



Identificación de las Prioridades de Investigación y Planes de Acción

J. Isabel López Arroyo



Vivir Mejor

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2. The Area-wide management project in Mexico

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XXII
Congreso
Nacional
de Control
Biológico

MEMORIAS

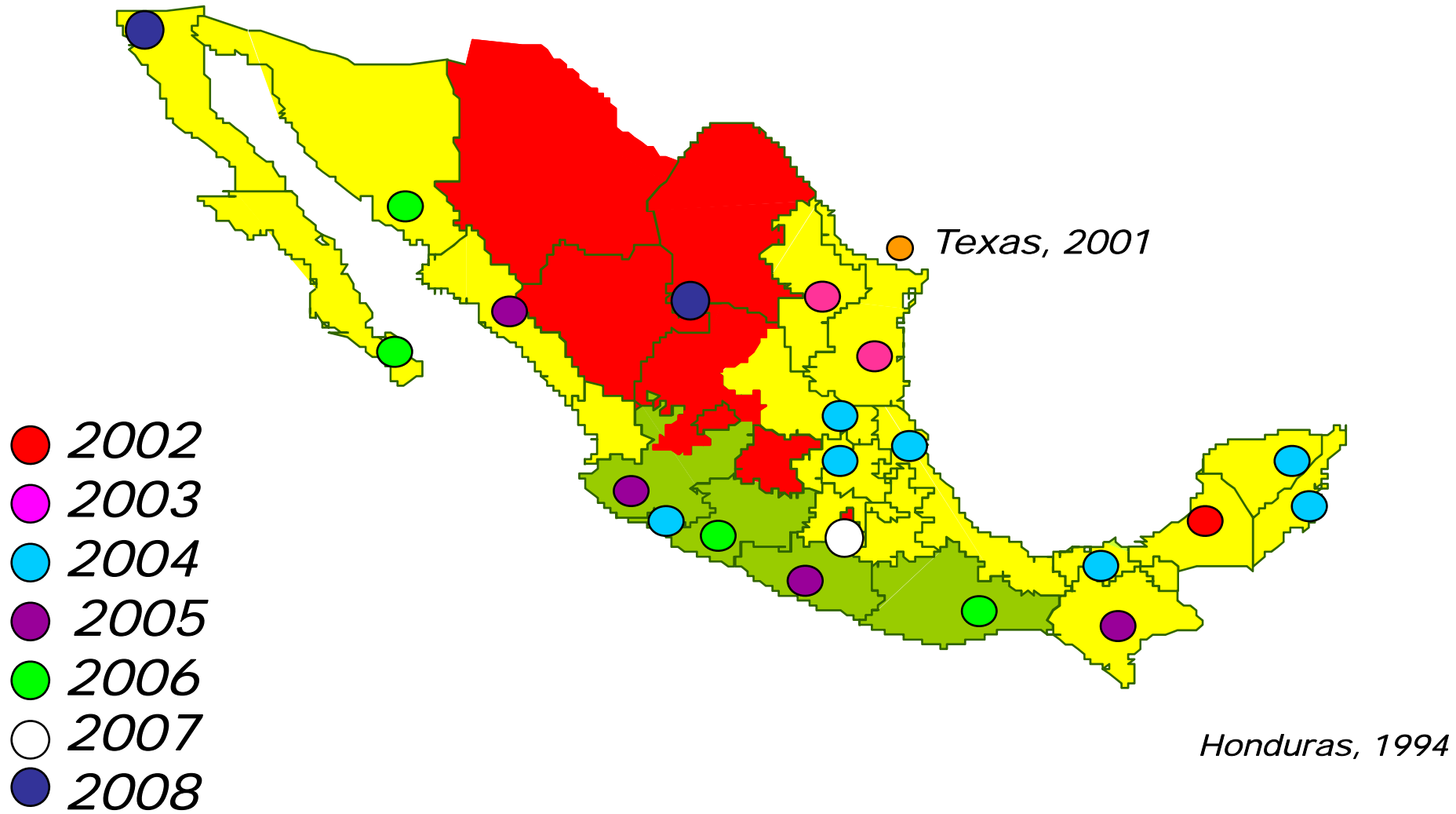
Colegio de Postgraduados
Montecillo, Edo. de Méx.
28 y 29 de octubre de 1999

Editores:
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Juan José Escobar Aguayo



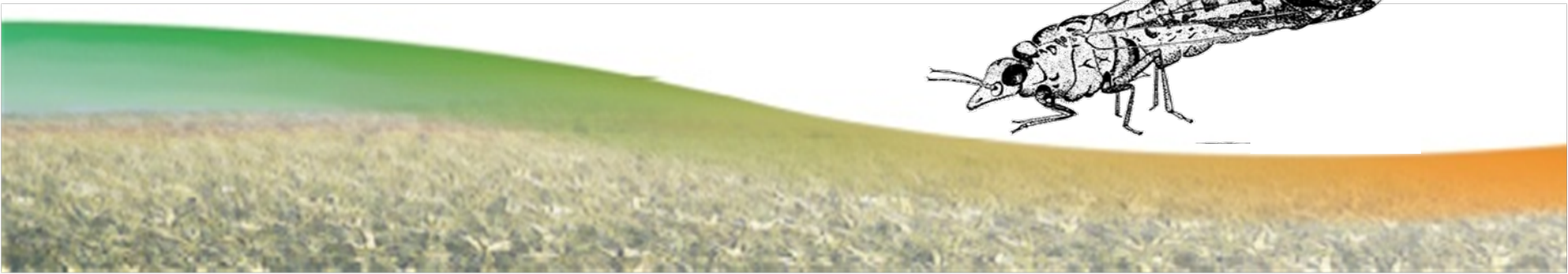
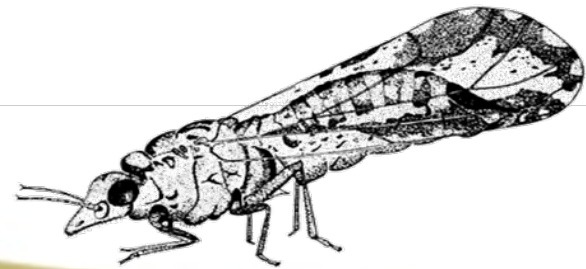
1999

Invasión de México por *D. citri*



PROYECTO
“MANEJO DE LA ENFERMEDAD
HUANGLONGBING (HLB) MEDIANTE EL CONTROL
DE POBLACIONES DEL VECTOR *Diaphorina citri*
(HEMIPTERA: PSYLLIDAE), EL PSÍLIDO ASIÁTICO
DE LOS CÍTRICOS”

J. Isabel López Arroyo



Objetivo General

- **Generar conocimiento y tecnología para integrar el manejo regional del psílido asiático de los cítricos, *Diaphorina citri*, en la citricultura nacional, que permitan reducir eficientemente las poblaciones y daño directo de la plaga, así como desarrollar estrategias para prevenir y manejar la enfermedad “Huanglongbing” causada por el patógeno *Candidatus Liberibacter* spp.**

Ejecución

Período: 2009 – 2012.

Áreas: **Ámbito de la citricultura nacional.**

Estados: Chiapas, Colima, Michoacán, N.L., S.L.P., Sinaloa, Sonora, Tamaulipas, Veracruz, Yucatán.

Investigadores: 45.

Instituciones: 1. INIFAP 2. CP 3. UANL 4. UAAAN 5. UNAM 6. UJED 7. UASL
8. IPN 9. ECOSUR 10. DGSV.

Coop. Intnal.: University of Florida, Texas A&M University, USDA-ARS.

Apoyo logístico: Citricultores, Viveristas, Comités Estatales de Sanidad Vegetal.

Líneas de Investigación

- 1. Análisis de riesgo por región agroecológica citrícola, acorde a la incidencia del vector.**
- 2. Técnicas de muestreo específicas a las condiciones agroecológicas y de fenología del cultivo.**
- 3. Validación, armonización y transferencia de técnicas de diagnóstico para la detección del HLB.**
- 4. Medidas de manejo del vector.**
- 5. Medidas de manejo del HLB.**
- 6. Técnicas para la remediación de la enfermedad en campo.**
- 7. Sistema informático para la vigilancia epidemiológica del HLB y su vector.**
- 8. Material para la capacitación y divulgación sobre el manejo del HLB y su vector.**

ZONAS AGROECOLÓGICAS DE ESTUDIO Y PARTICIPANTES EN EL PROYECTO



ARS Researchable Areas from ARS HLB Workshop: 4-23-08
CROP IMPROVEMENT

Plant host resistance to HLB

1. Transgenic/cisgenic resistant varieties and rootstocks.
2. Induced resistance w/ SAR.
3. Virus-based host resistance (to pathogen or vector) for screening/therapy.
4. Conventional breeding rootstocks.
5. Host response mechanisms. Direct RNA sequencing (compare healthy vs infected plants) e.g., role of miRNAs in disease development.

VECTOR BIOLOGY AND MANAGEMENT

Psyllid biology

1. Vector biology, seasonality, environmental effects, life history, dispersal.
2. Vector capacity, survival, transmission efficiency, propensity.
3. Vector genomics, including expression.

Psyllid resistance and response to the HLB pathogen

1. Transgenic psyllid
2. Endosymbiont targets (e.g., Wolbachia/virus transformation for Liberibacter control)
3. Strain diversity; host-compatibility groups
4. Histopathology of psyllid-vector relationship

Psyllid-based control

1. Urban areas? Homeowner psyllid management?
2. Area-wide program?
3. Symbiont manipulation for vector control
4. Novel pesticide development (RNAi targeted at specific psyllid genes for treatment of vector, BT library evaluation)
5. Insecticide resistance management
6. Insect repellent plant development ("virtual nets")
7. Mating disruption by...sound/pheromones, etc.
8. Plant Volatiles (attractants-Murraya, repellents)
9. Natural enemies, esp. for unmanaged citrus, e.g., parasites, predators, fungal/viral pathogens of insect
10. Kaolin clay applications
11. Barrier-based control (plants or netting impregnated with insecticide)
12. Wind breaks with lures and chemical treatment (trap plant)

PATHOGEN AND DISEASE BIOLOGY AND DETECTION

Pathogen biology and detection

1. Pathogen detection
2. Genome sequence
3. Culture organism to supplement current effort (high throughput methods)
4. Improved diagnostics in psyllid and plant tissue
5. Pathogen variability and diversity
6. ID factors critical to pathogenesis, virulence: Functional genomics, gene expression, proteomics, direct RNA sequencing
7. Population biology and spatial and temporal distribution in planta
8. Asymptomatic plant host range e.g., source of resistance, reservoir for inoculum

Disease biology

1. Etiology (Koch postulates for Liberibacter., metagenomics from phloem and psyllid endophytes)
2. Sources of variability in symptoms (interaction with other microbes, endophytes, stress interactions)
3. Early disease detection (non-pathogen based)
4. High throughput disease detection (e.g., imaging techniques, hyperspectral detection or laser or microwave detection, trained dogs)
5. Seed transmission and seed treatment
6. Rescue of infected germplasm/breeding material from field/seed

EPIDEMIOLOGY AND DISEASE MANAGEMENT

Epidemiology and Management of HLB

1. Symptom remission methods (antibiotics, others? Injection methodology)
2. Intercropping for disease management (e.g., guava)
3. Volatile effects and identification
4. Replacement tree protection via physical/chemical barriers (e.g., slow release "virtual net")
5. Repellents/avoidance factors (e.g., kaolin itself and as a carrier)
6. Cultural methods (e.g., nutrient delivery, early production systems, flush management, open hydroponic systems)
7. Effect of climate on disease biology (e.g. effect on range of endemic Liberibacter and psyllid, grow in Northern FL?)
8. Fruit/juice quality and abscission (productivity and quality in infected and treated trees)

Research Priorities in Mexico
25 out of 46



International Research Conference on Huanglongbing (IRCHLB)

Orlando, FL December 1-5, 2008

Research Priorities

<input checked="" type="checkbox"/> Researcher/Scientist	<input type="checkbox"/> Citrus Industry
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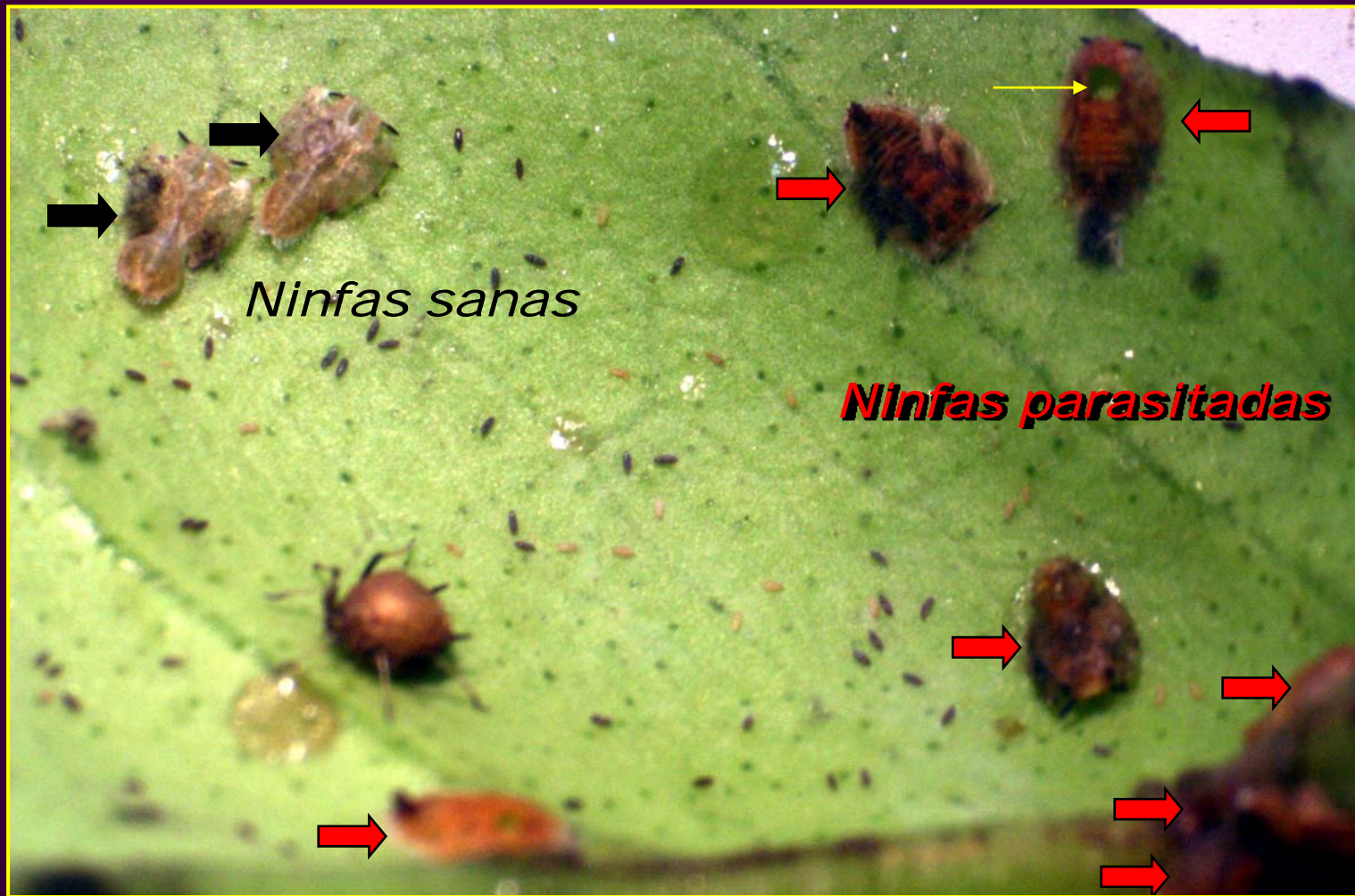
NAS Identified Research Priorities 2008

1. Perform Koch's postulates to conclusively demonstrate that Liberibacter is the causative agent of HLB and investigate biological properties of any relevant co-cultures.
2. ● Identify psyllid repellents from guava volatiles and other sources.
3. Construct Liberibacter genomic DNA library. Sequence and assemble full-length genome sequence.
4. ● Determine if modified spray techniques/applications etc. can improve psyllid control.
5. Develop transformation systems for mature tissue of commercial varieties (both rootstocks and scions).
6. ● Determine if RNA interference can be used to manipulate psyllid gene expression.
7. Develop improved (faster and more sensitive) assays for Liberibacter using PCR-, antibody-based, remote sensing, or other methods of detection.
8. ● Identify proteins or peptides that have anti-Liberibacter activity.
9. Examine the effects of oils and particle films on psyllids and HLB transmission.
10. Characterize the microbiome of citrus phloem tissue.
11. ● Identify attractants (chemical, color) in psyllid hosts and from other sources.
12. ● Examine effectiveness of flush management in controlling citrus psyllid interactions and HLB transmission. Ways to improve flush management include high-density planting using micrografting and tree dwarfing (with dwarfing factors, e.g. different rootstock).
13. ● Save Rutaceous germplasm; Screen citrus and Vepris germplasm for Liberibacter resistance and initiate citrus breeding to develop HLB resistant citrus.
14. Identify biological proteins (Bt-like) that affect the psyllid.
15. Examine the relationship between environmental factors (light intensity, temperature, etc.) and HLB symptoms and timing of host and pathogen responses.
16. Develop HLB resistant citrus by mutagenesis and selection.
17. Perform detailed Liberibacter transmission studies using psyllid, Liberibacter and citrus genotypes found in Florida.
18. ● Determine if Liberibacter is seed transmitted in citrus.
19. Develop a more facile (non-citrus) model system for HLB.
20. Identify factors related to variability of psyllid acquisition of pathogen; establish why less than 100% of psyllids will acquire Liberibacter.
21. ● Develop fast assay for detecting pathogen in the psyllid.
22. Determine the latency period between inoculation of citrus with Liberibacter and development of HLB symptoms, under different pathogen titers and different host conditions (phenology).
23. Study effects of anti-microbial compounds on HLB in citrus.
24. Examine transcript, protein and metabolite levels in Liberibacter infected citrus to better understand the plant's response to HLB.
25. ● Determine if citrus explants in tissue culture support high levels of Liberibacter.
26. ● Grow nursery and production stock under screen until fruit-bearing age.
27. ● Conduct economic feasibility studies of alternative citrus production systems (i.e. high density planting, hydroponics, screen house propagation/culture, intercropping with guava).
28. Develop a detailed understanding of the mechanism of HLB transmission, including Liberibacter acquisition time, mechanism of attachment to psyllid, inoculation period. Determine the mechanism and location of attachment of Liberibacter in psyllids.
29. ● Examine life cycle of psyllid in detail, including mating cues (acoustics), migration/dispersal patterns, stylus formation, etc.
30. ● Develop better methods for monitoring and determining the presence of psyllids in the environment.
31. Develop psyllids by breeding or transgenic approaches that do not vector Liberibacter.
32. Synthesize repellents or engineer microbes to synthesize repellants.
33. Sequence an EST library of psyllid genes.
34. ● Examine seasonal distribution of Liberibacter in citrus (both levels and location within a tree). Analyze the distribution of Liberibacter in symptomatic and asymptomatic citrus tissues.
35. Develop molecular research tools for Liberibacter including monoclonal antibodies to Liberibacter, reference strains of Liberibacter and psyllids, cDNA and genomic libraries of Liberibacter.
36. ● Examine effectiveness of different methods to stimulate systemic acquired resistance to HLB in citrus.

Research Priorities in Mexico
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Tamarixia en México



Aprovechamiento de *Tamarixia radiata*



Establecimiento de Pie de Cría de Tamarixia radiata

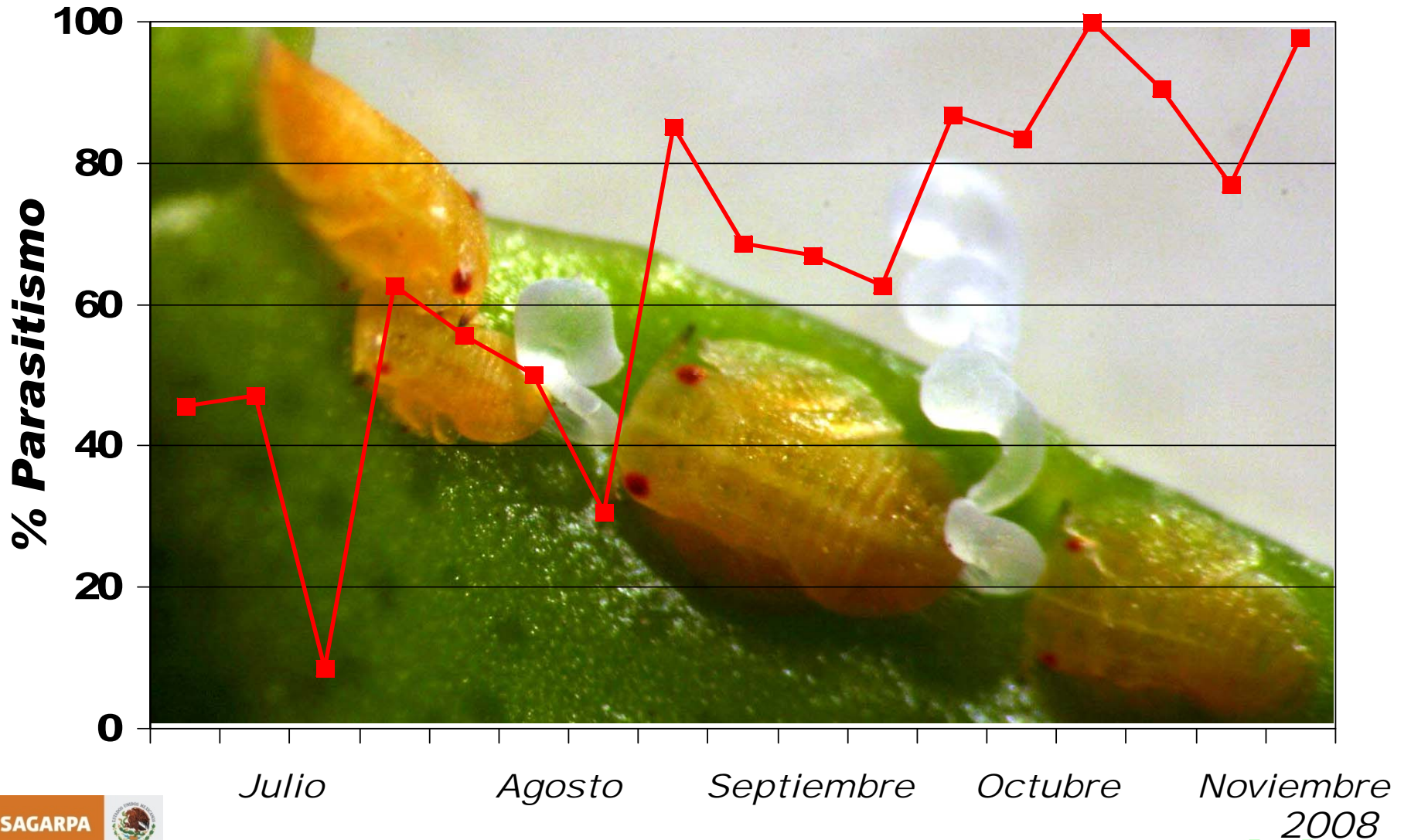
1. Colonia de Diaphorina citri



2. Producción de Tamarixia radiata



*Parasitismo de Diaphorina citri por Tamarixia radiata en laboratorio.
(26°C, 60% H.R., 16:8 h L:O) C*



Potencial para la Cría Masiva de *Tamarixia radiata*



inifap

ENEMIGOS NATURALES DE Diaphorina citri EN MÉXICO



Olla v-nigrum



Olla v-nigrum



Harmonia axyridis



Brachiacantha decora

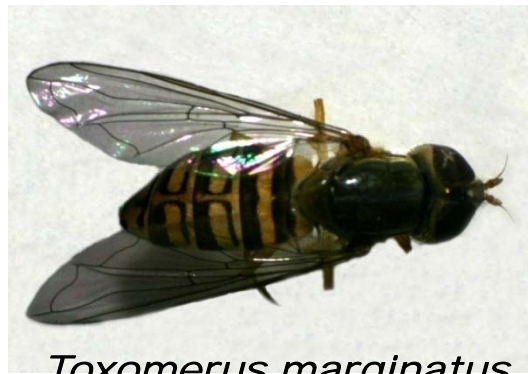


Cycloneda sanguinea



Hippodamia convergens

ENEMIGOS NATURALES DE Diaphorina citri EN MÉXICO



Toxomerus marginatus

Toxomerus politus



ENEMIGOS NATURALES DE *Diaphorina citri* EN MÉXICO



ENEMIGOS NATURALES DE Diaphorina citri EN MÉXICO



Chrysoperla externa

Chrysoperla comanche

Chrysoperla rufilabris

La Estrategia de los Generalistas



CENTROS REGIONALES DE ESTUDIOS Y
REPRODUCCION DE ORGANISMOS BENEFICOS

Control Biológico



Olla v-nigrum
(Coleoptera: Coccinellidae)



← 2000-2001. 452,000 ladybugs.

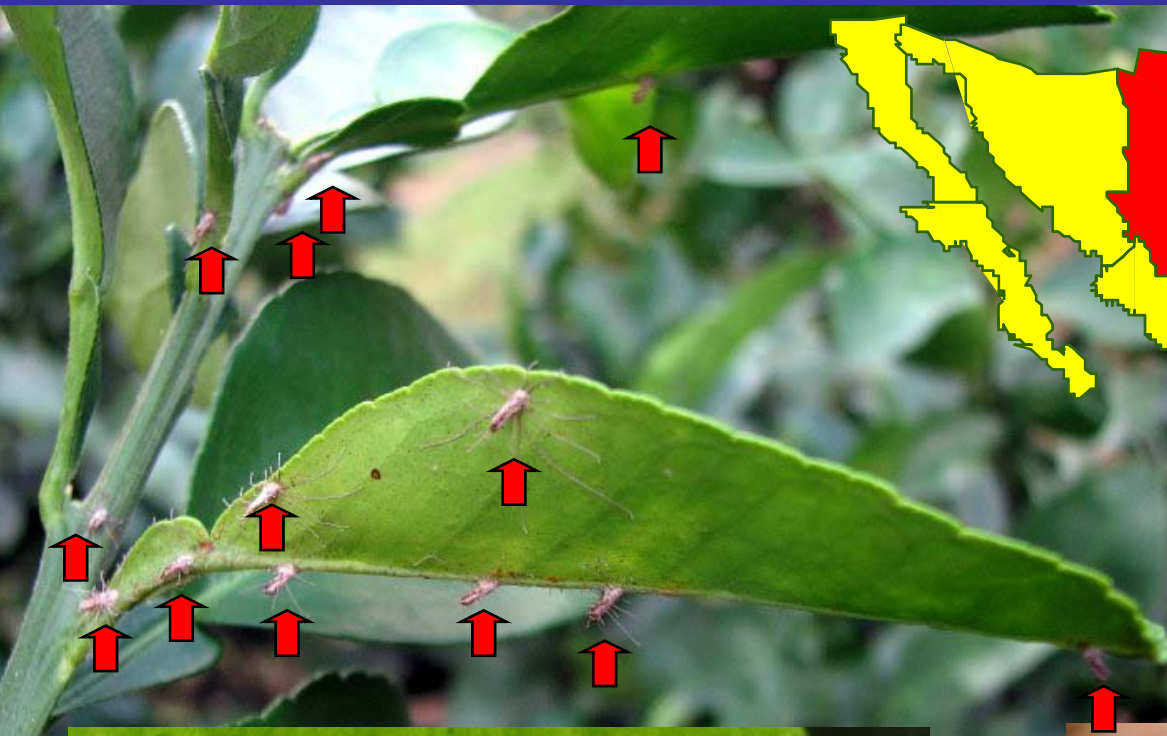
Ensayos con hongos entomopatógenos aislados de parientes cercanos a *D. citri*

HONGO	ORDEN/HUESPED	FAMILIA/HUESPED	ESPECIE/HUESPED	ORIGEN	% MORTALIDAD
<i>Metarhizium anisopliae</i> *	Homoptera	Cercopidae	<i>Aeneolamia</i>	Veracruz	90.00
<i>Paecilomyces fumosoroseus</i> *	Homoptera	Aleyrodidae	<i>Bemisia</i>	Armería, Col.	87.55
<i>Paecilomyces fumosoroseus</i> *	Homoptera	Aleyrodidae	<i>Bemisia</i>	Armería, Col.	78.35
<i>Paecilomyces fumosoroseus</i> *	Homoptera	Aleyrodidae	<i>Bemisia</i>	Armería, Col.	74.67
<i>Paecilomyces fumosoroseus</i>	---	---	---	---	69.66
<i>Metarhizium anisopliae</i>	Homoptera	Cercopidae	<i>Aeneolamia</i>	Veracruz	59.23
<i>Metarhizium anisopliae</i>	Homoptera	Cercopidae	<i>Aeneolamia</i>	Veracruz	56.54

*Candidatos a ser usados como agentes de control microbiológico.



Hirsutella citriformis Speare



National Committee for Biological Control of ACP

Short -term needs

1. *Surveying natural enemies attacking ACP in newly invaded areas* ✓
2. *Conducting host testing for strains of *T. radiata* currently in quarantine* ✓
3. *Developing methods of mass production of *T. radiata** ✓
4. *Begin release and evaluation of appropriate strain(s) of *T. radiata* when approved for field release* ✓
5. *Determine the distance that natural enemies will travel or disperse from an area* ✓
6. *Determine the impact of insecticide applications for psyllid control of natural enemies* ✓

National Committee for Biological Control of ACP

Long -term needs

- 1. Survey of natural enemies attacking ACP in other parts of the world newly invaded areas***
- 2. Studies of the optimal time and number of parasitoids to release in a given habitat ✓***
- 3. Studies on release type for optimal effect from the natural enemies ✓***
- 4. Studies on methods to conserve natural enemies ✓***
- 5. Determine the distance that natural enemies will travel or disperse from an area ✓***
- 6. Optimal incorporation of biological control in area-wide management programs ✓***