Epidemiology of HLB and potential pathways for introduction

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Pathway Risk Analysis

"Entails:

Identifying the components of pathways

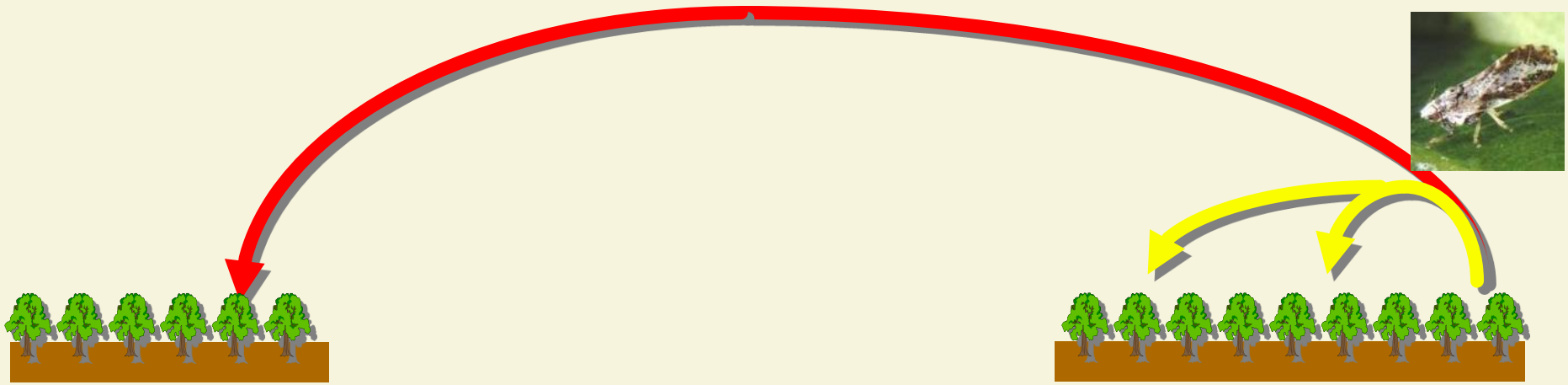
and

Assigning the probabilities *of entry, spread, introduction, establishment, and/or outbreak of a pest through the various pathways*

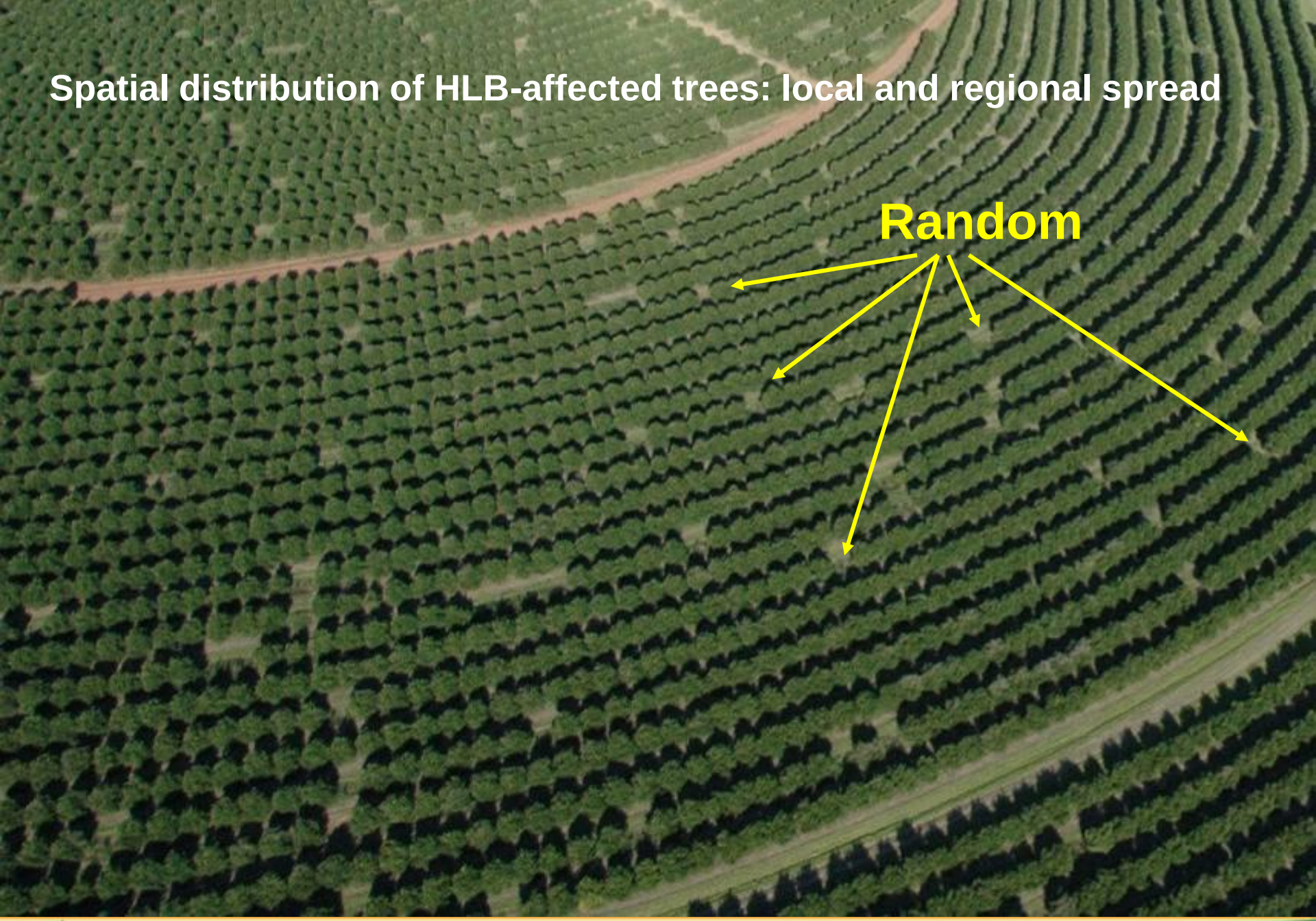
along with ***the consequences*** *of their introduction."*

Pathways for HLB Introduction

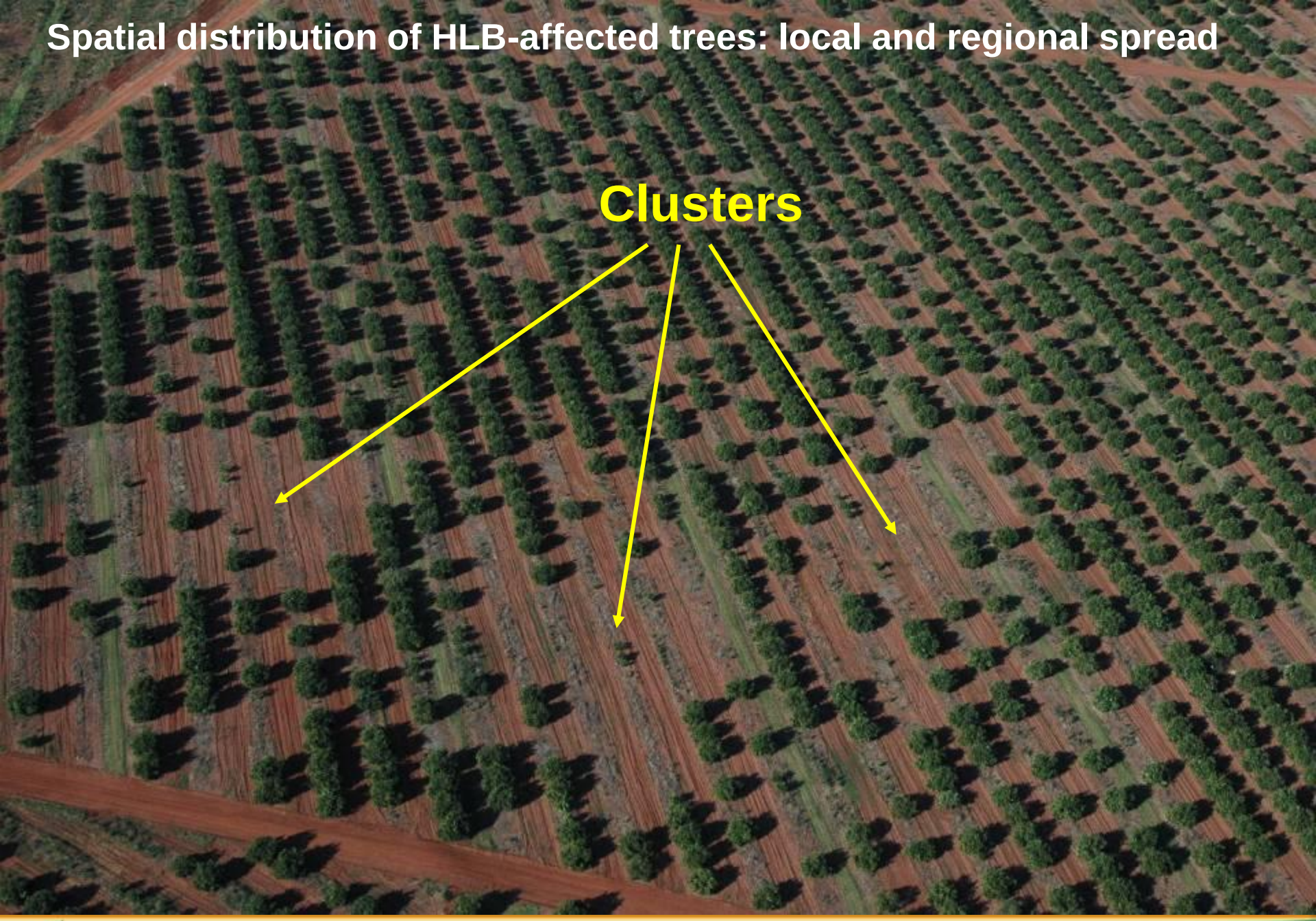
1) Infected Asian citrus psyllid transmission locally and regionally



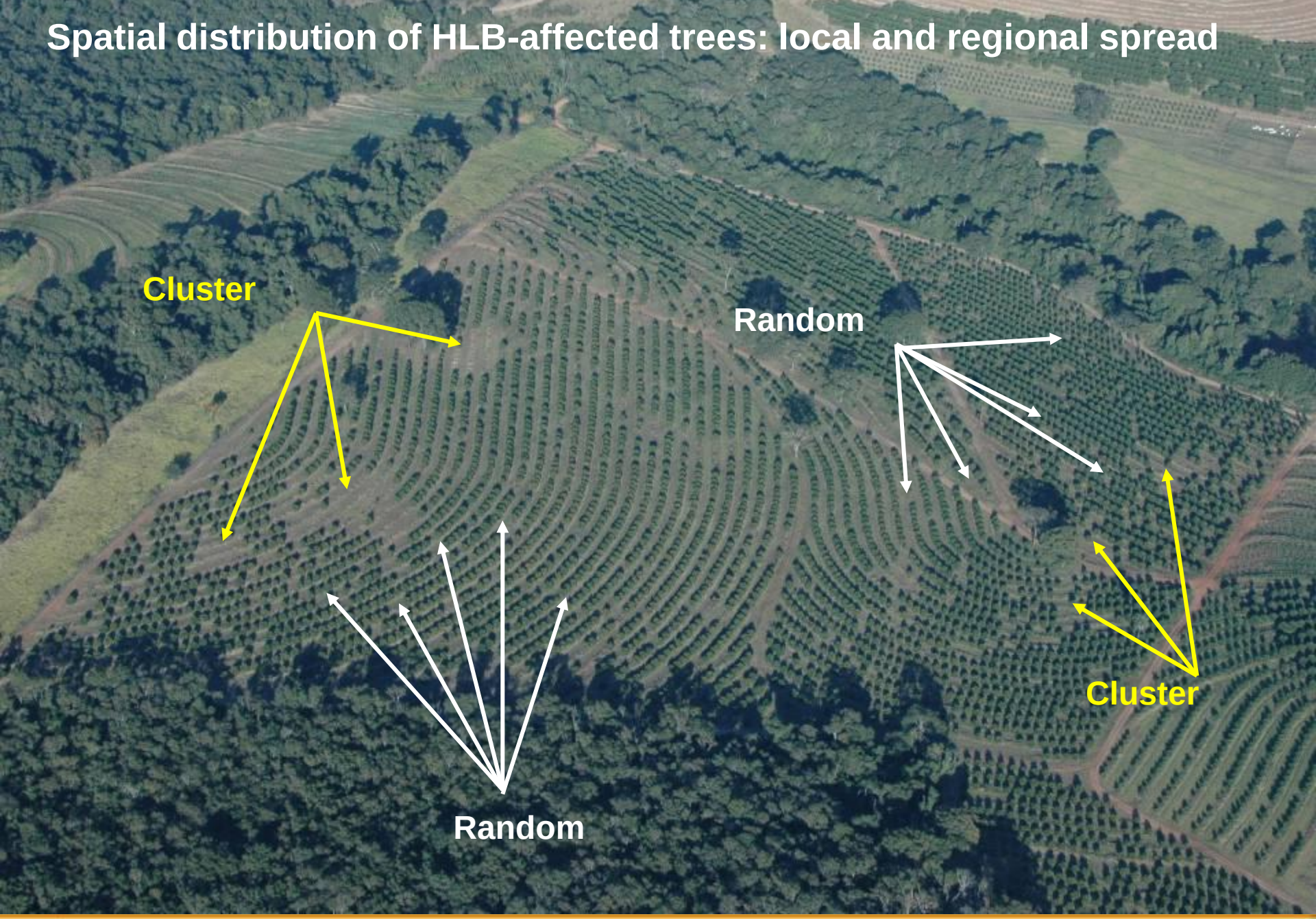
Spatial distribution of HLB-affected trees: local and regional spread

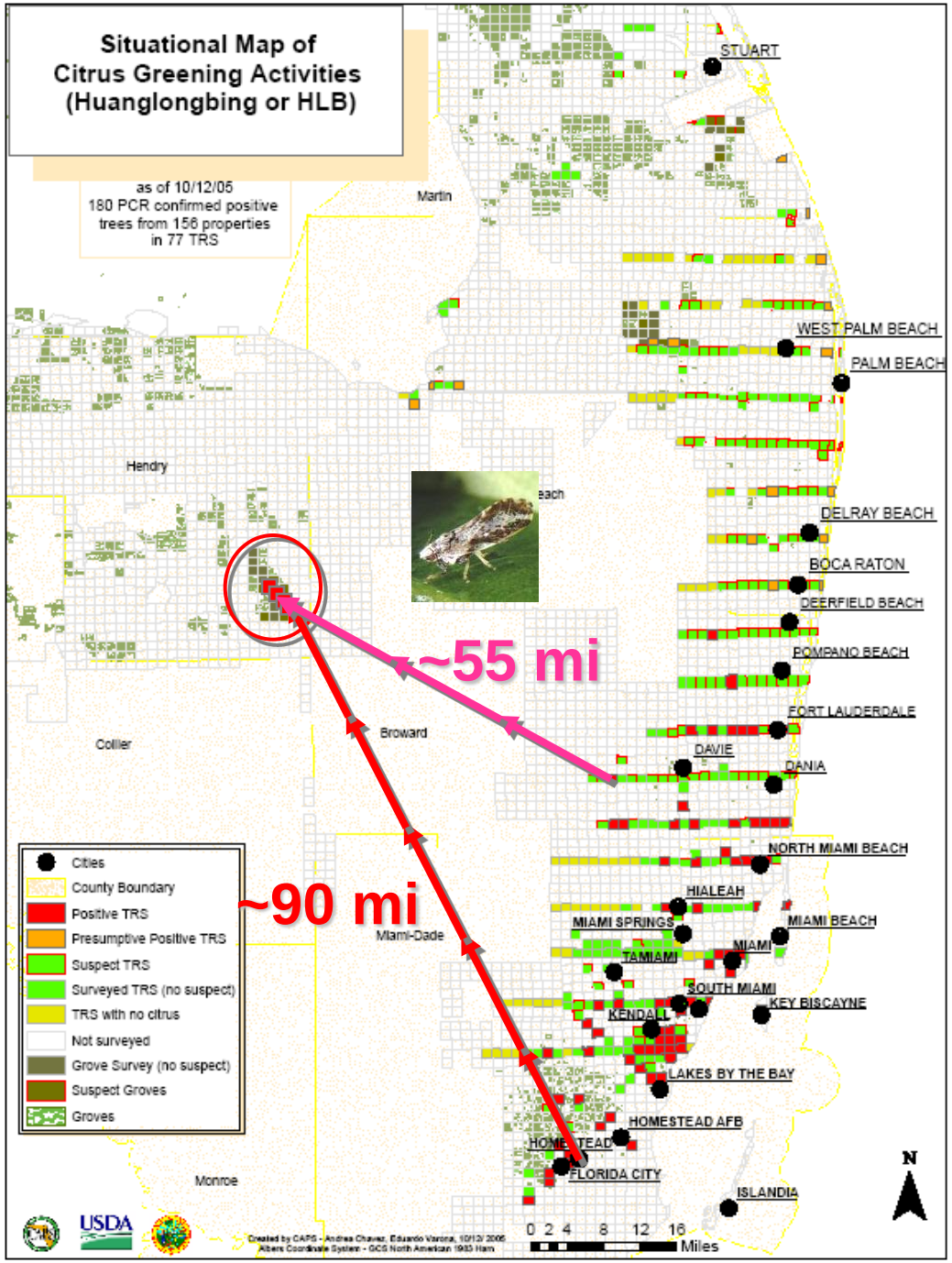


Spatial distribution of HLB-affected trees: local and regional spread



Spatial distribution of HLB-affected trees: local and regional spread

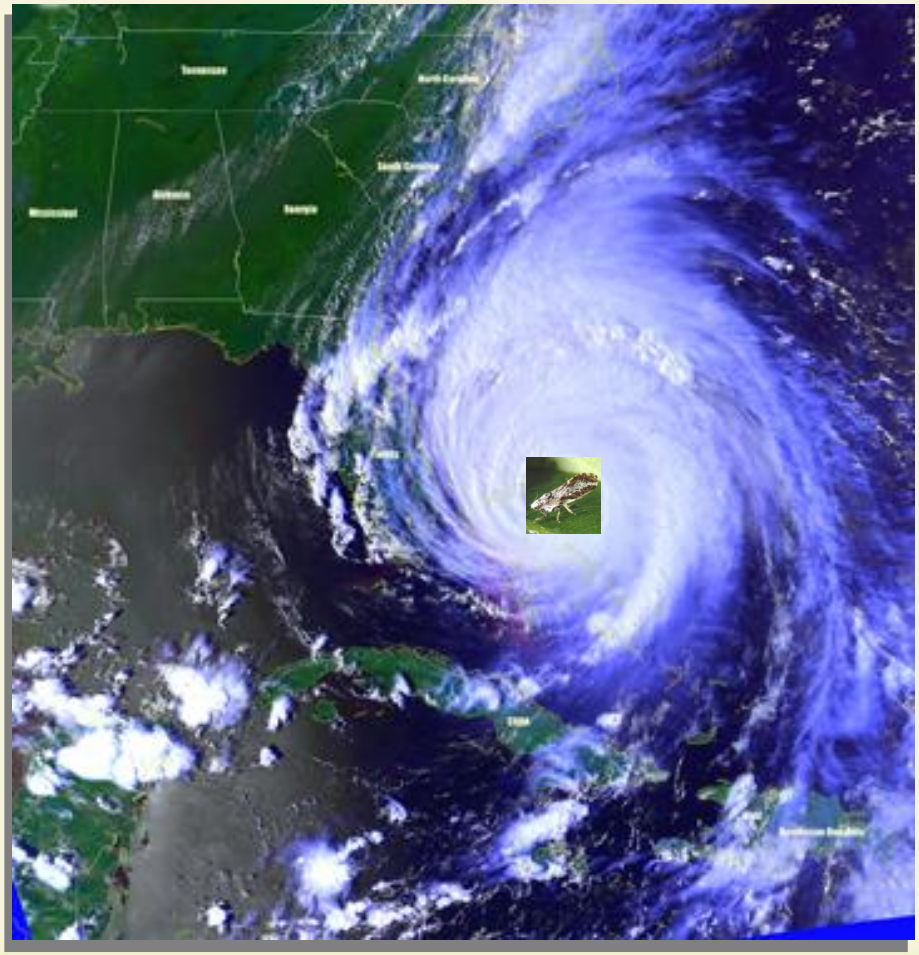




Evidence of long distance spread by vector

- Search for First Commercial Infections:
  - **~90 miles** from initial find to NW
  - **~55 miles** from nearest residential infections known
- No recent replants from outside source

Long distance spread by vector related to movement of air masses during hurricane or tropical storms



- Makes the prevention of infected ACP introduction and establishment in new areas very difficult to inhibit


South Carolina
2008
Mississippi
Alabama
Georgia
2009
Louisiana
Jacksonville
2007
Houston
Florida
2005
Great Abaco Island
Nassau
Bahamas
Golfo do México
La Habana
Ragged Island
2006
Cuba
2009
Mérida, YUC, México
2008
(Porto Principe)
Ciudad de México
Ilhas Cayman
Navassa Island
2009
Jamaica
Ambergris Cay
Belmopan

Pathways for HLB Introduction

2) Movement of infected Asian citrus psyllid on plant material

Host list for *Diaphorina citri* Kuwayama

TABLE 1. HOST LIST FOR *DIAPHORINA CITRI* KUWAYAMA.

Species	Source	Comments
Aegle marmelos (L.) Corr.	Viraktamath & Bhumannavar 2002	
Aeglopsis chevalieri Swingle	Koizumi et al. 1996	
Afraegle gabonensis Engl.	DPI Citrus Arboretum survey	
Afraegle paniculata (Schaum.) Engl.	DPI Citrus Arboretum survey	
Artocarpus heterophyllus Lamarck (Moraceae)	Shivankar et al 2000	
Atalantia missionis Oliver	Tirtawidjaja 1981	
Atalantia monophylla (L.) Corr.	DPI Citrus Arboretum survey	
Atalantia sp.	Koizumi et al. 1996; Aubert 1990a	adult feeding only (Aubert)
Balsamocitrus dawei Stapf	Koizumi et al. 1996	
Citropsis gilletiana Swingle & M. Kellerman	DPI Citrus Arboretum survey	
Citropsis schweinfurthii (Engl.) Swingle & Kellerm.	Chavan & Summanwar 1993	good host
Citrus aurantifolia (Christm.) Swingle	Aubert 1987, 1990a; Florida surveys	preferred host
Citrus aurantium L.	Florida surveys	
Citrus deliciosa Tenore	Aubert 1987	common
Citrus grandis (L.) Osbeck	Aubert 1987	occasional, *C. grandis* is considered a junior synonym of *C. maxima*
Citrus hystrix DC.	Aubert 1987; Lim et al. 1990	occasional
Citrus jambhiri Lushington	Florida surveys	
Citrus limon (L.) Burm. f.	Aubert 1987, 1990a	common
Citrus madurensis Loar.	Aubert 1990a	
Citrus maxima (Burm.) Merr.	Aubert 1990a	occasional, but observed nymphal development
Citrus medica L.	Aubert 1987, 1990a	common
Citrus meyeri Tan	Florida surveys	
Citrus × *nobilis* Lour.	Aubert 1987; Florida surveys	common
Citrus obovoidea Hort. ex Tanaka cv 'Kinkoji'	Florida surveys	
Citrus × *paradisi* Macfad.	Aubert 1987; Florida surveys; Tsai & Liu 2000	common; a preferred host in Florida (DPI); best host in laboratory assays (Tsai & Liu)
Citrus reticulata Blanco	Aubert 1987, 1990a, Koizumi et al. 1996; Florida surveys	common
Citrus sinensis (L.) Osbeck	Aubert 1987, 1990a; Florida surveys	common
Citrus spp.	Aubert 1990a; Florida surveys	common host
Clausena anisum-olens Merrill	Aubert 1990a	occasional host, observed nymphal development
Clausena excavata Burm. f.	Aubert 1990a; Lim et al. 1990	
Clausena indica Oliver	Aubert 1990a	adult feeding in laboratory
Clausena lansium (Lour.) Skeels	Koizumi et al. 1996; Aubert 1990; Florida surveys	poor host (Koizumi et al.); common host (Aubert); population highly variable (FL surveys)

TABLE 1. (CONTINUED) HOST LIST FOR *DIAPHORINA CITRI* KUWAYAMA.

Species	Source	Comments
Eremocitrus glauca (Lindley) Swingle	Koizumi et al. 1996	poor host, but plant died
Eremocitrus hybrid	DPI Citrus Arboretum Survey	
Fortunella crassifolia Swingle	DPI Citrus Arboretum Survey	
Fortunella margarita (Lour.) Swingle	DPI Citrus Arboretum Survey	
Fortunella polyandra (Ridley) Tanaka	DPI Citrus Arboretum Survey	
Fortunella spp.	Aubert 1987, 1990a	occasional; nymphal development, laboratory only (Aubert 1990)
Limonia acidissima L.	Koizumi et al. 1996	
Merrillia caloxylon (Ridley) Swingle	Lim et al. 1990; Aubert 1990a	cage in laboratory only (Lim et al.); adult feeding in laboratory (Aubert)
Microcitrus australasica (F.J. Muell.) Swingle	Koizumi et al. 1996; Aubert 1987, 1990a; DPI Citrus Arboretum survey	common; observations in laboratory (Aubert 1990a)
Microcitrus australis (Planch.) Swingle	DPI Citrus Arboretum survey	
Microcitrus papuana H.F. Winters	DPI Citrus Arboretum survey	
Microcitrus sp. 'Sidney'	DPI Citrus Arboretum survey	
Murraya exotica L.	Aubert 1990a	adult feeding in laboratory
Murraya koenigii (L.) Sprengel	Koizumi et al. 1996; Aubert 1987; 1990a; Lim et al. 1990; Florida surveys	good host (Koizumi); occasional host; no eggs observed (Aubert 1987); good host with nymphal development (Aubert 1990a); not an excellent host but will support a small population, including eggs (FL surveys)
Murraya paniculata (L.) Jack	Koizumi et al. 1996; Aubert 1987, Florida surveys	a preferred host
Naringi crenulata (Royb.) Nicholson	DPI Citrus Arboretum survey	
Pamburus missionis (Wight) Swingle	DPI Citrus Arboretum survey	
Poncirus trifoliata (L.) Raf.	Koizumi et al. 1996; Aubert 1987, 1990a)	occasional; eggs, but no nymphs (Aubert 1987, 1990a)
Severinia buxifolia (Poiret) Ten.	Koizumi et al. 1996; Florida surveys	
Swinglea glutinosa (Blanco) Merr.	Garnier & Bové 1993; Florida surveys	
Toddalia asiatica (L.) Lam	Aubert 1987, 1990a	occasional; no eggs observed
Triphasia trifolia (Burm. f.) P. Wilson	Koizumi et al. 1996; Aubert 1987; DPI Citrus Arboretum survey; Aubert 1990a	poor host (Koizumi); occasional host (Aubert); all stages and damage evident (FL surveys)
Vepris lanceolata G. Don	Aubert 1987, 1990a	occasional; no eggs observed
Zanthoxylum fagara (L.) Sarg.	DPI Citrus Arboretum Survey	plenty of suitable new shoots; very few *D. citri* found; possible non-host.
Apparent non-hosts:		
Casimiroa edulis Llave & Lex.	DPI Citrus Arboretum Survey	plenty of suitable new shoots; no *D. citri* found
Zanthoxylum clava-herculis L.	DPI Citrus Arboretum Survey	plenty of suitable new shoots; no *D. citri* found

Halbert & Manjunath (2004)

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Pathways for HLB Introduction

3) Movement of infected citrus plants

Citrus trees for sale

Open field nursery trees

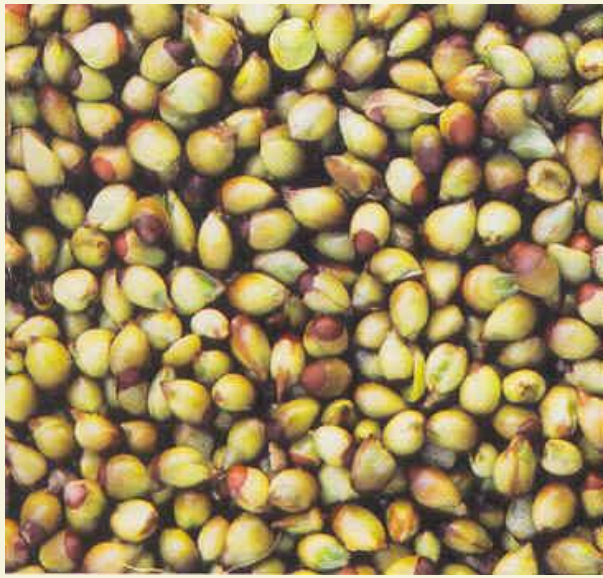
“Protected” nursery trees

Pathways for HLB Introduction

3) Movement of infected citrus plants

Scion budwoods

Rootstock seedlings



Seeds ?

Citrus host list for *Candidatus Liberibacter* spp.

Species	Source	Comments
Citrus amblycarpa Ochse	Tirtawidjaja 1981	
Citrus aurantifolia (Christm.) Swingle	Miyakawa & Yuan 1990; Tirtawidjaja 1981	mild symptoms
Citrus aurantium L.	Miyakawa & Yuan 1990	symptoms
Citrus depressa Hayata	Miyakawa & Yuan 1990	symptoms
Citrus grandis (L.) Osbeck	Miyakawa & Yuan 1990; Su & Huang 1990	symptoms; pomelo-infecting strain prevalent since 1970s (Su & Huang). *C. grandis* is considered a junior synonym of *C. maxima*
Citrus hassaku Hort. ex Tanaka	Miyakawa & Yuan 1990	symptoms
Citrus hystrix DC.	Miyakawa & Yuan 1990	symptoms
Citrus ichangensis Swingle	Miyakawa & Yuan 1990	symptoms
Citrus jambhiri Lushington	Tirtawidjaja 1981	
Citrus junos Sieb. ex Tanaka	Miyakawa & Yuan 1990	symptoms
Citrus kabuchi Hort. ex Tanaka	Miyakawa & Yuan 1990	symptoms
Citrus limon (L.) Burm. f.	Miyakawa & Yuan 1990	symptoms, presence of putative pathogen in tissue; plant reported tolerant to disease, but source of vectors (Lee 1996)
Citrus × *limonia* Osbeck	Miyakawa & Yuan 1990; Tirtawidjaja 1981	symptoms
Citrus × *nobilis* Lour. 'Ortanique'	Koizumi et al. 1996	symptoms
Citrus × *nobilis* Lour.	Koizumi et al. 1996	symptoms
Citrus oto Hort. ex Tanaka	Miyakawa & Yuan 1990	symptoms
Citrus × *paradisi* Macfad.	Miyakawa & Yuan 1990	symptoms
Citrus reticulata Blanco	Miyakawa & Yuan 199; Tirtawidjaja 1981	symptoms
Citrus sinensis (L.) Osbeck	Miyakawa & Yuan 1990	symptoms, presence of putative pathogen in tissue
Citrus sunki Hort. ex Tanaka	Miyakawa & Yuan 1990	symptoms
Citrus unshiu (Mack.) Marc	Miyakawa & Yuan 1990	symptoms
Citrus sp. (mandarins)	Miyakawa & Yuan 1990	symptoms
Citrus sp. (pomelo/shaddock)	Miyakawa & Yuan 1990	symptoms

Halbert & Manjunath (2004)

Pathways for HLB Introduction

4) Movement of infected citrus relatives used as ornamentals in the urban landscape

Host list for *Candidatus Liberibacter* spp.

Species	Source	Comments
Poncirus trifoliata (L.) Raf.	Miyakawa 1980; Miyakawa & Yuan 1990; Nariani 1981; Koizumi et al. 1996	back inoculations (Miyakawa, Miyakawa & Yuan)
Severinia buxifolia (Poiret) Ten.	Hung et al. 2000; Koizumi et al. 1996	DNA hybridization with specific probe; symptoms
Swinglea glutinosa (Blanco) Merr.	Tirtawidjaja 1981	symptoms only, vector transmission
Toddalia lanceolata Lam	Korsten et al. 1996	DNA/DNA hybridization, PCR
Triphasia trifolia (Burm. f.) P. Wilson	Koizumi et al. 1996	severe stunting, vector transmission
Possible non-hosts:		
Citrus indica Tanaka	Bhagabati 1993	no symptoms in the field in endemic area
Citrus limetta Risso	Nariani 1981	no symptoms; laboratory inoculation (does not specify how)
Citrus macroptera Montrons	Bhagabati 1993	no symptoms in the field in endemic area
Cuscuta australis R. Br. (Convolvulaceae (Cuscutaceae))	Su & Huang 1990	observed to multiply in stems, haustoria and flower stalks
Fortunella spp.	Miyakawa & Yuan 1990	symptoms
Limonia acidissima L.	Koizumi et al. 1996; Su et al. 1995; Hung et al. 2000	symptoms only; vector transmission; DNA hybridization (Su et al.); infection apparently temporary (Hung et al.)
Microcitrus australasica (F. J. Muell.) Swingle	Koizumi et al. 1996	stunting
Murraya koenigii (L.) Sprengel	Hung et al. 2000	no detection by dot hybridization after attempted graft transmission; no symptoms (Hung et al.)
Murraya paniculata (L.) Jack	Tirtawidjaja 1981; Aubert et al. 1985; Miyakawa 1980; Hung et al. 2000, Koizumi et al. 1996; Toorawa 1998	Mixed results: symptoms only (external and internal), vector transmission (Tirtawidjaja); can harbor greening organism (Aubert et al.). EM negative (Miyakawa); No detection by dot hybridization after attempted graft transmission (Hung et al.); no symptoms (Koizumi et al.); not a host (Toorawa)
Nicotiana tabacum L. 'Xanthii' (Solanaceae)	Garnier & Bové 1993	symptoms, dodder transmission only
Aeglopsis chevalieri Swingle	Koizumi et al. 1996	questionable symptoms
Atalantia missionis Oliver	Tirtawidjaja 1981	symptoms only, vector transmission
Balsamocitrus dawei Stapf.	Koizumi et al. 1996	symptoms only; vector transmission
Calodendrum capensis Thunb.	Garnier et al. 2000	molecular characterization
Catharanthus roseus (L.) G. Don (Apocynaceae)	Tirtawidjaja 1981	symptoms, electron microscopy; (dodder transmission only)
X *Citroncirus webberi* J. Ingram & H. E. Moore	Miyakawa & Yuan 1990; Nariani 1981	symptoms (few) stunting, seed abortion (Miyakawa & Yuan); symptoms fairly intense (Nariani)
Clausena indica Oliver	Miyakawa & Yuan 1990	symptoms (stunting)
Clausena lansium (Lour.) Skeels	Tirtawidjaja 1981; Koizumi et al. 1996	symptoms only, vector transmission

Halbert & Manjunath (2004)

Host for *Candidatus Liberibacter* asiaticus

Vepris lanceolata

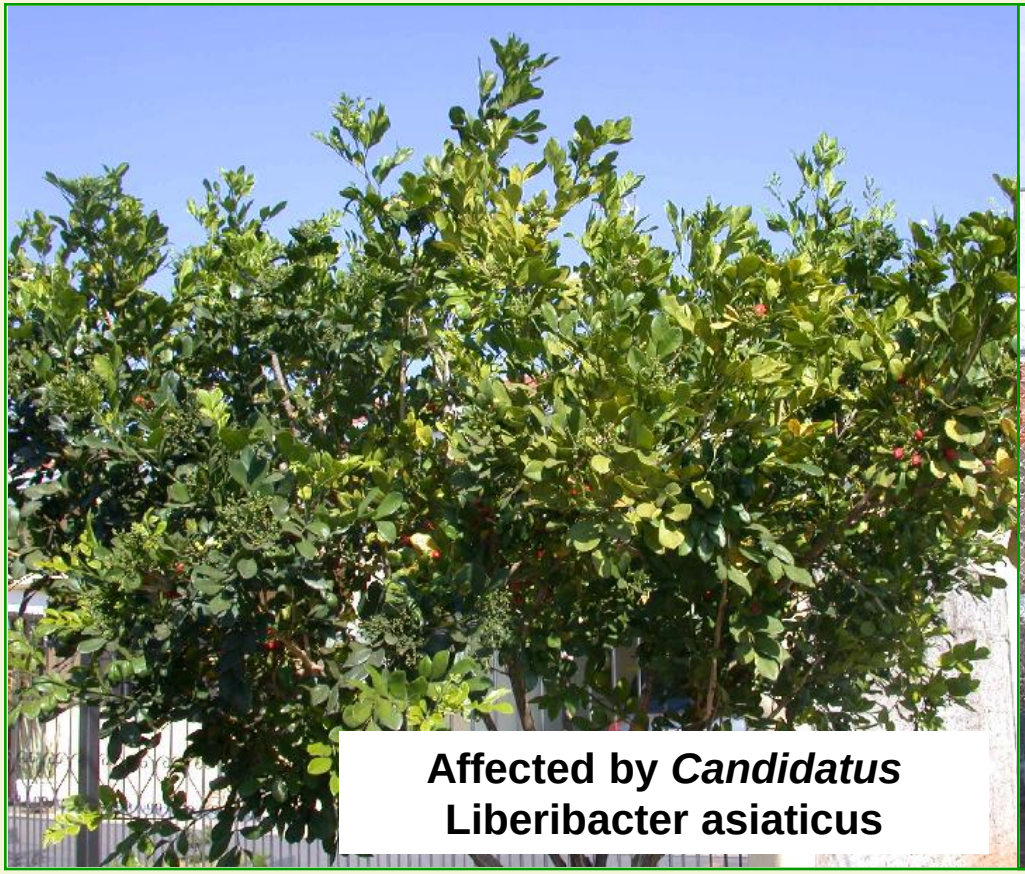
Limonia acidissima

Host for *Candidatus Liberibacter* asiaticus

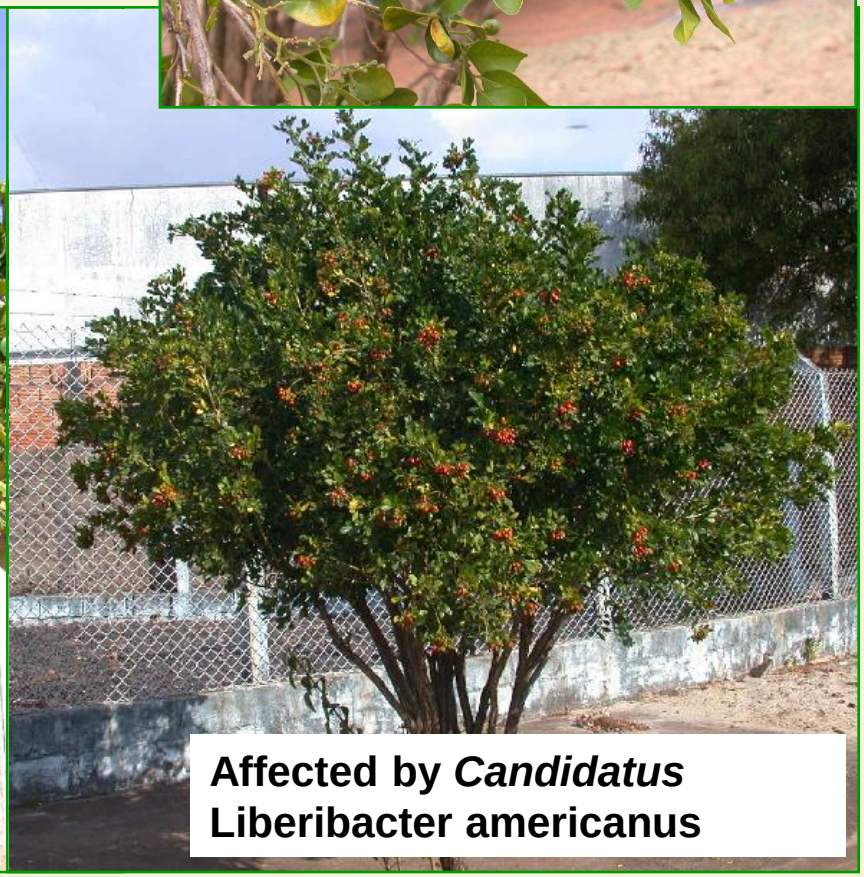
Murraya paniculata

Severinia buxifolia

Murraya paniculata (orange jasmine)
Host of bacterium and vector



Affected by *Candidatus* Liberibacter asiaticus



Affected by *Candidatus* Liberibacter americanus

Detection of '*Candidatus* Liberibacter asiaticus' in *Diaphorina citri* and Its Importance in the Management of Citrus Huanglongbing in Florida

K. L. Manjunath, S. E. Halbert, C. Ramadugu, S. Webb, and R. F. Lee

doi:10.1094/PHYTO-98-4-0387

TABLE 1. Analysis of selected psyllid samples from Florida by conventional polymerase chain reaction (cPCR) and quantitative real-time PCR (qPCR) cycle threshold (Ct) values in real-time PCR tests for '*Candidatus* Liberibacter asiaticus' (Ct Las) and *Diaphorina citri* (Ct DC) are shown

ID[a]	Date of collection	Host	Category[b]	County	Psyllids/extraction[c]	Ct Las	Ct DC	qPCR[d]	cPCR
F0001	8.10.2005	*Murraya paniculata*	Nursery	Miami-Dade	3	32.13	27.14	N	N
F0003	8.10.2005	*M. paniculata*	Nursery	Miami-Dade	6	0.00	24.36	N	N
F0003n	8.10.2005	*M. paniculata*	Nursery	Miami-Dade	10	35.70	24.98	N	N
F0004	9.29.2005	*Citrus aurantifolia*	Grove	Miami-Dade	6	39.65	25.15	N	N
F0005	10.5.2005	*C. aurantifolia*	Residential	Palm Beach	2	33.57	26.12	N	N
F0005n	10.5.2005	*C. aurantifolia*	Residential	Palm Beach	10	29.03	24.12	P	W
F0006	9.30.2005	*C. limon*	Residential	Broward	5	37.15	24.90	N	N
F0007	9.30.2005	*C. aurantium*	Nursery	Miami-Dade	4	0.00	25.75	N	N
F0007n	9.29.205	*C. aurantium*	Nursery	Miami-Dade	7	36.85	22.61	N	N
F0008	8.10.2005	*M. paniculata*	Nursery	Miami-Dade	1	36.13	28.80	N	N
F0009	9.30.2005	*C. sinensis*	Residential	Broward	2	35.12	27.24	N	N
F0010	10.1.2005	*Citrofortunella*	small farm	Miami-Dade	1	31.49	27.45	N	N
F0010n	10.1.2005	*Citrofortunella*	small farm	Miami-Dade	5	34.52	27.33	N	N
F0011	10.5.2005	*C. aurantium*	Residential	Palm Beach	3	24.56	31.80	P	P
F0012	10.7.2005	*M. paniculata*	Nursery	Miami-Dade	5	0.00	25.81	N	N
F0013	10.5.2005	*M. paniculata*	Nursery	Miami-Dade	2	35.15	27.21	N	N
F0014	10.5.2005	*Bergera koenigii*	Nursery	Miami-Dade	3	34.71	23.17	N	N
F0015	9.9.2005	Suction trap	Suction trap	Polk	1	25.66	31.60	P	P
F0016	9.15.2005	*M. paniculata*	Garden Center	Nassau	2	24.75	25.56	P	P
		M. paniculata	Residential	Palm Beach	2	24.27	30.84	P	P
		C. sinensis	Grove	Polk	2	25.48	27.40	P	P
		C. sinensis	Grove	Polk	5	18.75	29.94	P	P
		Citrofortunella	Grove	Polk	1	24.26	28.91	P	nd
		M. paniculata	Nursery	Miami-Dade	3	27.39	25.60	P	P
		M. paniculata	Nursery	Miami-Dade	5	22.16	26.01	P	P
		C. sinensis	Grove	Collier	50	23.20	29.50	P	P
		C. sinensis	Grove	De Soto	50	24.30	26.17	P	P
		Citrus sp.	Nursery	Marion	2	28.55	25.71	P	nd
		C. sinensis	Garden Center	Marion	10	30.49	20.93	P	nd
		Citrus sp.	Grove	Marion	5	29.70	24.71	P	nd
		C. sinensis	Garden Center	Marion	5	27.65	22.23	P	nd
		C. sinensis	Garden Center	Marion	10	25.86	23.24	P	nd
		C. sinensis	Grove	Marion	5	26.71	23.74	P	nd
		C. sinensis	Grove	Marion	11	27.05	23.21	P	nd
		Citrus sp.	Nursery	Marion	1	27.34	28.65	P	nd
		Citrus sp.	Nursery	Marion	5	25.50	23.51	P	nd
		Citrus sp.	Nursery	Marion	4	29.36	28.01	P	nd
		Citrus sp.	Nursery	Marion	4	28.42	23.74	P	nd
		Citrus sp.	Nursery	Marion	10	27.62	22.83	P	nd
		Citrus sp.	Nursery	Marion	5	24.46	22.34	P	nd
F0552n	6.5.2006	*Citrus* sp.	Nursery	Marion	11	28.05	23.51	P	nd

PCR positive psyllid samples collected from *Murraya* or *Citrus* nurseries or garden centers

"The study suggests that discount garden centers and retail nurseries may have played a significant role in the widespread distribution of psyllids and plants carrying HLB pathogens in Florida."

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HLB Epidemiology: difficult study

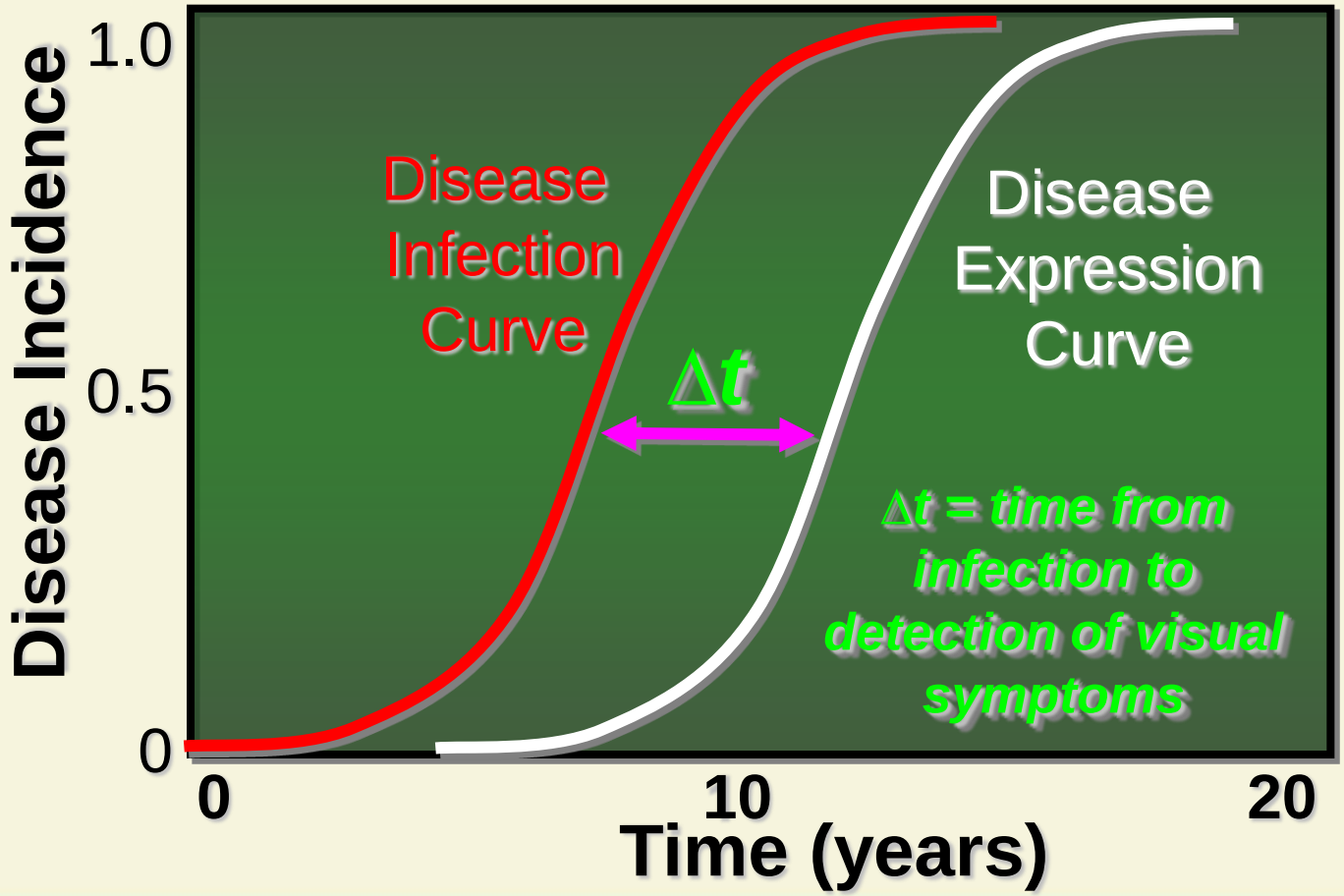
- **Locate study sites where the disease can be allowed to progress without intervention of control activities for multiple years**

Severe damages on fruit yield and quality

HLB Epidemiology: difficult study

- **LONG and VARIABLE Incubation Period:**

lag in time between pathogen transmission by ACP and the onset of visual symptoms



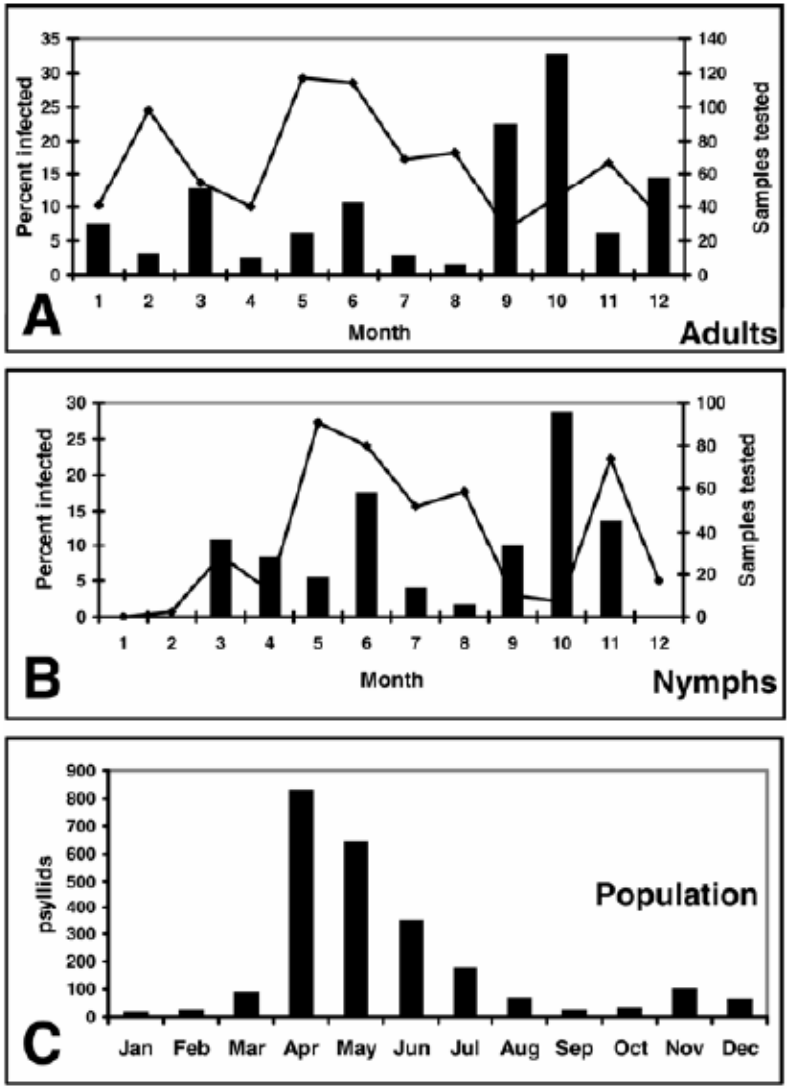


Fig. 4. Detection of '*Candidatus* Liberibacter asiaticus' in **A,** psyllid adult and **B,** nymph samples collected from different parts of Florida in different months. Total number of samples tested each month (line graph) and percent samples with huanglongbing incidence (bar graph) are shown in each graph. Monthly data on the number of psyllids collected from a suction trap from March 2006 to February 2007, located in a citrus grove in Palm Beach is shown in graph **C**.

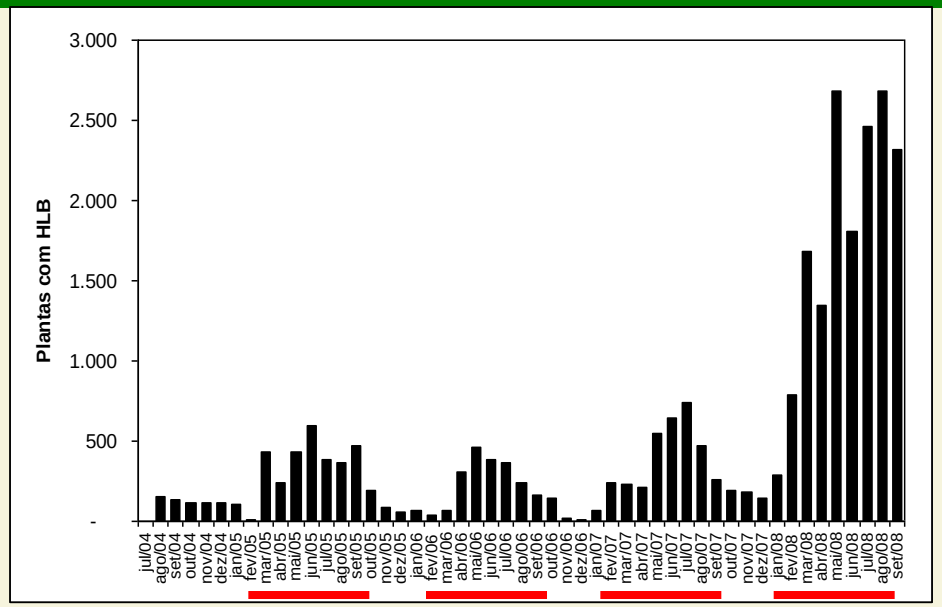
Infected psyllid were found throughout the year

No correlation between % of infected adults and nymph with highest population

Highest psyllid population when new flush is available (spring and summer)

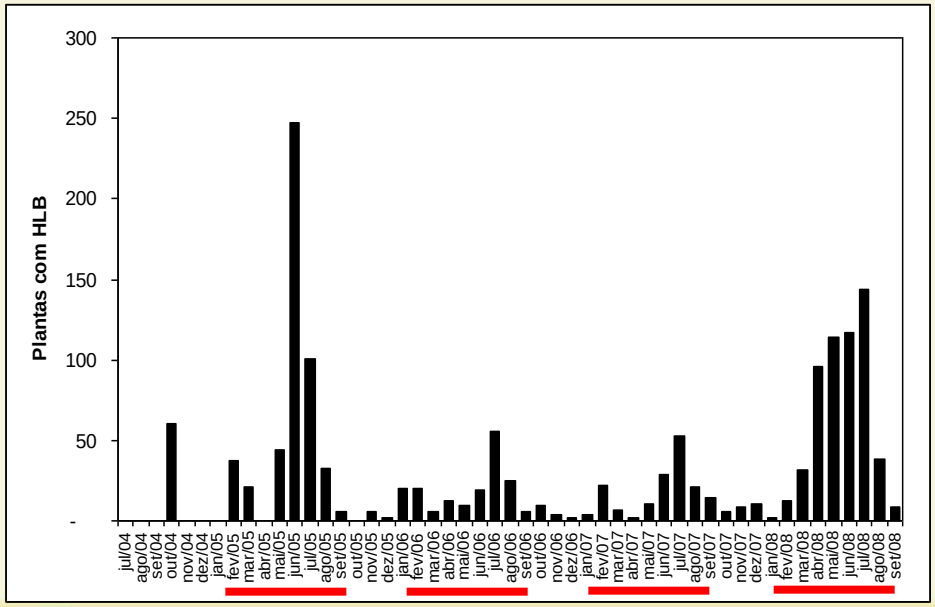
Manjunath et al. (2008)

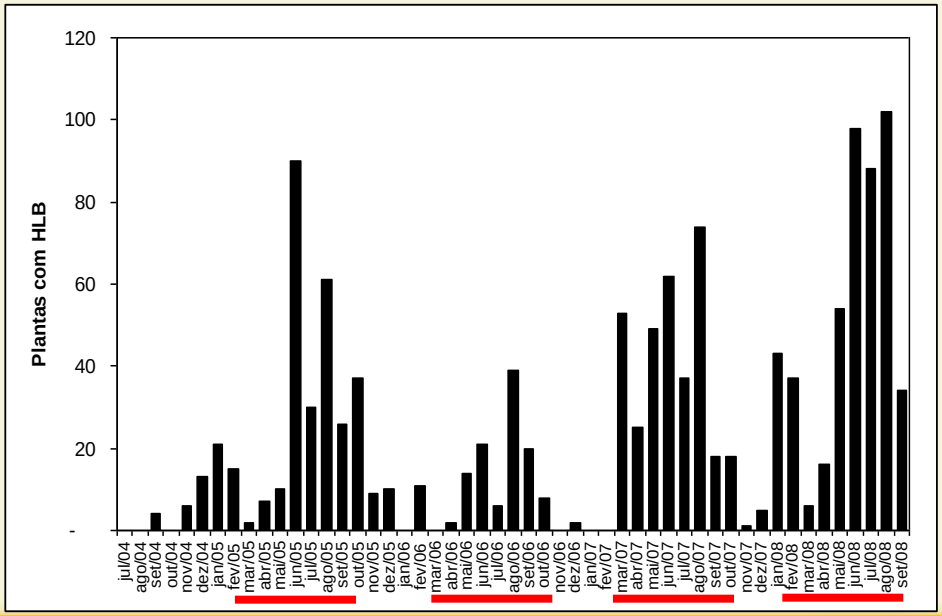
Seasonality of HLB symptom expression



End of summer and beginning of spring
Maximum between autumn and winter

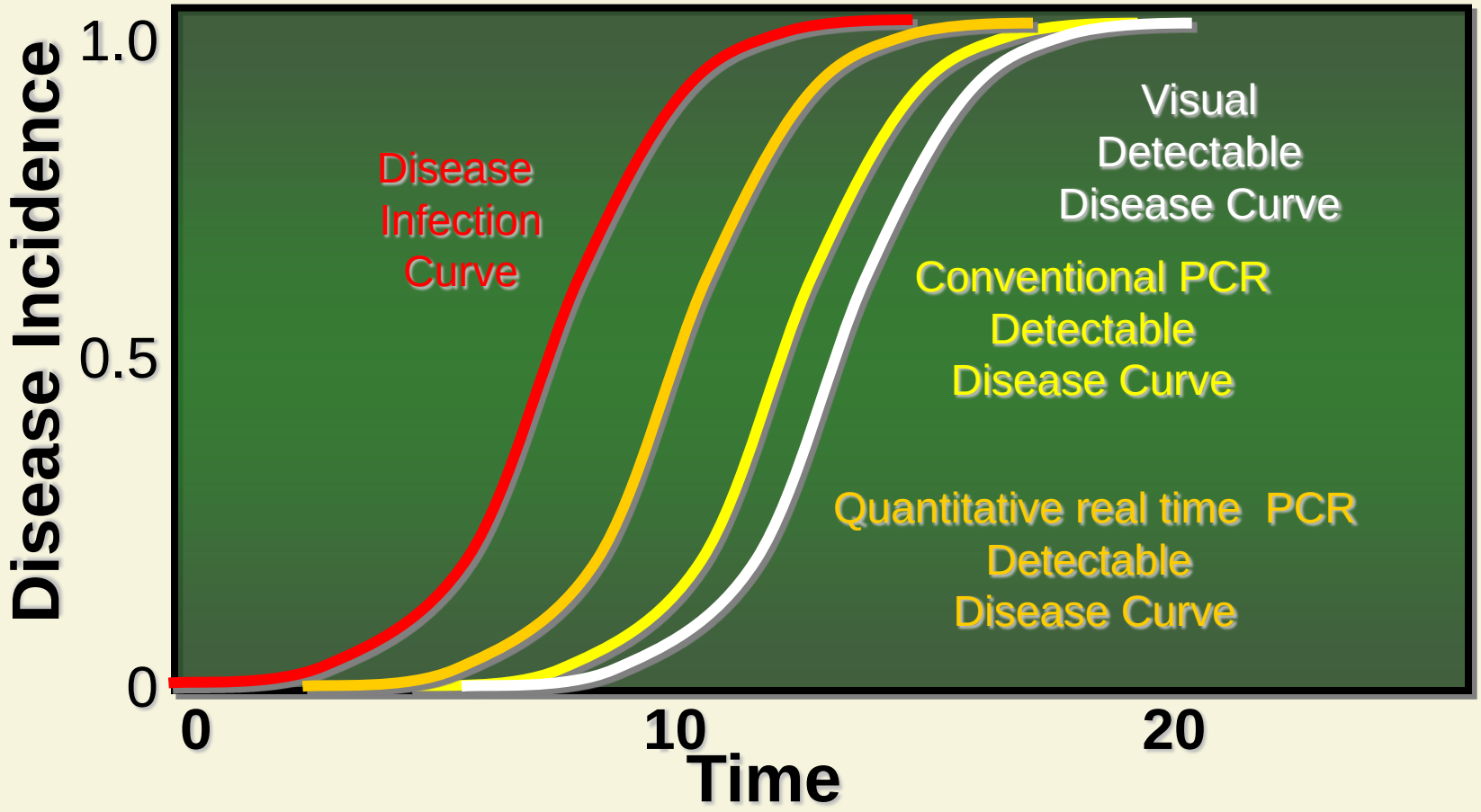
Trees expressing the onset of infection at the same time may have been infected at different times in the past





HLB Epidemiology: difficult study

- **NO ability to detect an infection for some time after vector transmission:**
  Takes time for the bacteria becomes systemic and reaches detectable titer



HLB Epidemiology: difficult study

Proc. Fla. State Hort. Soc. 119:89-93. 2006.

A REFEREED PAPER

COMPARISON OF VISUAL ASSESSMENT AND POLYMERASE CHAIN REACTION ASSAY TESTING TO ESTIMATE THE INCIDENCE OF THE HUANGLONGBING PATHOGEN IN COMMERCIAL FLORIDA CITRUS

MICHAEL S. IREY[1]*, TIM GAST[2] AND TIM R. GOTTWALD[3]

Table 1. Percentage of trees with vi
for HLB in five 14 row by 14 tre

Grid designation (block: tree numbers evaluated)	Total nu of tre evaluat
C3: trees 20-33	189
C3: trees 71-84	187
C4: trees 11-24	196
C4: trees 61-74	196
C4: trees 81-94	195

New data estimated 2 to 56 HLB-positive but asymptomatic trees for every symptomatic tree!

(Gottwald unpublished)

trees in Florida. Declining young tree (A), mottled leaves (B), leaves with green and yellow islands

*"The incidence of infection based on real-time PCR testing may be up to **two times** the incidence of infection estimated by visible symptoms alone"*

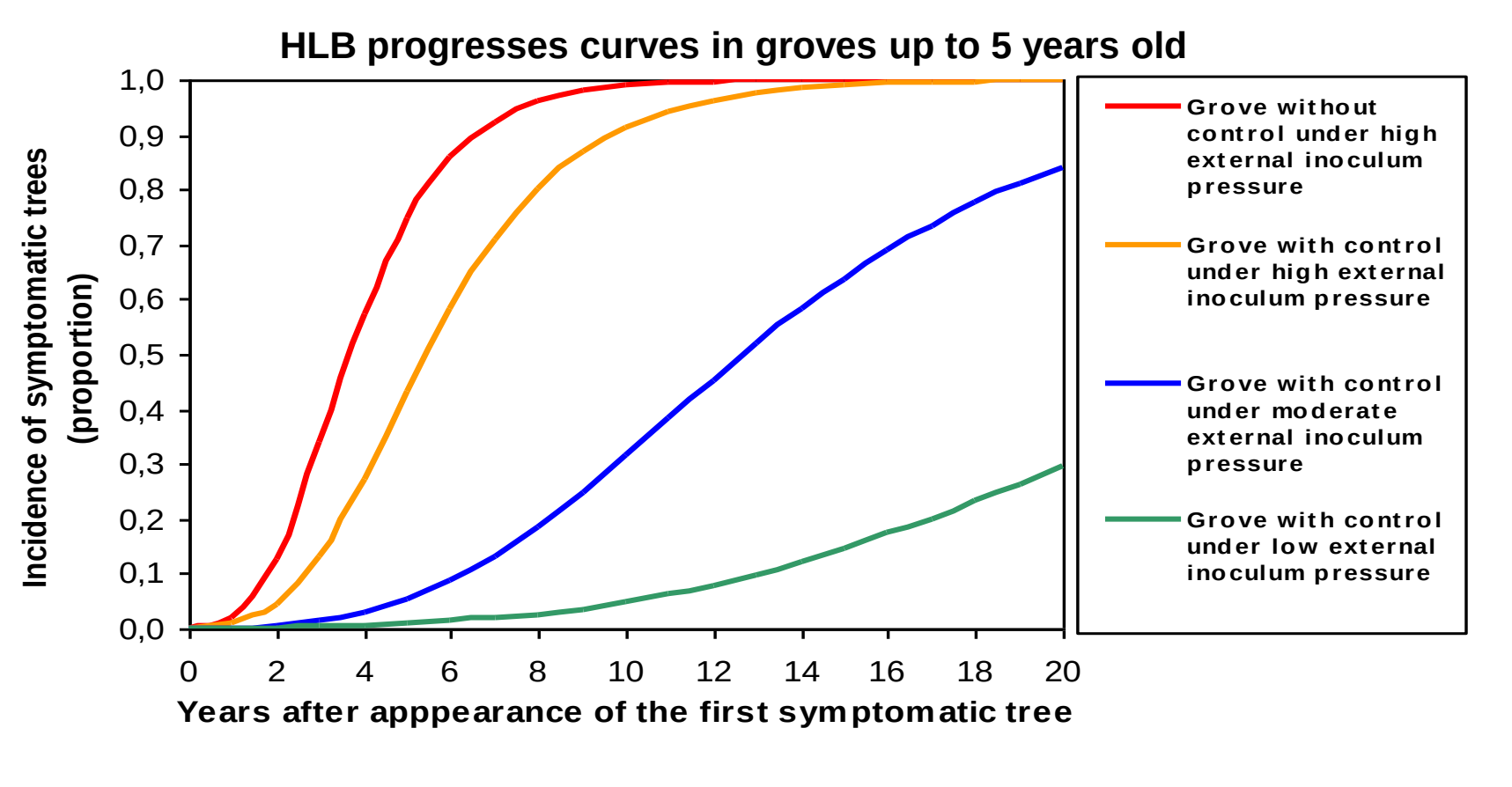
Assessments during the winter

HLB Epidemiology: temporal

Disease incidence progress is dependent on:

(i) Extent of inoculum reservoir

(ii) Local vector population



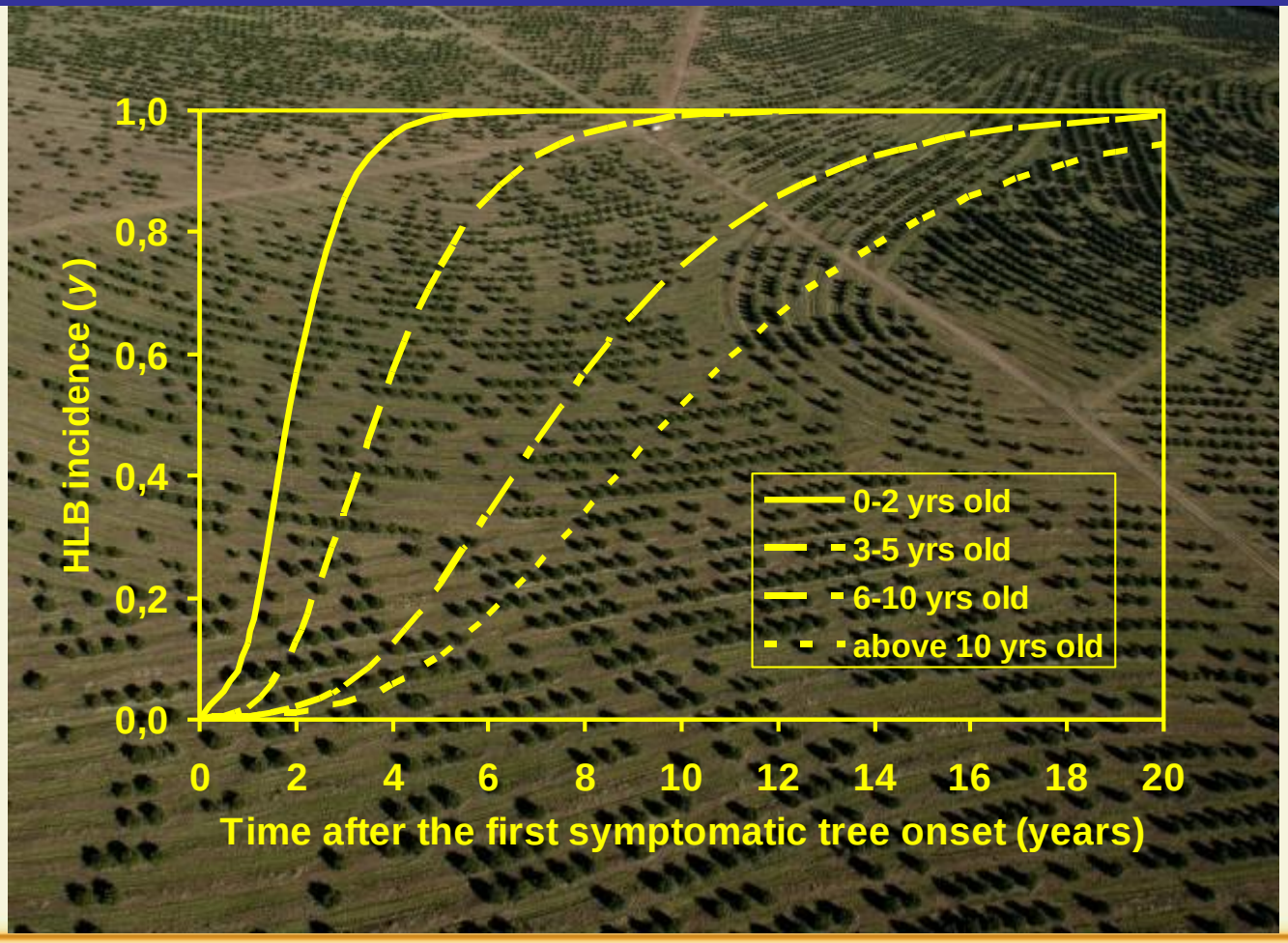
Control: monthly inspections and eradication + vector control when present

Bassanezi, 2008

HLB Epidemiology: temporal

Disease incidence progress dependent on:
(iii) Age of the grove at first infection

HLB incidence progress curves according to the age at first symptoms appearance



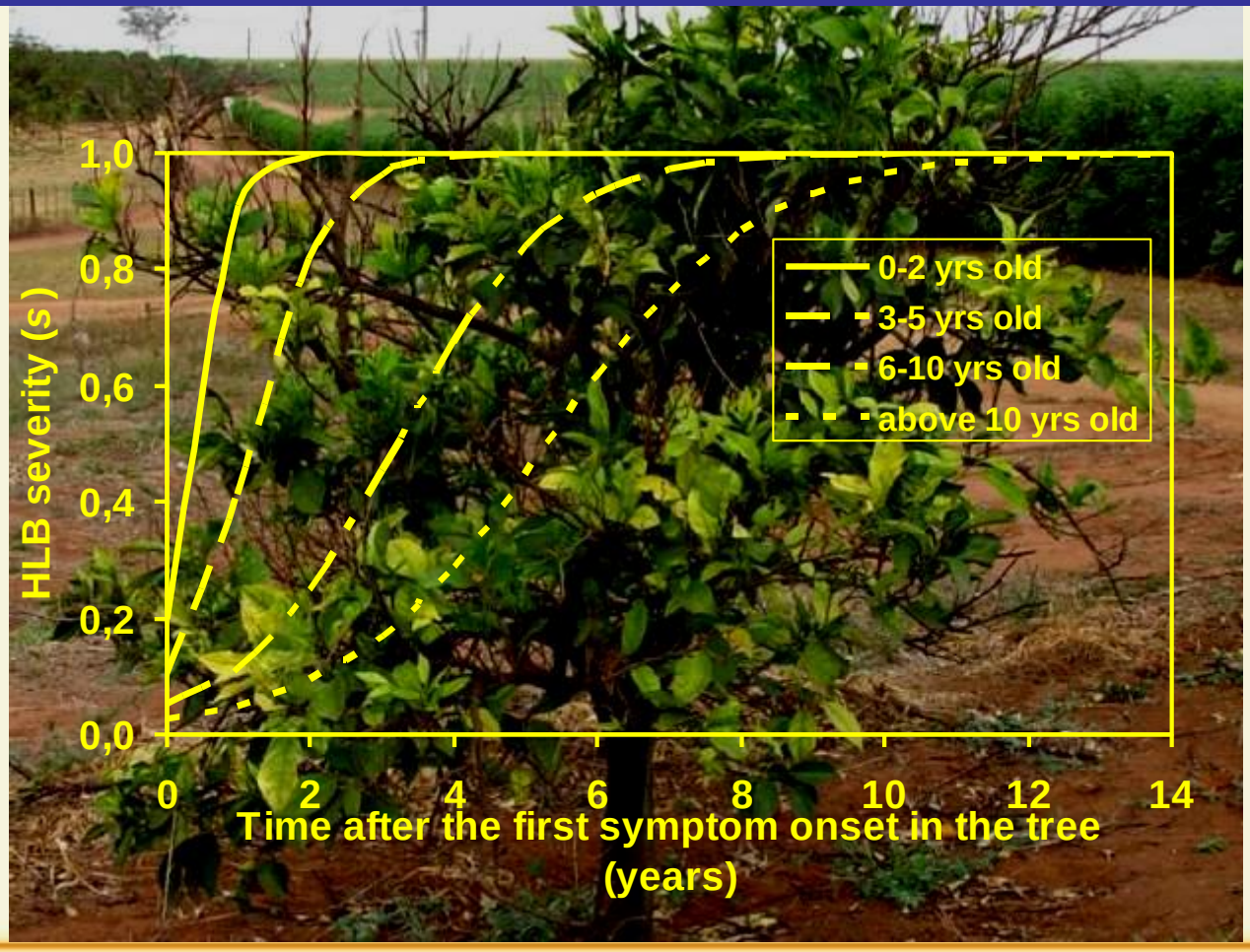
HLB Epidemiology: temporal

Disease severity progress dependent on:

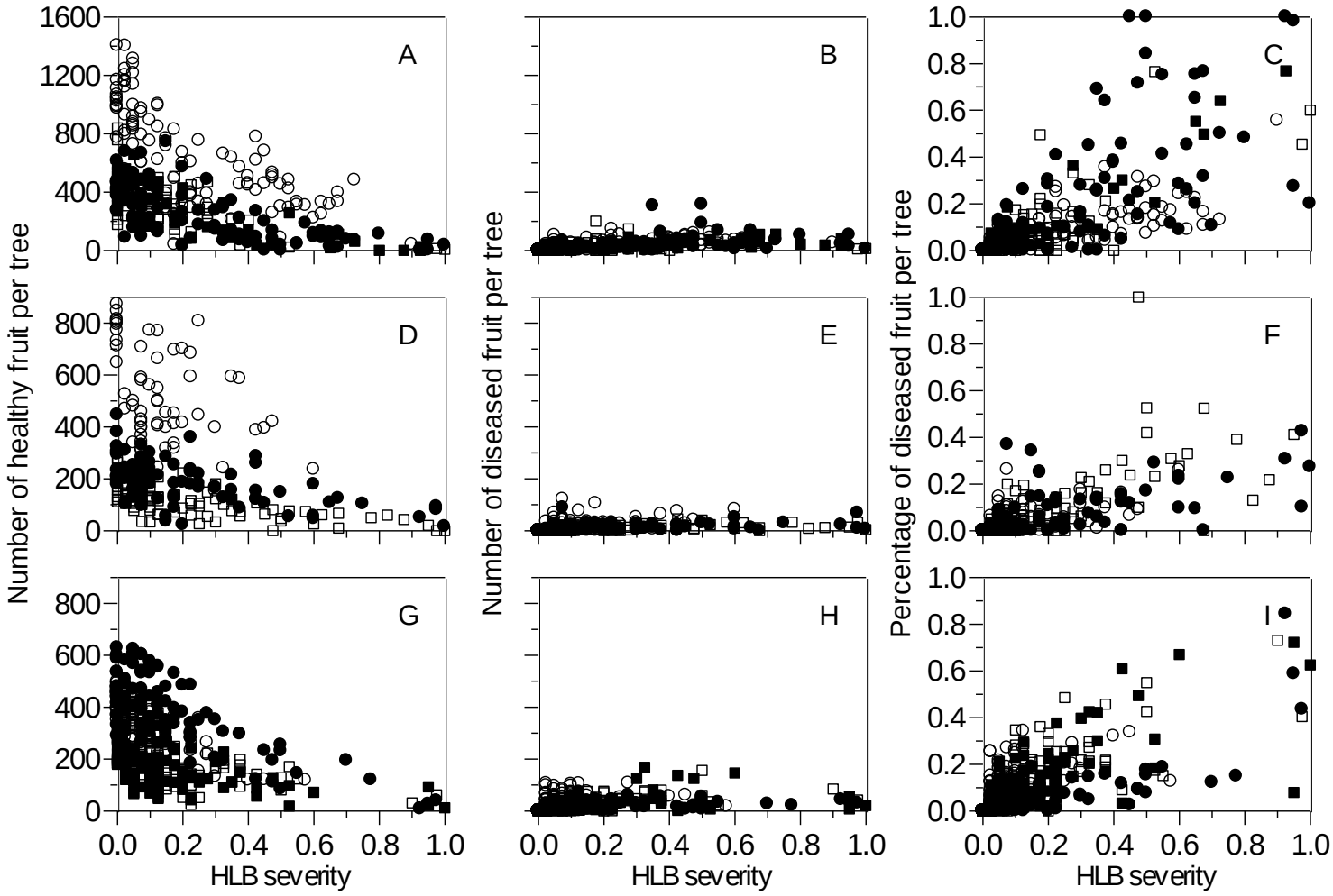
(i) Age of the grove at first infection

(ii) Cultivar

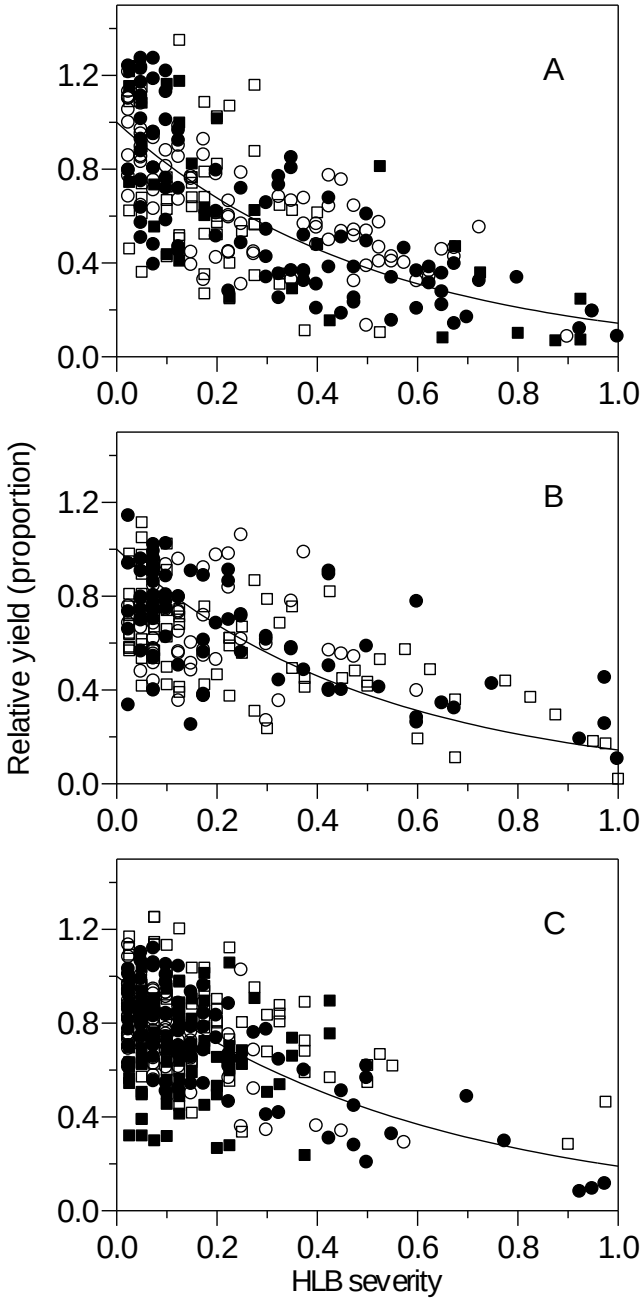
HLB severity progress curves according to the age at first symptoms appearance



HLB Epidemiology: damage



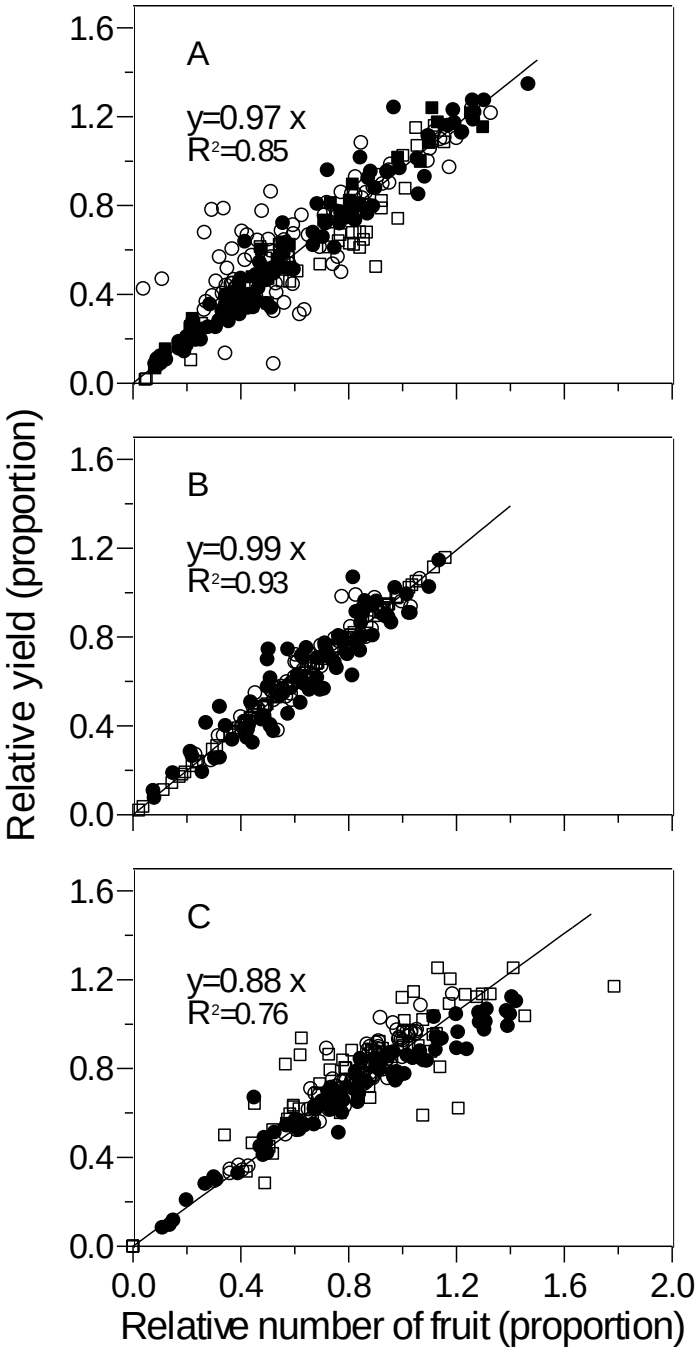
Relationship between HLB severity and the number of healthy (A, D, G) and diseased fruit (B,E,H) per tree, and the percentage of diseased fruit per tree (C,F,I), in early (A-C), mid-season (D-F) and late (G-I) sweet oranges cultivars in São Paulo State. Different symbols refer to different blocks. Bassanezi et al. (2009)



Relationship between HLB severity and relative yield in early (A), mid-season (B) and late (C) sweet oranges cultivars in São Paulo. Different symbols refer to different blocks.

Cultivars	R^2	*b* (± std error)
Early	0.44	1.95 ± 0.115
Mid-season	0.10	1.94 ± 0.134
Late	0.20	1.66 ± 0.087
All together	0.33	1.85 ± 0.063

Bassanezi et al. (2009)



Relationship between the relative number of fruit (number of fruit from diseased trees / average number of fruit from healthy trees) and relative yield (yield from diseased trees / average yield from healthy trees) in early (A), mid-season (B) and late (C) sweet oranges cultivars in São Paulo State. Different symbols refer to different blocks.

Most of reduction in yield is due to the early fruit drop or by lack of new fruit in affected branches

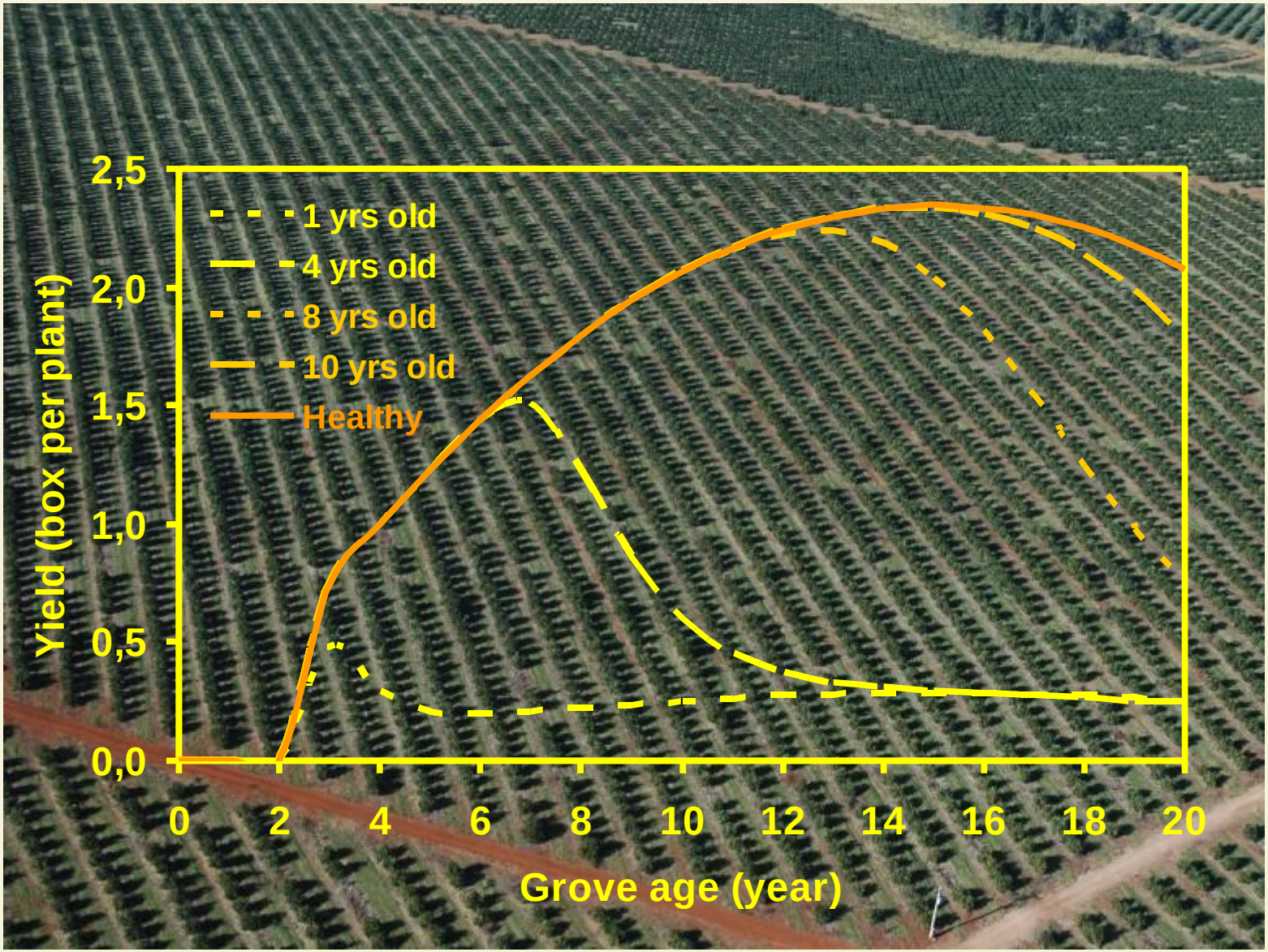
Bassanezi et al. (2009)

Table 3 Reduction (-) or increase (+), in percentage, on fruit quality variables of symptomatic fruit from huanglonbing symptomatic branches and normal fruit from asymptomatic branches for five sweet orange cultivars

Fruit quality variables	Reduction or increase (%)[y]				
	Val. Am. (*n*=40)	**Hamlin** (*n*=156)	**Pera** (*n*=130)	**Westin** (*n*=80)	**Valencia** (*n*=100)
Weight	-17.49 a	-27.53 b	-26.79 b	-39.62 c	-42.27 c
Diameter	-7.25 a	-12.51 b	-11.94 b	-18.32 c	-18.95 c
Height	-7.43 a	-12.73 b	-11.15 ab	-17.60 c	-16.96 c
Juice content	+4.78 a	-5.86 bc	-2.21 b	-6.97 cd	-10.40 d
Brix	-0.18 a	-6.39 a	-5.40 a	-16.14 b	-17.02 b
TSS/box	+4.32 a	-10.65 b	-7.09 b	-21.80 c	-25.15 c
TSS/fruit	-13.84 a	-32.66 b	-31.84 b	-51.82 c	-56.36 c
Acidity	+5.55 a	+20.35 b	+18.54 b	+17.83 b	+45.24 c
Ratio	-4.27 a	-18.91 b	-17.90 b	-27.40 c	-41.37 d

[y] Values with the letter in row were not different by Tukey highly significant difference test ($P>0.05$).

HLB Epidemiology: damage



Bassanezi et al. (2009)

HLB Epidemiology: spatial distribution

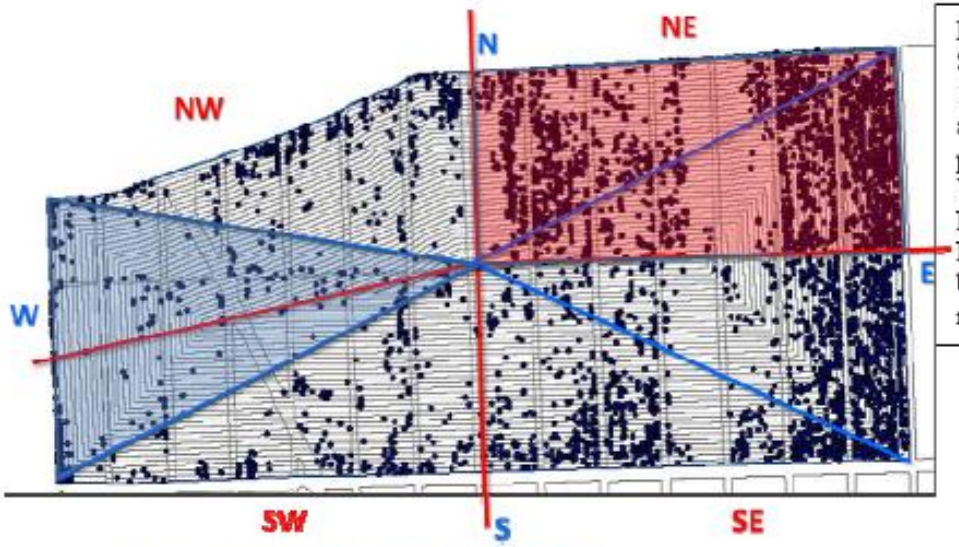


Figure 1. Portion of the SG plantation showing 10-m concentric annuli and parsing of plantation into N, E, S, W, and NE, SE, SW, NW quadrants. W and NE quadrates shown by blue and red shading, respectfully.

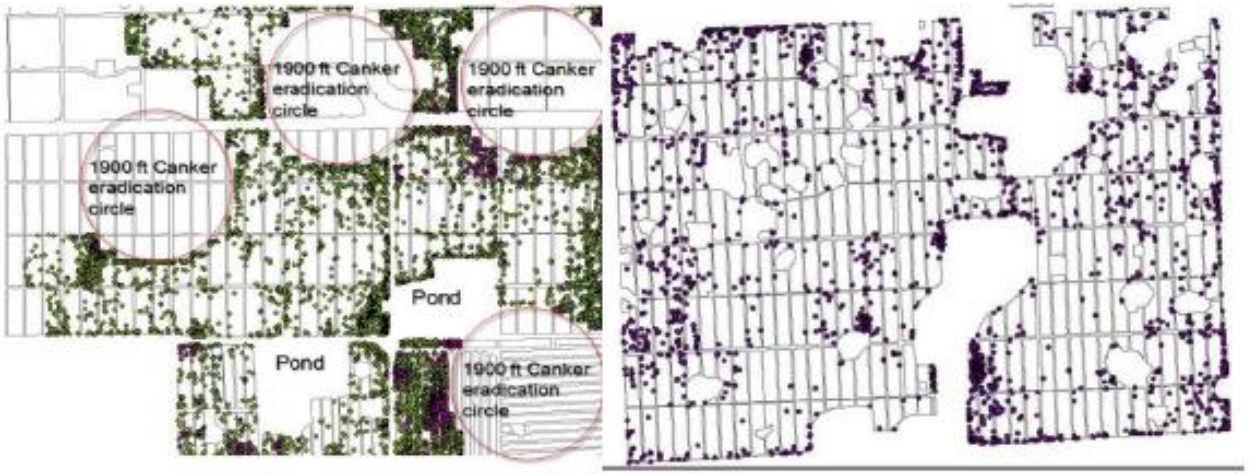


Figure 2. Demonstration of two additional plantations with perimeter edge effects. For the plantation to the right, note accumulation of HLB-positive trees in the plantation adjacent to voids caused by 1900-ft radus circles of tree removals to accomplish eradication of citrus canker.

Edge effects:
accumulation of HLB-positive trees at the interface of the planting with zones of non-citrus, not only at the perimeter of the planting, but also at voids internal to the planting created by roads, canals, and ponds)

Gottwald & Irey (2008)

HLB Epidemiology: spatial distribution

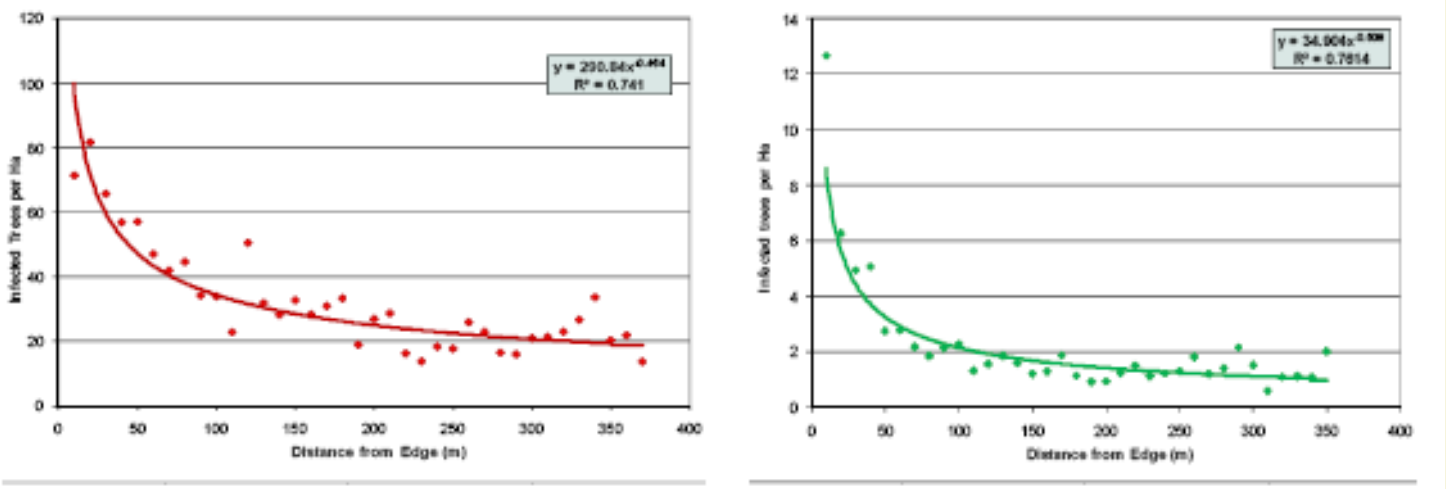


Figure 3. Perimeter edge effects of two commercial plantings in South Florida.

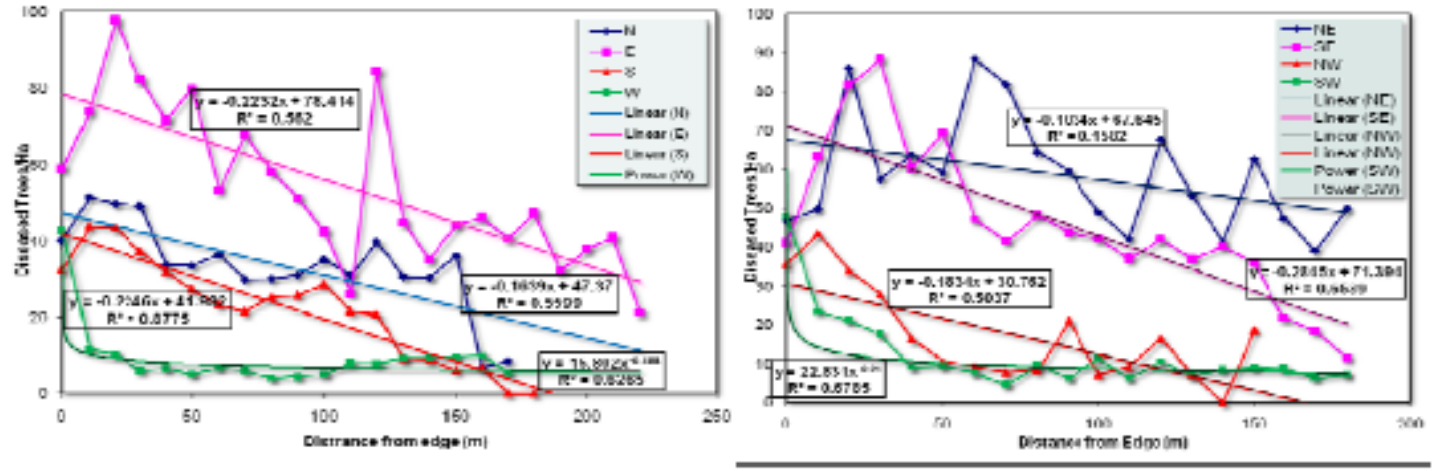


Figure 4. Directional disease gradients a large for a large commercial planting in south Florida demonstrating perimeter edge effects in all directions.

Edge effects:

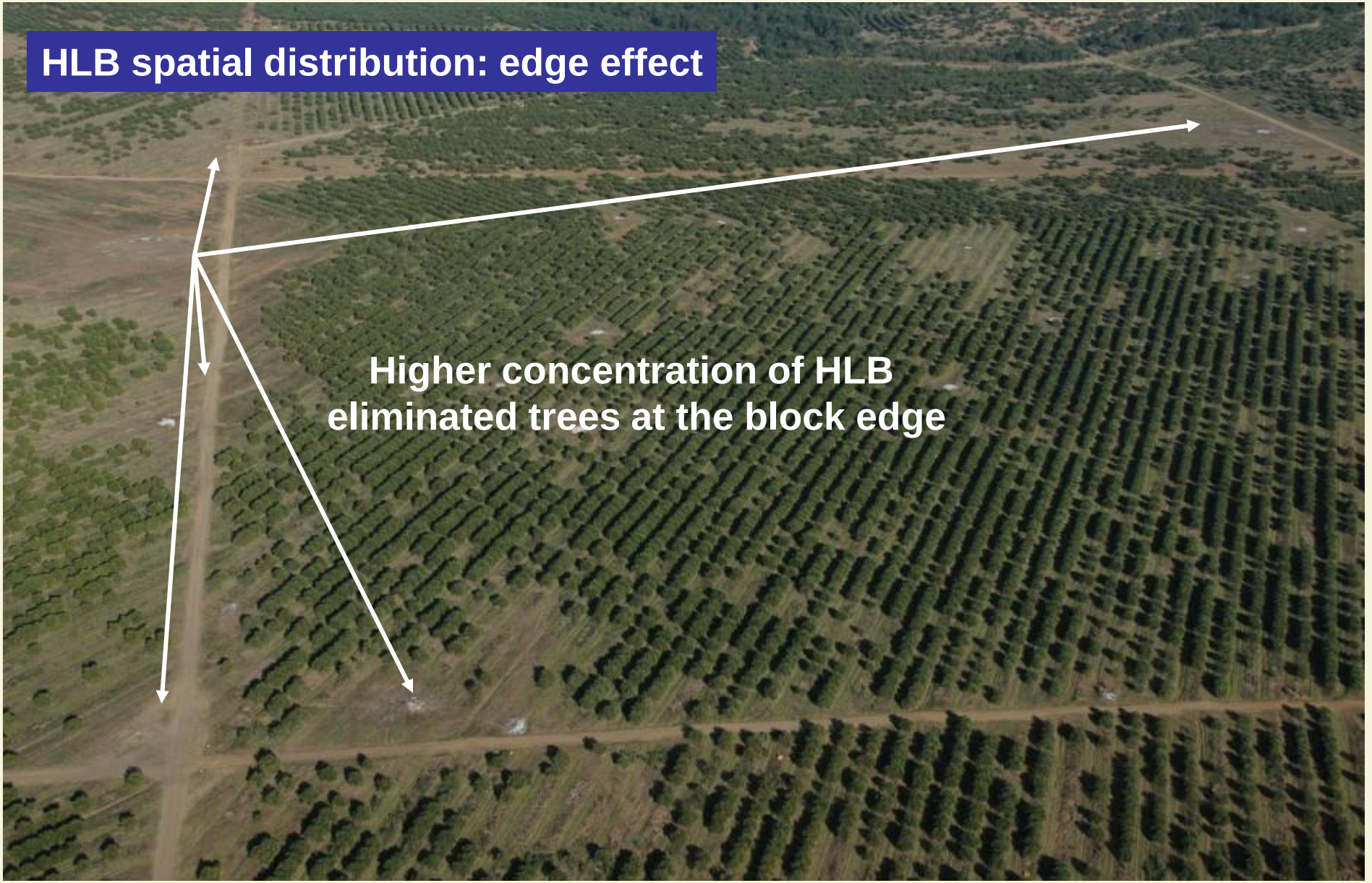
-Disease gradient from the perimeter (planting and block)

-Higher concentration at first 30 m

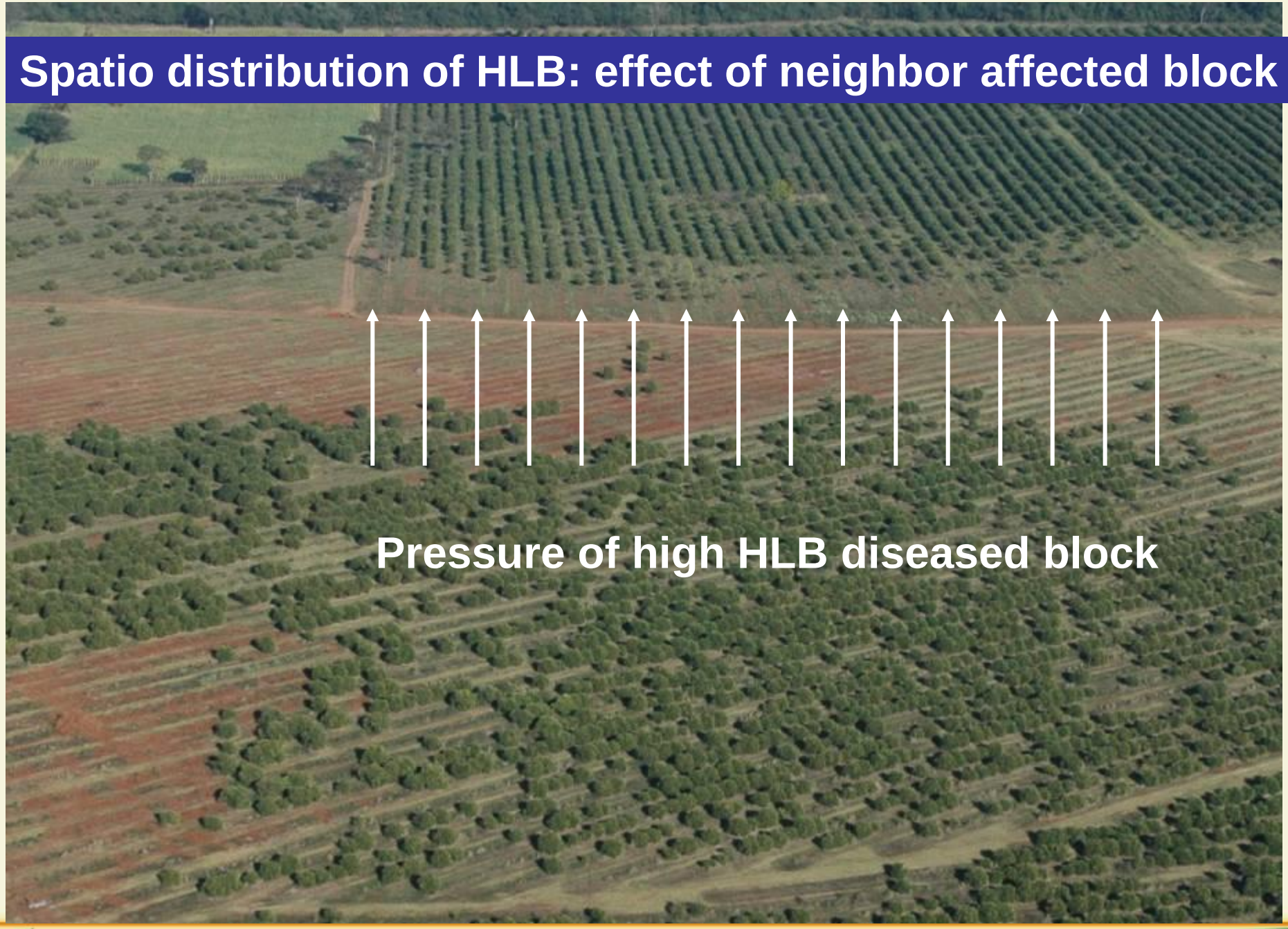
-Diseased trees at long distances

Gottwald & Irey (2008)

HLB spatial distribution: edge effect



Spatio distribution of HLB: effect of neighbor affected block



HLB Epidemiology: spatial distribution

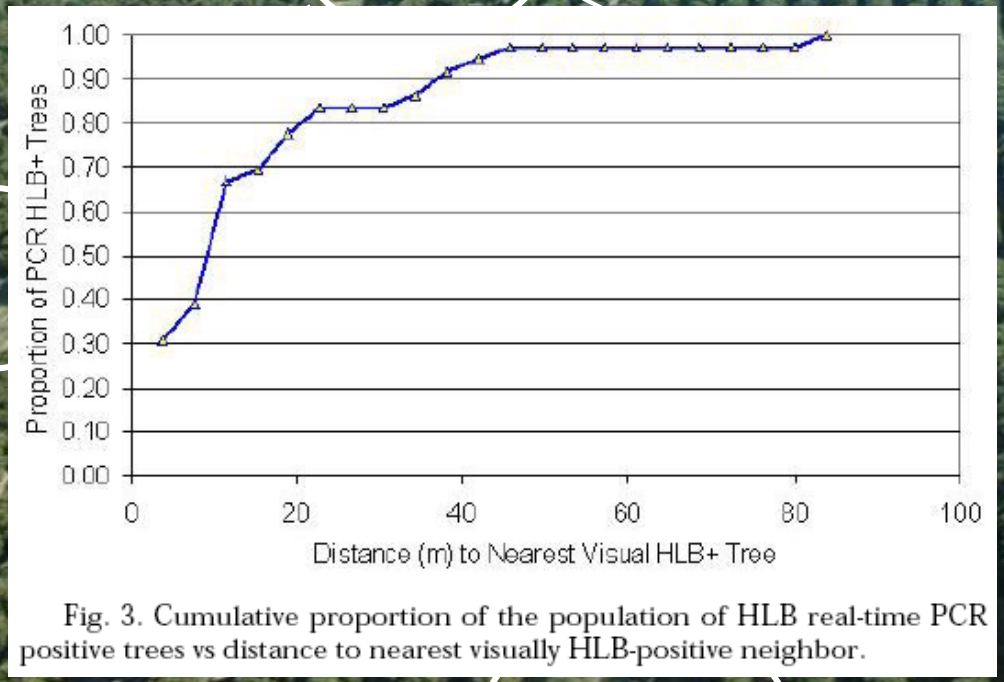


Fig. 3. Cumulative proportion of the population of HLB real-time PCR positive trees vs distance to nearest visually HLB-positive neighbor.

Aggregation:

- Some evidence of clustering among immediately adjacent trees, but not strong;

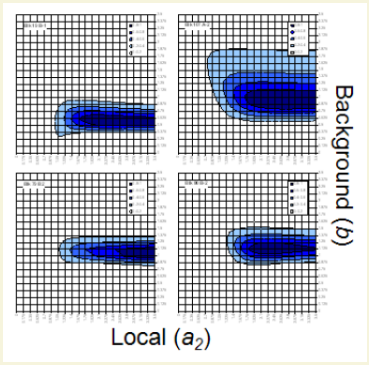
- Within-row aggregation slightly stronger than across-row aggregation;

- Core clusters found to be associated with secondary clusters as far as **25 to 50 m** apart

HLB Epidemiology: spatio-temporal

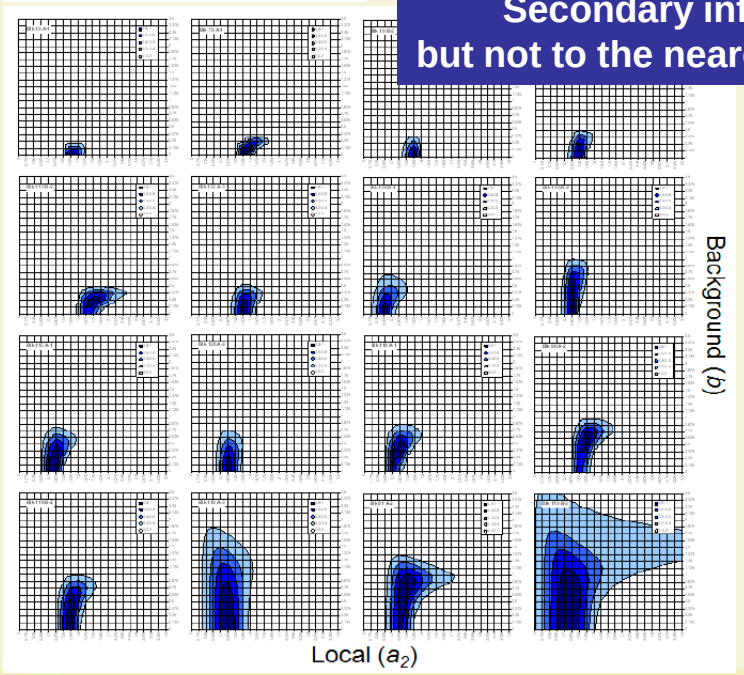
10.2 A Stochastic Spatiotemporal Analysis of the Contribution of Primary versus Secondary Spread of HLB.

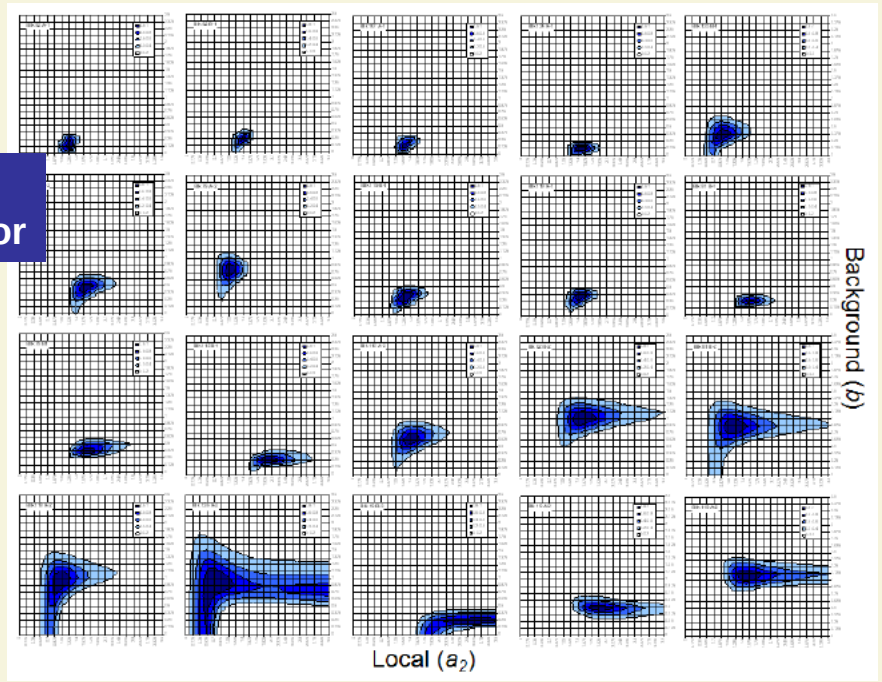
[1]*Gottwald, T.,* [2]Irey, M., [3]Bergamin-Filho, A., [4]Bassanezi, R, and [5]Gilligan, C.



Primary infection

Secondary infection, but not to the nearest neighbor





Mixed processes of primary and secondary infections

HLB Epidemiology: spatial distribution

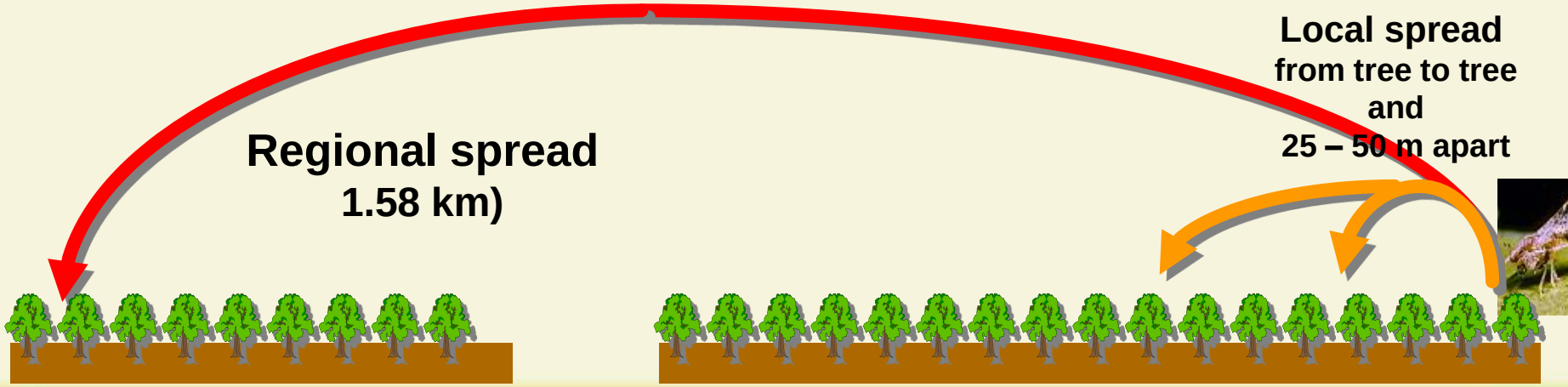
Local spread:

- From one tree to those within the immediate vinicity as well as over larger scale to trees at 25 to 50 m distance, the latter initiating new foci of infection.

Regional spread:

- Continuous relationship among HLB-diseased trees over a broad range of spatial distances up to 3.5 km;

- The most common distance between pairs of HLB-diseased trees ranged from 0.88 to 1.61 km (median 1.58 km);



HLB Epidemiology: spatial distribution

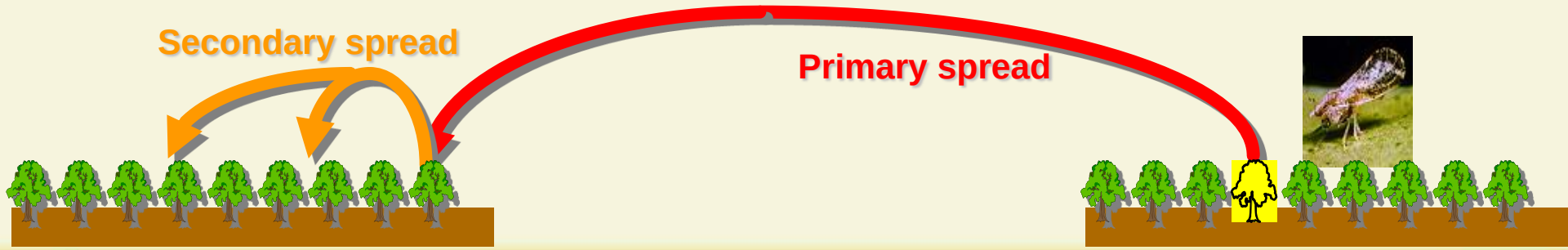
Secondary spread:

- Can be more or less mitigated by local insecticide applications and removal of symptomatic trees.

Primary spread:

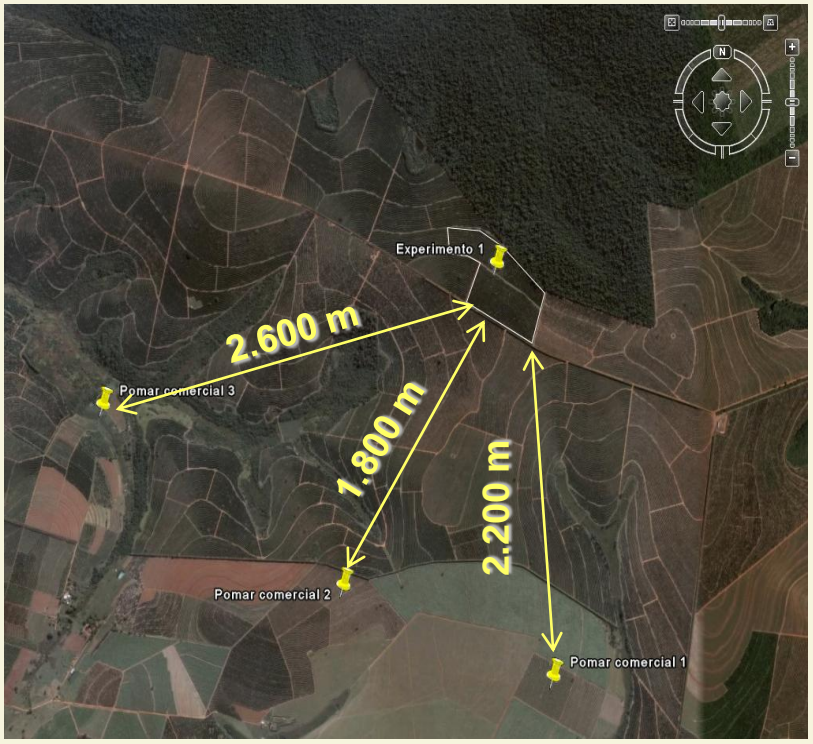
- Is the most hazardous kind of spread because even with large amount of local insecticide applications is difficult to stop psyllids from feeding on distant HLB-positive sources, migrating to uninfected trees at some distance, and transmitting the pathogen before they die from insecticide applied to the new trees they settle on.

Significant control of HLB will likely only be achieved from regional disease management



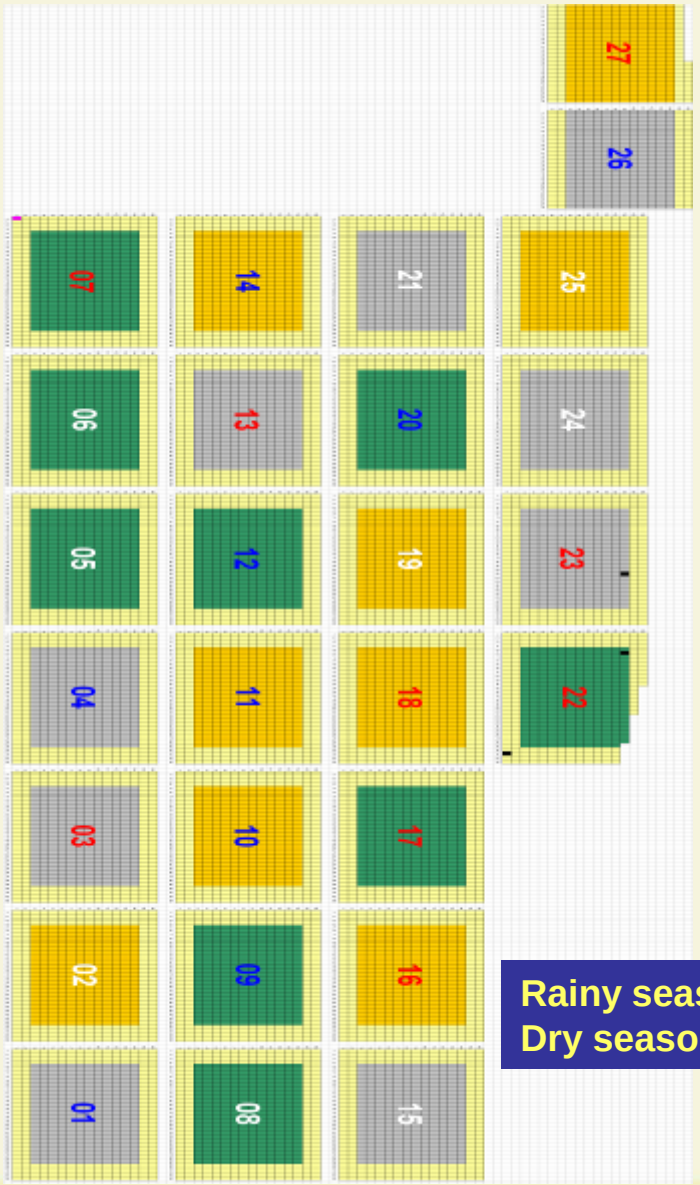
Study the effect of different frequencies of removal of HLB-symptomatic trees and vector control on HLB progress

EXPERIMENT 1



- In the middle of a large citrus farm with rigorous HLB control program since 2004
- Planting: October/2005
- 27 2.0-acres plots with 528 trees of Valencia Am/Swingle (16 rows with 33 trees)
- Spacing 6.00 m x 2.50 m

EXPERIMENT 1



TREATMENTS

3x3 Factorial design with 3 replications

Factor "Inoculum reduction"

- every 28 days
- every 56 days
- every 112 days

X

Factor "Vector control"

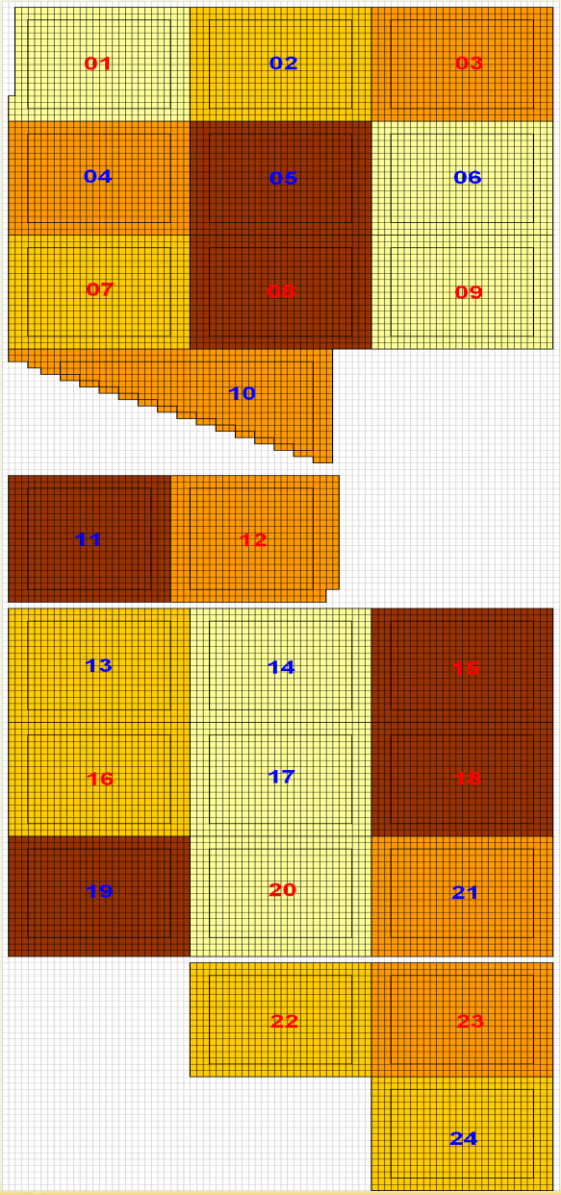
- no psyllid control
- psyllid control program A (every 28 days)
- psyllid control program B (every 14 days)

Rainy season: Aldicarb and Thiamethoxam (soil application)
Dry season: Imidachloprid, Dimethoate and Lambda-cyhalothrin (foliar spray)

EXPERIMENT 2

- In a small farm surrounded by severe HLB-affected farms until Jan/2007 and by many affected non-commercial citrus groves without control of HLB and psyllid.
- Planting: April/2006
- 24 2.4-acres plots with 504 Valencia/Rangpur lime (18 rows with 28 trees)
- Spacing 6.65 m x 2.90 m

EXPERIMENT 2



TREATMENTS

4x2 Factorial design with 3 replications

Factor "Inoculum reduction"

- every 14 days
- every 28 days
- every 84 days
- every 182 days

X

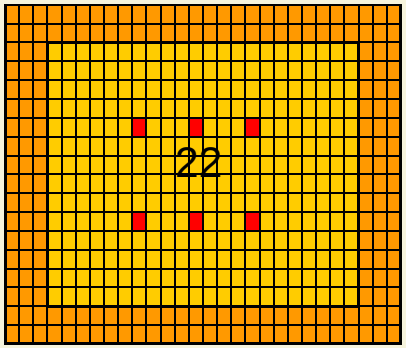
Factor "Vector control"

- no psyllid control
- psyllid control program C (every 14 days)

Rainy season: Aldicarb and Thiamethoxam (soil application)
Dry season: Imidachloprid, Dimethoate and Etofenprox (foliar spray)

ASSESSMENTS

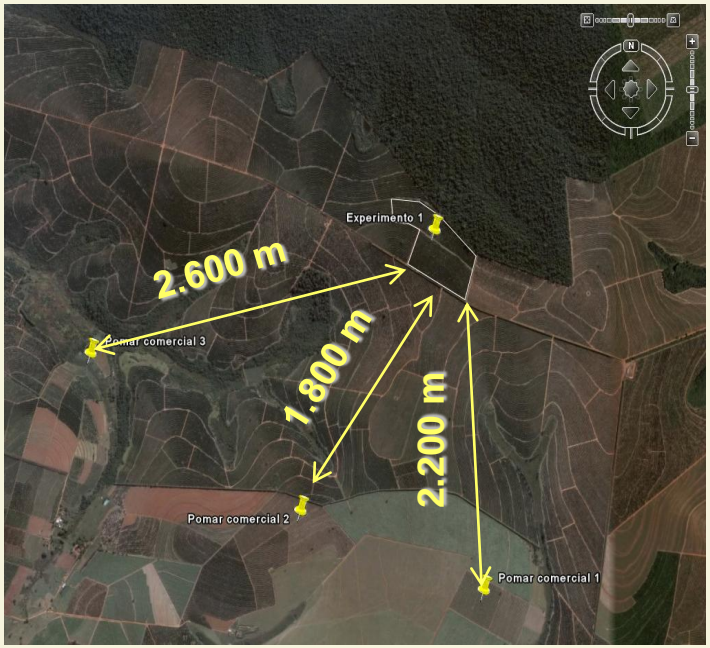
•**Psyllid population**:
- Fortnightly
- Adults counting in 6 yellow stick traps / plot



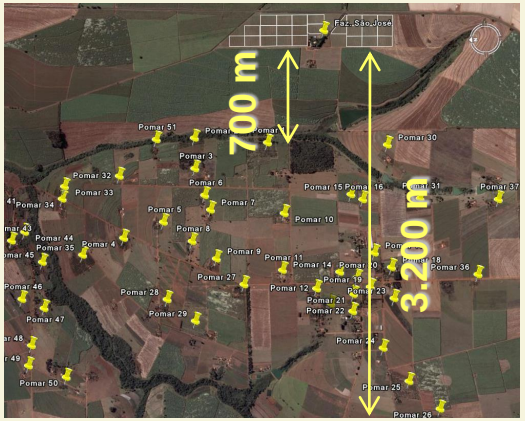
•**HLB incidence**
-Monthly (exp. 1) or fortnightly (exp. 2) inspection
-Visual observation of HLB symptoms
and PCR confirmation

PARTIAL RESULTS

EXPERIMENT 1



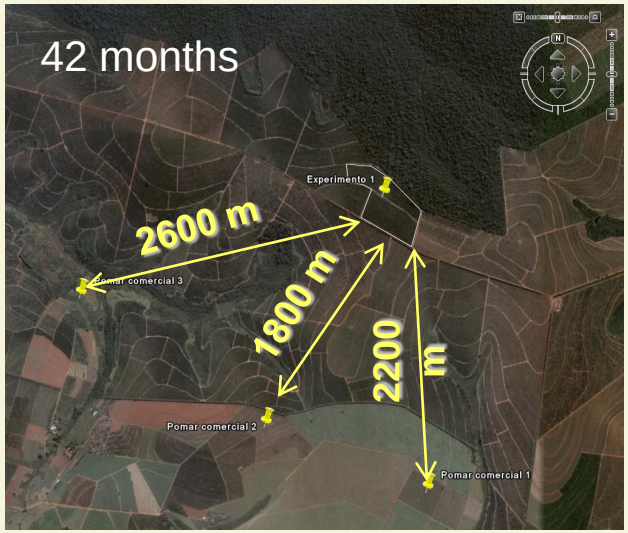
EXPERIMENT 2



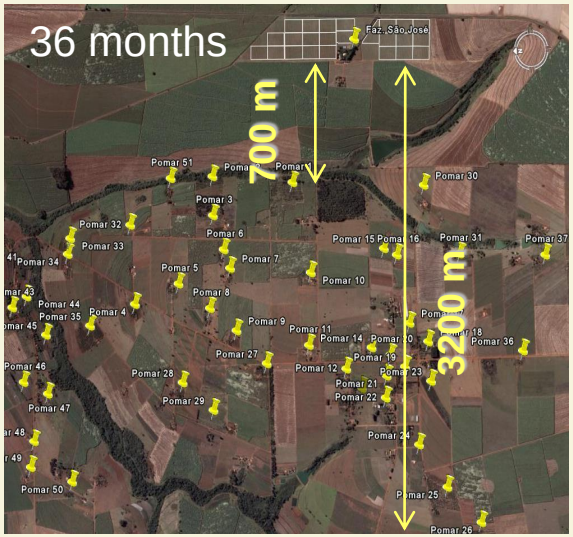
	Exp. 1	Exp. 2
Distance from external inoculum	>1800 m	~700 m
First HLB symptomatic tree	22 m.a.p.	13 m.a.p.
Psyllid population without vector control	2.4/month	7.7/month
Accum. HLB incid. without vector control	1.1%	36.0%

PARTIAL RESULTS

EXPERIMENT 1



EXPERIMENT 2



Vector control	Psyllid population*		HLB incidence (%)	
	Exp.1	Exp.2	Exp.1	Exp.2
No control	99.0 a	276.8 a	1.1 a	36.0 a
Program A (28d)	65.0 a		0.9 a	
Program B (14d)	71.9 a		0.7 a	
Program C (14d)		58.8 b		16.1 b

Treatments with the same letter in the column were not different by HSD Tukey´s test (*P* > 0.05)
***Mean of total number of caught adult psyllid**

Final Remarks

Movement of infected vegetative material can be controlled by quarantine regulations:

- Not easy with increasing of globalized trade and travel.

Presence of ACP in many countries of South, Central and North Americas and the recent reports of HLB in Brazil, USA, Cuba, Dominican Republic, Belize and Mexico:

- Risk of HLB introduction and spread within Western Hemisphere citrus producing areas or countries can be considered HIGH.

Final Remarks

Preventive strategies to avoid HLB introduction and to stop the spread:

(i) Inspection of host plants at the international ports, state borders, airports and mail-sorting facilities;

(ii) Establish quarantines for HLB, the Asian citrus psyllid, or both for areas with known HLB infection

(iii) Removal of HLB-infected trees to prevent further spread to healthy trees;

(iv) Confiscation of illegally shipped plants;

(v) Implementation of awareness campaigns to educate the public about this serious threat.

(vi) In order to ship *D. citri* host plants from locations under quarantine for the Asian citrus psyllid to areas where the Asian citrus psyllid is not present, the plant must be treated, inspected and accompanied by a limited permit that prevents distribution to any psyllid-free citrus-producing states or territories. In locations under quarantine for HLB, host plants of HLB (including all live plants, budwood, and cuttings) are prohibited from being shipped or moved outside of the counties or states.

(vii) Additionally the production of young citrus plants are now restricted to nurseries with insect-proof screenhouses and frequent inspection surveys for early detection of the disease are required.

Final Remarks

Suppression Program for introduced HLB:

(i) Inoculum reduction;
(ii) Vector control;
(iii) Healthy nursery trees;
(iv) Resistant or tolerant variety (NOT YET).

The efficacy of HLB control can be greatly increased with the establishment of a regional approach and policy to HLB management


Thank you
Gracias