Universidad Central del Ecuador

Potato zebra chip disease in Ecuador: Situation and perspectives

Jorge David Caicedo Chávez

Professor - Researcher

WORKSHOP

Phytosanitary emergency in the potato crop in Ecuador and implications for Peru and the Andean region: Purple top, potato psyllid and zebra chip

Lima, January 20-22, 2020

inia

SENASA
Servicio Nacional de Sanidad Agraria
PERÚ

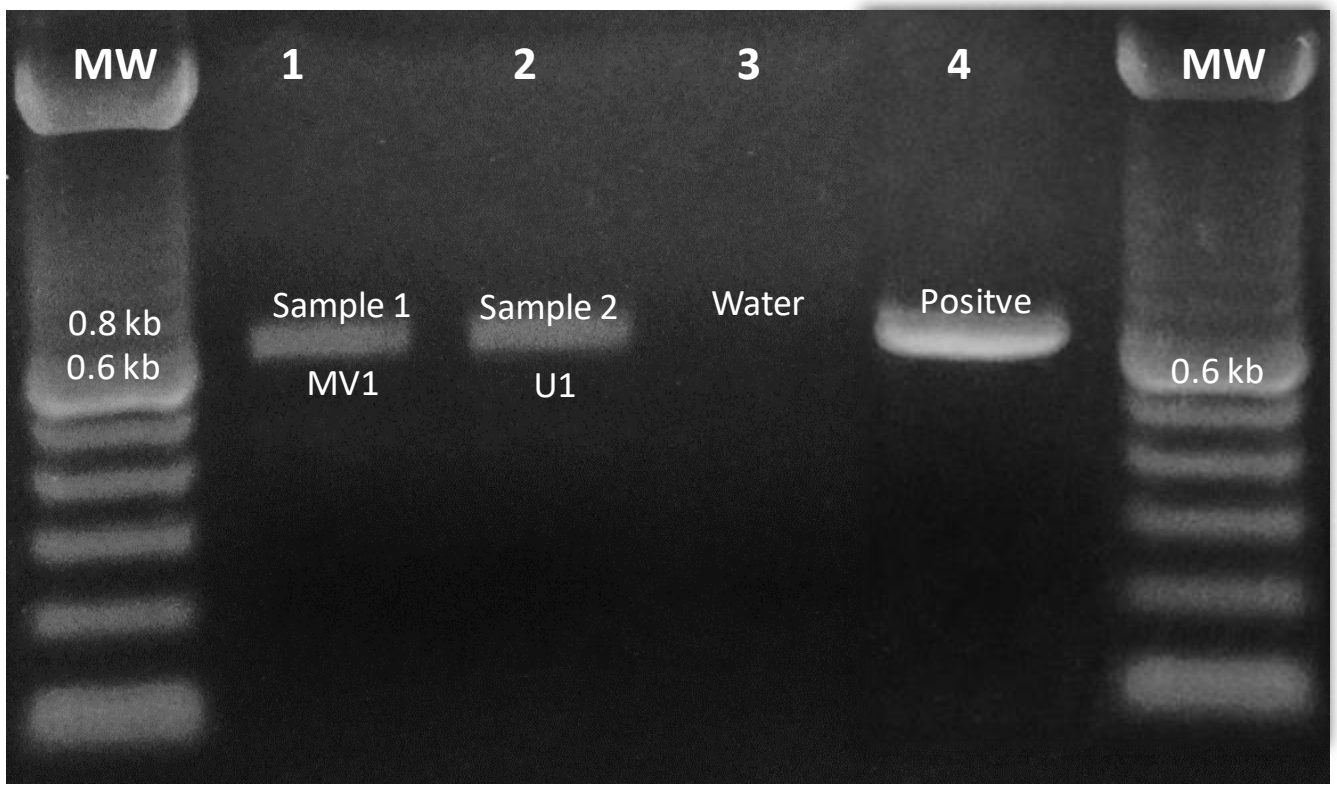
CIP
INTERNATIONAL POTATO CENTER

Background of PPT in Ecuador

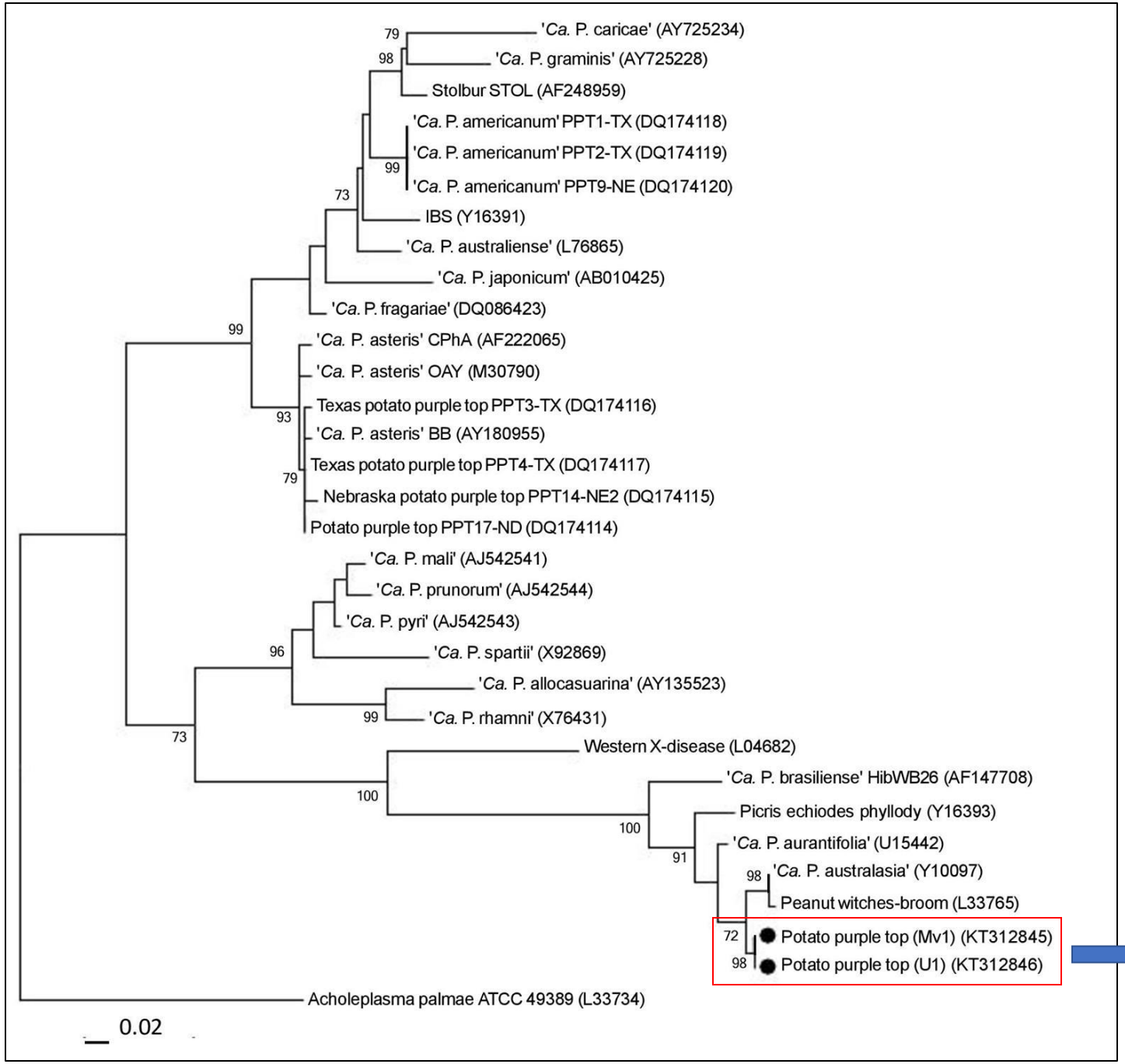
On 2014 in potato plants (var. Super Chola) appeared symptoms that described a possible phytoplasma infection (San Gabriel-Carchi).

Were taken some infected samples for PCR analysis. Using a sweep net were detected the presence of common leafhoppers in plants affected by a possible phytoplasma.

After DNA extraction, by nested PCR reaction was amplified the 16S rRNA gene of phytoplasmas, obtaining amplicons with an expected size of 0.8 kb using the primers fU5/rU3.



Electrophoresis on 1 % agarose gel: 16SrDNA gene amplified with primers fU5/rU3 for phytoplasma.



The sequencing and the phylogenetic analysis of the 16S rRNA gene, confirmed that the symptoms like purple top, aerial tubers, etc., in San Gabriel-Carchi is caused by '*Candidatus* Phytoplasma aurantifolia' 16SrII.

Caicedo et al. (2015). First report of 'Candidatus Phytoplasma aurantifolia' (16SrII) associated with potato purple top in San Gabriel-Carchi, Ecuador.

Common symptoms observed in potato plants from 2017-2019

“Potato purple top (PPT) disease” caused by *Phytoplasmas*

Aceratagallia sp.

“Zebra Chip” disease caused by *Liberibacter*

Bactericera cockerelli

‘*Ca.* P. aurantifolia’

High antioxidant concentration

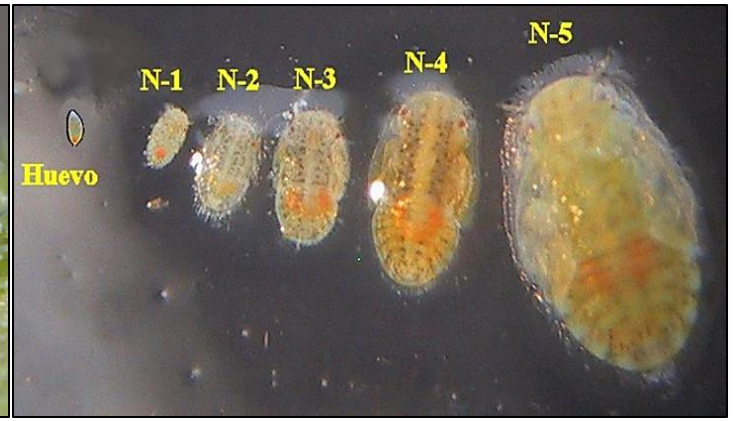
(purpling leaves)

‘*Ca.* L. solanacearum’

High phenolic compounds concentration

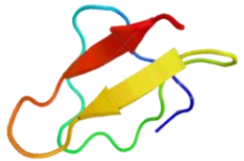
(reddish leaves)

What damage does *Bactericera cockerelli* cause?



CaLso

Toxin in saliva



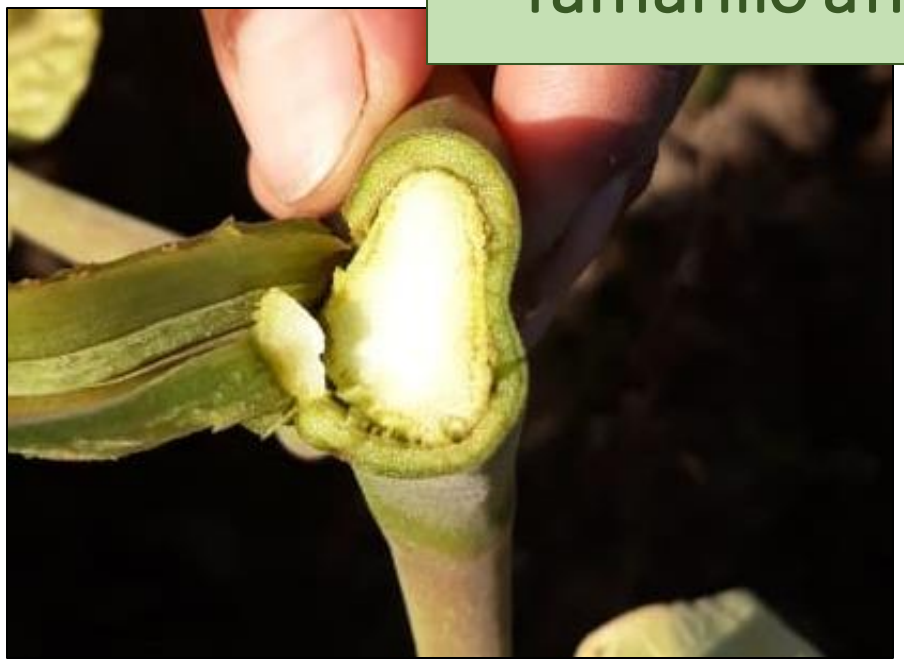
Zebra Chip disease

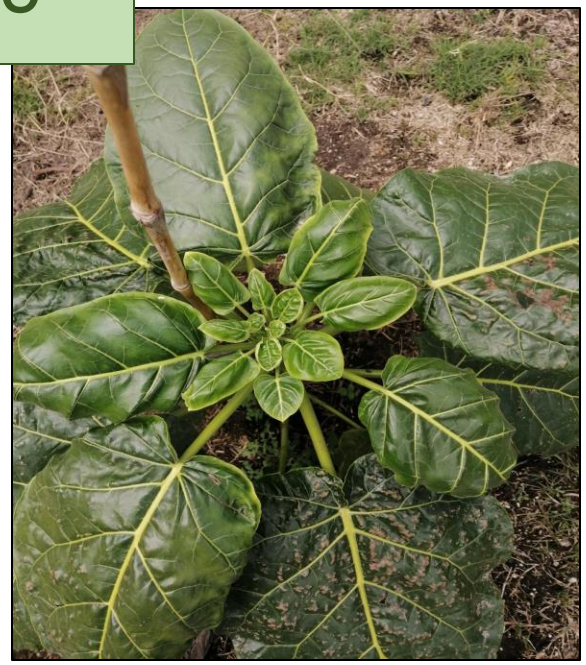
Damage caused by toxins

Main crops affected by *Bactericera cockerelli* in Ecuador

Potato (*Solanum tuberosum*)	Tamarillo (*Solanum betaceum*)	Pepper (*Capsicum annuum*)	Cape Gooseberry (*Physalis peruviana*)

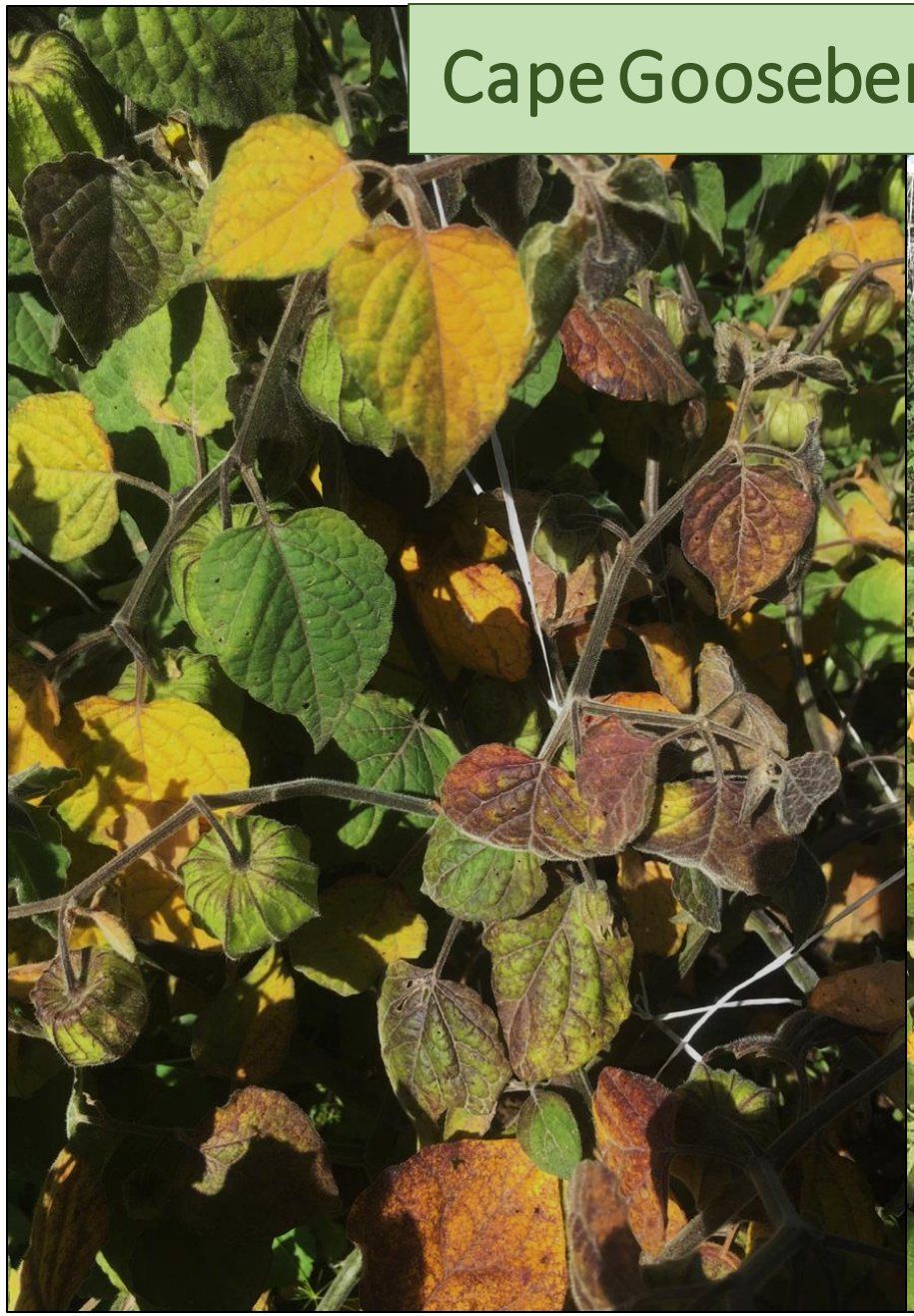
Tamarillo affected by *B. cockereli* and CaLso





Pepper affected by *B. cockerelli* but not CaLso

Cape Gooseberry affected by *B. cockereli* and CaLso



International market for fruits from Ecuador, has been affected by new important pests such as *B. cockerelli* and CaLso

Ecuador realiza su primer envío de uvillas a EE.UU.

December 2019

9267

Según el MAG, la exportación se dio luego de 11 años de negociaciones con el Servicio de Inspección de Sanidad Animal y Vegetal del Departamento de Agricultura de los Estados Unidos. Foto: Archivo / EL COMERCIO

Ecuador envía 600 kilos de tomate de árbol por primer vez a EE.UU.

January 2019

17263

La Agencia de control dijo que el tomate de árbol exportado recibió una certificación que lo acreditaba como un producto "libre de mosca de fruta". Foto: Archivo EL COMERCIO

Facing this problem, Ecuador needed to implement imperative research projects:

- **To determine the main strategies for *B. cockerelli* and CaLso management.**
- **To detect and identify the presence of CaLso in several crops belonging to Solanaceae family.**
- **To characterize the haplotype of CaLso present in Ecuador.**
- **To identify the other host of *B. cockerelli* and CaLso in Ecuador.**

Solanaceae family

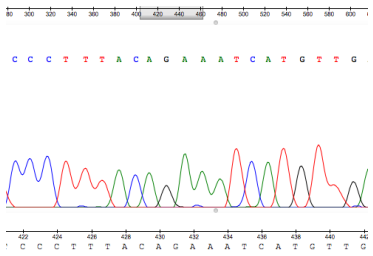
Background management of *B. cockerelli* and PPT in Ecuador (2017-2018)

- **Higher susceptibility of Superchola variety compared with other potato varieties.**
- **Experiences showed an inefficient action of insecticides for the control of *B. cockerelli* in potato fields.**
- ***B. cockerelli* populations showed a possible metabolic resistance to some insecticides.**
- **High relationship between the presence of *B. cockerelli* and the appearing of ZC disease in the Pichincha province.**
- **Reduction of the potato yield and cultivated area in Ecuador.**
- **Presence of *B. cockerelli* in other important crops in Ecuador.**

Main research project (UCE-FCA 2019-2021)

First step

1) Detection
2) Identification
3) Characyerization

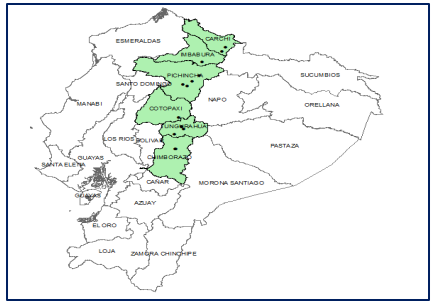


Second step

1) Integrated pest managemet (IPM)
2) Tolerance

Third step

1) Secondary host
2) Spreading in Ecuador.



Research Project 1

UNDERGRADUATE THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF ENGINEER AGRONOMIST

TITLE:

Evaluation of an integrated management system of *Bactericera cockerelli* associated to Potato Purple Top disease in Tumbaco, Pichincha. (2019-2019)

Autor: María Soledad Dalgo Nicolalde (Undergraduate student)

Advisor: Jorge David Caicedo Chávez, I.A., M.Sc. (Professor)

Status of the research: **Finished (unpublished data)**

Soledad Dalgo

OBJECTIVES

General objective

To evaluate the implementation of an integrated management system of *Bactericera cockerelli* associated to potato purple top disease, in three potato varieties with different precocity levels in Tumbaco-Pichincha.

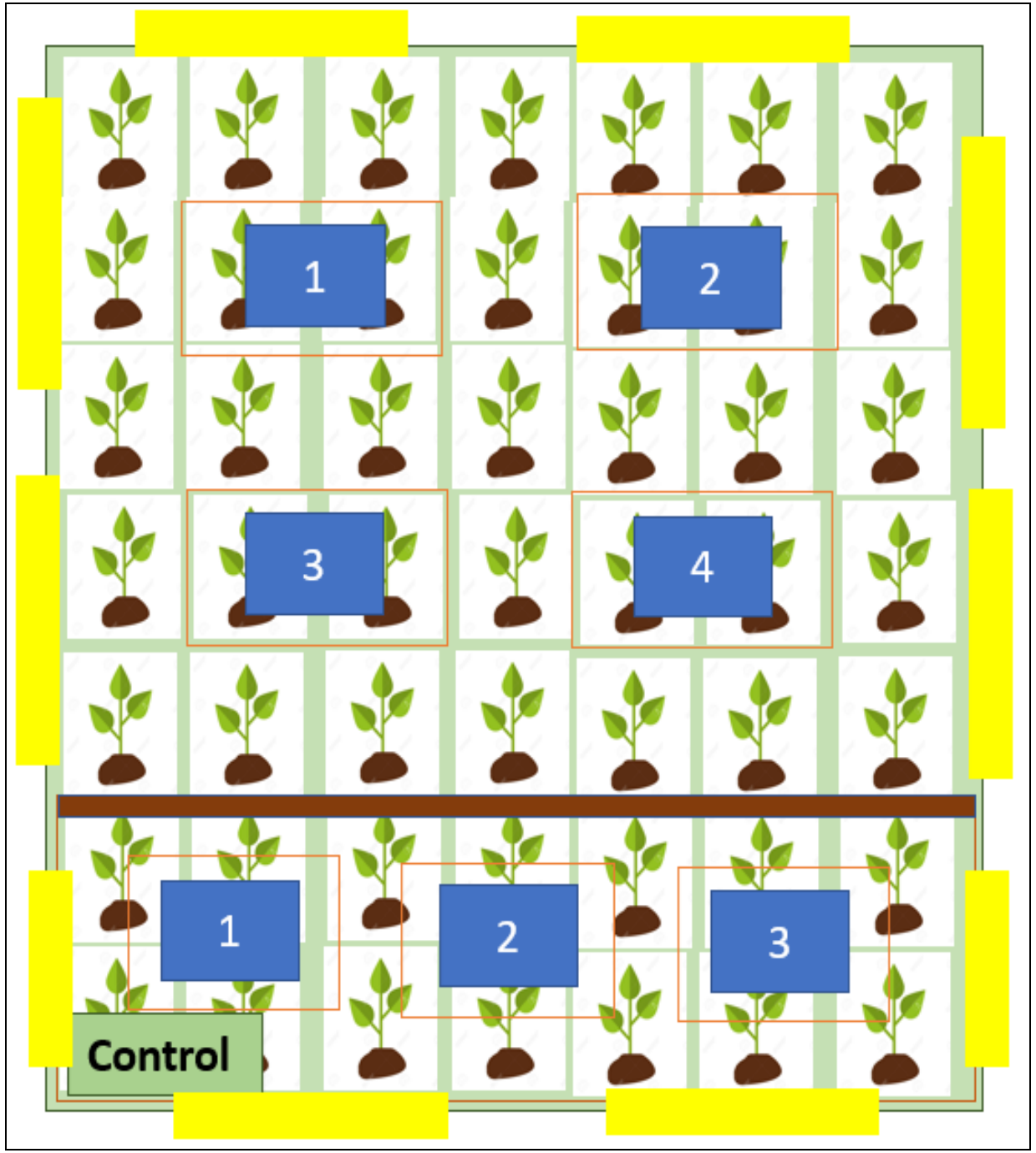
Specific objectives

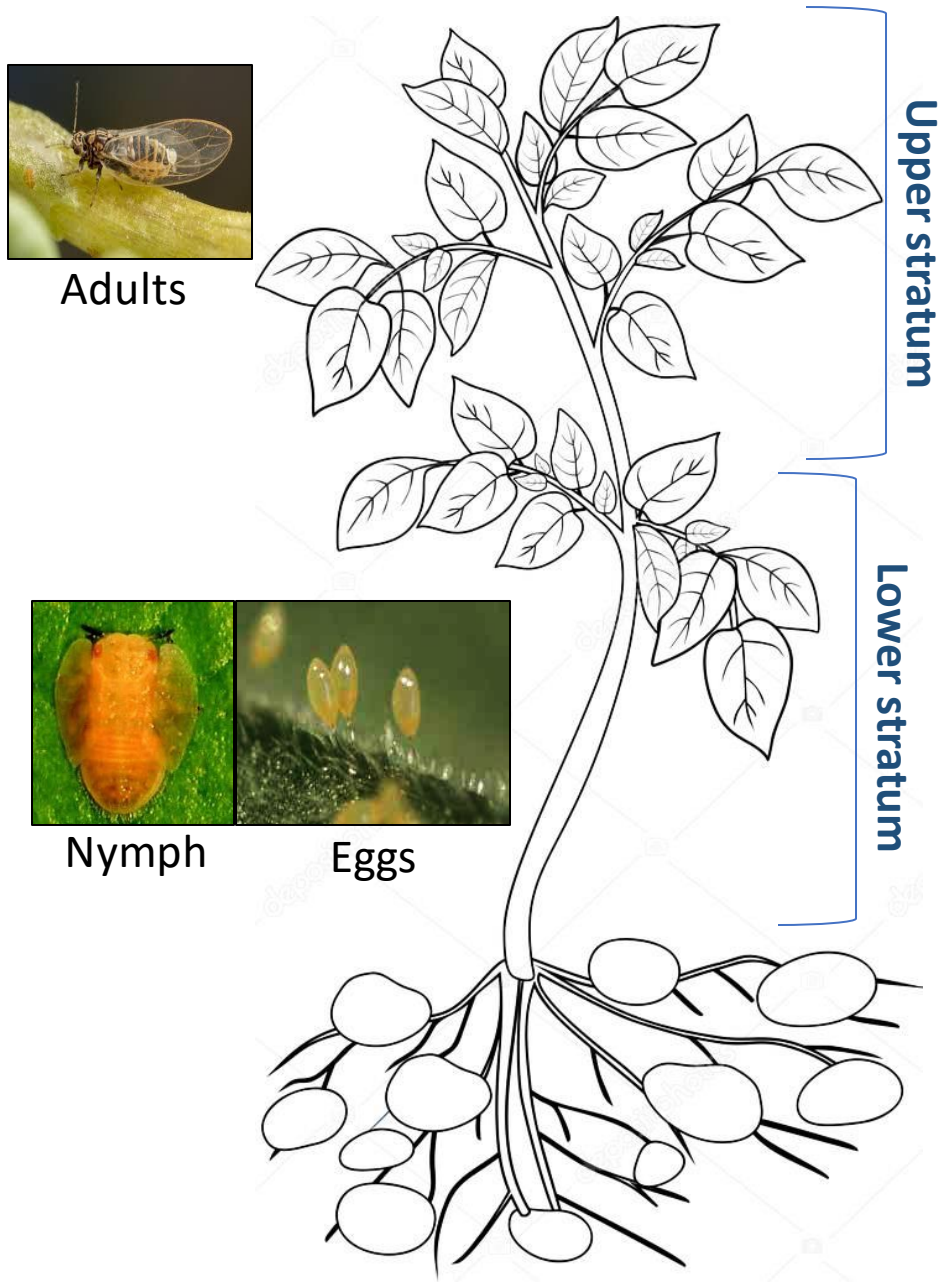
- Monitoring and evaluate the population dynamics of *Bactericera cockerelli* in three potato varieties with different precocity levels.
- To determine the main chemical control times of *Bactericera cockerelli.*
- To determine the effect of supplementary nutrition on potato plants affected with PPT.
- To determine the potato yield and quality on three potato varieties under an integrated management system of *Bactericera cockerelli* and PPT.

Schematic representation of the experiment in the field

Study Factor: Precocity varieties
Suprema (early variety) 120 days
Fripapa (semi late variety) 150 days
Superchola (late variety) 180 days

Monitoring scheme





Population dynamics of *B. cockerelli*

Management plan of *B. cockerelli* based on the monitoring

Stage	Active ingredient	Complementary fumigation		pH fumigation	Frecuency*
Eggs	Thiocyclam	Teblubenzuron, Diflubenzuron		6 to 7	7 days
	Spinetoram			5 to 9	7 to 15 days
Nymph	Spirotetramat + Thiacloprid	Paraffinic, cycloparaffinic hydrocarbons	*Beauveria bassiana*	5 to 9	7 to 15 days
	Spinetoram				
	Sulfoxaflor				
Adult	Dimetoato			4 to 5	15 days
	Bifentrina + Z cipermetrina			5 to 9	15 days
*Depending on monitoring					

Fumigation type	Water volumen/ha	Days	Product
Drench	600 - 800 liters	Emerging to 60 days	Systemic insecticide
Foliar	200 - 600 liters	Emerging to 150 days	Contact insecticide and foliar fertilizers

Complementary fertilization under a management system of *B. cockerelli*

Development stage	Product	Days	Precocity
Emerging	Potassium phosphite + salicylic acid	30-45	Early and semi late variety
	Pyraclostrobin		
Vegetative growth	Metalosate K, Ca and Zn + Potassium phosphite + salicylic acid	45	
	Metalosate K, Ca and Zn + Pyraclostrobin	60	
Flowering y tuberization	Metalosate K, Ca and B + Potassium phosphite + salicylic acid	60-90	
	Pyraclostrobin		
Filling tubers	Metalosate K and Ca + Potassium phosphite + salicylic acid	90-120	
Maturing	Metalosate K	120-180	Late variety

Management of the experiment

Sowing

Complementary fertilization

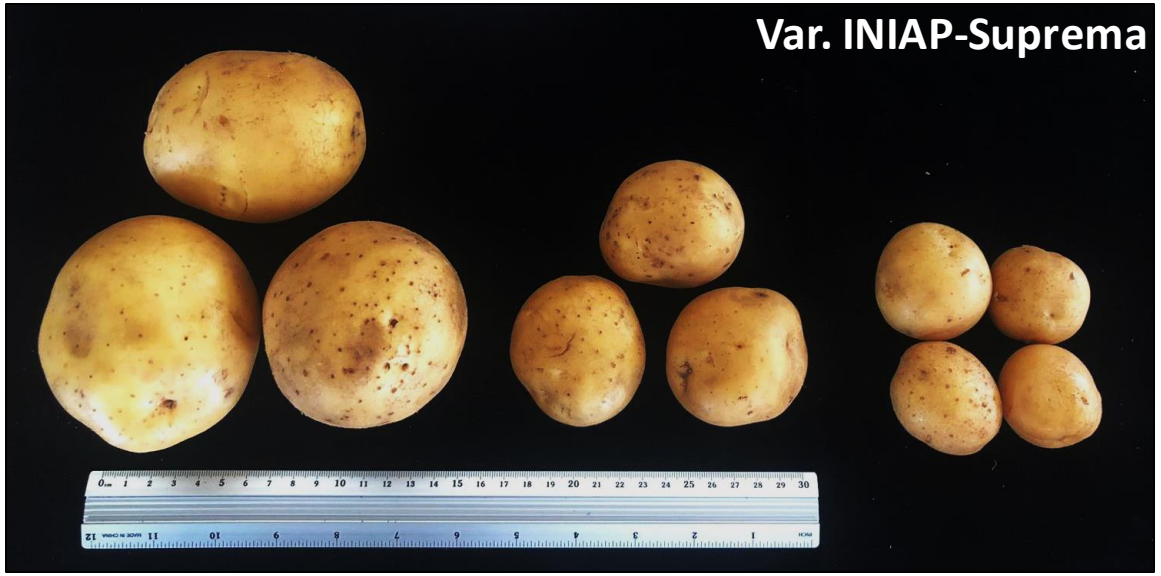
Monitoring

Insecticide aplication

Harvested

Yield classification

RESULTS

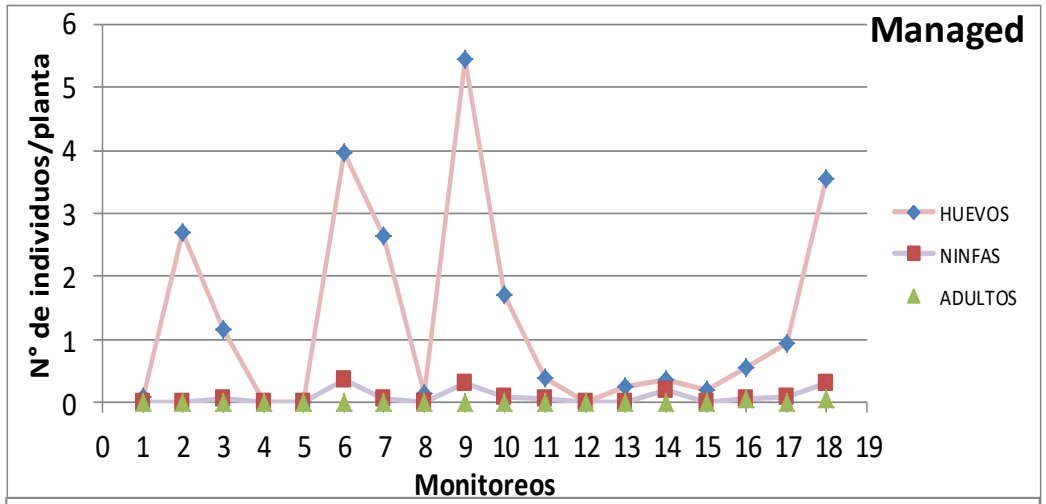


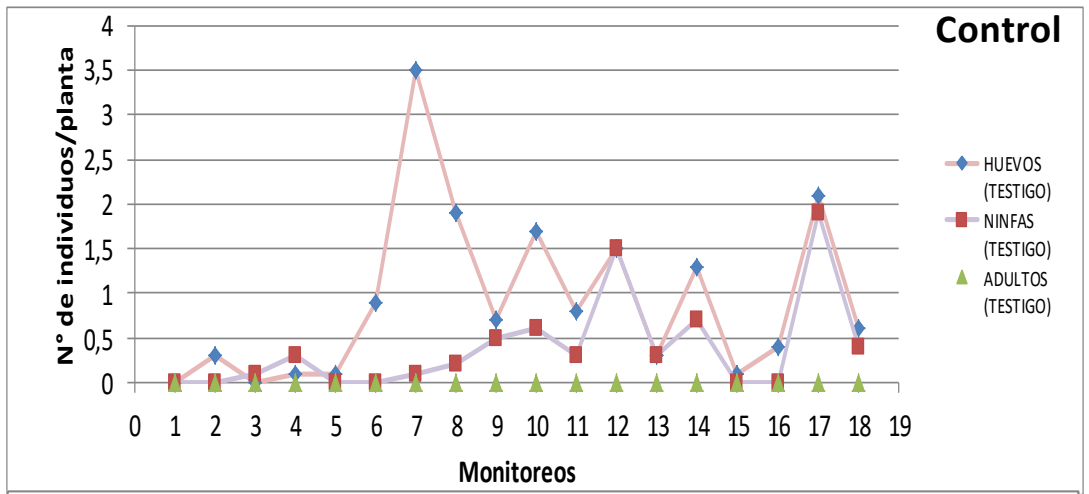
First objective

To determine the main strategies for *B. cockerelli* and PPT management.

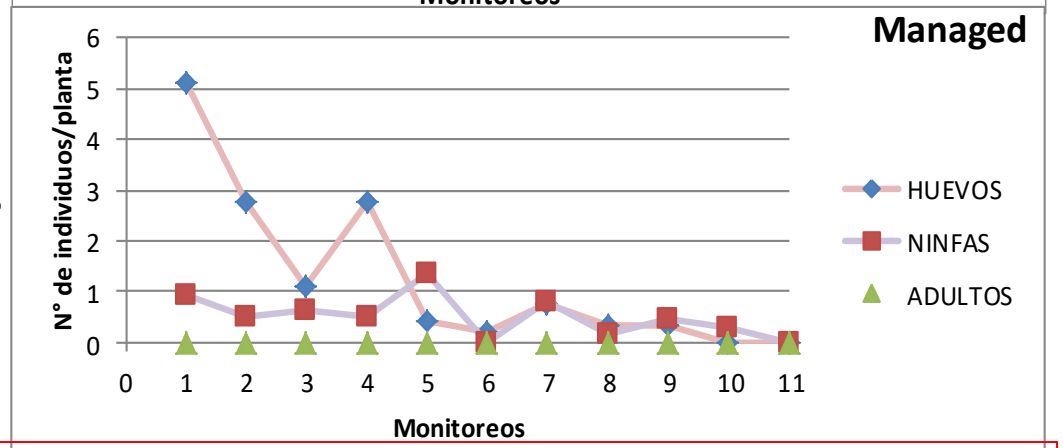
Population dynamics of *B. cockerelli*

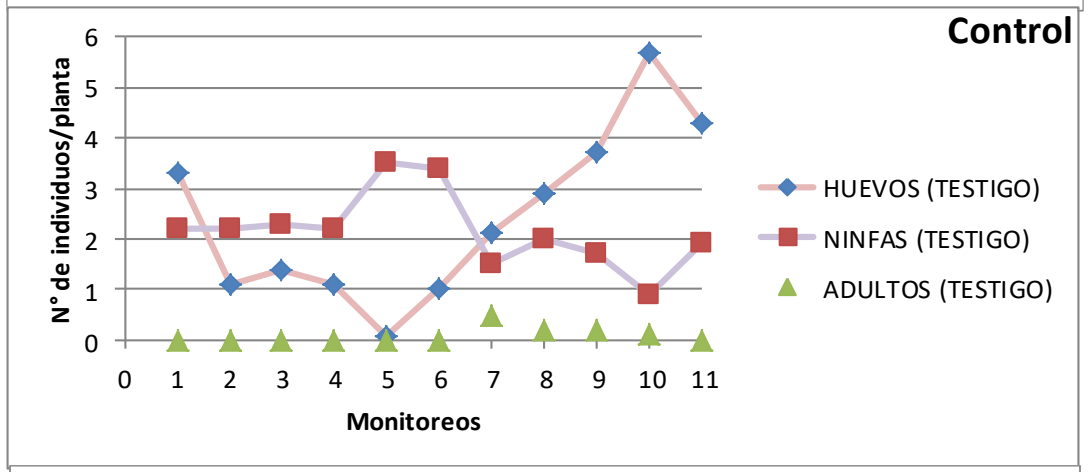
Superchola



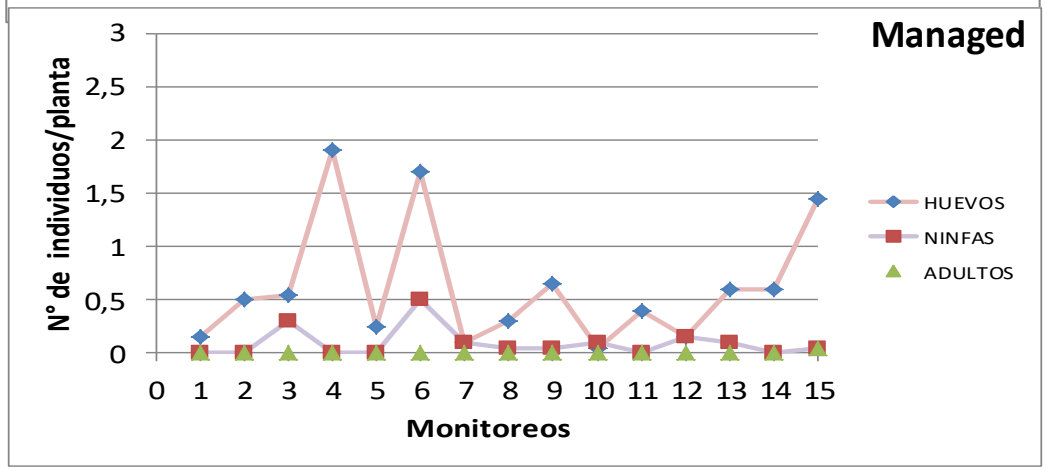


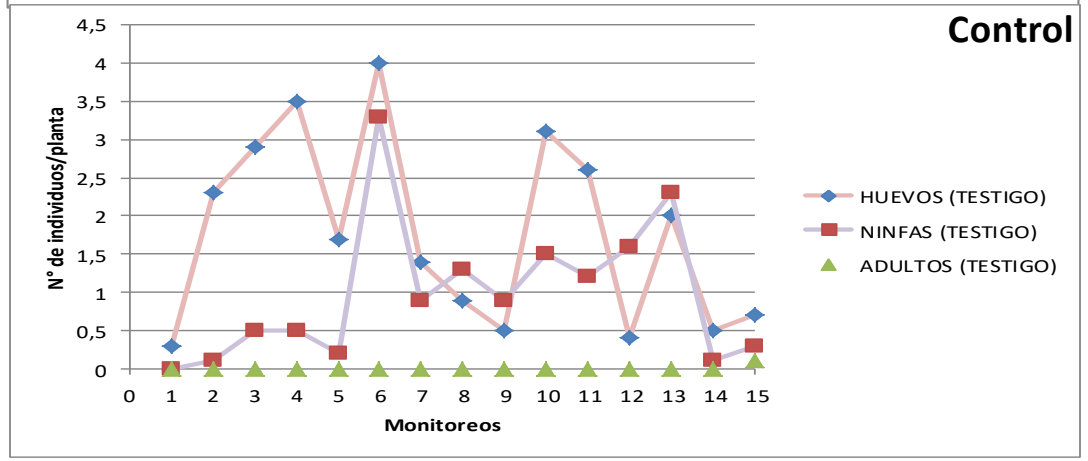
INIAP-Suprema





INIAP-Fripapa

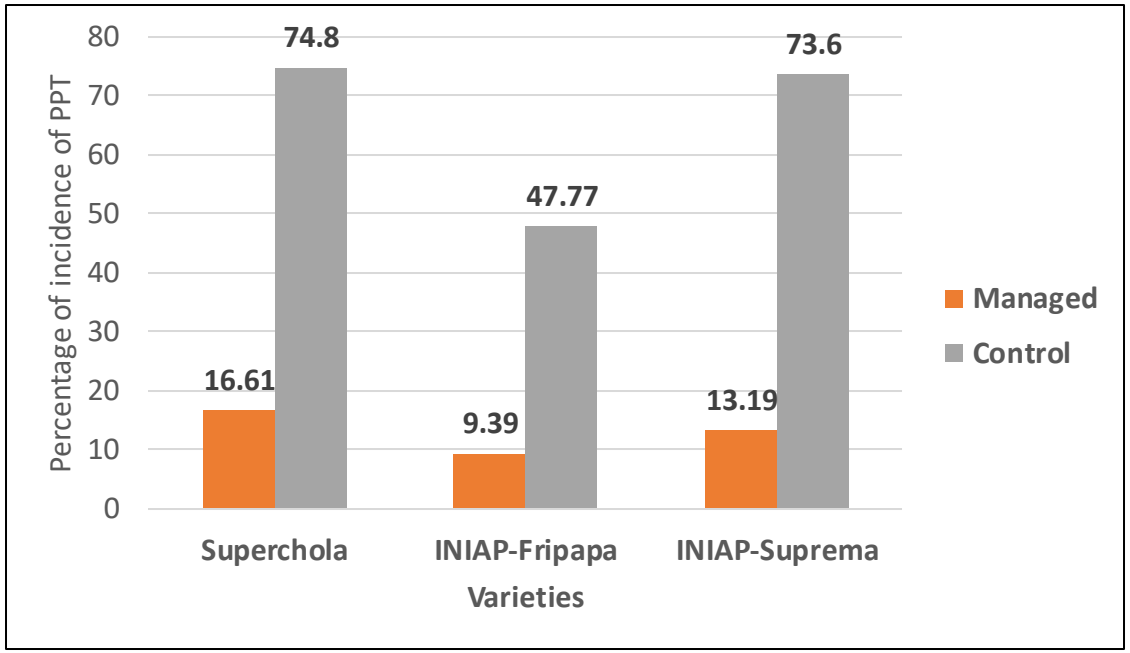


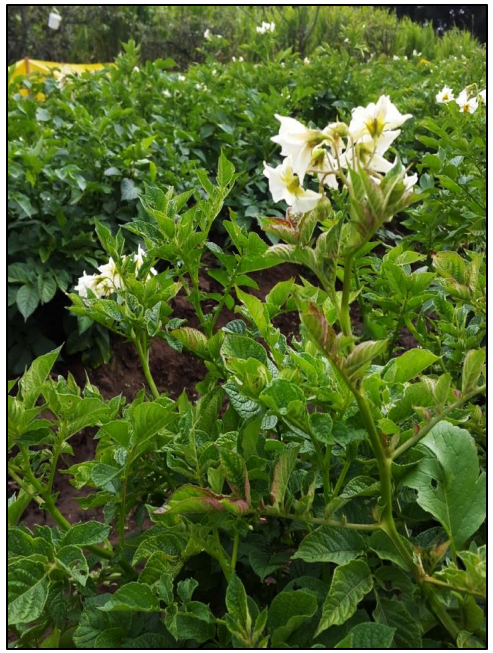


Efficiency of the insecticides used for the control of *B. cockerelli*

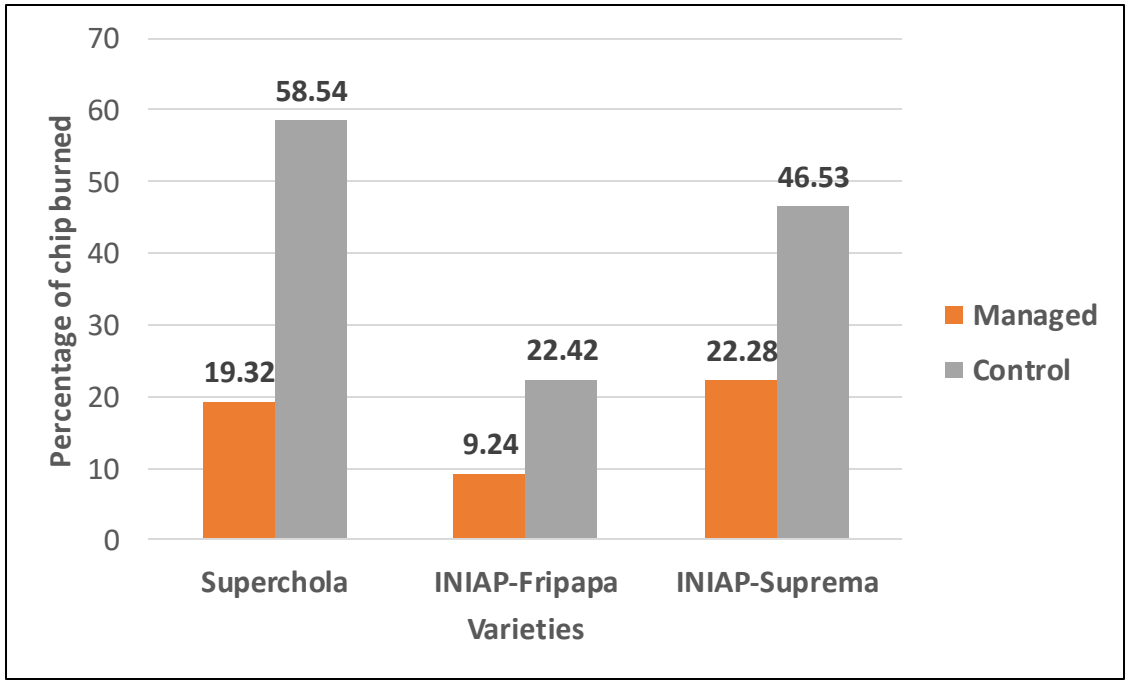
Stage	Active ingredients	Application number	Control percentage	General efficiecy of the insecticide
Eggs	Thiocyclam	5	74 %	75,5 %
	Hexythiazox	6	77 %	
Nymph	Sulfoxaflor	6	70 %	81,3 %
	Spirotetramat + Thiacloprid	4	74 %	
	Spinetoram	2	100 %	
Adults	Bifentrina + Zeta cypermitrina	2	95 %	91,0 %
	Dimetoato	2	87 %	

Incidence of PPT in three potato varieties comparing two treatments (managed and control)

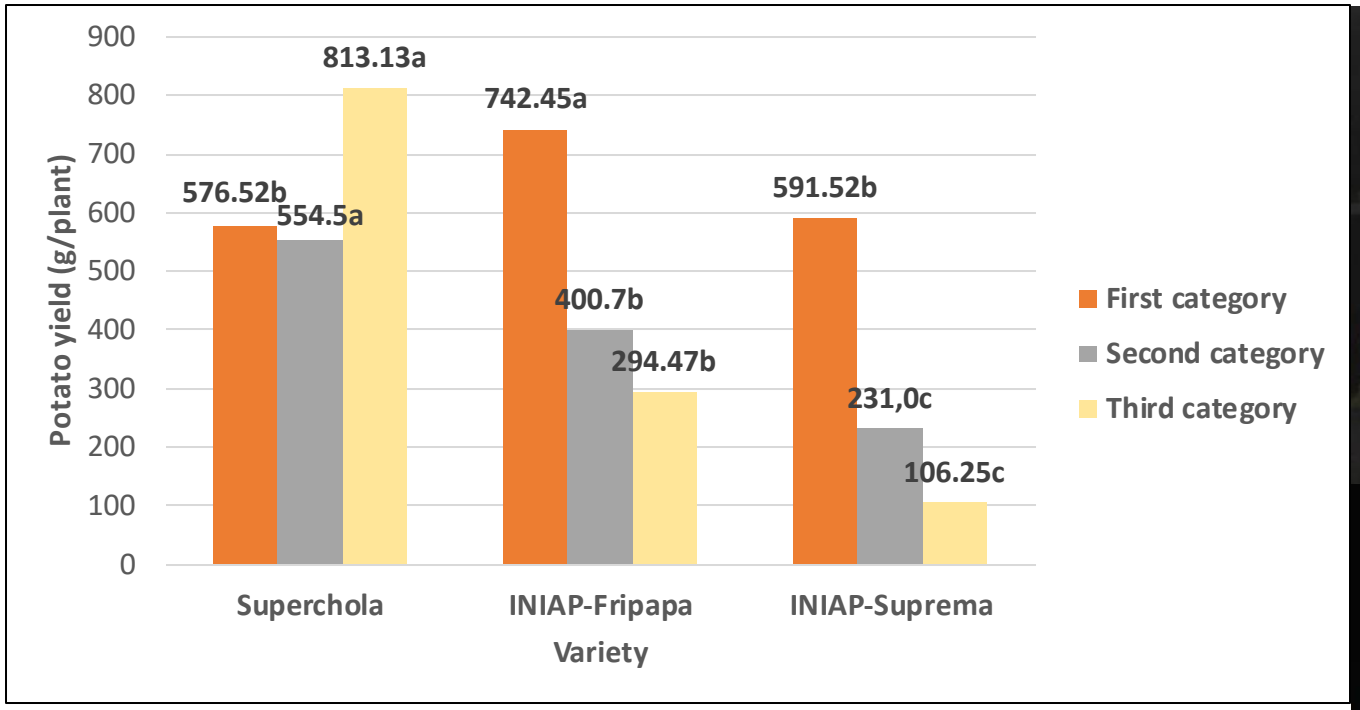




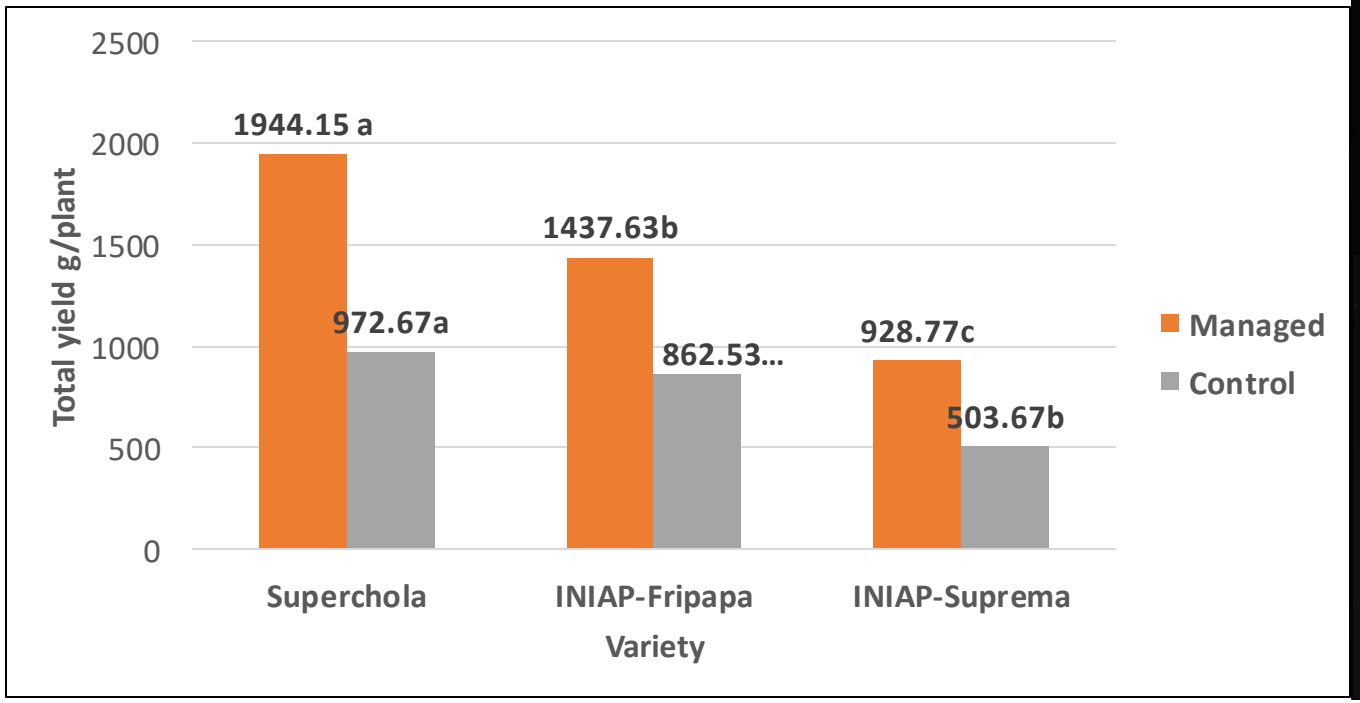
Percentage of chip burned in three potato varieties comparing two treatments (managed and control)



Potato yield in first, second and third category in three potato varieties



Total potato yield in three varieties comparing two treatments (managed and control)

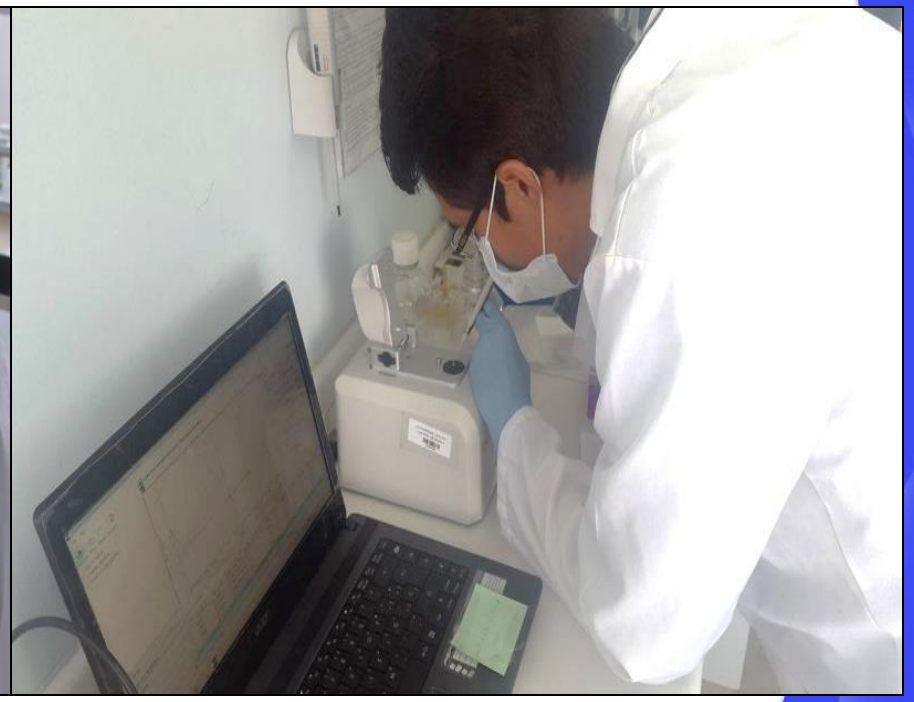


First conclusions

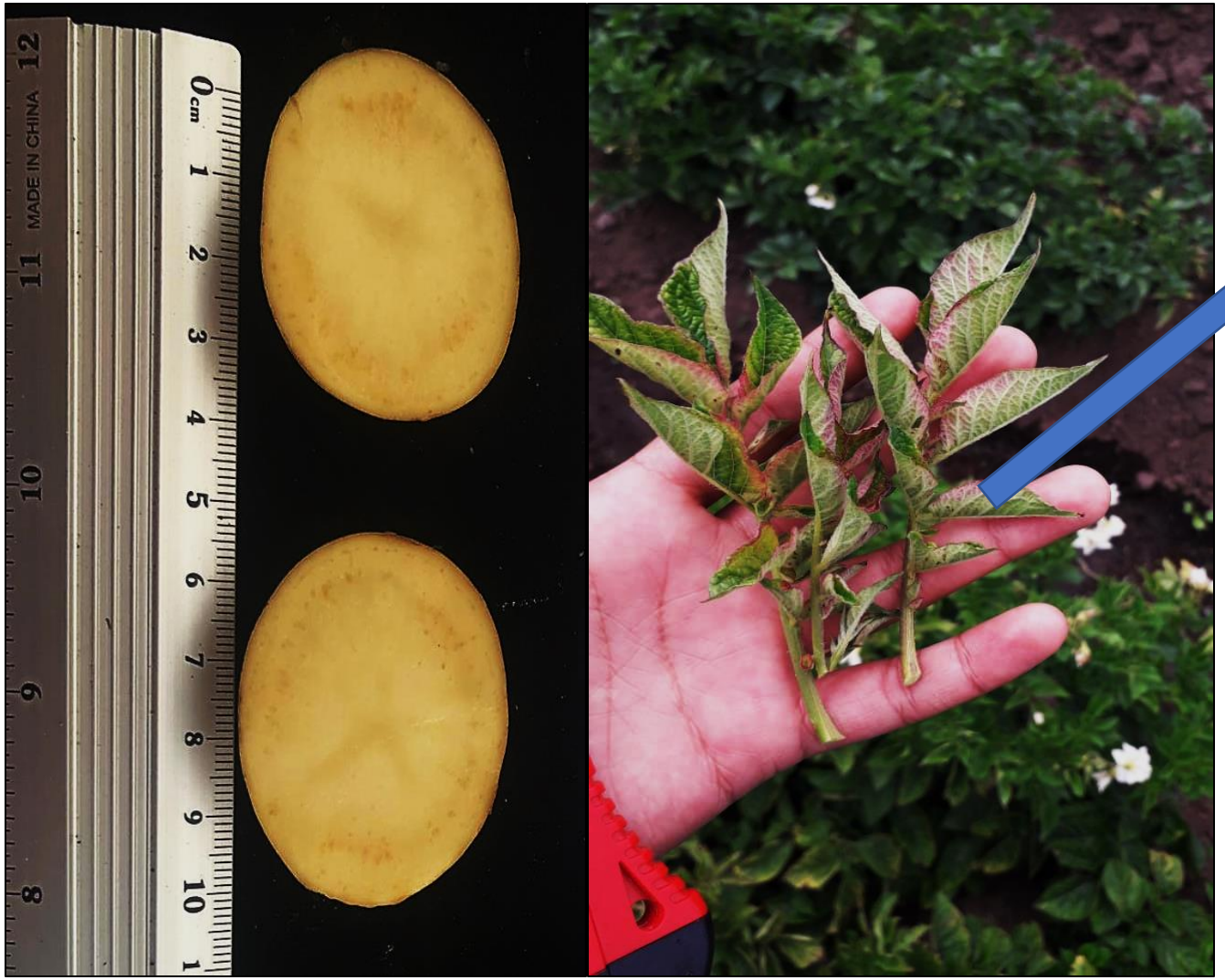
- Integrated management of *B. cockerelli* based on monitoring is the key to insure the potato yield.
- Supplementary fertilization helps to obtain better categories of tuber potato.
- INIAP-Fripapa (semi late variety) showed higher tolerance to ZC and PPT diseases in all variables evaluated, compared with Superchola (late variety) and INIAP-Suprema (early variety).

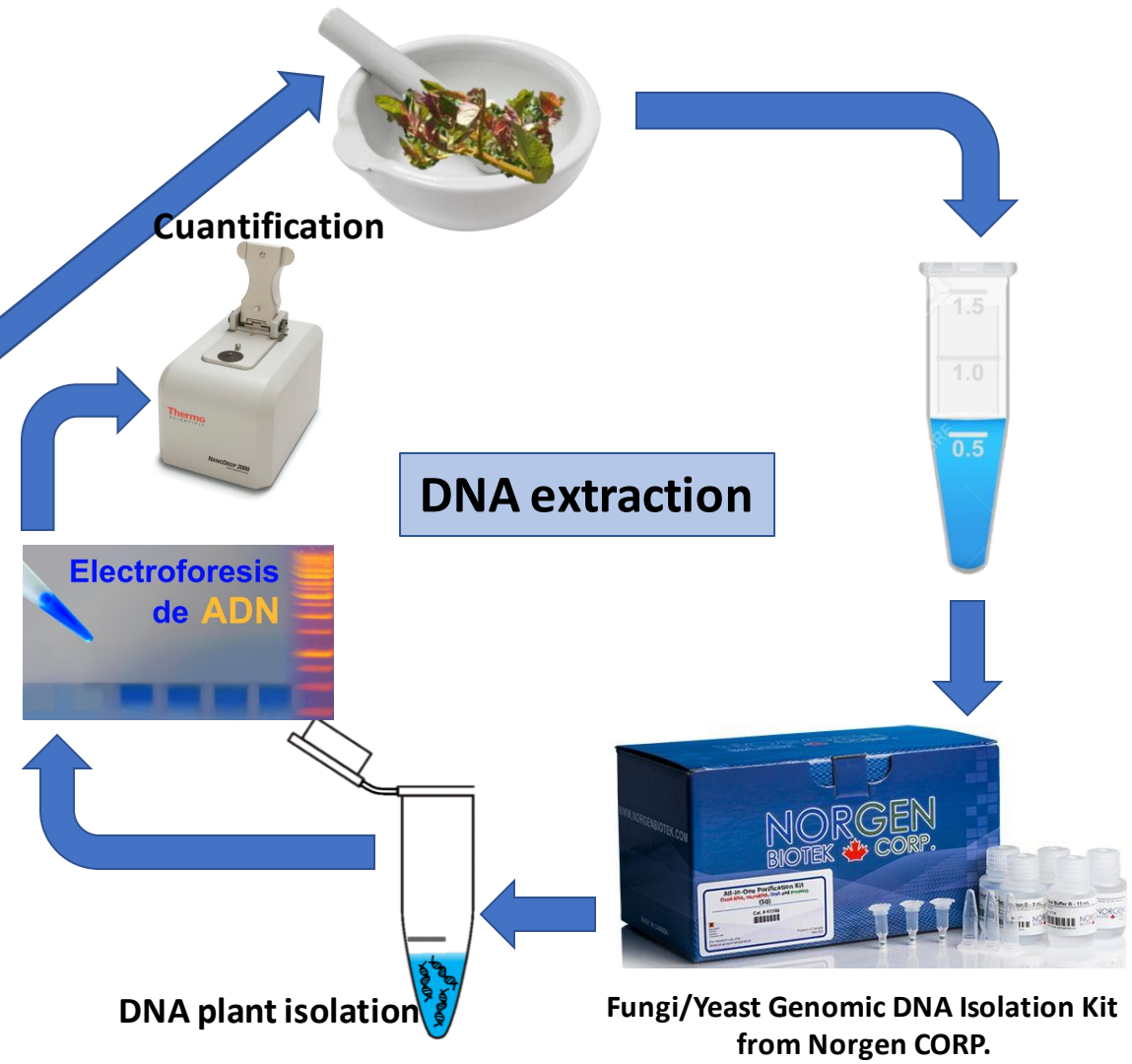
Second objective

To detect and identify the presence of CaLso in several crops belonging to Solanaceae family.

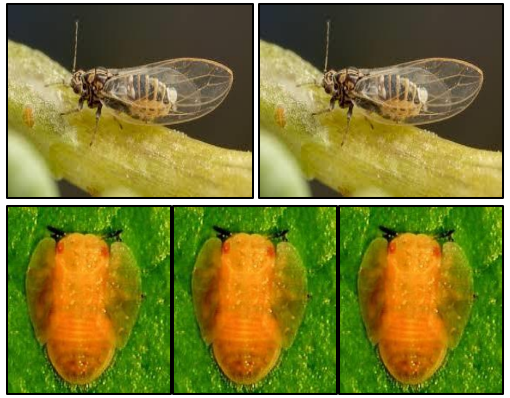


Identification of *'Candidatus* Liberibacter solanacearum' in potato plants



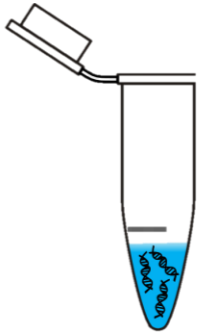


DNA extraction of *Bactericera cockerelli*

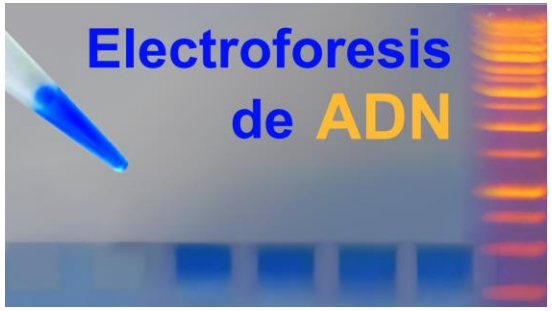


Adults and nymph of *B. cockerelli*

Purelink Genomic DNA mini kit. from Invitrogen



DNA insect isolation



Cuantification

Store

PCR amplification of taxonomic genes from CaLso

Primer	Sequence 5' - 3'
CLi.po.F	TACGCCCTGAGAAGGGGAAAGATT
OI2c	GCCTCGCGACTTCGCAACCAT
CL514F	CTCTAAGATTTCGGTTGGTT
CL514R	TATATCTATCGTTGCACCAG

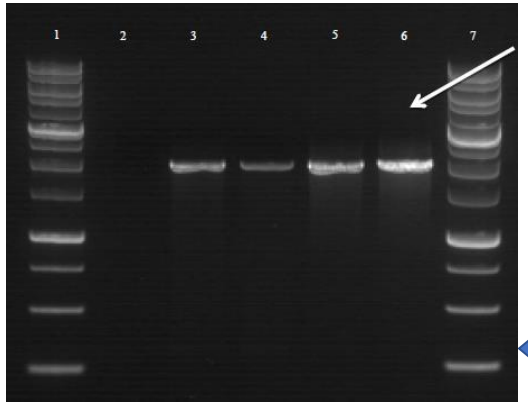
Component	Volumen (µl)
2X Platinum Super Fi Master Mix	12,5
Primer Forward (10 uM)	1,25
Primer reverse (10 uM)	1,25
SuperFi GC Enhancer	5
Agua ultrapura	3
ADN (20 ng/ul)	2

PCR program

Etapa	Temperatura (ºC)	Tiempo
Desnaturalización inicial	98	30 seg
Desnaturalización	98	10 seg
Hibridación	68	10 seg
Extensión	72	23 seg
Extensión final	72	5 min

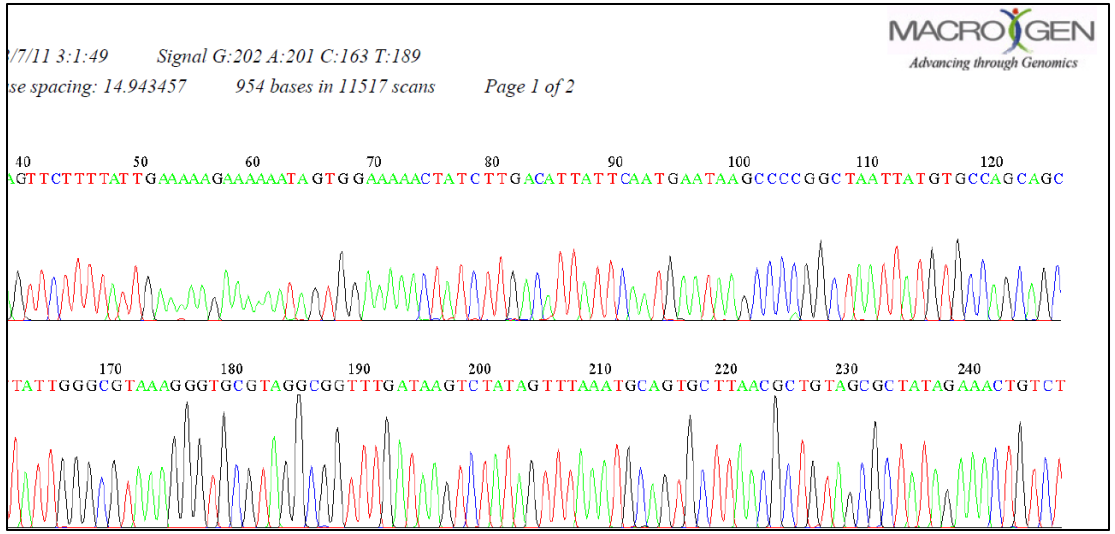
PCR amplification

Electrophoresis gel of PCR products



Purification of positive PCR products

Sequencing of PCR products



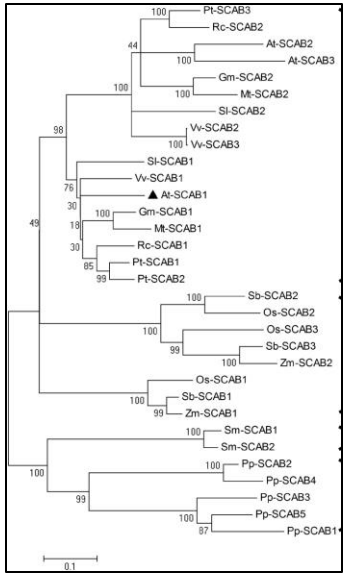
Quality control of the sequences

Bioinformatics

Edition of the sequences

Blast analysis

Phylogenetic reconstruction



Phylogenetic tree

Results of Blast Analysis

BLAST » blastn suite » results for RID-25XS7E25016

Home | Recent Results | Saved Strategies | Help

Edit Search | Save Search | Search Summary

How to read this report? | BLAST Help Videos | Back to Traditional Results Page

Job Title	Nucleotide Sequence
RID	25XS7E25016 *Search expires on 01-19 19:16 pm* Download All
Program	BLASTN Citation
Database	nt See details
Query ID	lcl|Query_8813
Description	None
Molecule type	dna
Query Length	1120
Other reports	Distance tree of results MSA viewer

Filter Results

Organism *only top 20 will appear* exclude

Type common name, binomial, taxid or group name

+ Add organism

Percent Identity [ ] to [ ] E value [ ] to [ ] Query Coverage [ ] to [ ]

Filter Reset

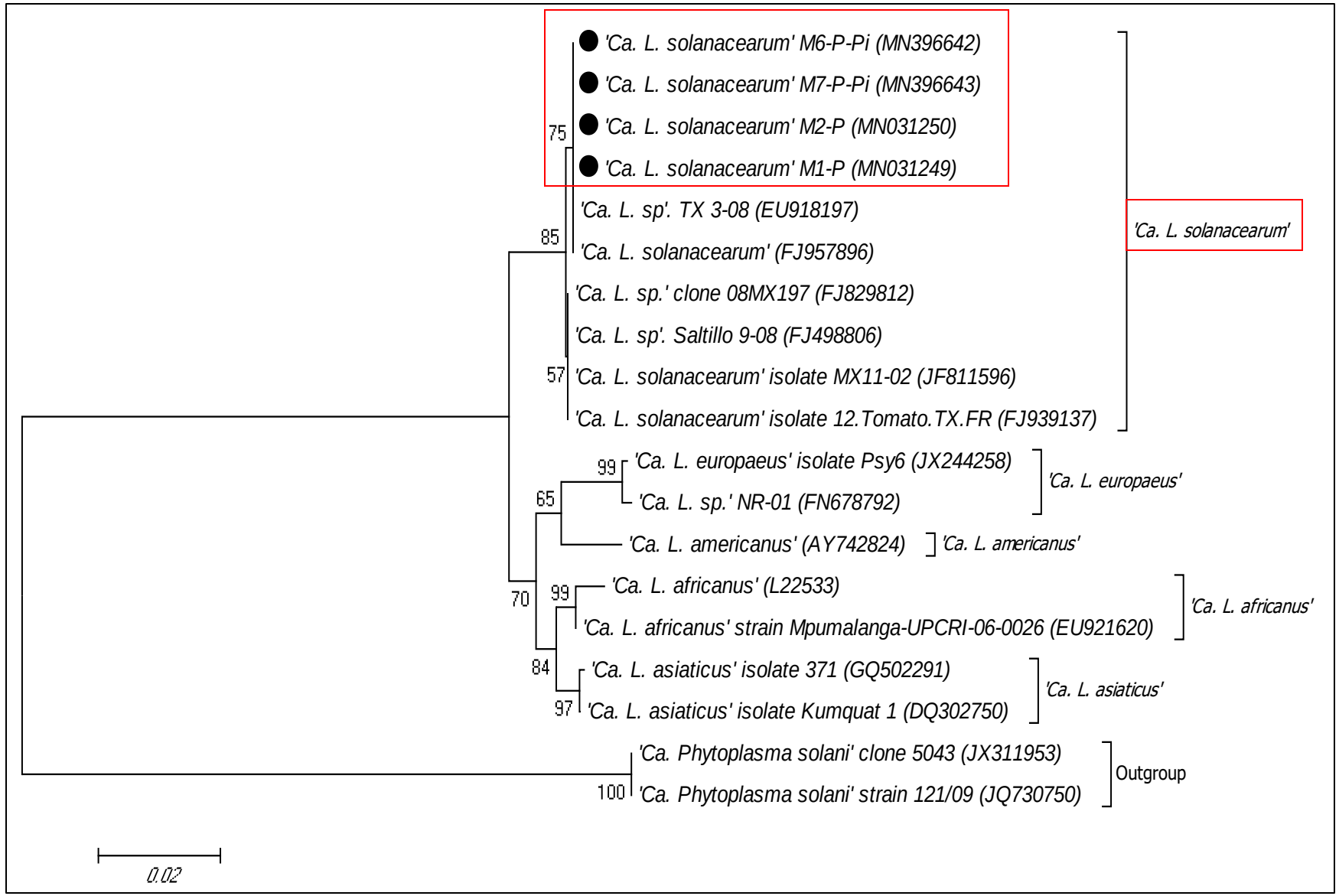
Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments Download Manage Columns Show 100

select all *100 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Candidatus Liberibacter solanacearum clone M7-P-Pi 16S ribosomal RNA gene, partial sequence	2069	2069	100%	0.0	100.00%	MN396643.1
Candidatus Liberibacter solanacearum clone M6-P-Pi 16S ribosomal RNA gene, partial sequence	2069	2069	100%	0.0	100.00%	MN396642.1
Candidatus Liberibacter solanacearum clone M5-T-Im 16S ribosomal RNA gene, partial sequence	2069	2069	100%	0.0	100.00%	MN396641.1
Candidatus Liberibacter solanacearum clone M4-T-Im 16S ribosomal RNA gene, partial sequence	2069	2069	100%	0.0	100.00%	MN396640.1

Results of Phylogenetic reconstruction

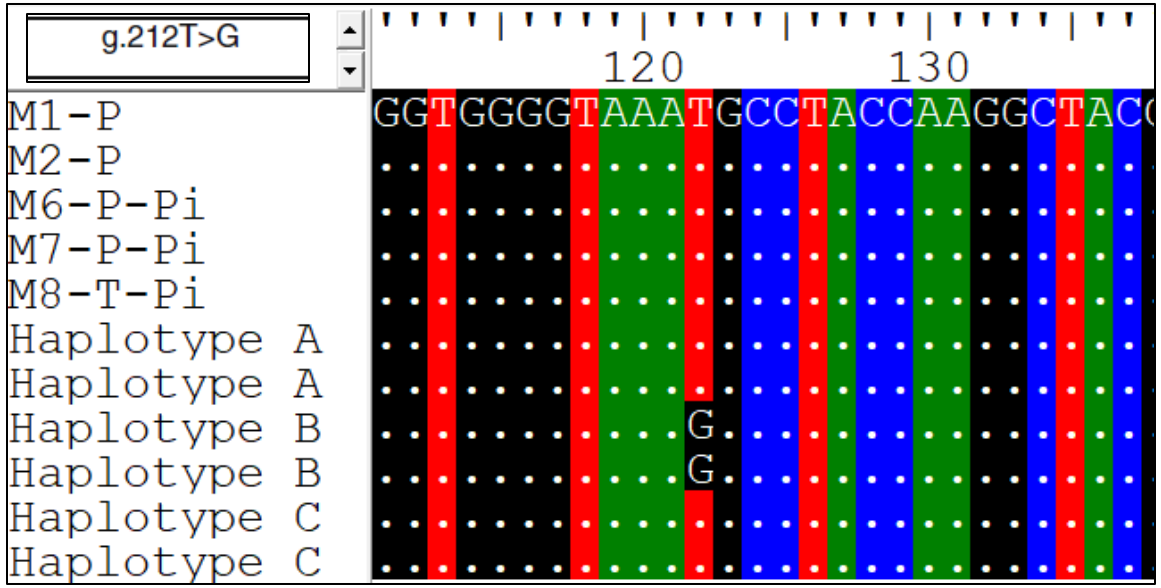


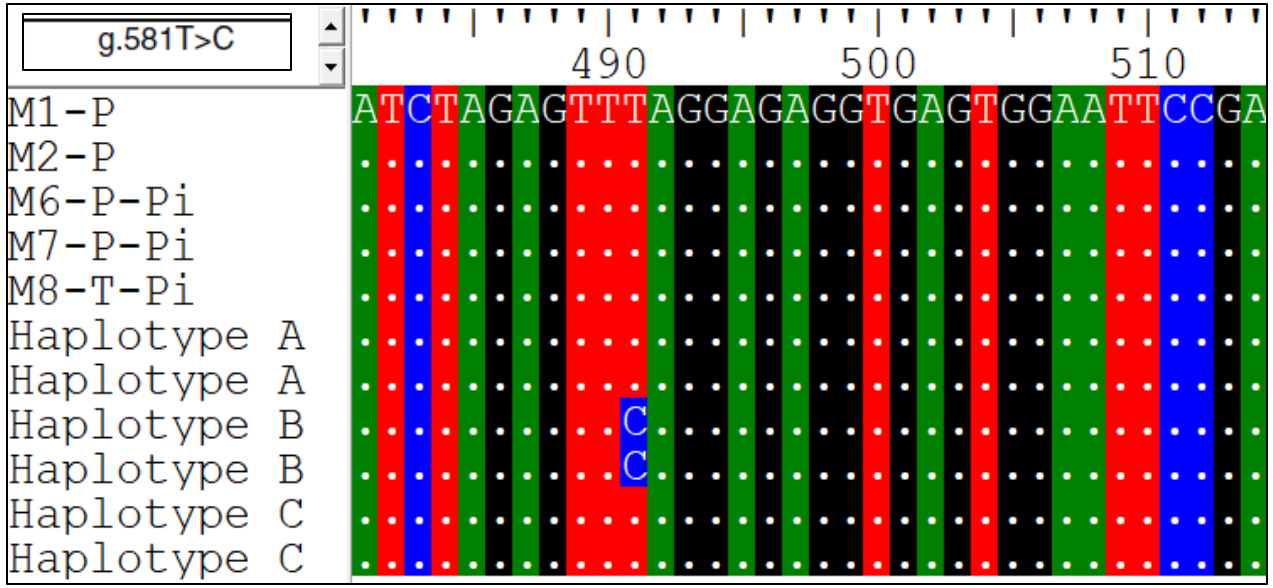
Phylogenetic tree inferred from 16S rDNA sequences using the Maximum Likelihood method,. Codon-based MUSCLE alignment function was used to align the sequences. Bootstrap values are shown next to the branches. GenBank accession numbers are indicated in parentheses, black dots indicate samples analyzed in this study

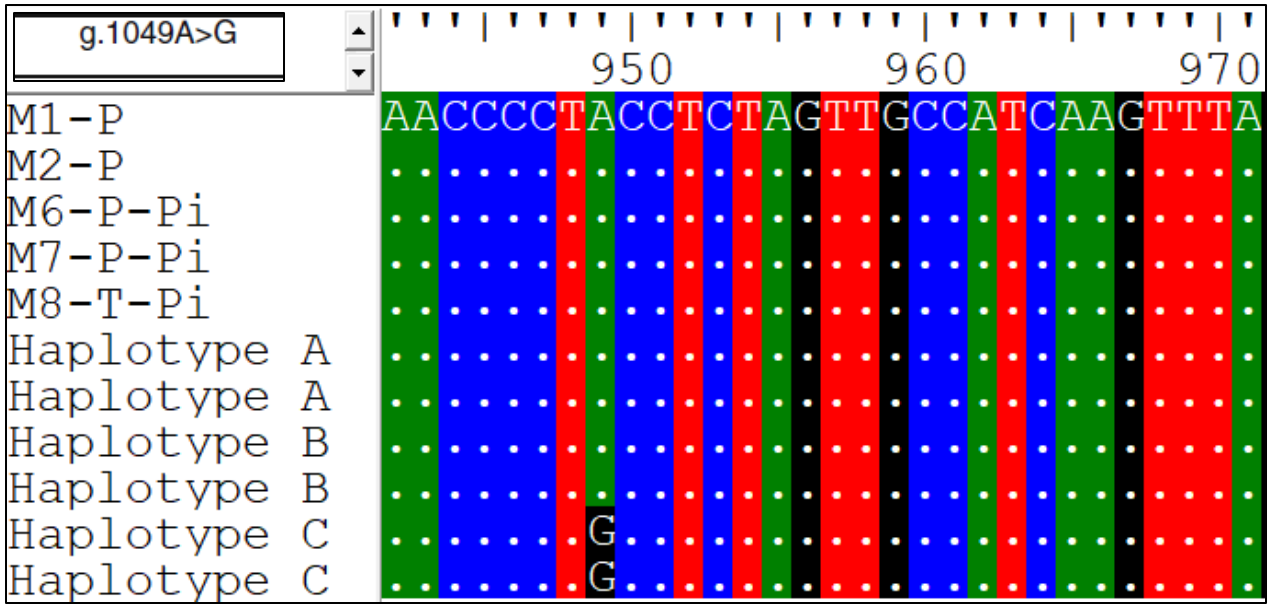
Third objective

To characterize the haplotype of CaLso present in Ecuador.

Haplotype characterization







Description	SS#	Gene region	Haplotypes		
			a	b	c
g.212T>G	161109903	16s	T	G	T
g.581T>C	161109904		T	C	T
g.1049A>G	244233404		A	A	G

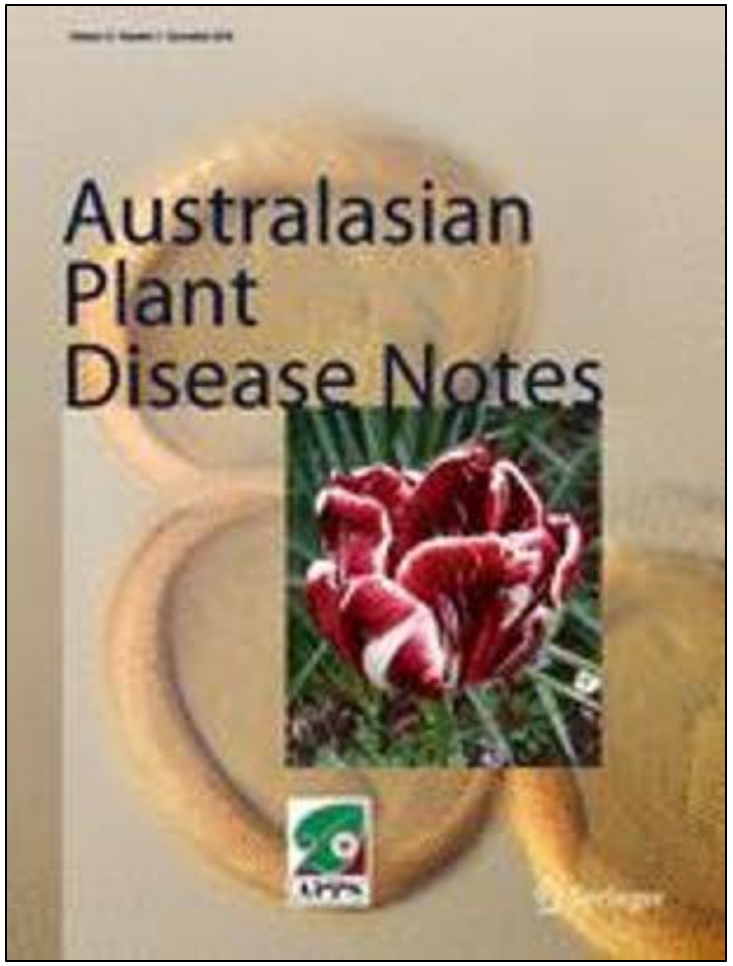
Eur J Plant Pathol (2011) 130:5–12
DOI 10.1007/s10658-010-9737-3

Publication accepted in the Journal "Australassian Plant Diseases Notes"

First report of '*Candidatus* Liberibacter solanacearum' in Ecuador and in South America

Abstract

'*Candidatus* Liberibacter solanacearum' (CaLso) haplotypes A and B, is a phloem-limited proteobacterium widely associated with Zebra chip (ZC) disease in potato tubers. In June 2019, symptoms such as yellowing and upward rolling in leaves and browning of vascular tissues and streaks at the medullary area in tubers were observed at potato fields located at the Pichincha province, Ecuador. In addition, high populations of *Bactericera cockerelli* were associated with the occurrence of these symptoms. CaLso haplotype A was detected in infected potato fields and the psyllid, *B. cockerelli* using conventional PCR. DNA sequence analysis confirmed CaLso in infected potato plants and insect tissues. To our knowledge this is the first report of CaLso haplotype A associated with potato and *B. cockerelli* at the Pichincha province, Ecuador and South America, where potato represents an important



Authors: Jorge D. Caicedo; Lorena L. Simbaña; Daniela A. Calderón; Karina P. Lalangui; Lydia I. Rivera-Vargas

Second conclusions

- For first time in Ecuador and South America, it was detected and identified CaLso in infected potato plants and in *B. cockerelli*.
- The haplotype A of CaLso was identified as the causal agent of ZC disease.
- It was associated the presence of CaLso with ZC disease in infected potato plants, and was determine some relationship with symptoms of PPT disease.

Fourth objective

To identify the other hosts of *B. cockerelli* and CaLso in Ecuador

Tamarillo

Pepper

Cape Gooseberry

Research Project 2

UNDERGRADUATE THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF ENGINEER AGRONOMIST

TITLE:

Molecular and phylogenetic characterization of '*Candidatus* Liberibacter solanacearum' in four species of the Solanaceae family in the Ecuadorian highlands. (2019-2020)

Autor: Marjorie Patricia Vallejo Borja (Undergraduate student)

Advisor: Jorge David Caicedo Chávez, I.A., M.Sc. (Professor)

Status of the research: **In process (unpublished data)**

Marjorie Vallejo

OBJECTIVES

General objective

To characterize molecular and phylogenetically '*Candidatus* Liberibacter solanacearum' in four species of the Solanaceae family in the Ecuadorian highlands.

Specific objectives

- To identify and characterize molecularly the presence of CaLso in potato, tamarillo, pepper and cape gooseberry plants.
- To locate phylogenetically the CaLso haplotype present in potato, tamarillo, pepper and cape gooseberry plants, comparing to the CaLso haplotypes present in other parts of the world.
- To determine the relationship between CaLso and *Bactericera cockerelli* in potato, tamarillo, pepper and cape gooseberry plants.
- To create a thematic map of the presence of the CaLso in the Ecuadorian highlands.

First objective

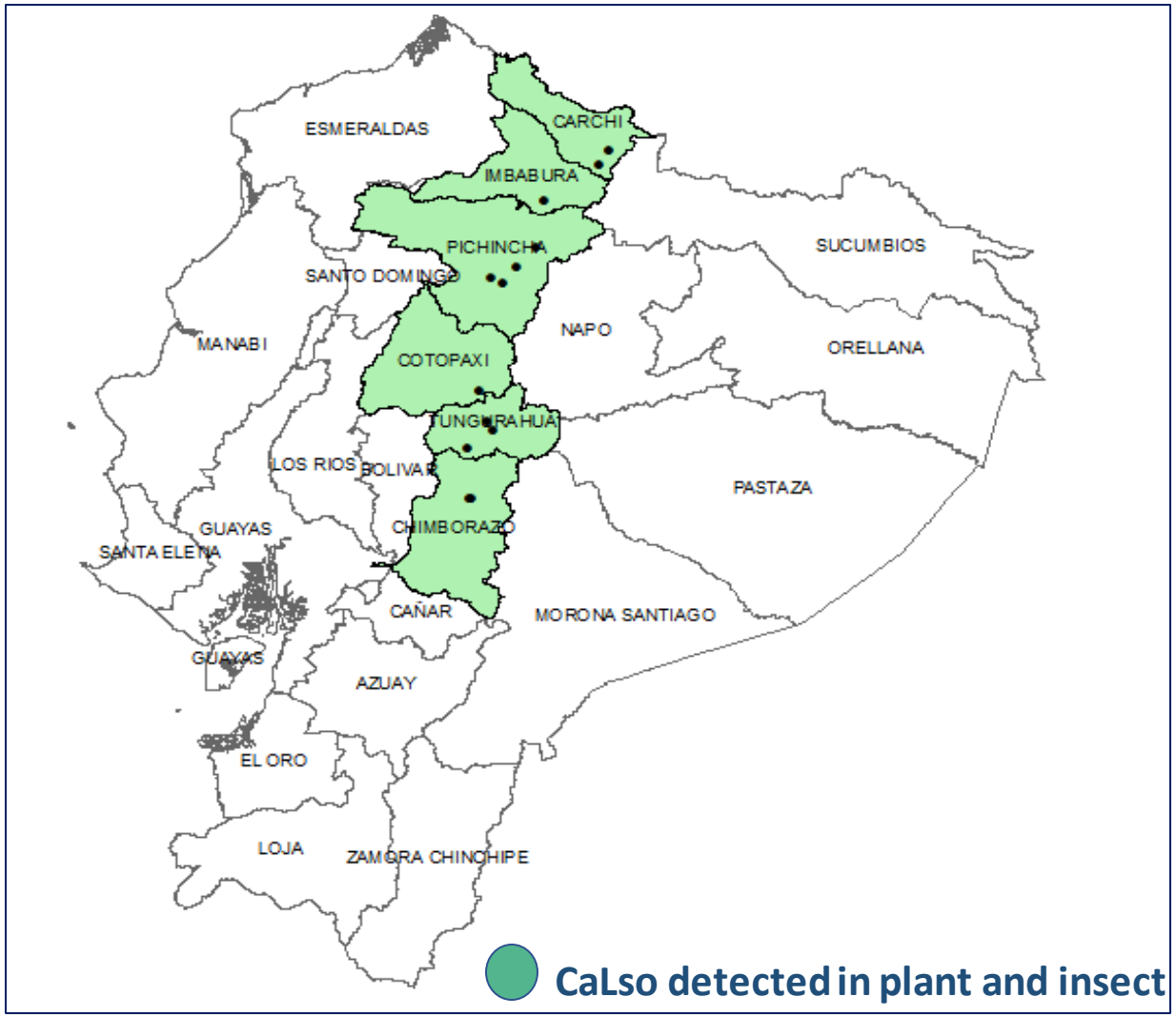
To identify and characterize molecularly the presence of CaLso in potato, tamarillo, pepper and cape gooseberry plants.

Preliminary results of CaLso detection in the Ecuadorian highlands

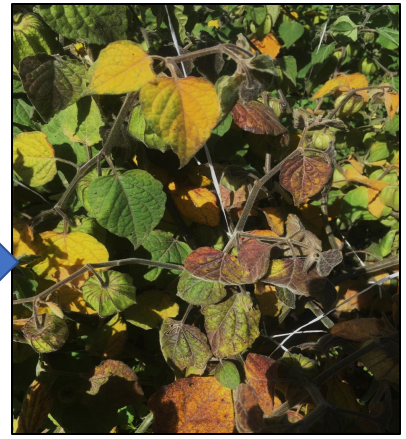
Province	Crop	Samples	Positive CaLso*	Negative CaLso	Identity
Carchi	Tamarillo	3	3	0	CaLso A
	Pepper	1	0	1	
Imbabura	Tamarillo	6	2	4	CaLso A
	Pepper	2	0	2	
Pichincha	Potato	7	1	6	CaLso A
	Tamarillo	9	3	6	CaLso A
	Cape gooseberry	1	1	0	CaLso A
Cotopaxi	Tomate	1	1	0	CaLso A
	Potato	1	0	1	
Chimborazo	Tomate	2	1	1	CaLso A
	Potato	1	1	0	CaLso A
Tungurahua	Potato	2	0	2	
	Tamarillo	6	3	3	CaLso A
	Cape gooseberry	1	1	0	CaLso A
Total samples		**43**	**17**	**26**	

*** Additionally, all samples tested were negative for phytoplasma infection using nested PCR.**

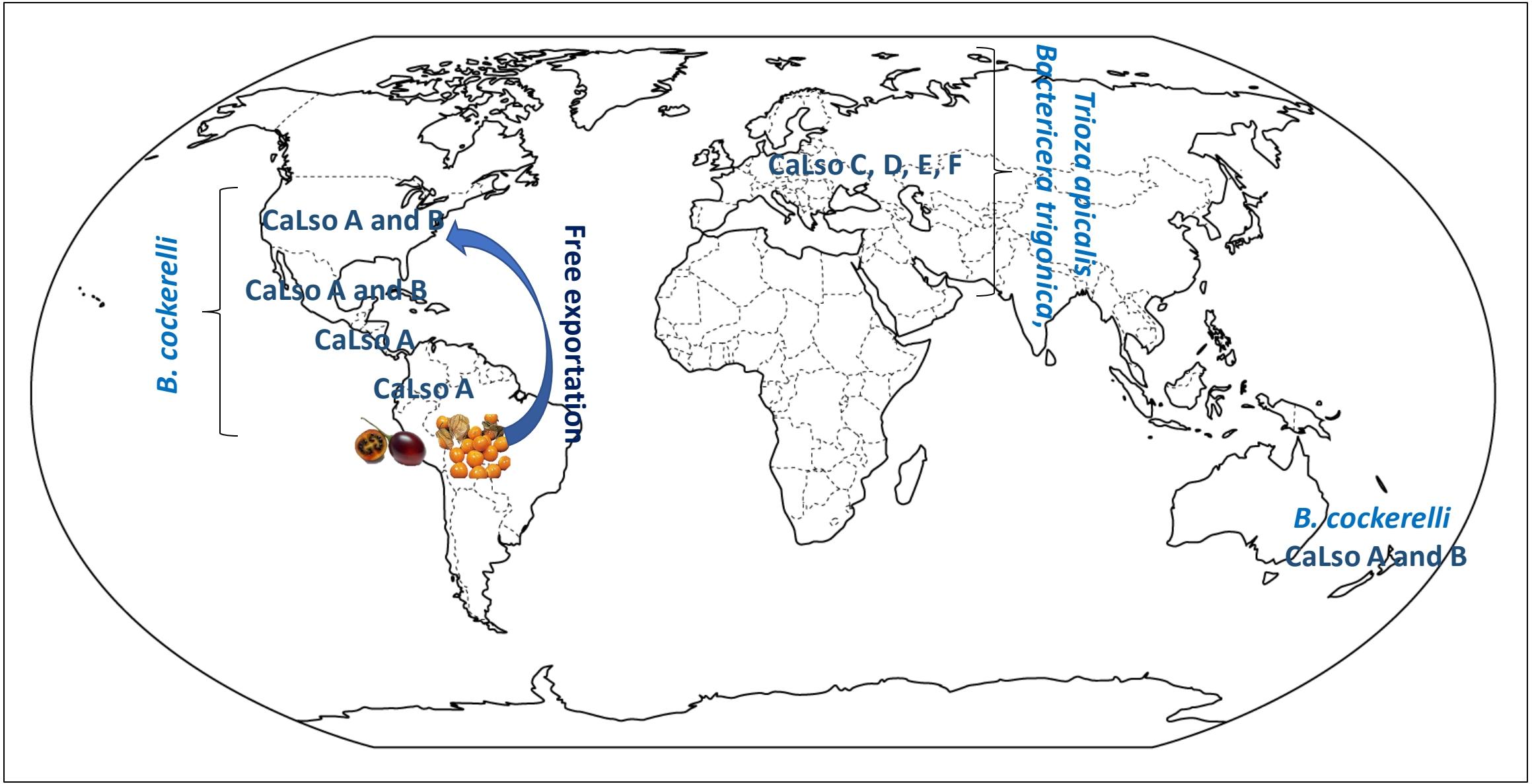
Thematic map of CaLso distribution in the Ecuadorian highlands



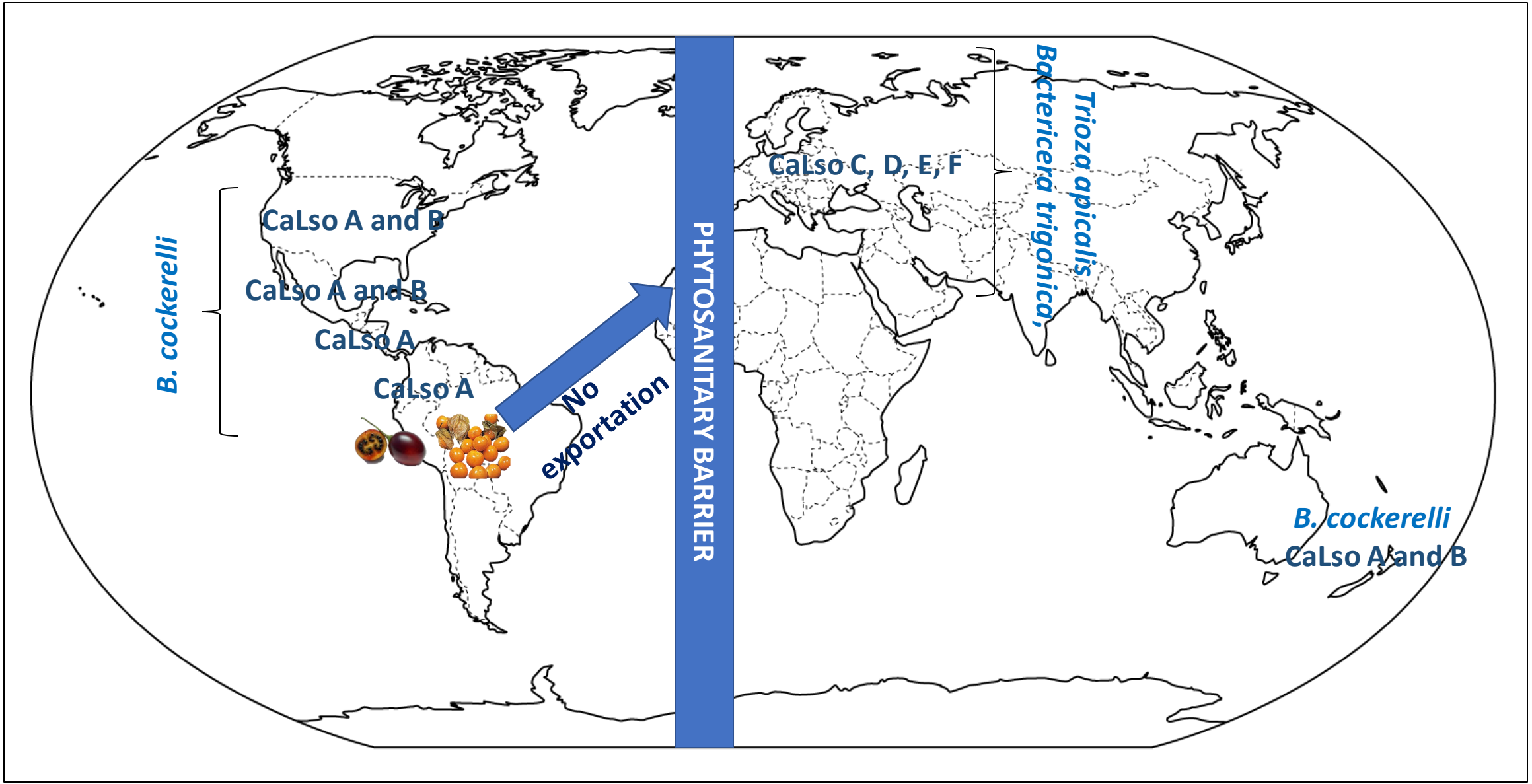
CaLso + *B. cockerelli*



Spreading of CaLso around the world



Spreading of CaLso haplotypes around the world



Third conclusions (preliminary)

- It was observed high populations of *B. cockerelli* affecting to potato, tamarillo, pepper and cape gooseberry plants, causing serious economic losses to producers.
- It was detected the presence of CaLso in potato, tamarillo and cape gooseberry, causing different symptoms.
- It was not detected CaLso in pepper plants, although high populations of *B. cockerelli* has been observed in this crop.
- CaLso was detected in five provinces of Ecuadorian highlands.

Acknowledgment

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External collaborators

Lydia Rivera PhD
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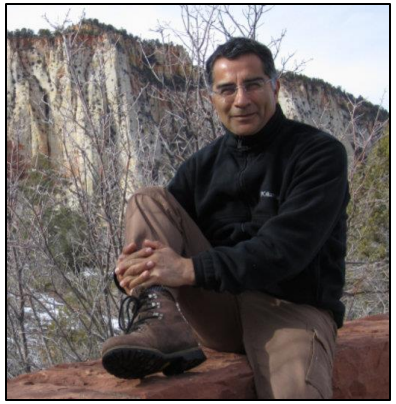
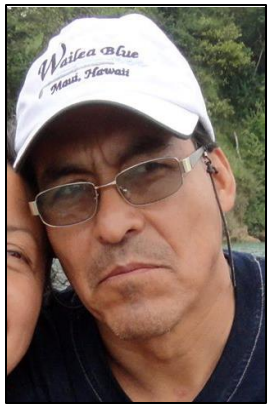


Ing. Héctor Andrade
UCE-FCA



Ing. Manuel Pumisacho
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Thanks for your attention!

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WORKSHOP

Phytosanitary emergency in the potato crop in Ecuador and implications for Peru and the Andean region: Purple top, potato psyllid and zebra chip

Lima, January 20-22, 2020