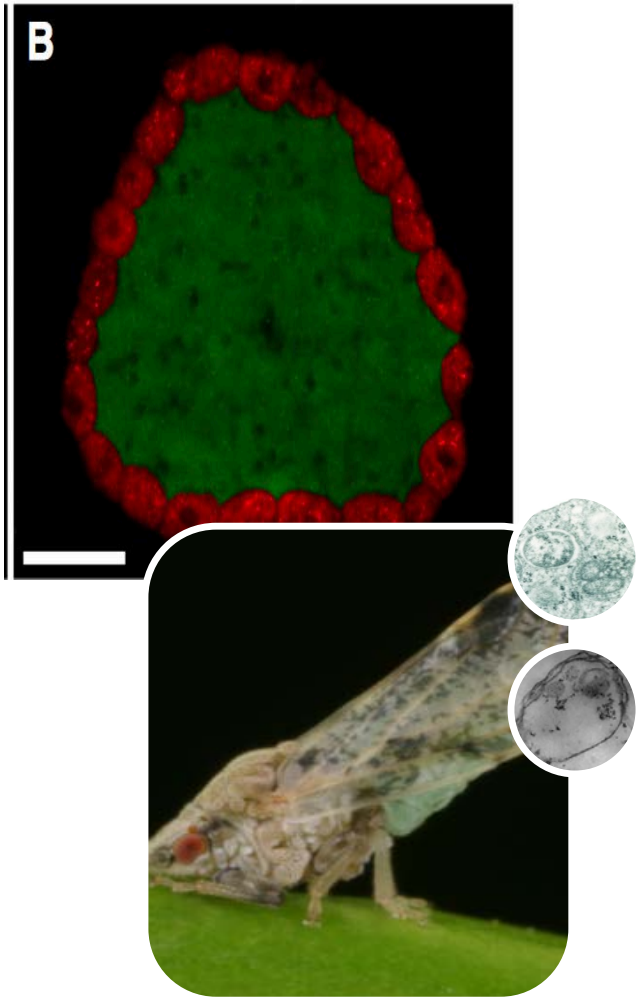

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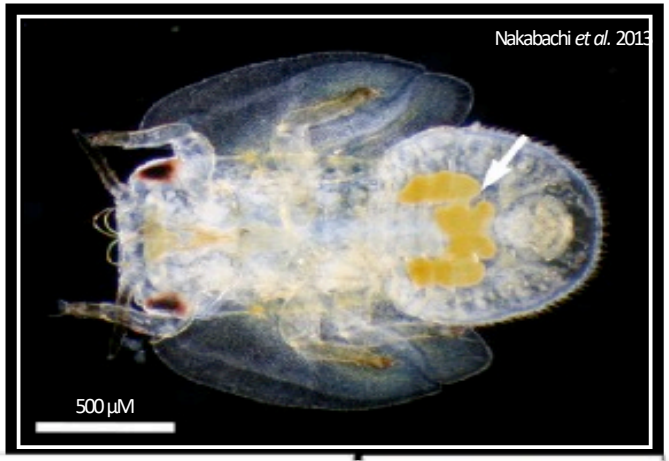
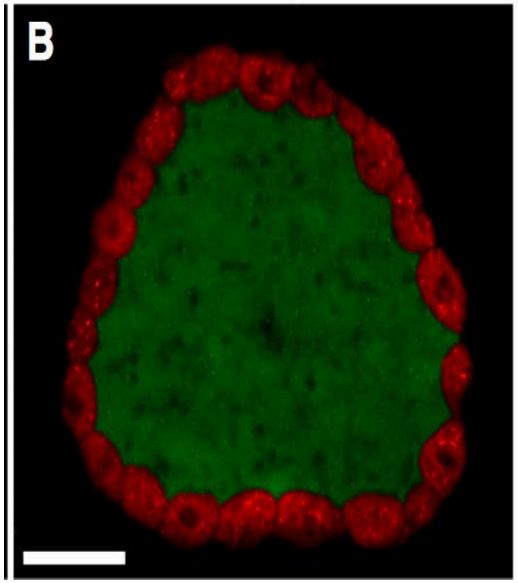
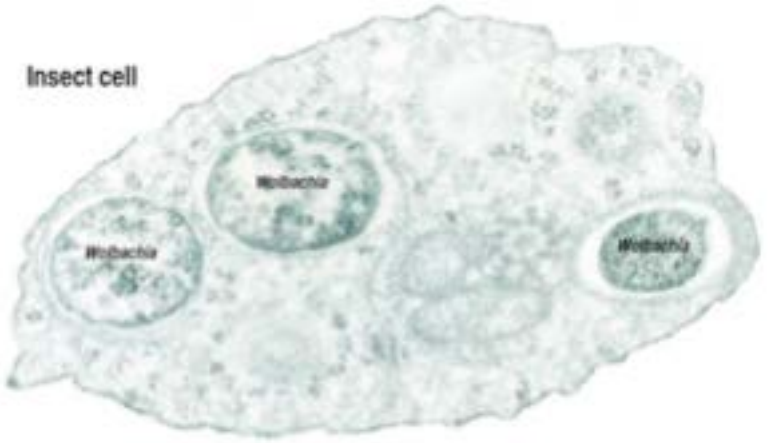






Antimicrobials for reducing ACP survival and Las transmission

Antibiotic treatments:

- Plants (Las)
- ACP (Las, endosymbionts)

Antisense RNA technologies:

- Reduce Las in plants
- Selectively eliminate Las symbionts

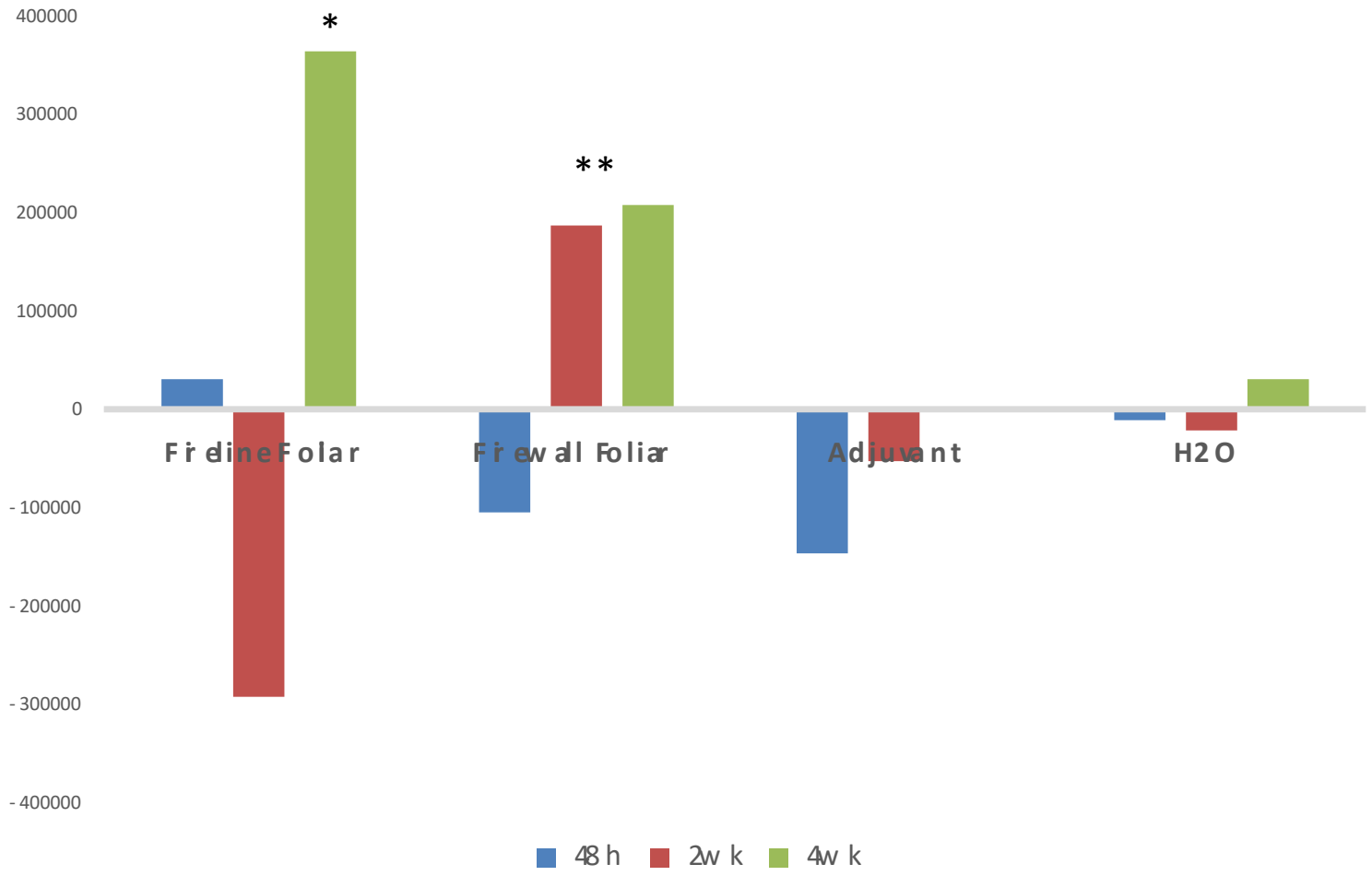
Management of Huanglongbing: EPA crisis declaration

Florida citrus lost an estimated $7.80 billion between 2006-2014.

Three commercially available bactericides for Florida citrus:

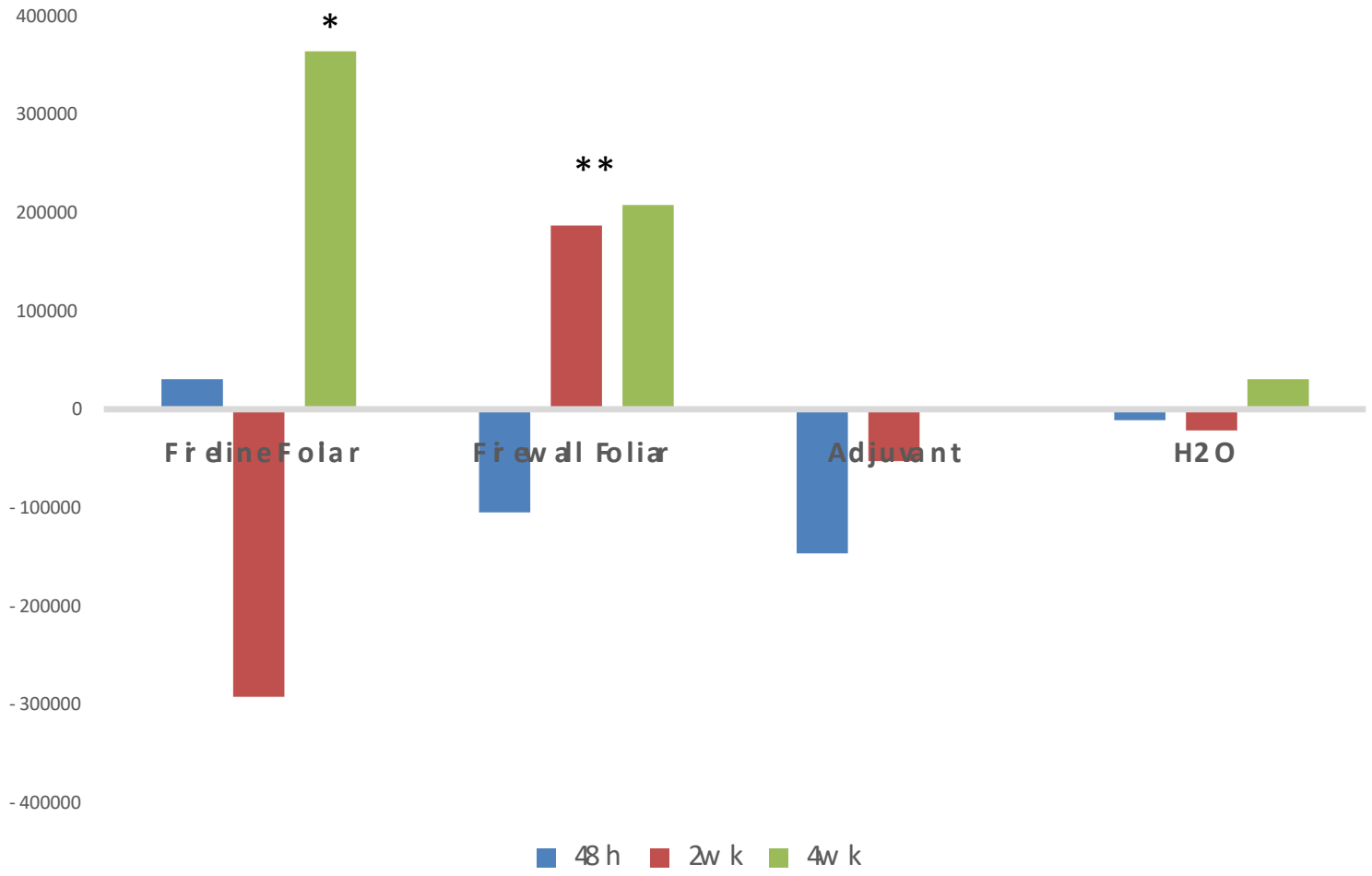
- FireWall™ 50 WP: Streptomycin sulfate
- FireLine™ 17 WP: Oxytetracycline hydrochloride
- Mycoshield®: Oxytetracycline calcium complex base

Greenhouse Plant Assay: Firewall Vs. Fireline Foliar Spray Efficacy



FireWall and Fireline applied at label rate (200 PPM)

Greenhouse Plant Assay: Firewall Vs. Fireline Foliar Spray Efficacy



Positive values indicate reductions in CLas titer compared to day 0

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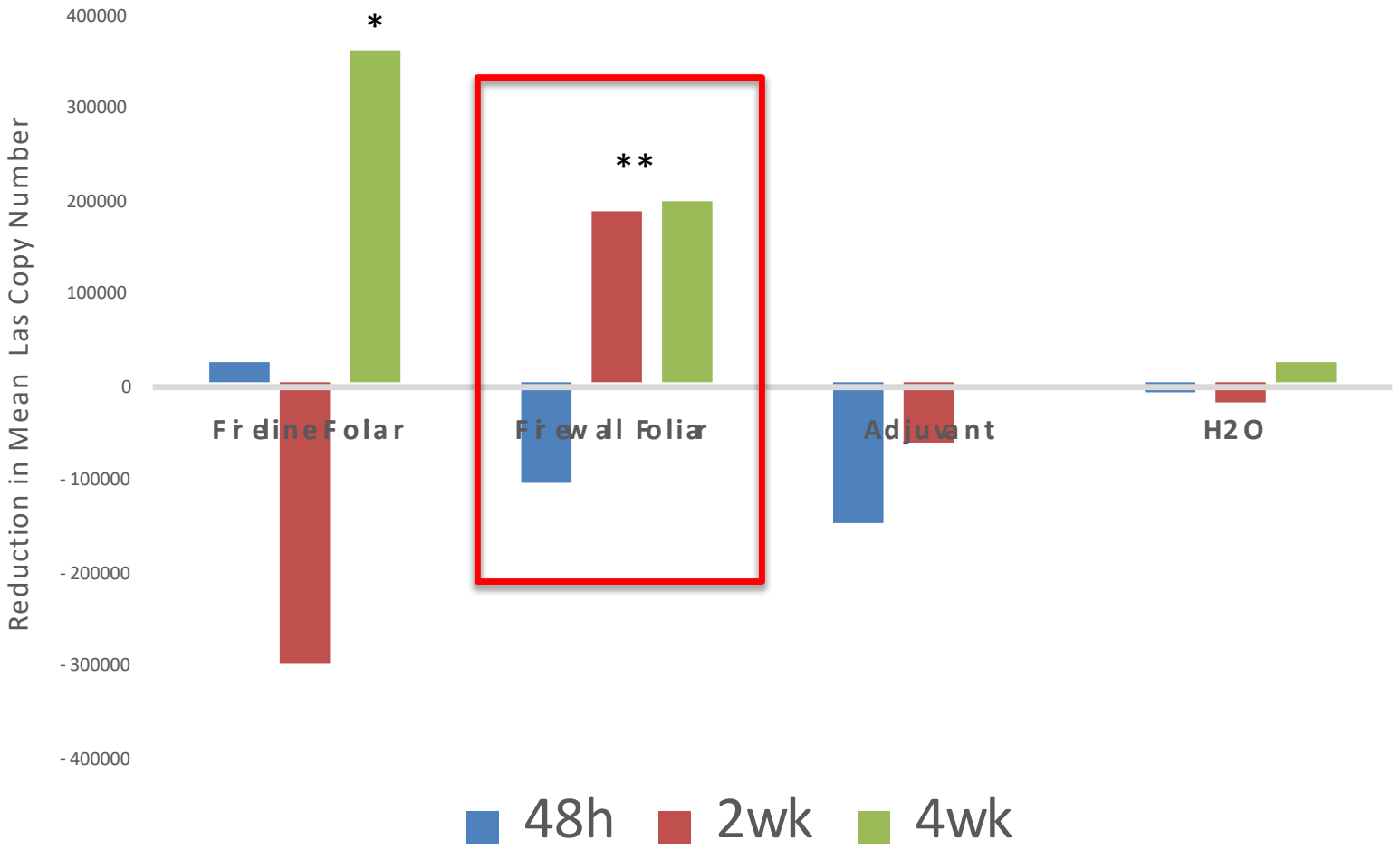
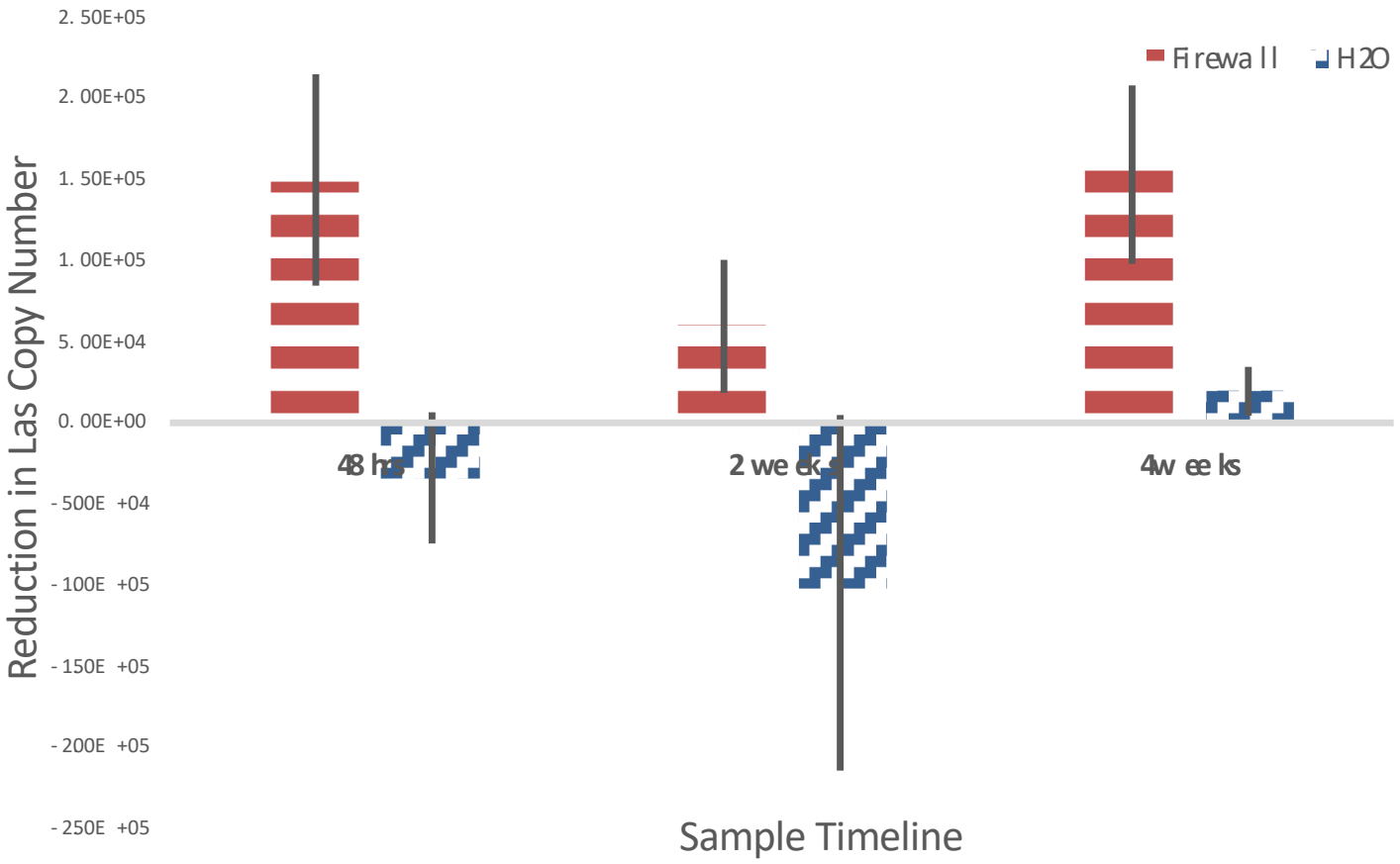


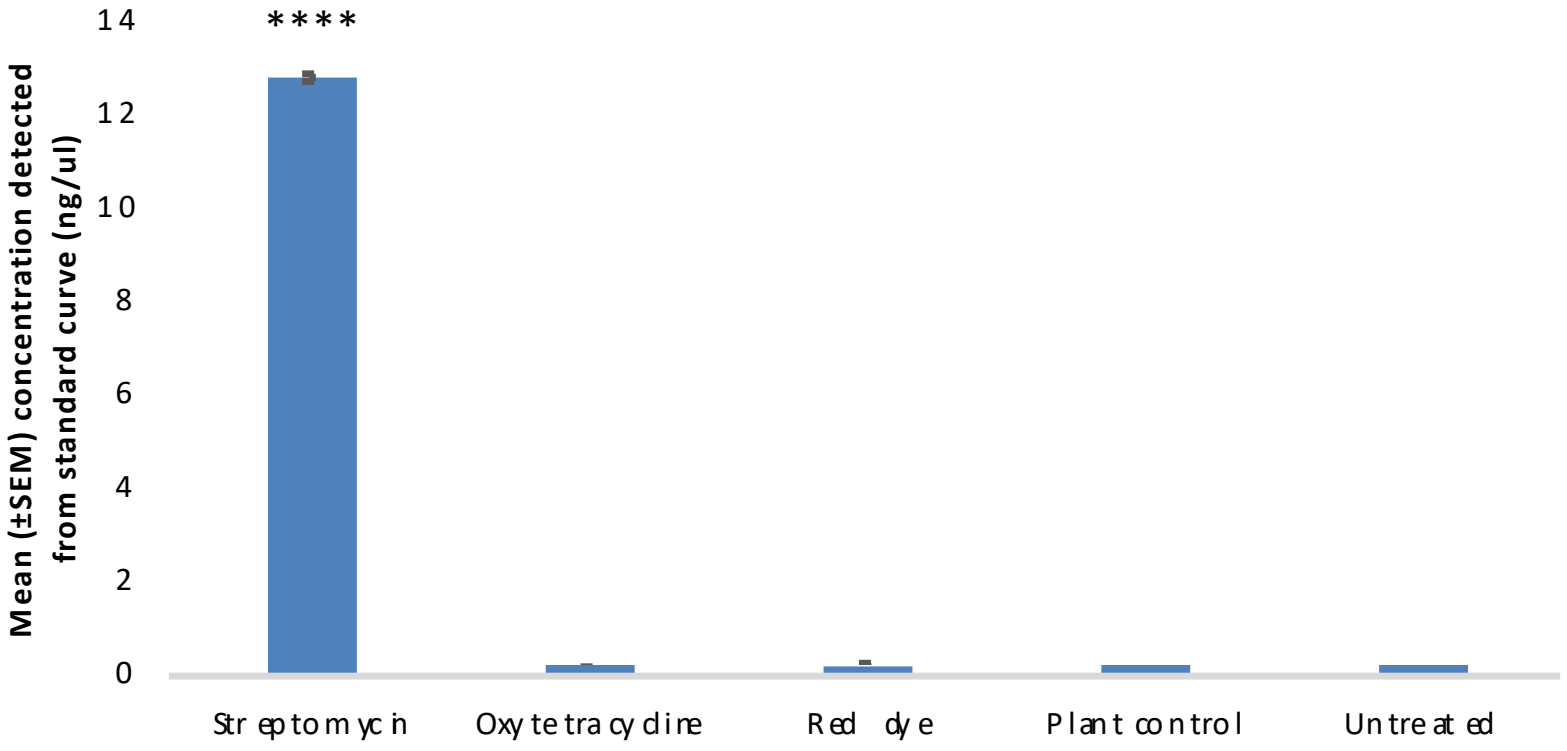
- **Positive values indicate reductions in CLas titer compared to day 0 in response to Firewall** (stremptomycin)
- Reduction in CLas after 4 weeks with Fireline (oxytetracycline)

Greenhouse Plant Assay: Firewall Soil Drench Efficacy



Positive values indicate reductions in CLas titer compared to day 0 in response to Firewall (streptomycin)

Streptomycin in leaf samples



- Plants treated by soil drench
- Leaves sampled day 6
- Concentrations determined from standard curve in ELISA assay

**** = $P \leq 0.0001$

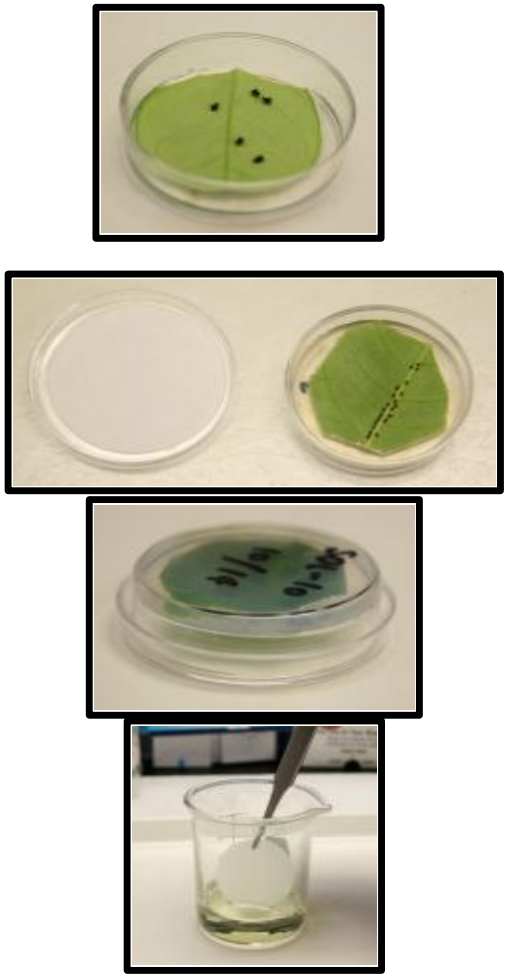
Do antibiotics reduce ACP feeding?

10 adults (mixed age) released onto a artificial diet ring

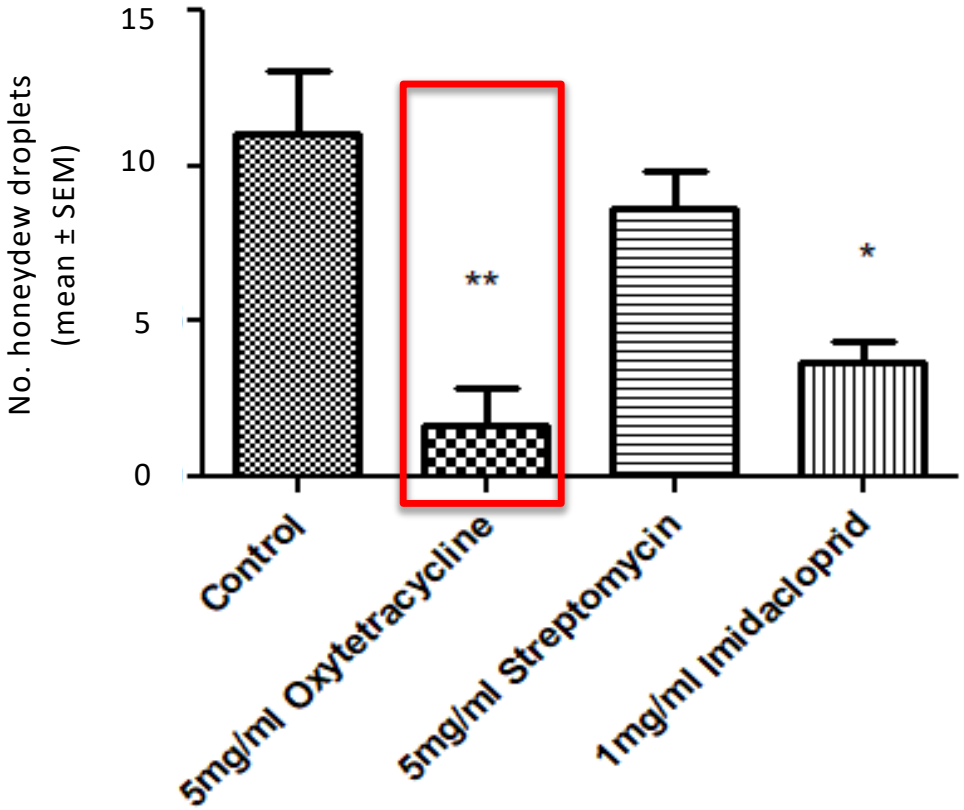
After 3 days, adults removed filter paper kept under the lid

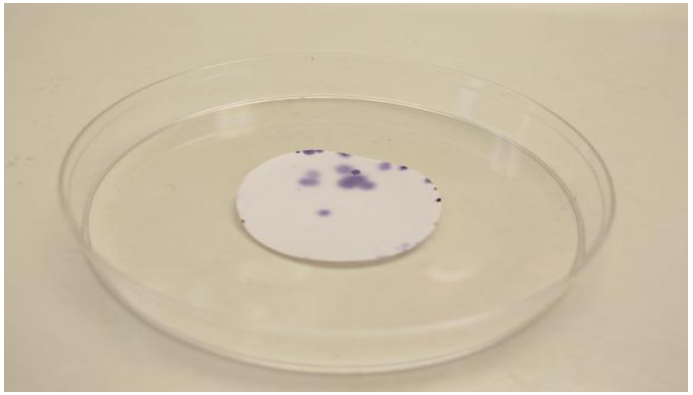
Petri plate inverted to collect honeydew droplets on filter paper

Filter paper dipped in ninhydrin (1%) for 3 min and dried



Oxytetracycline inhibits ACP feeding

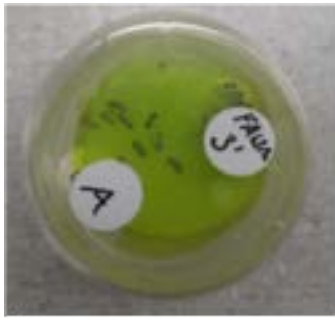


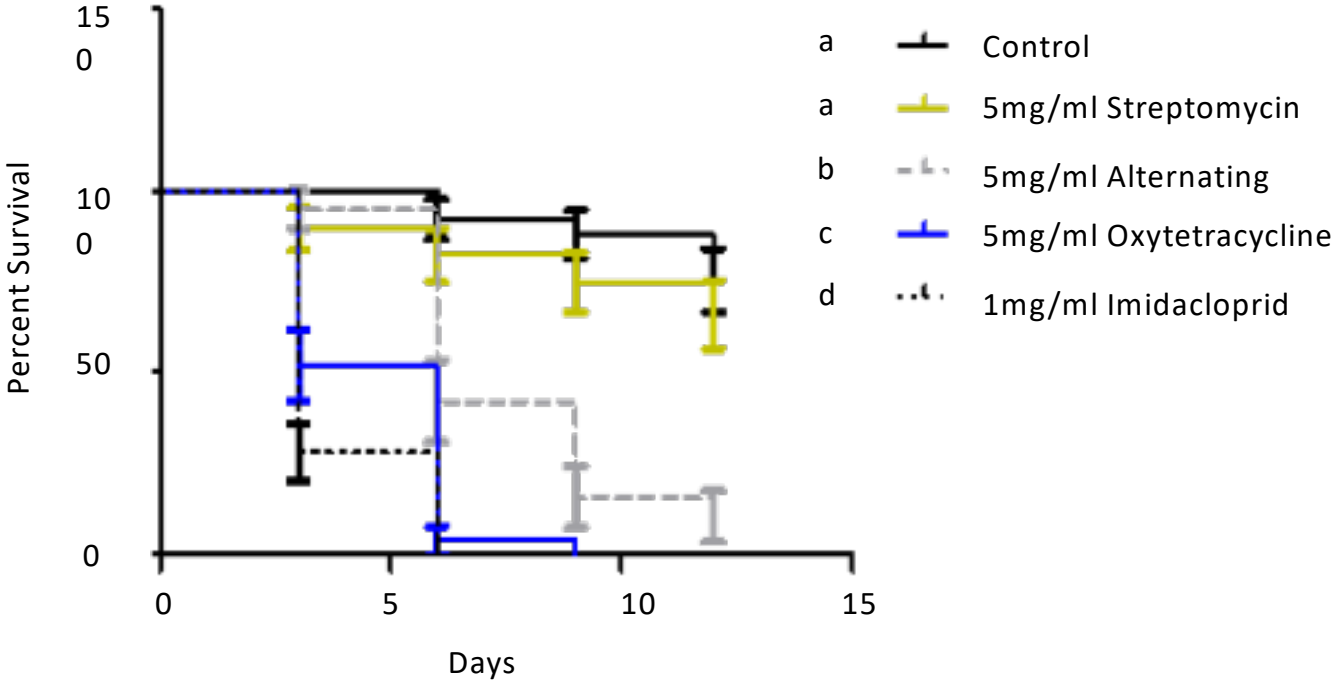


Purple spots quantified

ANOVA: $P = 0.005$, F = 9.75, df= 3, 11

ACP survival in response to bactericides





Mantel-Cox: $P = <0.0001$, $F = 5.468$, $df = 4$,

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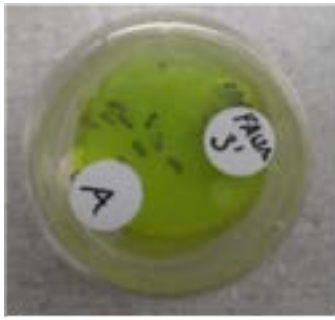
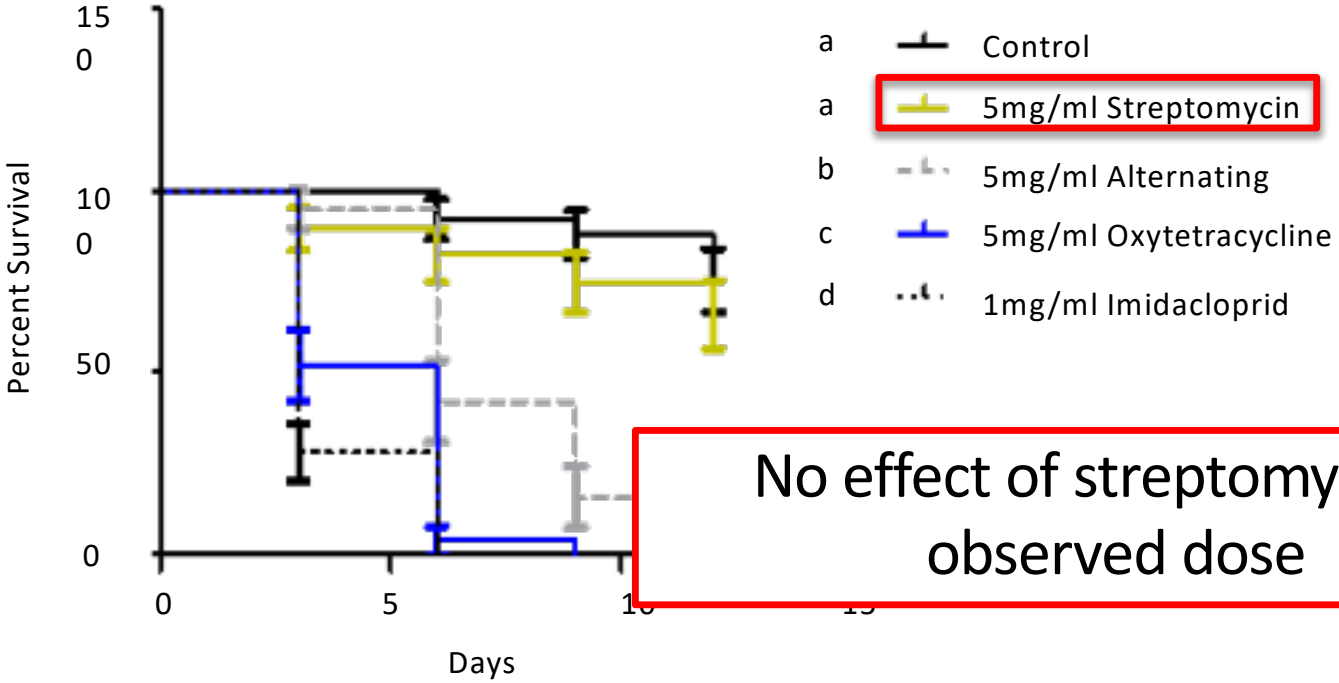
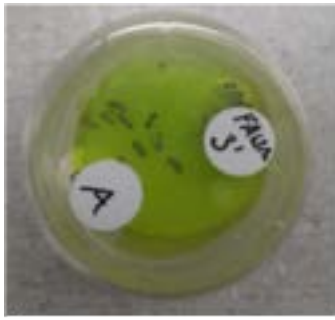


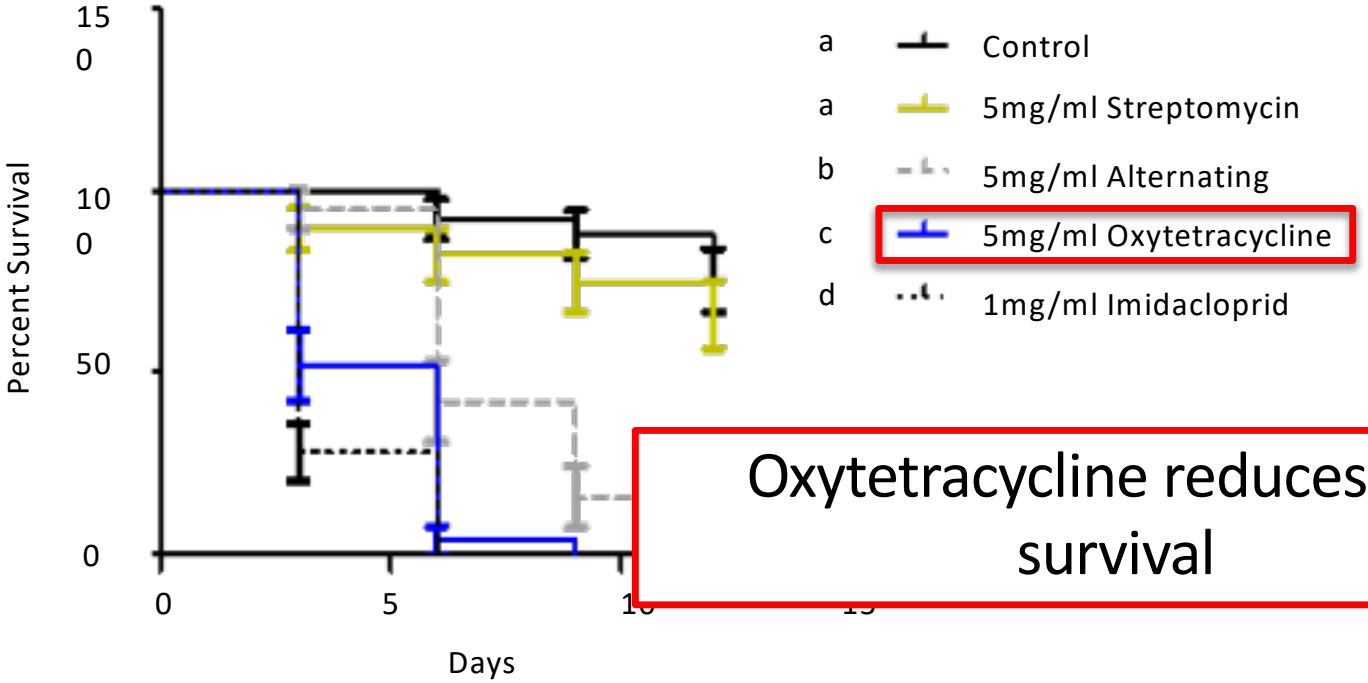


No effect of streptomycin at observed dose

Mantel-Cox: $P = <0.0001$, $F = 5.468$, $df = 4$,

ACP survival in response to bactericides





Oxytetracycline reduces ACP survival

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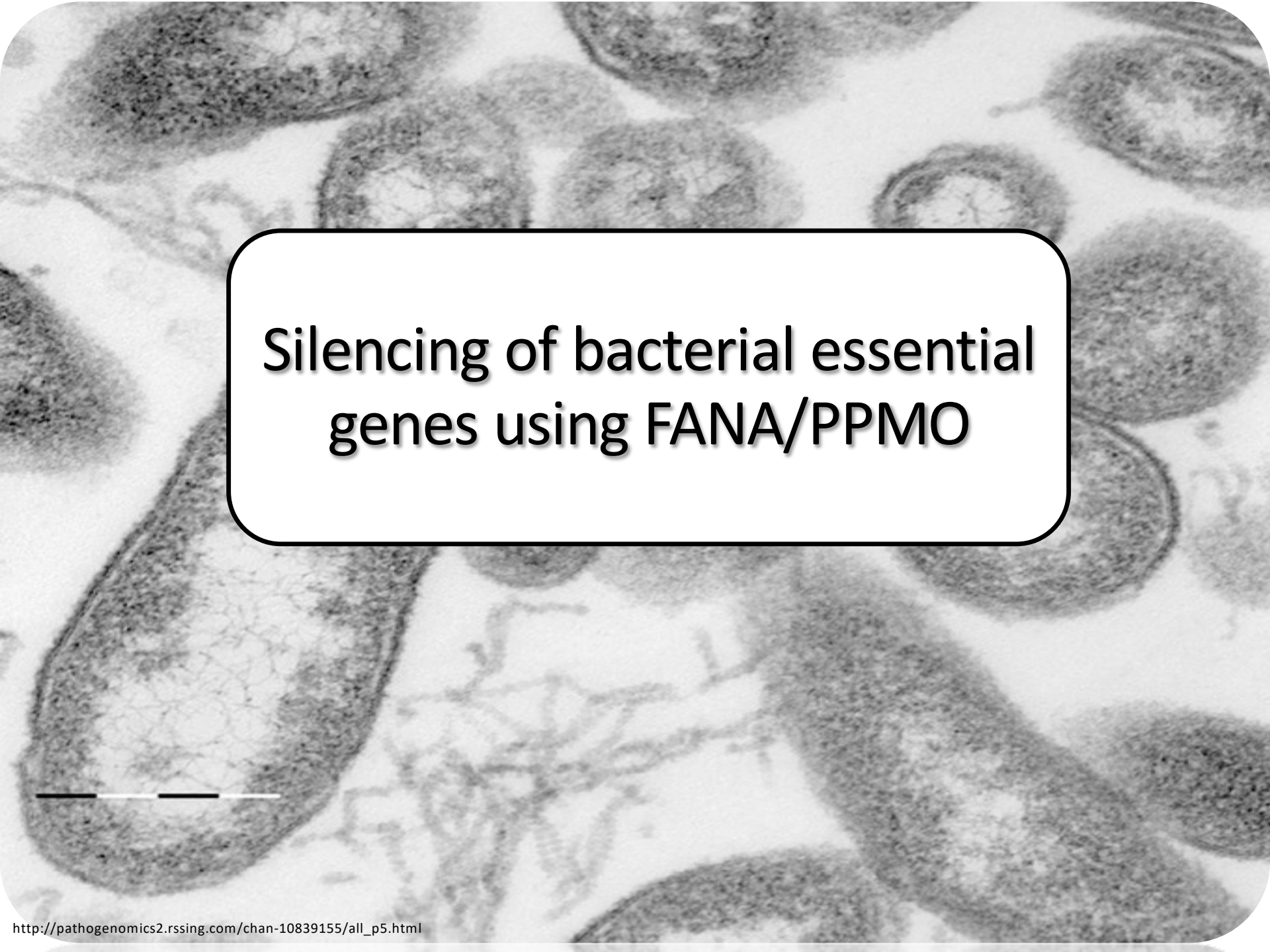
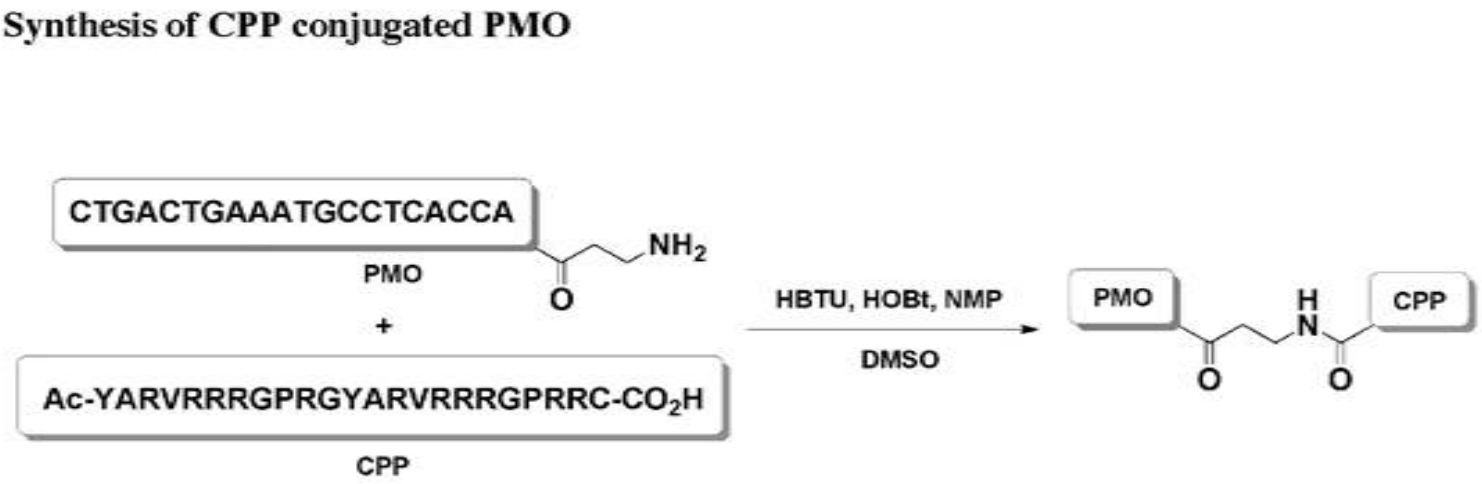


http://pathogenomics2.rssing.com/chan-10839155/all_p5.html

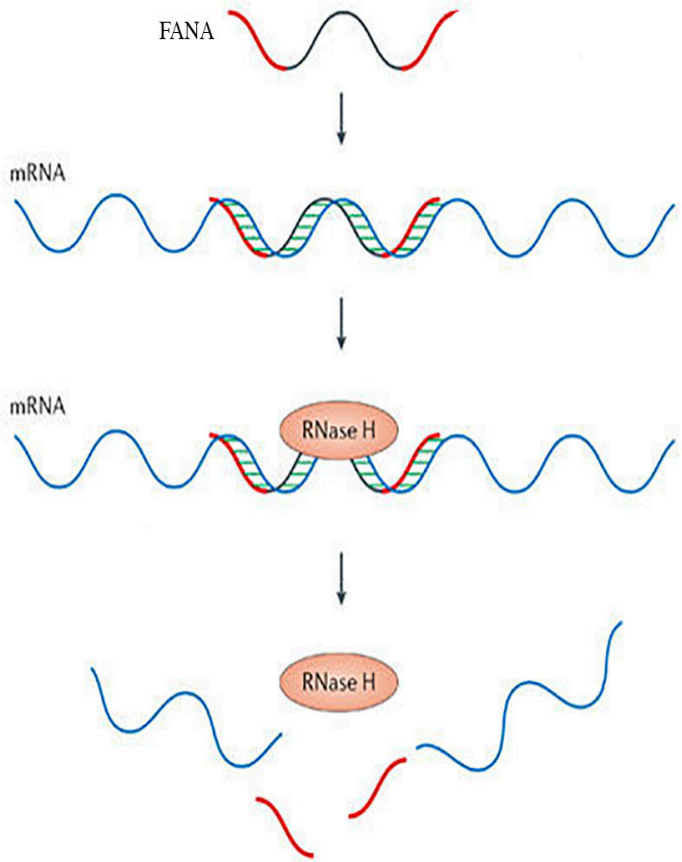
. Knock out endosymbiont to reduce transmission/vector fitness

- Development of FANA and morpholino (PPMO)-EGS technology targeting *C*Las and bacterial symbionts in ACP
- Synthetic molecules that mimic DNA and inhibit bacterial gene expression.
- These engineered molecules can be delivered specifically to target bacteria based on gene sequence, avoiding the problems of effecting non-target bacteria.



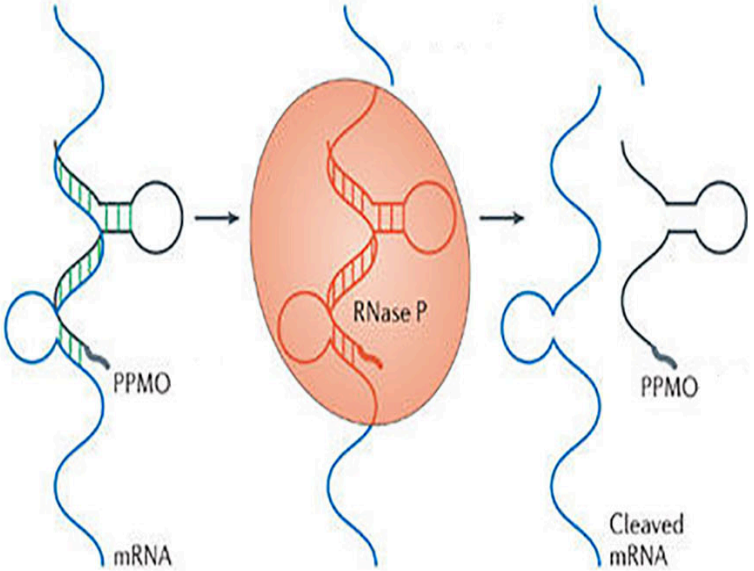
RNA-based bactericidal agents: selectively bind to bacterial mRNA

FANA antisense oligonucleotides (FANA)



- High stability
- Stable hybridization with the target mRNA

Peptide conjugated morpholino (PPMO)



- Endonuclease resistant
- Proven track record of limiting bacterial populations

Image Kole *et al.* 2012

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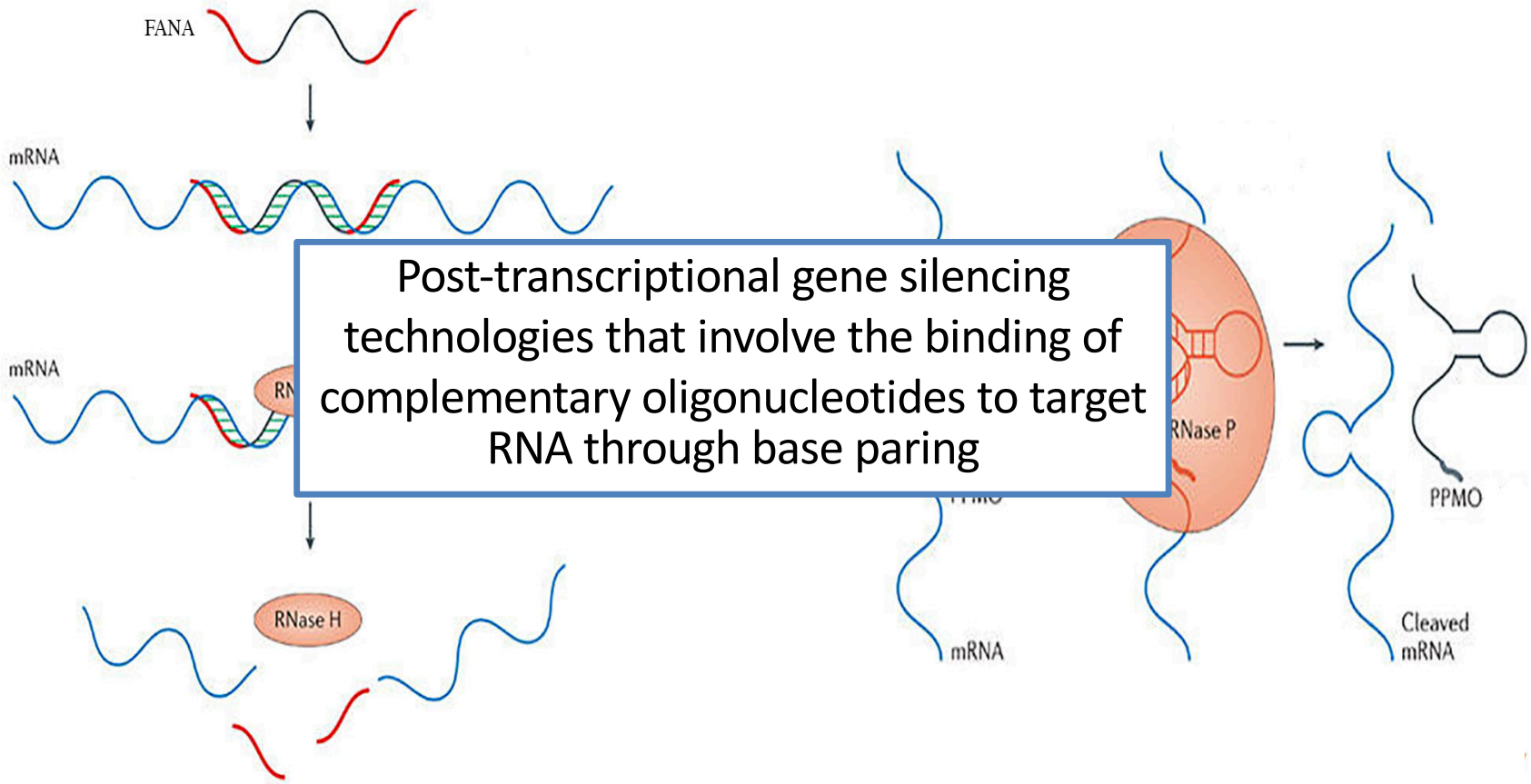


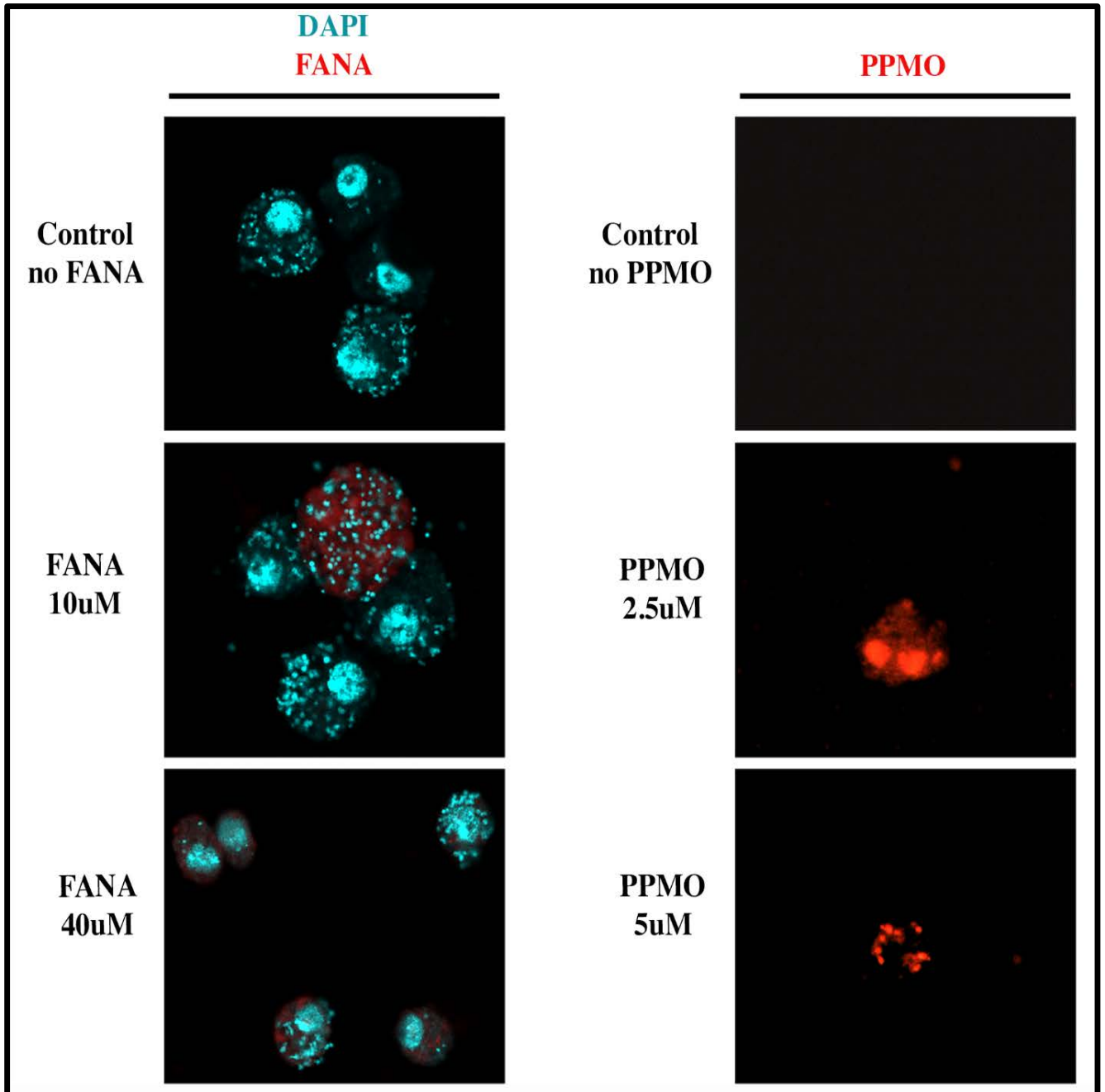
Post-transcriptional gene silencing technologies that involve the binding of complementary oligonucleotides to target RNA through base paring

- High stability
- Stable hybridization with the target mRNA

- Endonuclease resistant
- Proven track record of limiting bacterial populations

Image Kole *et al.* 2012

Penetration of FANA and PPMO in insect cells

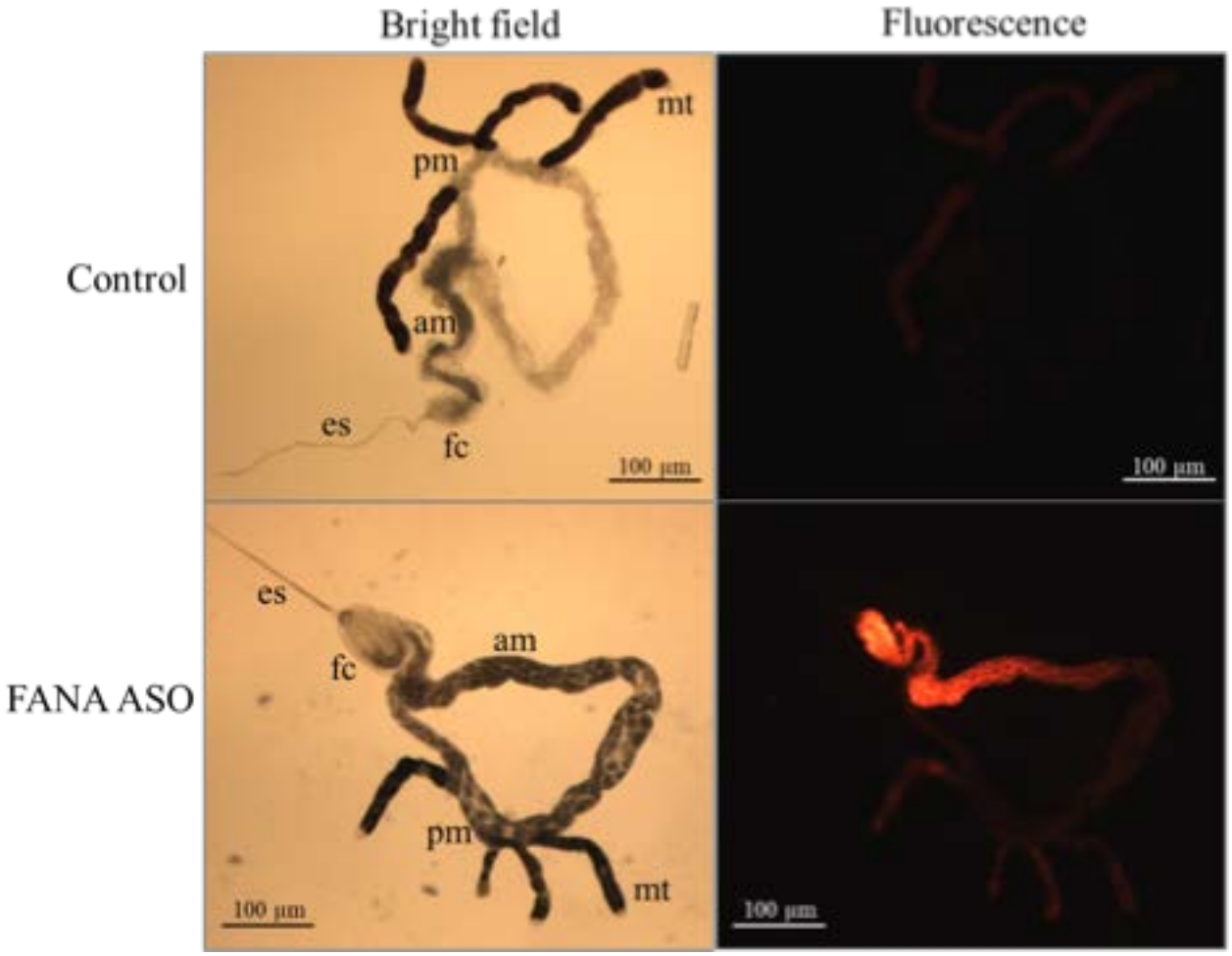


Red indicates penetration of oligonucleotides inside cells

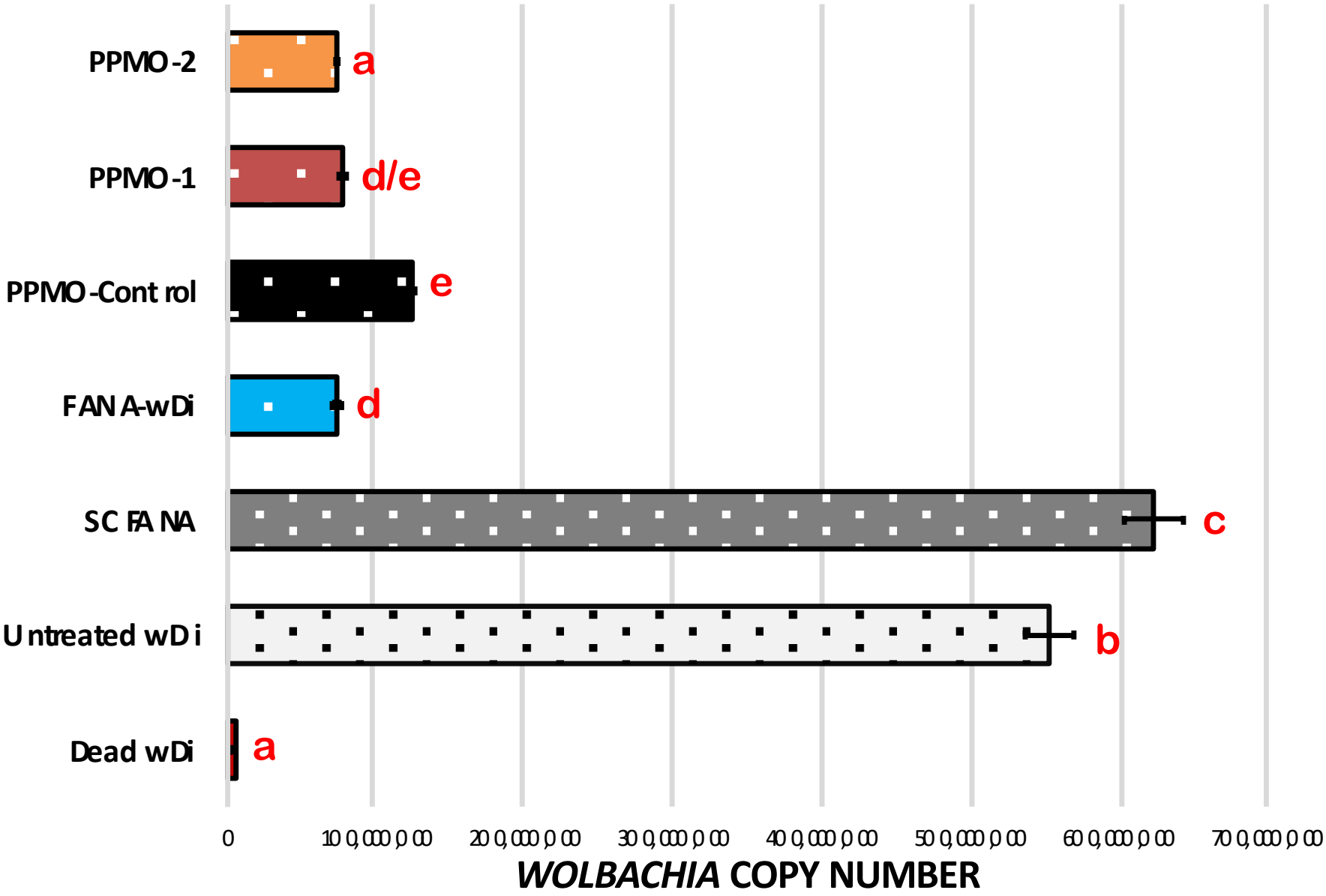
FANA fluorophore: Alexa647

PPMO fluorophore: Lissamine

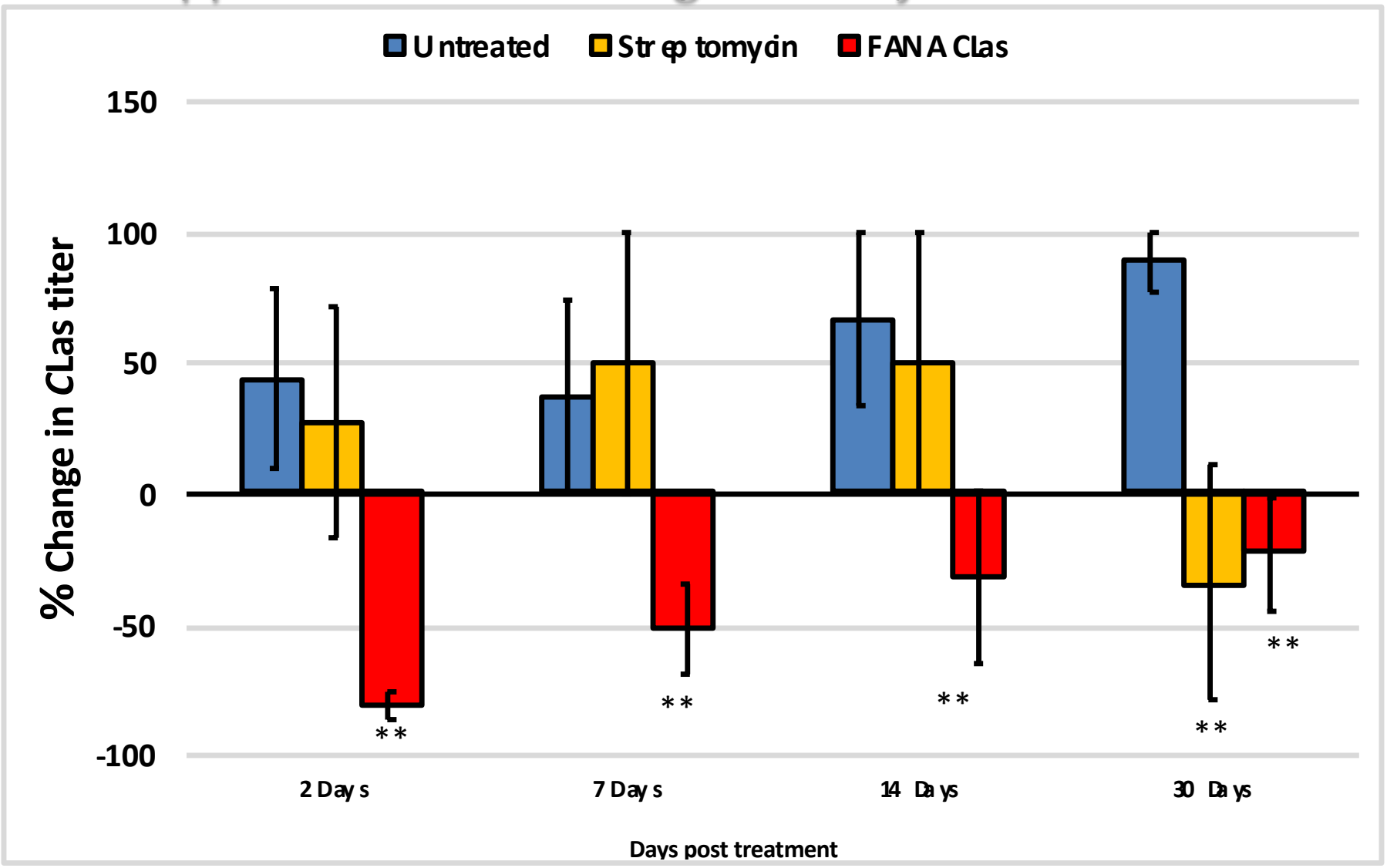
Localization of FANA oligo in the alimentary canal of ACP adults



FANA and PPMOs reduce *Wolbachia* survival in cell culture



FANA applied to citrus through root injection reduces CLas



Negative values represent reduction of Las titer

Summary of antisense efficacy

	FANA	PPMO
Las (Citrus)		
Las (ACP)		
Wolbachia (cell culture)		
Proftella endosymbiont	NA	
ACP Survival		TBD
Transmission	TBD	TBD

Conclusions

- Some reduction of Las titers in response to FireWall and FireLine in greenhouse assays
- Antisense FANAs more effective than streptomycin at reducing Las
- Ingestion of antimicrobials reduces feeding (tetracycline) survival (tetracycline, FANA) of ACP
- Antimicrobial treatments (antibiotics or antisense-RNA technologies) can specifically target Las and microorganisms in ACP

Future Strategies

- Antimicrobials currently available appear to may be effective in reducing ACP populations and CLas transmission
- Targeted antimicrobials likely to be more effective in reducing CLas transmission by ACP
  - Reduces potential for non-target effects
  - Multiple target sites reduce potential for resistance

Thanks!

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Alex Arp (USDA)

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