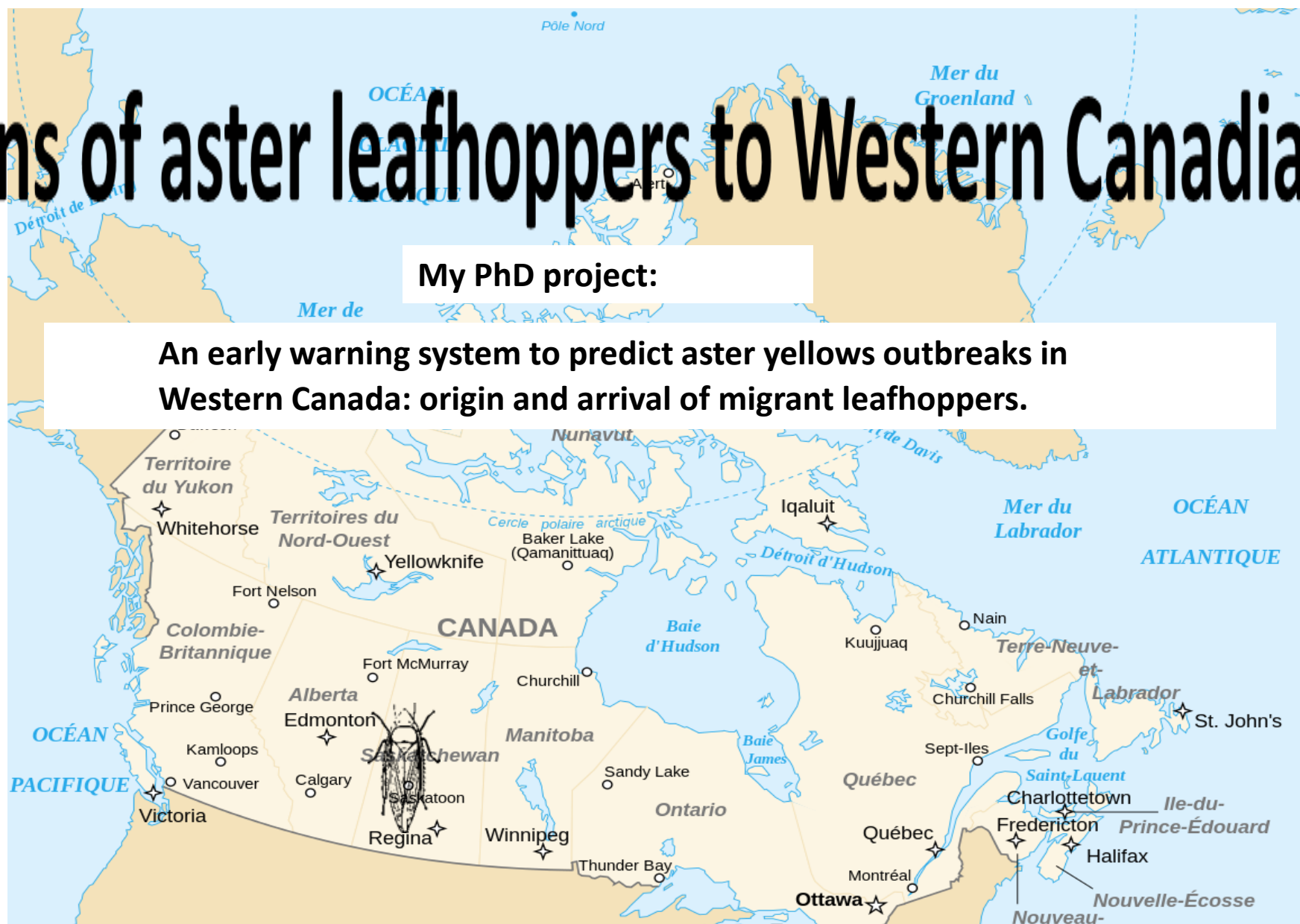


The origins of aster leafhoppers to Western Canadian prairies

My PhD project:

An early warning system to predict aster yellows outbreaks in Western Canada: origin and arrival of migrant leafhoppers.

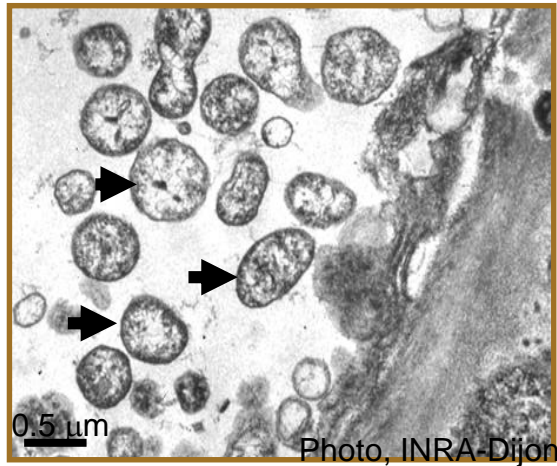


Karolina Pusz-Bochenska, Tyler Wist, Tim Dumonceaux, Erl Svendsen, Sean Prager, Chrystel Olivier, Keith Hobson
Agriculture and Agri-Food Canada
Saskatoon Research and Development Centre

Aster yellows disease

- caused by the nonculturable, Gram-positive bacterium

***'Candidatus Phytoplasma'* spp.**



- The **aster leafhopper** *Macrostoteles quadrilineatus* is the main vector of the AY phytoplasma.
- AY infects over 300 species of plants, including: **canola, barley, and the pulses.**
- **Symptoms: Phyllody** can lead to **very small malformed seeds or no seeds at all.**



(Tyler Wist)

In 2012 in SK **loss** was estimated at around **\$270 million.**



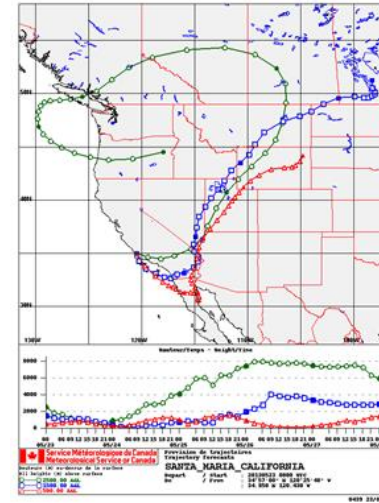


Tyler Wist

Project objectives:

- Evaluate the origin of migrant aster leafhoppers to better understand AY risk each year.

1. Wind trajectories monitoring.



2. Genetic profiling of leafhoppers sampled in SK and from Southern populations.

>KPB2_LCO_MP8014_19 KPB2-SK3k6-Aberdeen_CO1.0.2
 atgtgatcgttacatcccacgcattattataatcttcttatagttataccaattataa
 ttgggggttttgtaattgactactccactataattggagctcctgatatagcattcc
 cagcactaaataataagattctgacttctcccccttcattaacactattaatattaa
 gatcaatagtagaaatgggggtagggacgggtgaacagtataccccccctatctagaa
 atatcgcacatgcgggccaagagttgatatatcaattttctttacatttagccggtga

3. Stable isotopes.

¹ H 1.00784 99.985%	² H 2.0141 0.015%	³ H E _{1/2} =12.32yrs Cosmogenic anthropogenic
¹⁶ O 15.9949 99.76%	¹⁷ O 16.9991 0.04%	¹⁸ O 17.9991 0.20%
¹² C 12.00000 98.89%	¹³ C 13.00335 1.11%	¹⁴ C 14.0 E _{1/2} =5715yrs Radioactive Cosmogenic anthropogenic

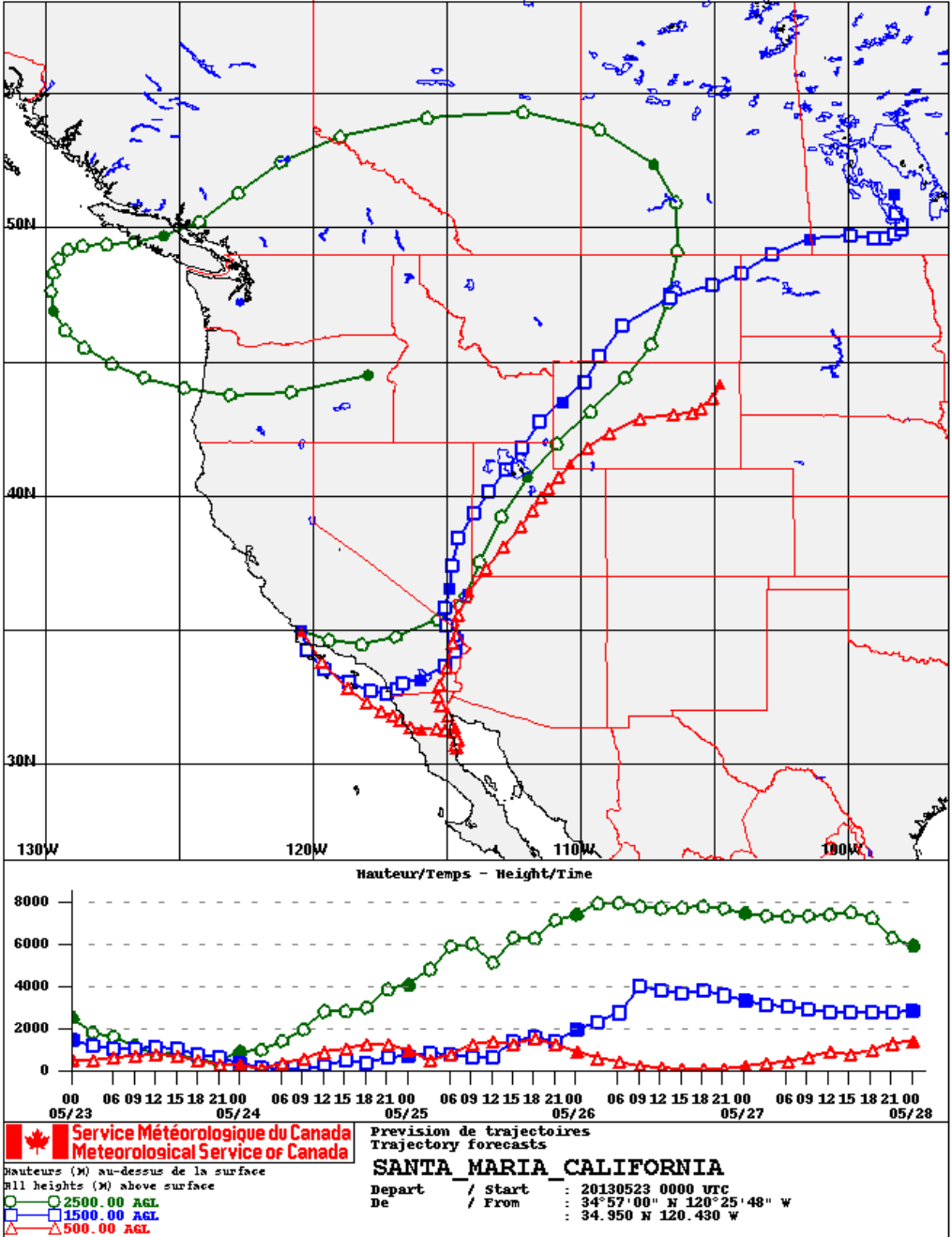


Aster Yellows predictions

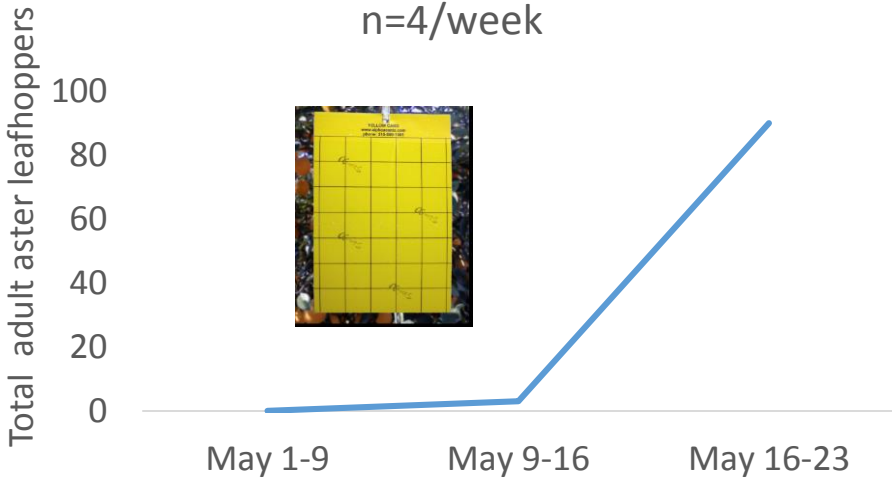
- South Wind: Reverse Trajectories
- Prairie Pest Monitoring Network (PPMN) monitors winds
- Environment Canada

Owen Olfert¹, Ross Weiss¹, Meghan Vankosky¹ and Serge Trudel²

- 1 - AAFC
- 2 - ECCC



MooseJaw SK 2018 Yellow Sticky Cards
n=4/week



- 2018, May 9th -16th **1st** arrival of aster leafhoppers (Southern Sask)
- 2018, May 23rd **2nd** arrival. Southern and Northern coverage

RT wind from Washington



RESEARCH ARTICLE

Molecular data reveals California as the potential source of an invasive leafhopper species, *Macrosteles* sp. nr. *severini*, transmitting the aster yellows phytoplasma in Hawaii

J.J. Le Roux^{1,2} & D. Rubinoff¹

¹ Department of Plant and Environmental Protection Sciences, University of Hawaii, Honolulu, HI, USA

² DST-NRF Centre of Excellence for Invasion Biology, Department of Botany and Zoology, Faculty of Science, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa

Keywords

CO1; invasion history; *Macrosteles*; *NADH1*; phylogenetics; *Wg*.

Correspondence

J.J. Le Roux, DST-NRF Centre of Excellence for Invasion Biology, Department of Botany and

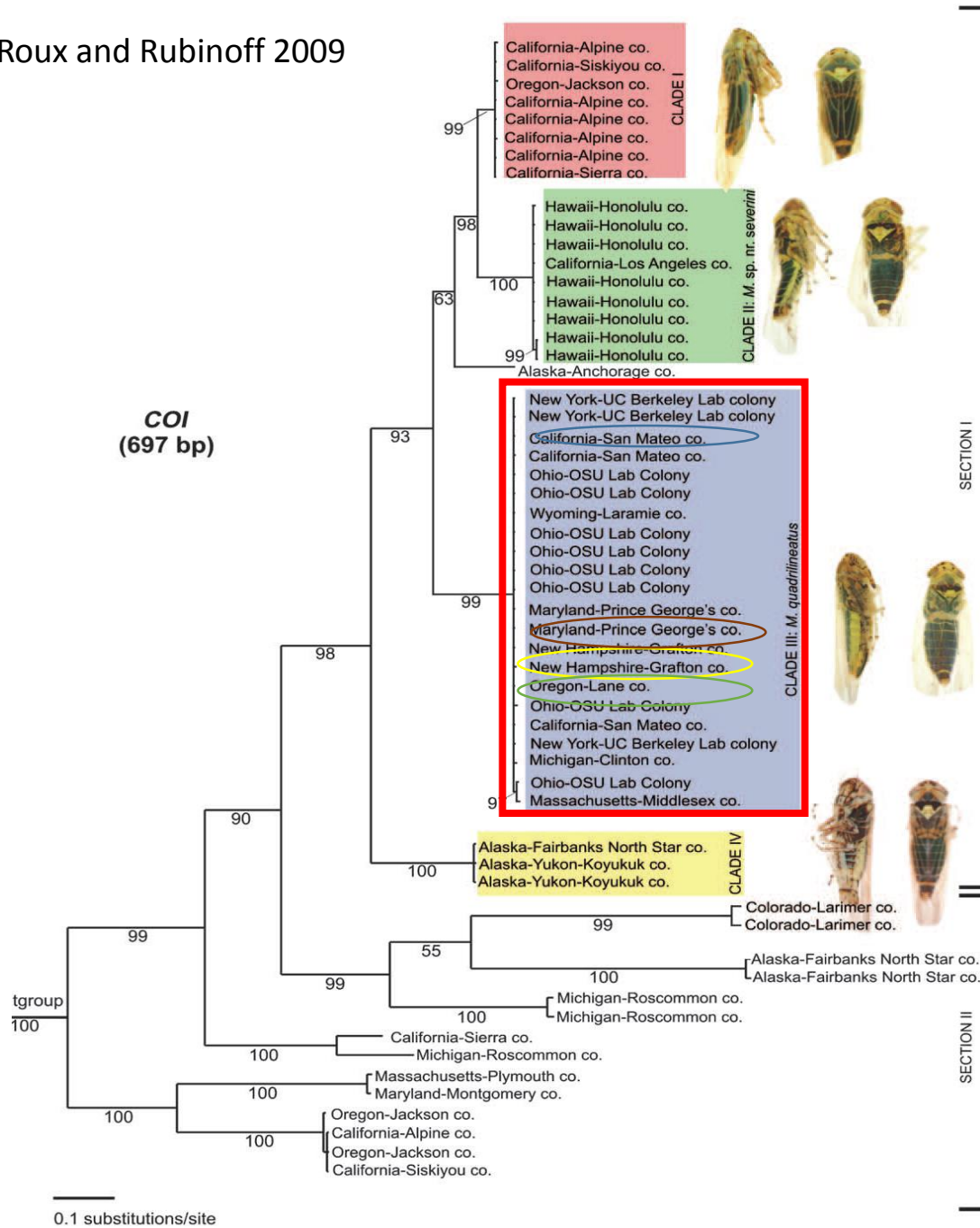
Abstract

A species of aster leafhopper (*Macrosteles* sp.) became established in 2001 on Oahu, Hawaii, and through the transmission of the aster yellows phytoplasma, caused devastating losses to the island's watercress industry. DNA sequence data were analysed from two mitochondrial genes [cytochrome oxidase sub-

Genetic markers to ID
popns of aster
leafhoppers: CO1 and
NADH1

Le Roux and Rubinoff
2009 created
GenBank records

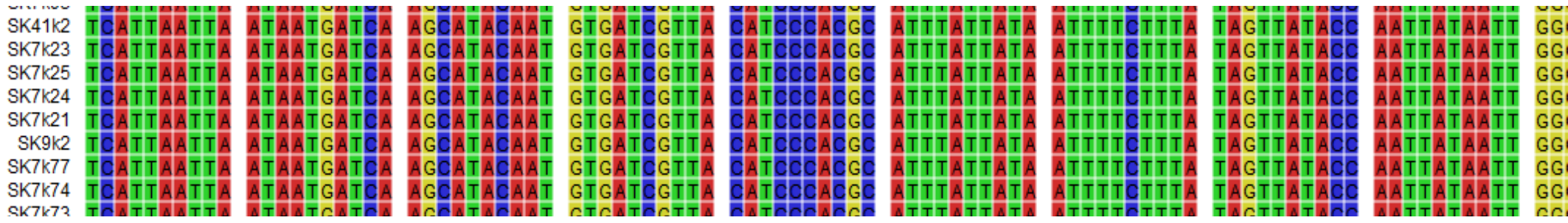




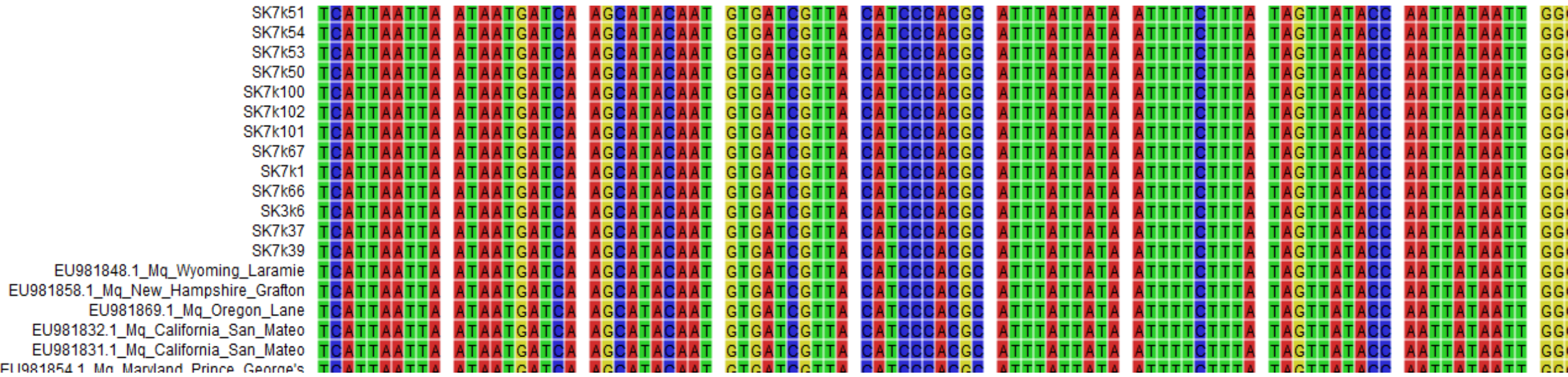
Sask leafhoppers from 2018



- SK7k68
- SK6Mell
- EU981848.1_Mq_Wyoming_Laramie
- SK7k102
- SK7k56
- SK7k74
- SK7k93
- SK7k13
- SK7k41
- SK7k104
- SK7k60
- EU981831.1_Mq_California_San_Mateo
- SK7k66
- SK7k54
- SK7k88
- SK7k24
- SK7k98
- SK7k15
- SK7k38
- SK7k65
- SK7k48
- EU981869.1_Mq_Oregon_Lane
- SK7k67
- SK7k106
- SK7k70
- SK7k23
- SK7k95
- SK7k42
- EU981859.1_Mq_New_Hampshire_Grafton
- EU981884.1_Mq_California_San_Mateo
- EU981854.1_Mq_Maryland_Prince_Georges
- SK3k6
- SK7k53
- SK4k1
- SK7k21
- SK7k92
- SK7k18
- SK7k35
- SK7k85
- SK7k8
- EU981832.1_Mq_California_San_Mateo
- SK7k1
- SK7k51
- SK7k71
- SK7k25
- SK7k10
- SK7k19
- SK7k62
- SK37k
- EU981858.1_Mq_New_Hampshire_Grafton
- SK7k101
- SK7k55



- little sequence variation in regions tested
- Next: look for SNPs across the genome (Illumina)



Project objectives:

- Create a fast, diagnostic tool to determine if leafhoppers are infected with AY.

Aster yellows detection:

- Molecular diagnostics

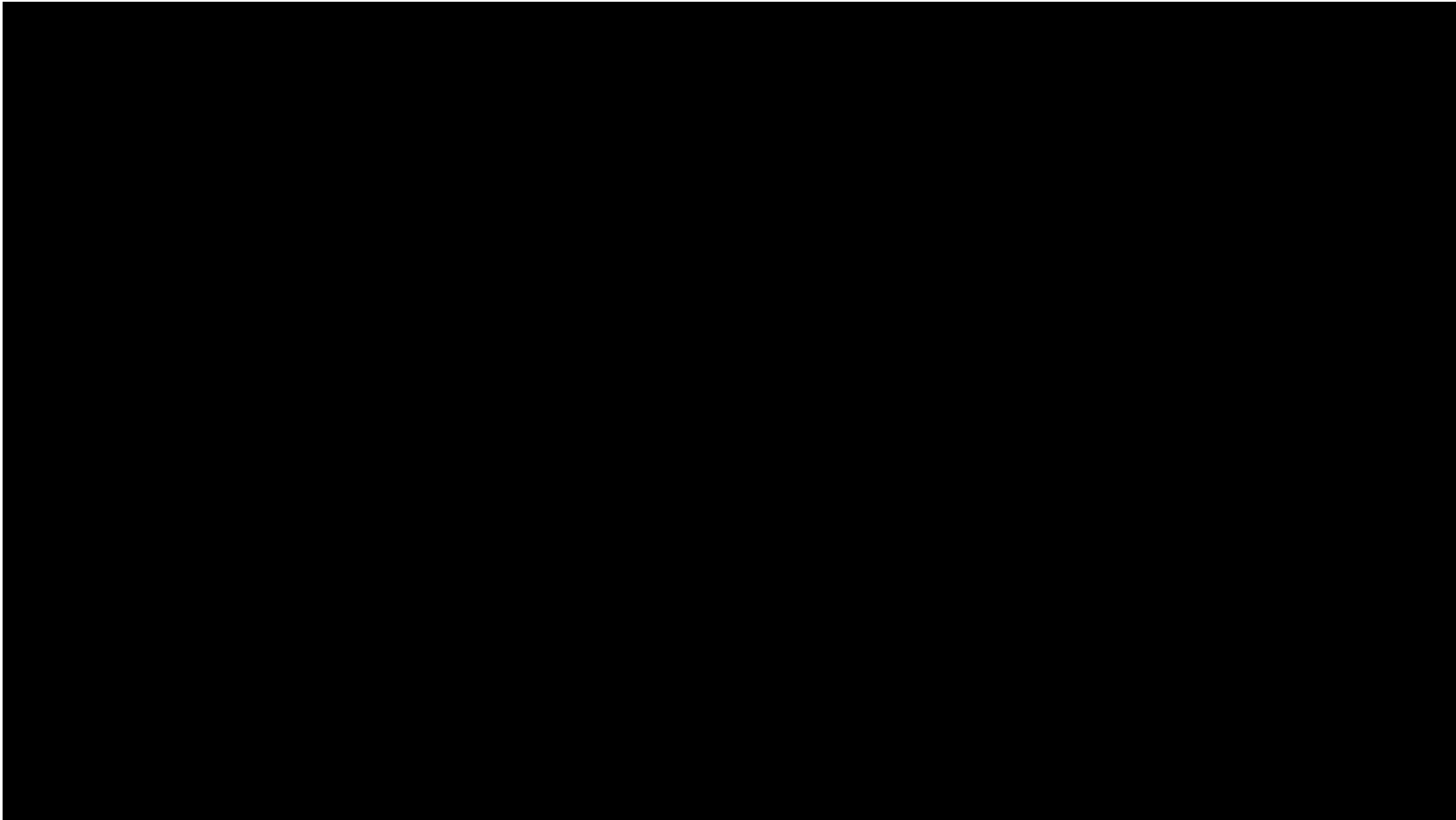


1. DNA extraction



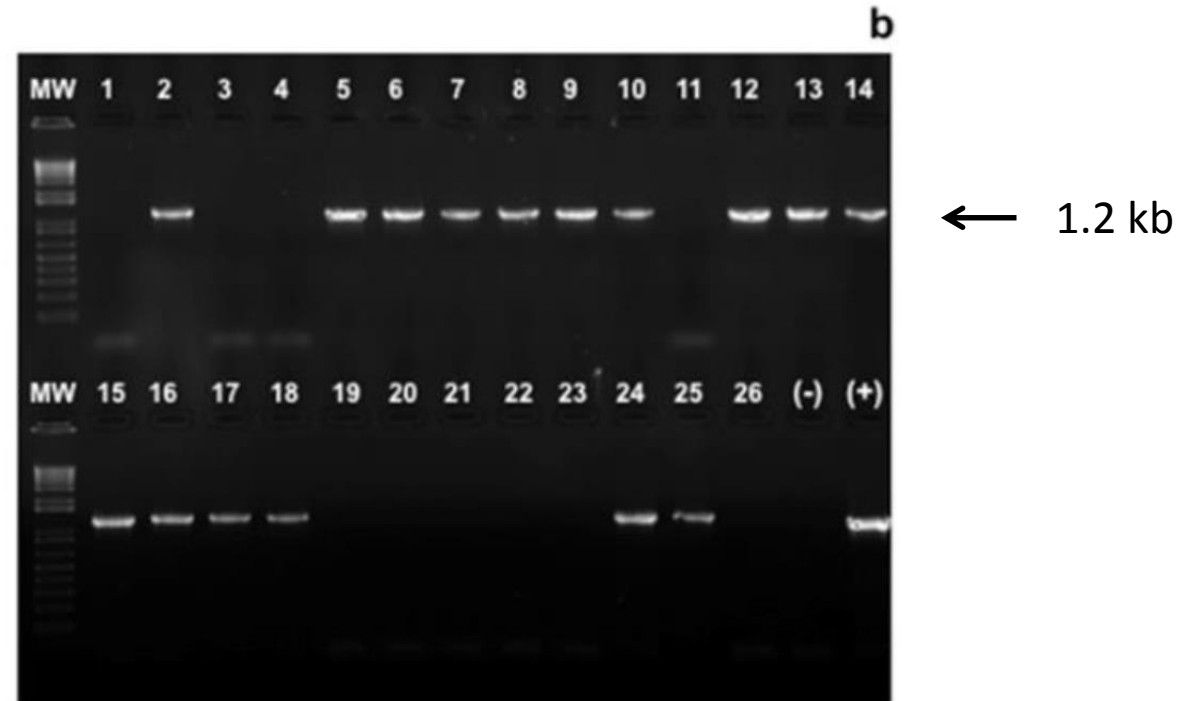
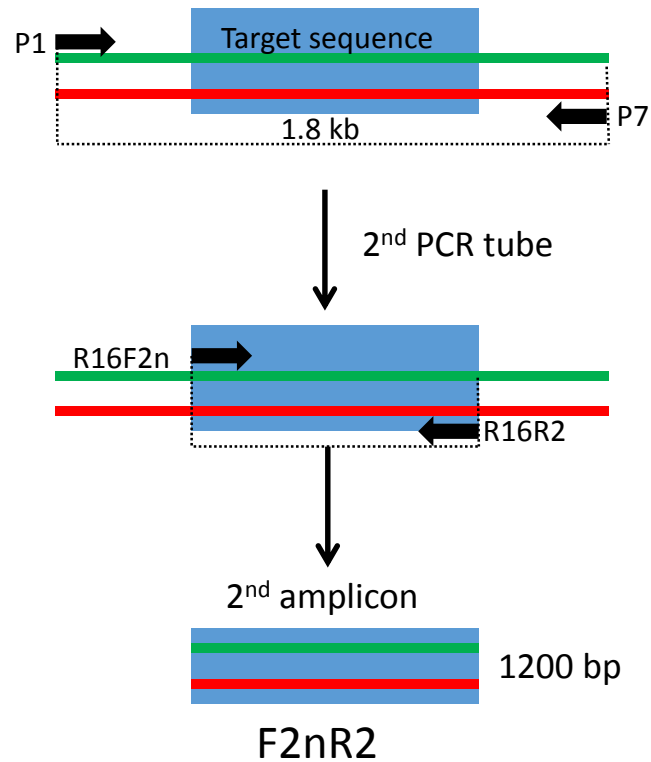
2. Molecular detection

Rapid AY diagnostics (RAYD)



Starring Rebecca Green working out of Tim Dumonceaux's lab

“nested” PCR based on 16S rRNA-encoding locus (F2nR2)



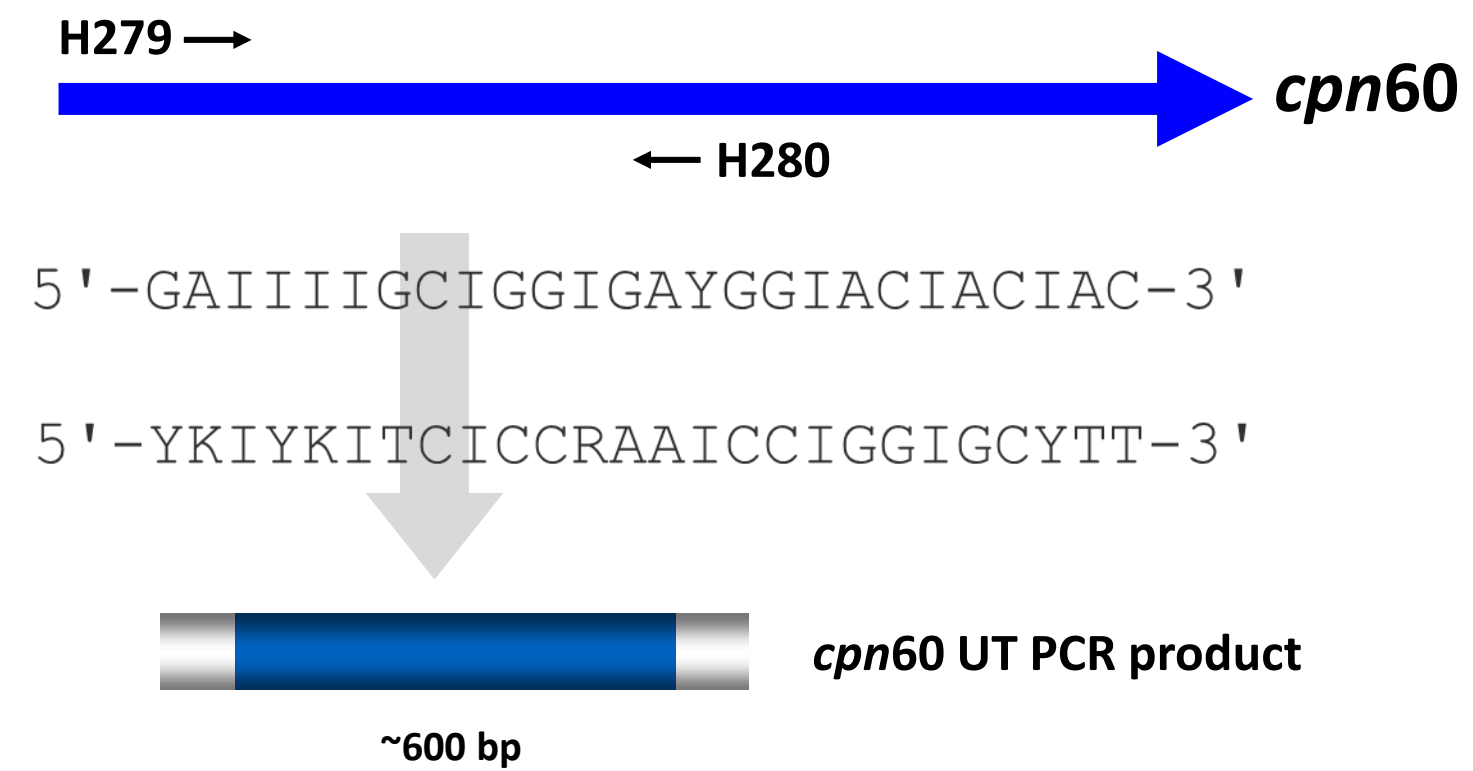
~5hr

~45 min

Gundersen, D. E., & Lee, I. M. (1996). Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopathologia Mediterranea*, 35(3), 144-151. doi:10.2307/42685262

- second “nested” PCR based on different encoding gene- rp ribosomal protein

“Universal” PCR amplification tools targeting *cpn60*

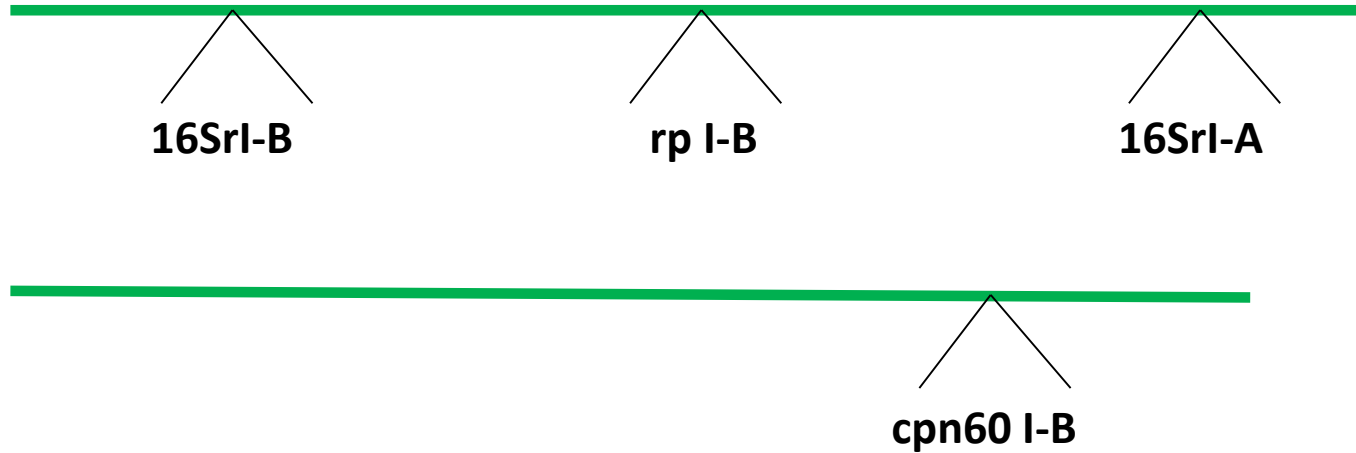


549-567 bp of template-derived sequence

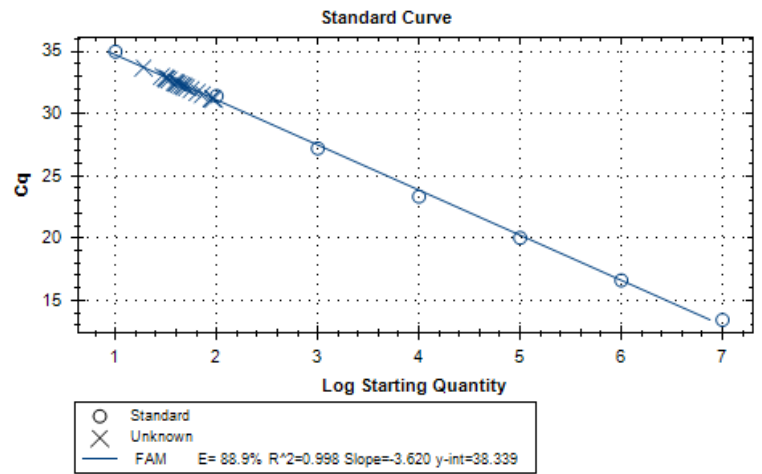
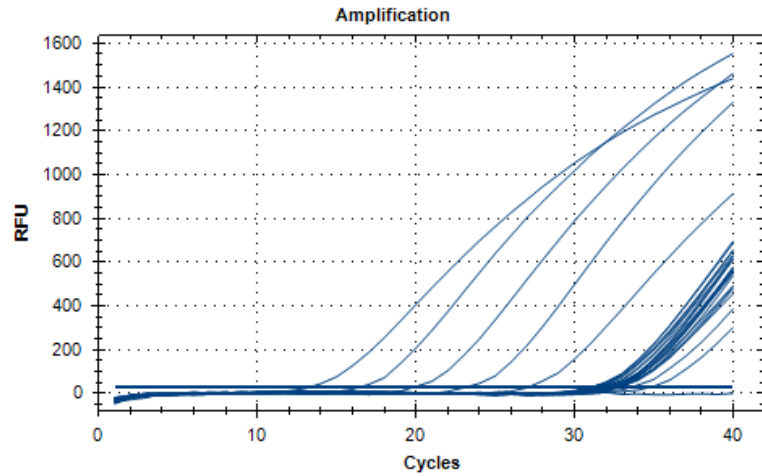
Phytoplasma genome

AY-canola (Saskatoon, SK)

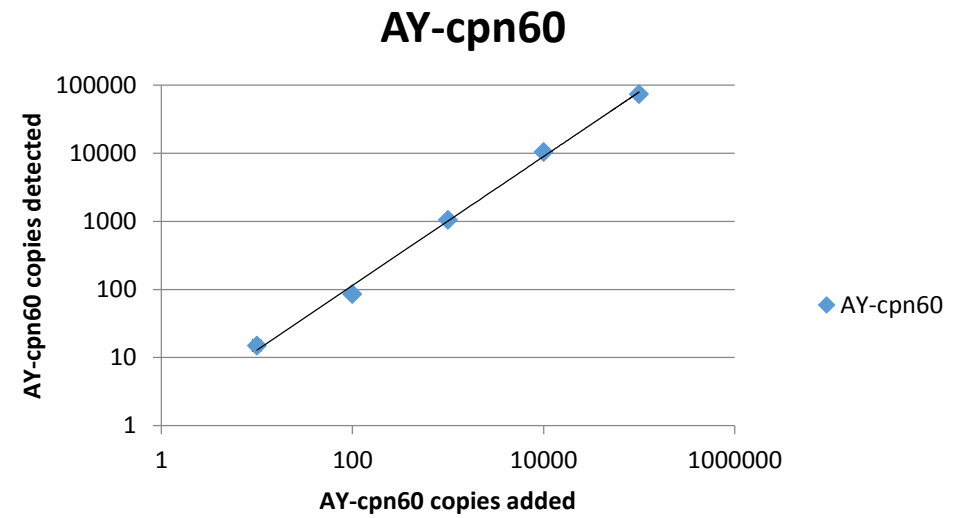
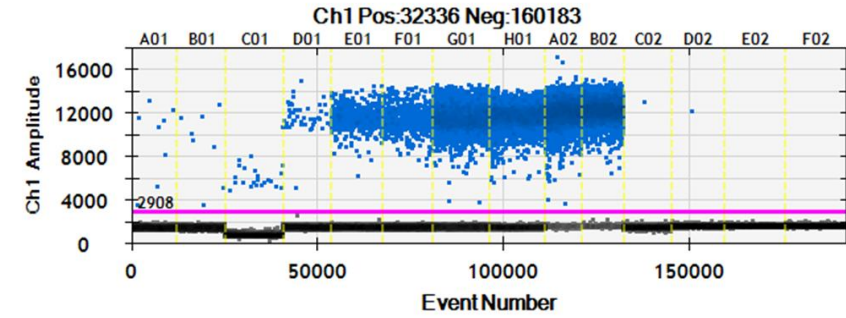
Sequencing results



qPCR targeting cpn60



ddPCR



LAMP- rapid molecular test of Aster yellows detection in a single leafhopper.

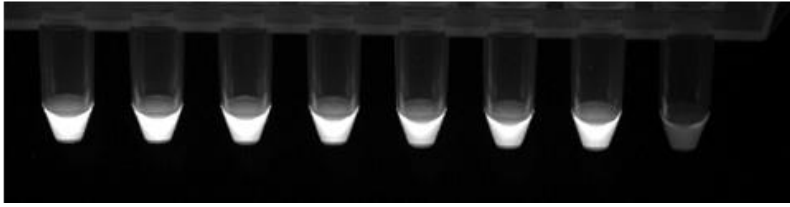


Rapid, non-PCR amplification

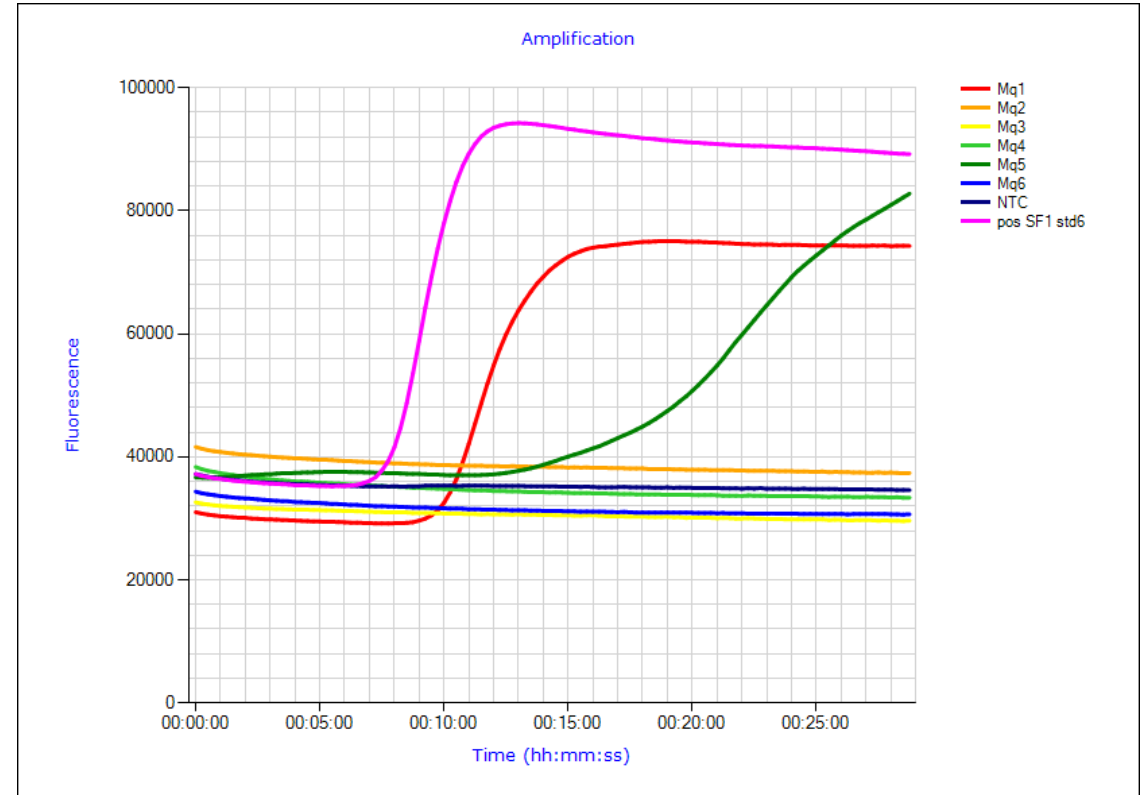
Loop-mediated isothermal DNA amplification (**LAMP**)

cpn60-targeted LAMP assay

BbSP-1 BbSP-2 BbSP-3 BbSP-4 STD7 STD6 STD5 NTC



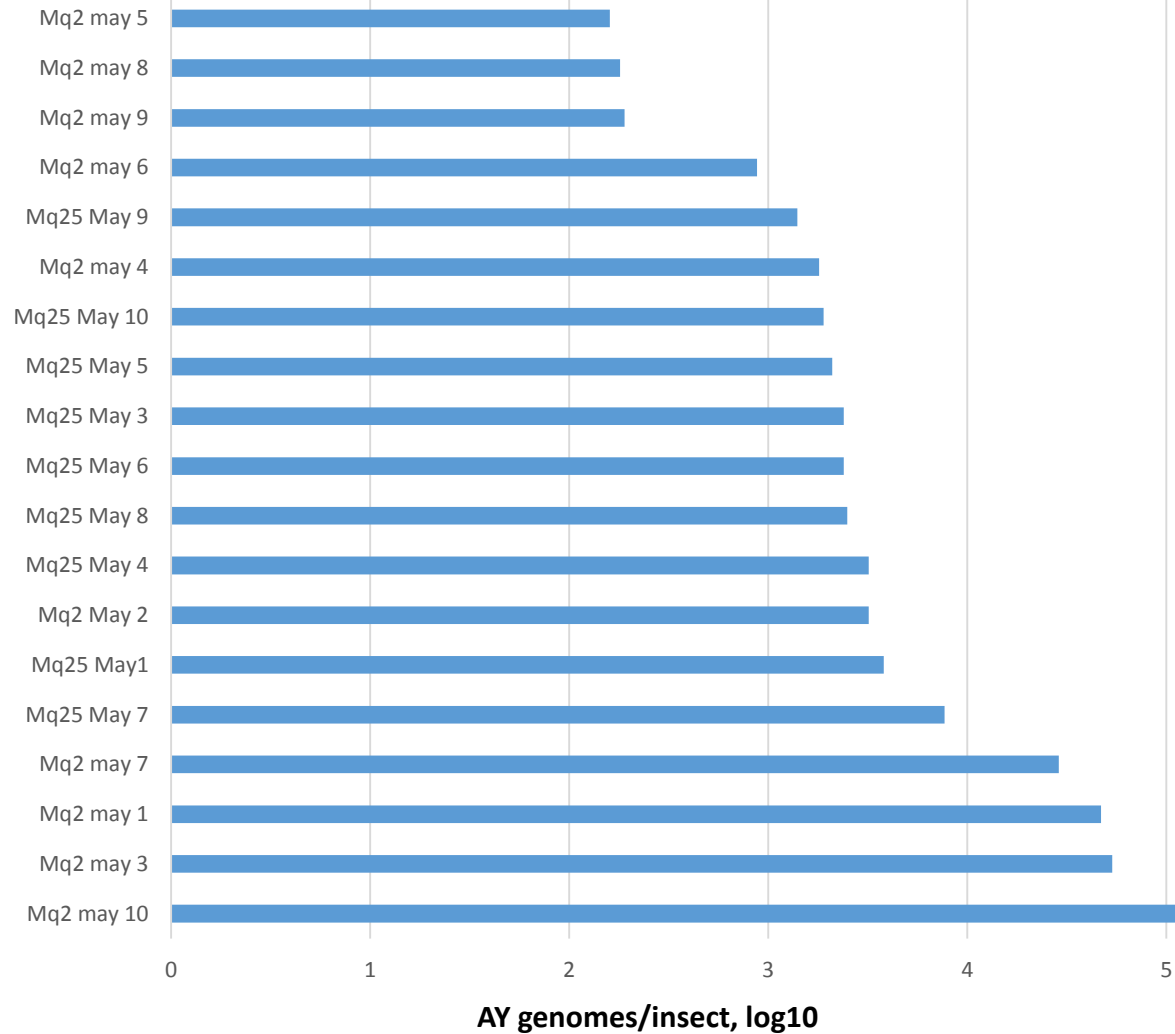
Binomial (pos/neg)



Time to positive (T_p)



AY- ddPCR copies per insect



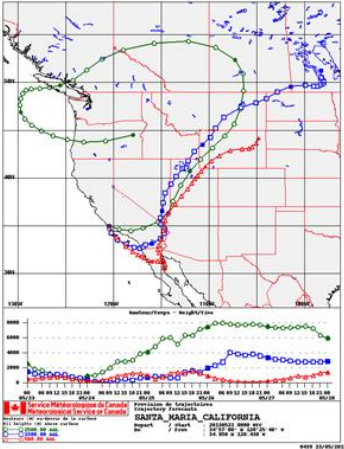
	Nested PCR- 16S	Nested PCR- rp	qPCR	LAMP
-	+	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	+	+	+	+
-	+	-	-	-
-	-	+	+	+
-	+	+	+	+
-	+	+	+	+
-	+	+	+	+
-	-	+	+	+
-	-	+	+	+
+	+	+	+	+
+	+	+	+	+
+	-	+	+	+
+	-	+	+	+
+	+	+	+	+

LAMP= ~ 1-2 hr total time

Nested PCR= ~ 1 week total time



Take-home message



+

```
>KPB2_LCO_MP8014_19 KPB2-SK3k6-Aberdeen_CO1.0.2
atgtgatcgttacatcccacgcatttattataatcttcttatagttataccaattataa
ttgggggtttggtaattgactactccacttataattggagctcctgatatagcattcc
cagactaaataataagattctgacttctcccccttcattaactattaatattaa
gatcaatagtagaaatgggggtagggacgggttgaacagtataccccccctatctagaa
atatcgacatgctggggccaagattgatataatcttctttacatttagccggtgta
```

+

¹ H 1.00784 99.985%	² H 2.0141 0.015%	³ H 3.01605 0.000135% (t _{1/2} = 12.32yrs) Cosmogenic/anthropogenic
¹⁶ O 15.9949 99.76%	¹⁷ O 16.9991 0.04%	¹⁸ O 17.9991 0.20%
¹² C 12.00000 98.89%	¹³ C 13.00335 1.11%	¹⁴ C 14.00324 1.0% (t _{1/2} = 5730yrs) Radioactive Cosmogenic/anthropogenic

= Origin of leafhoppers to Western Canadian Prairies

- quick, cheap, and reliable procedures to detect infected canola



+



=



Acknowledgments



- Funding from Western Grains Research Foundation
- Christine Hammond, Edel Lopez, Dana Leedahl
- Owen Olfert¹, Ross Weiss¹, Meghan Vankosky¹ and Serge Trudel²
 - 1 - AAFC
 - 2 - ECCC



Thank you

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