Use of molecular markers for plant DNA to determine host plant usage for potato leafhopper, *Empoasca fabae*

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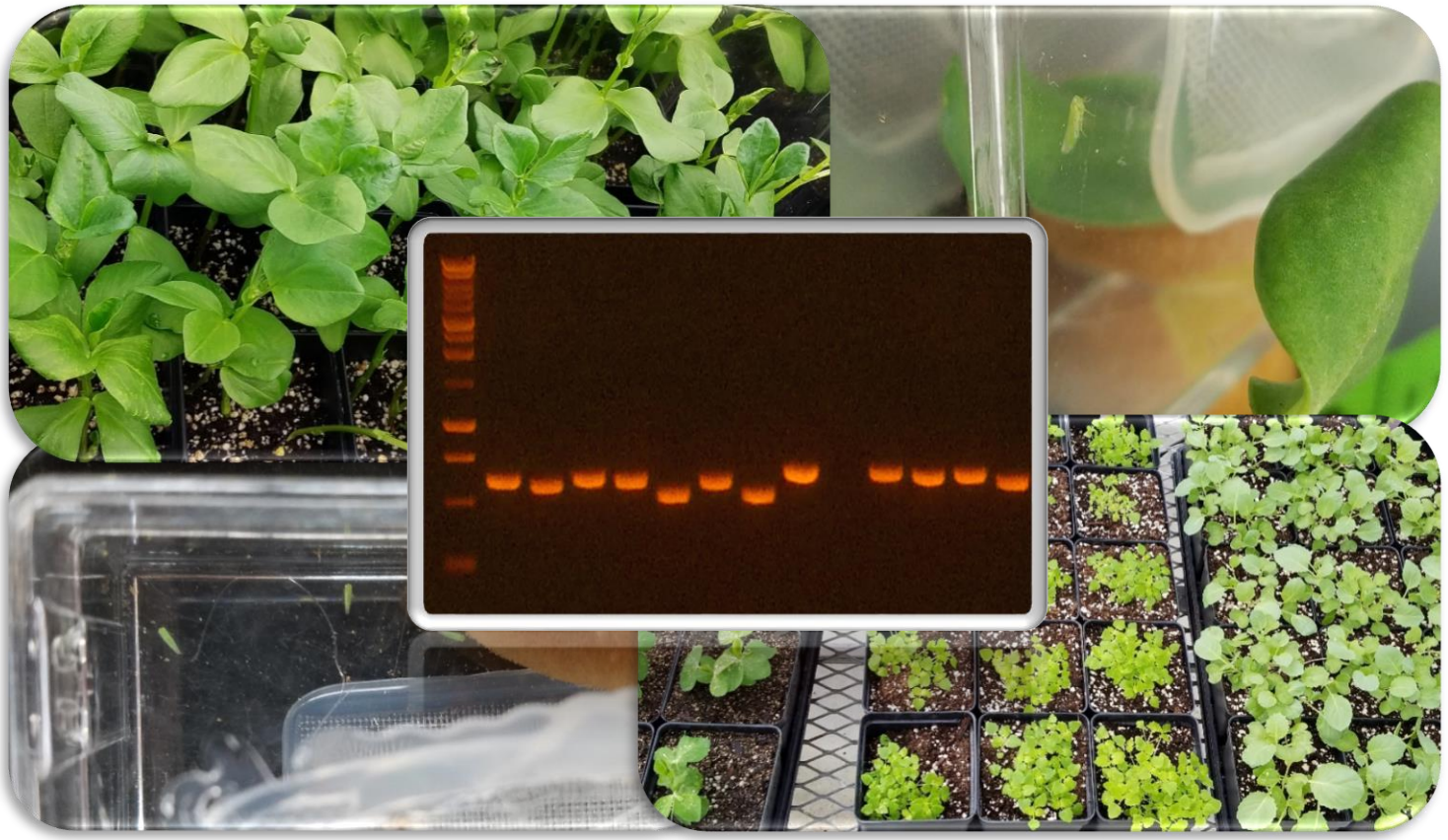
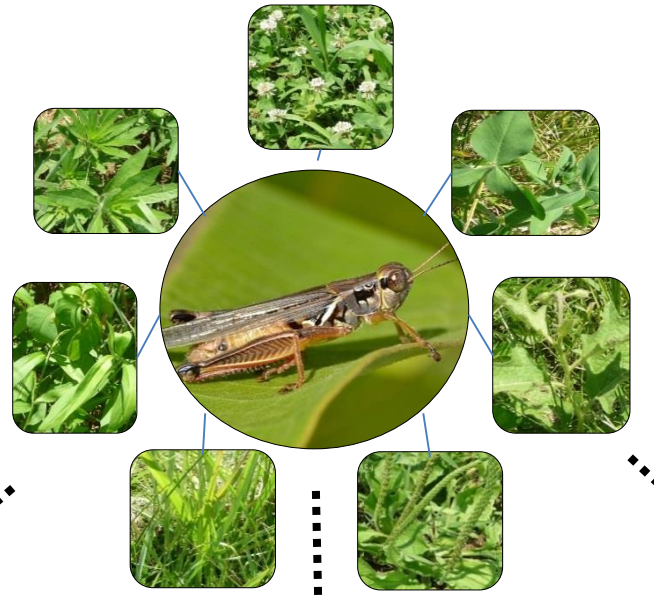


2018 ESA, ESC, and ESBC Joint Annual Meeting

Detecting plant meals in insects guts

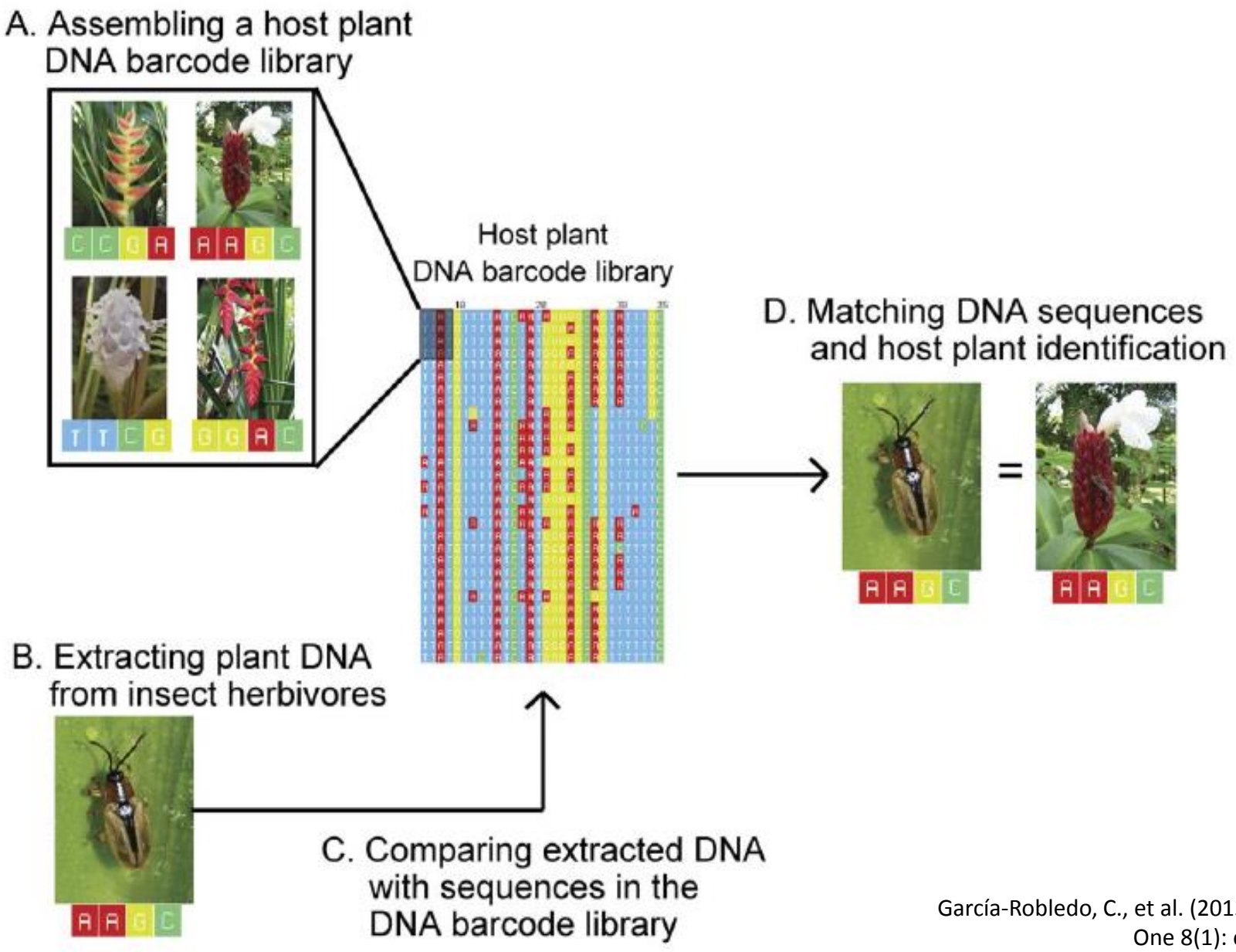
- understanding insect feeding preferences



- detecting and predicting plant-insect associations

Accurate confirmation of plant food digestion is critical

Molecular confirmation of diet



García-Robledo, C., et al. (2013) PLoS One 8(1): e52967

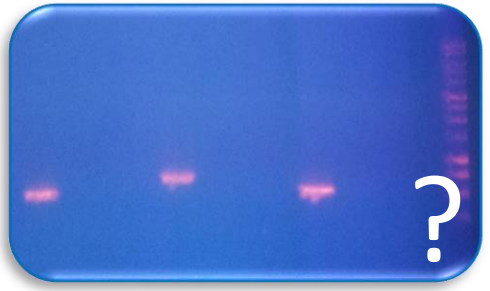
Existing protocols for plant DNA detection within insect guts

Limitations:

- Protocols have been developed for a limited number of insect species (mostly grazers) – protocols for sap-feeders?

- Information about insect body surface contamination with plant material is limited – plant DNA on the surface vs. ingested plant DNA?

- Plant DNA detectability over time – how long plant DNA can be detectable in the gut contents?



Research questions

Exp.1. Developing the protocol: Which region of plant DNA can be reliably detected in potato leafhoppers guts?

Exp.2. Is the detected plant DNA ingested or is it present on the insect body surface?

Exp.3. How long can plant DNA be detected in potato leafhoppers gut contents?

Study species

Potato leafhopper, *Empoasca fabae*

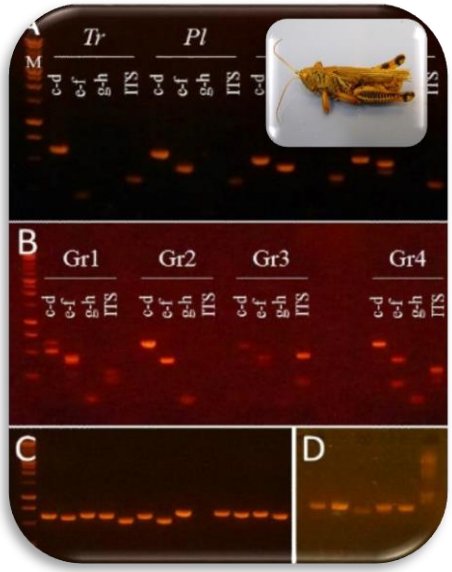
Adults

Nymphs

Fava bean, *Vicia faba*

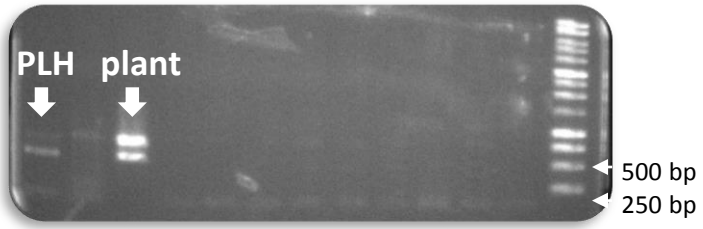
Exp.1. Developing a protocol for plant DNA detection within potato leafhopper guts

Step 1. DNA extraction and PCR amplification of the chloroplast *trn*L gene



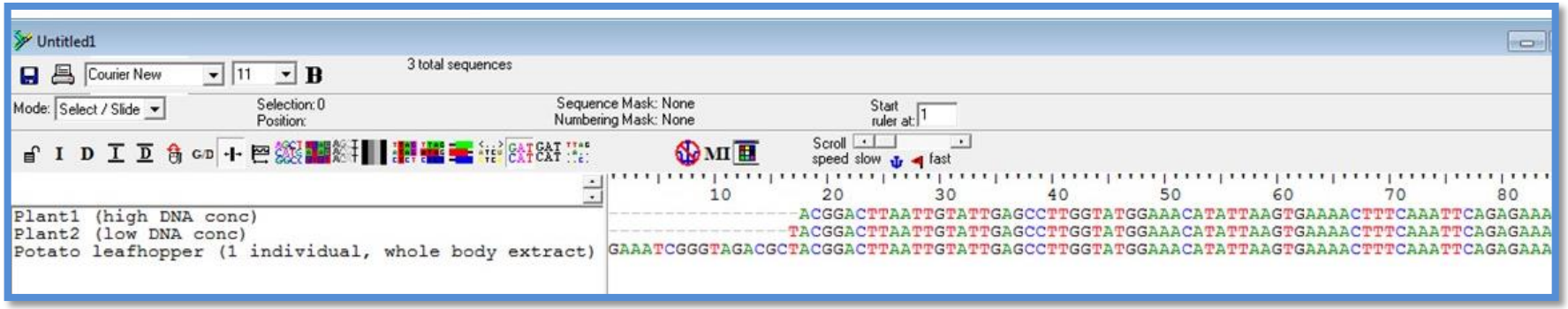
Target region	bp
Chloroplast *trn*L (UAA) intron	~ 550 bp

Avanesyan (2014),
Application in Plant Sciences
2(2):1300082

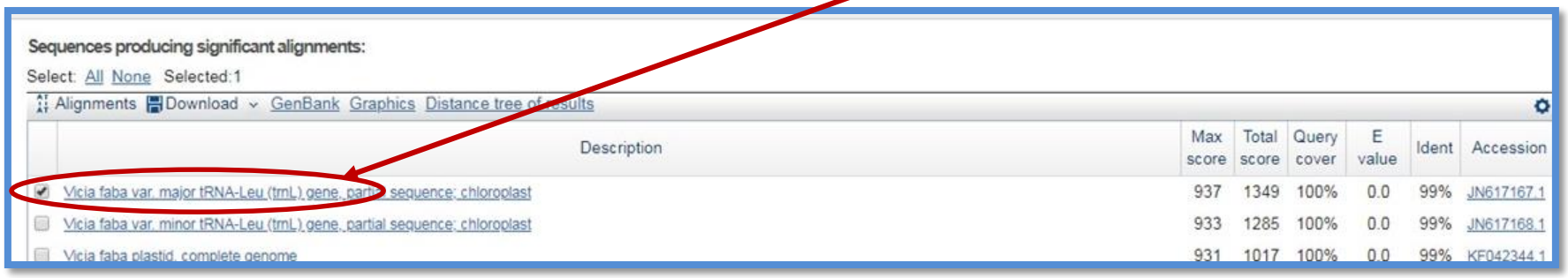


Step 2. Plant DNA barcoding

Sequence analysis



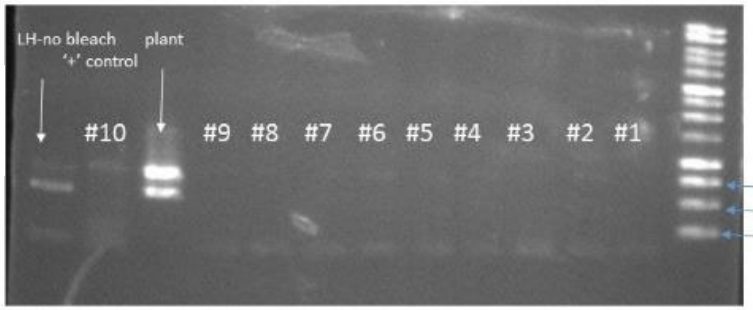
BLAST results (NCBI GenBank): 99% identity with *Vicia faba*, 100% sequence cover, 511 bp



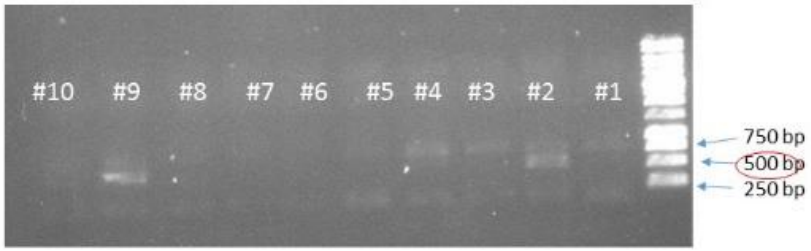
Exp.2. Ingested plant DNA vs. plant DNA on the leafhoppers body surface

Step 1. Bleach treatment

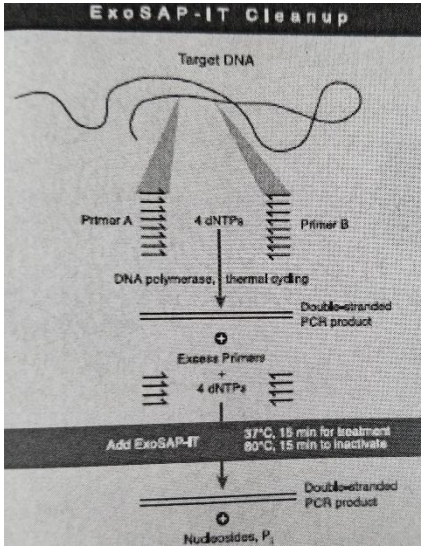
Bleach (2%)
n=10



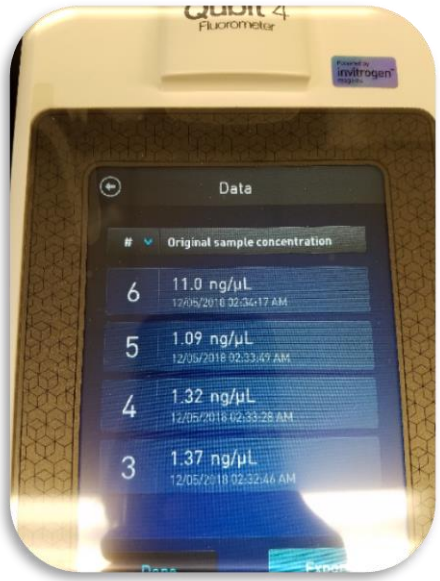
No bleach
n=10

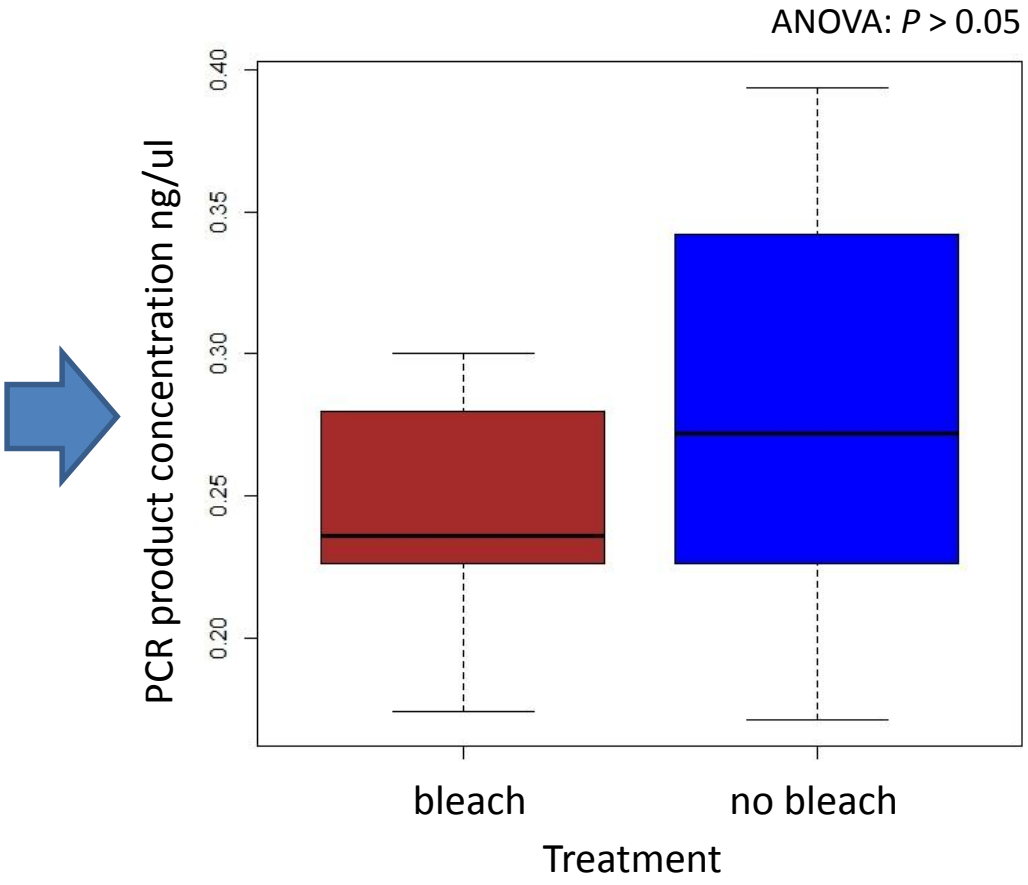


Step 2. PCR purification



Step 3. Recording final PCR product concentration





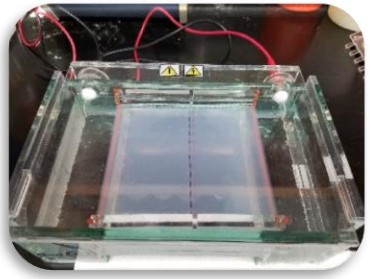
Exp.3. How long can plant DNA be detected in potato leafhoppers gut contents?

Step 1. Setting up feeding experiment

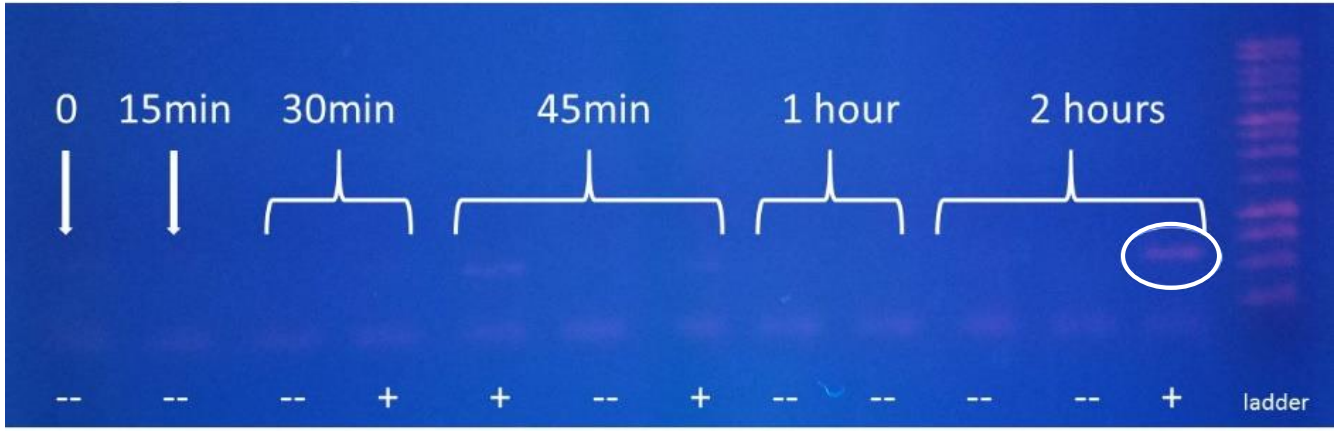


- 3-5 adult leafhoppers per cage
- Cages clipped, leaves removed after 24 hours of feeding
- Frozen at 0 min, 15 min, 30 min, 45 min, 1 hour, and 2 hours post ingestion

Step 2. PCR amplification



Gel electrophoresis results



- different individuals, some might not feed
- plant DNA detected up to 2 hours post ingestion

Conclusions

Exp.1. We have developed an effective protocol for plant DNA detection from potato leafhoppers guts: fragments (~511 bp) of the non-coding region of the chloroplast *trn*L (UAA) gene were successfully amplified

Exp.2. We have found that the contamination of the leafhopper body surface with the plant material is insignificant (~1.5%; $p > 0.05$), and almost all the detected plant DNA is ingested

Exp.3. We have demonstrated the utility of this protocol for determining time of digestion of plant material

Current work / Future directions

<u>Using the developed protocol:</u>

- Confirm utilization of different host plants

- Detect plant DNA in the gut contents of field collected individuals

This PCR method has important applications such as determining host usage of the potato leafhopper, as well as its potential migration

Thank you!

The Lamp lab:
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