Could 16SrIX phytoplasmas associated with almond witches'-broom disease represent an actual risk for Euro-Mediterranean Countries?

Quaglino F.[1], Abou-Yawdah Y.[2], Molino Lova M.[3], Al Achi R.[4], Choueiri E.[5], Tedeschi R.[6], Casati P.[1], Alma A.[6], Bianco P.A.[1]*

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DIPARTIMENTO DI SCIENZE AGRARIE E AMBIENTALI - PRODUZIONE, TERRITORIO, AGROENERGIA

MLO =mycoplasma like organism now *Candidatus* Phytoplasma sspp

- wall less prokaryotes (size variable from 200 and 800 nm)
- polymorphic, phloem inhabiting (companion cell)
- multiplying in hysotonic habitat (plant and insects)
- strictly host-dependent (not yet cultivable in axenic substrate)
- low CG content
- 600 -1600 kb genome size
- tetracycline sensitive
- transmitted by insect
- wide plant host range including species of cultivated crop

Phytoplasma Disease Symptoms on Periwinkle (*Chataranthus roseus*)

Yellowing

Virescence

Phyllody

Witches'-broom


Grapevine Yellows

Grapevine Yellows and dessication of the bunch


proliferation on
apple tree


Ca. Phytoplasma aurantifolia,
witches' broom of lime (WBDL)

MLO =mycoplasma like organism now *Candidatus* Phytoplasma sspp

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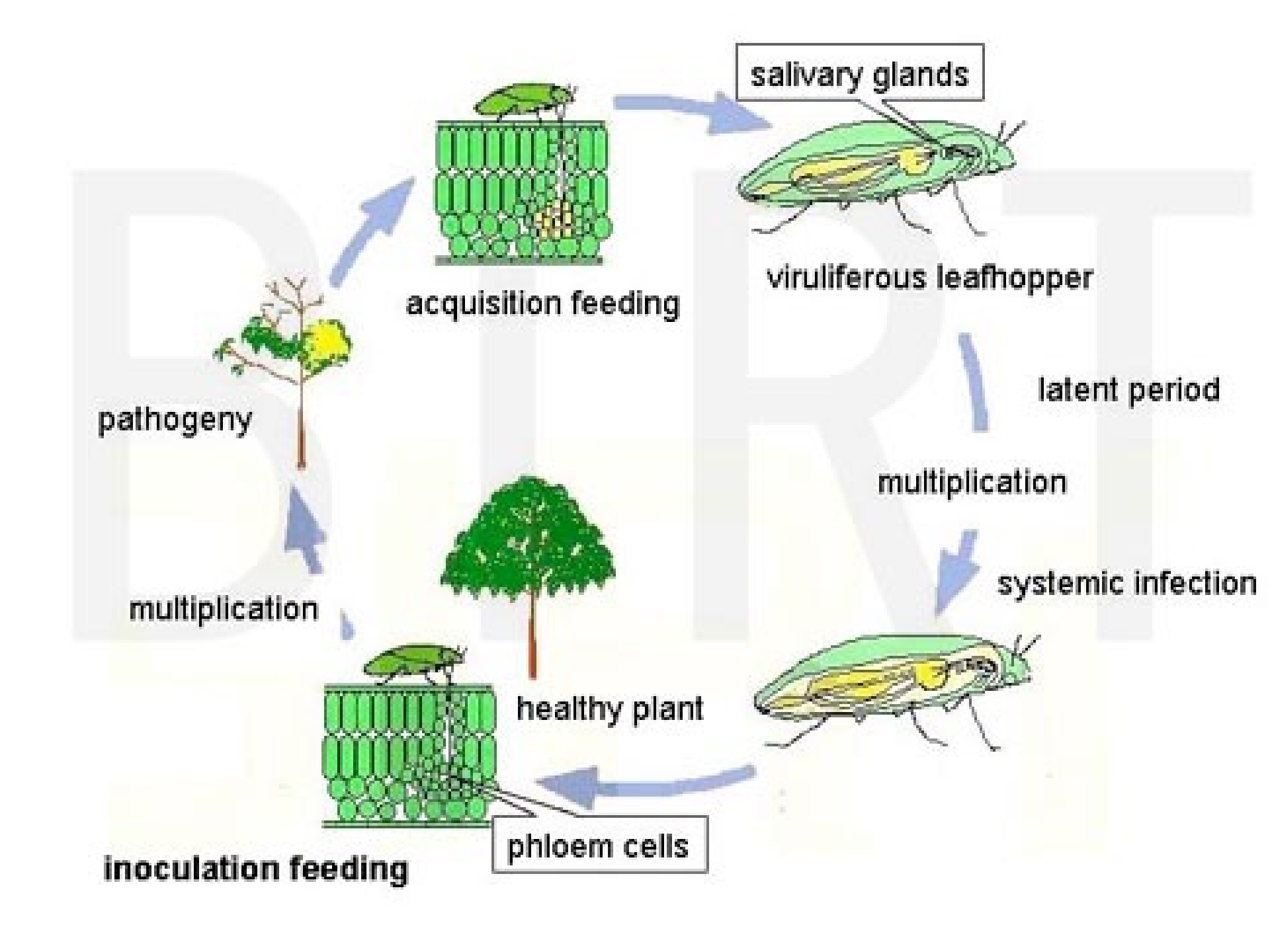

salivary glands
acquisition feeding
viruliferous leafhopper
latent period
multiplication
systemic infection
pathogeny
multiplication
healthy plant
phloem cells
inoculation feeding

Almond Witches' Broom Phytoplasma, An Emerging Lethal Disease of Stone Fruits with Potential Threat to the Mediterranean Area

Threat to major economic for the stone fruit crops

Almond producing countries	Area harvested (ha)	Quantity produced (tons)
United states	315,590	720,000
Lebanon	5,000	26,000
Syria	51,575	86,271
Tunisia	19,000	70,000
Morocco	151,109	99,067
Turkey	23,395	75,055
Italy	68,437	89,865
Spain	53000	215,100
World		1,934,817

Peach and Nectartine producing countries	Area harvested (ha)	Quantity produced (tons)
China	772,100	12,027,600
Lebanon	3,650	37000
Syria	6,674	59,095
Tunisia	16,000	128,000
Morocco	5,636	70,720
Turkey	28,362	575,730
Italy	71,012	1,331,621
Spain	50000	747,200
World	1,499,872	21,083,151

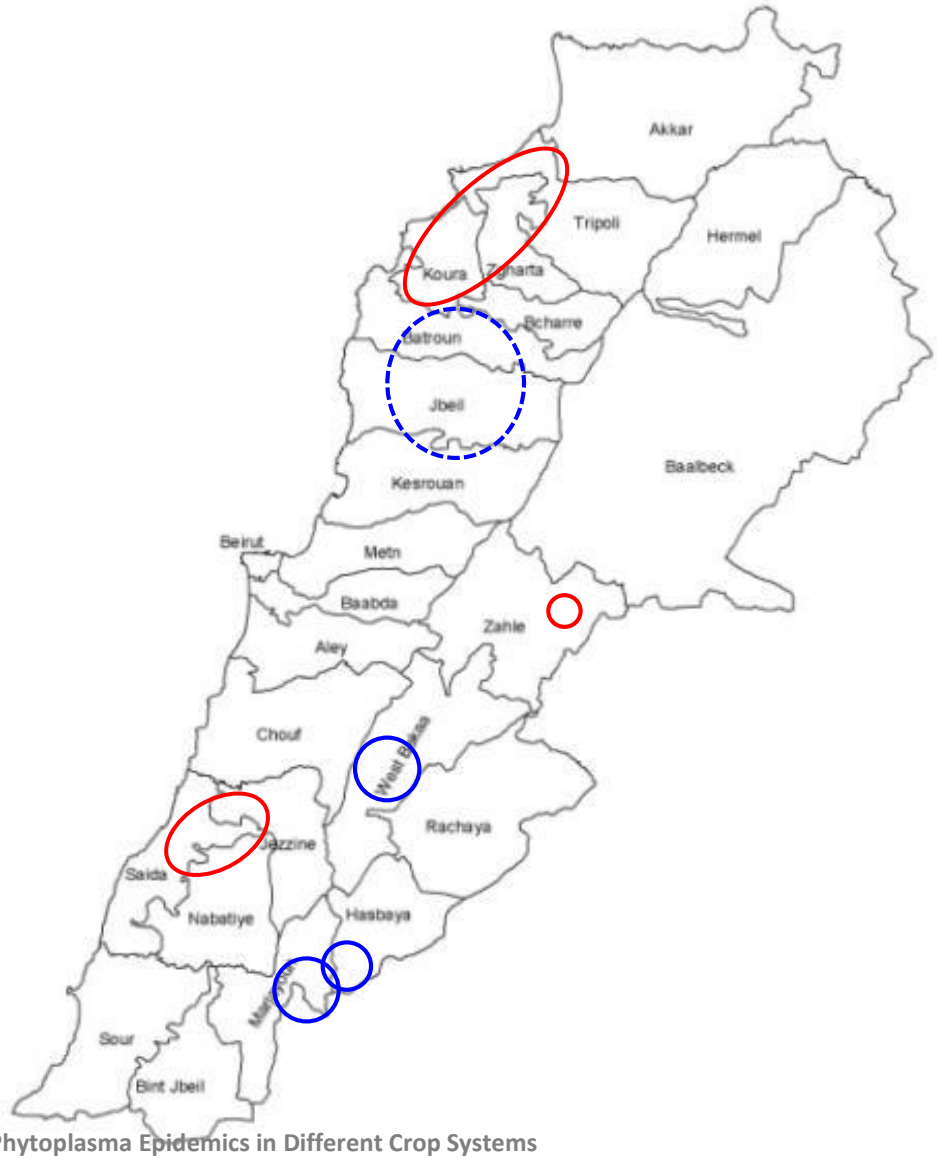
The past surveys

2001

(Choueiri *et al*., 2001: Abou-Jawdah et al., 2002; Verdin et al., 2003)

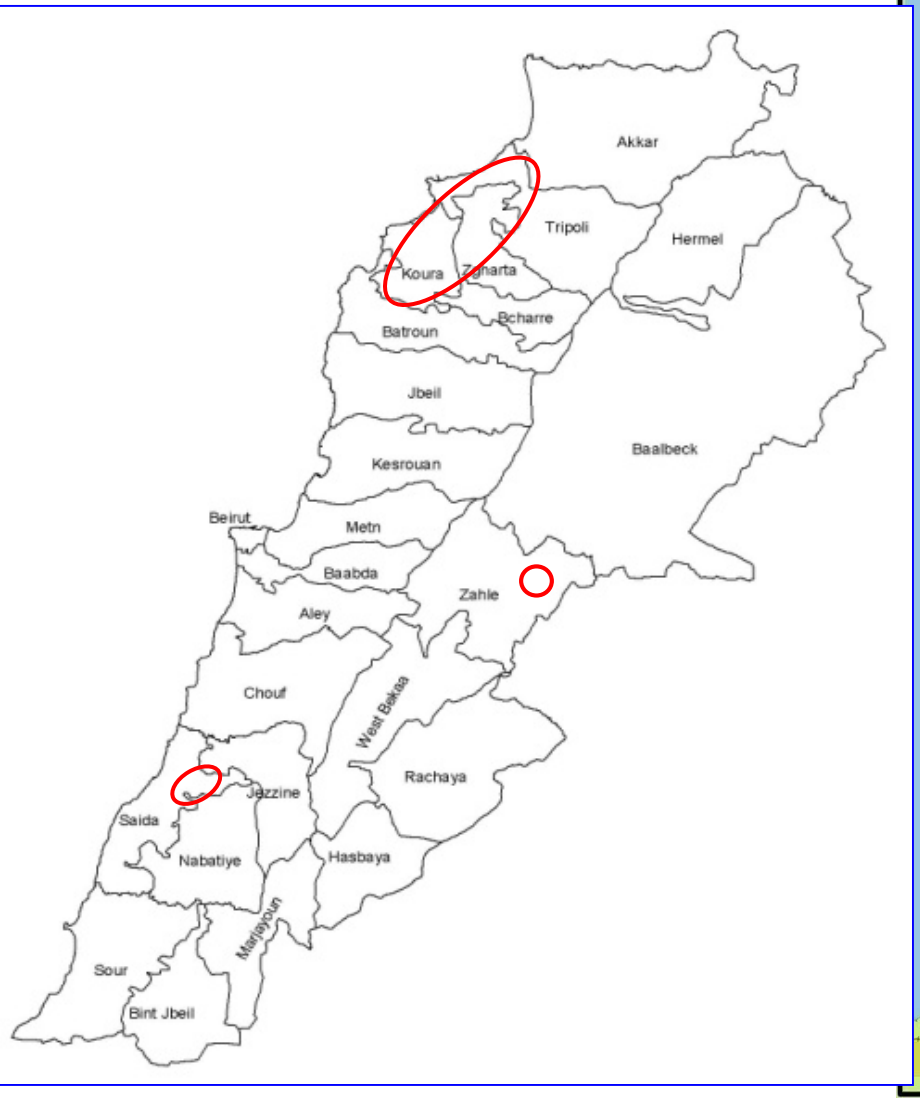
2009

(Abou Jawdah et al., 2009)

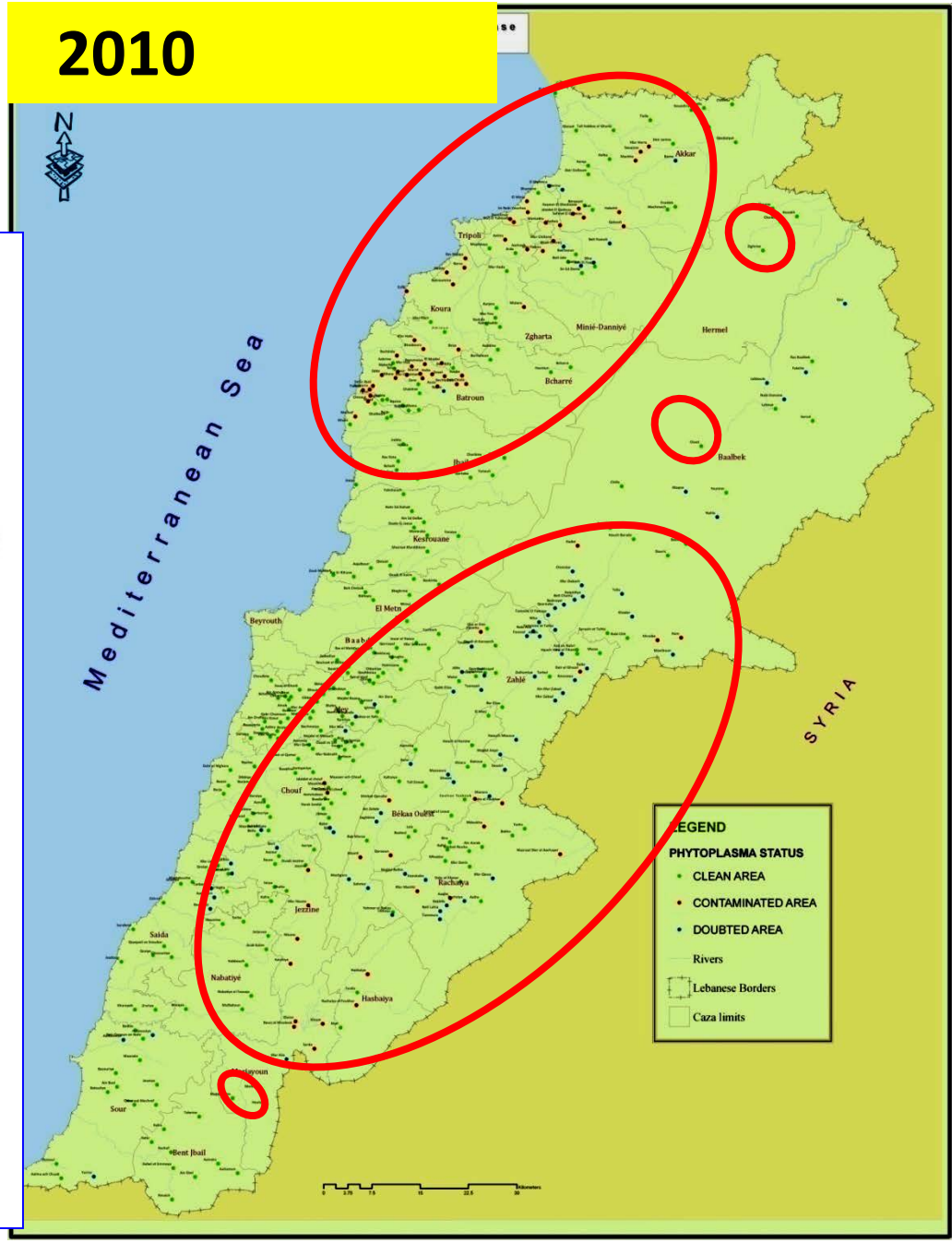


Rapid spread of AlmWB

2001→



2010



Fast spread of the Disease

AlmWB spread rapidly from coastal areas to >1200 m,

AlmWB spread :

- properly managed or abandoned orchards,
- isolated trees

→ One or more efficient aerial vector(s) ?

Symptoms on Almond

AlmWB: NEW HOSTS

Identification of
'*Ca.* P. phoenicium'
in peach
and nectarine

National Survey 2009-2010

Joint work among Italian and Lebanese Universities and the international cooperation, for the common CAPACITY BUILDING.

26 researchers worked on this project:

1) Training on symptom observation and sample collection by University of Milan.

2) Training on diagnostic tools (DNA extraction and PCR) by the American University of Beirut.

3) Training on insect identification by the Lebanese University – Faculty of Sciences and the University of Turin.

DATA COLLECTION ABOUT THE AlmWB SPREAD IN LEBANON

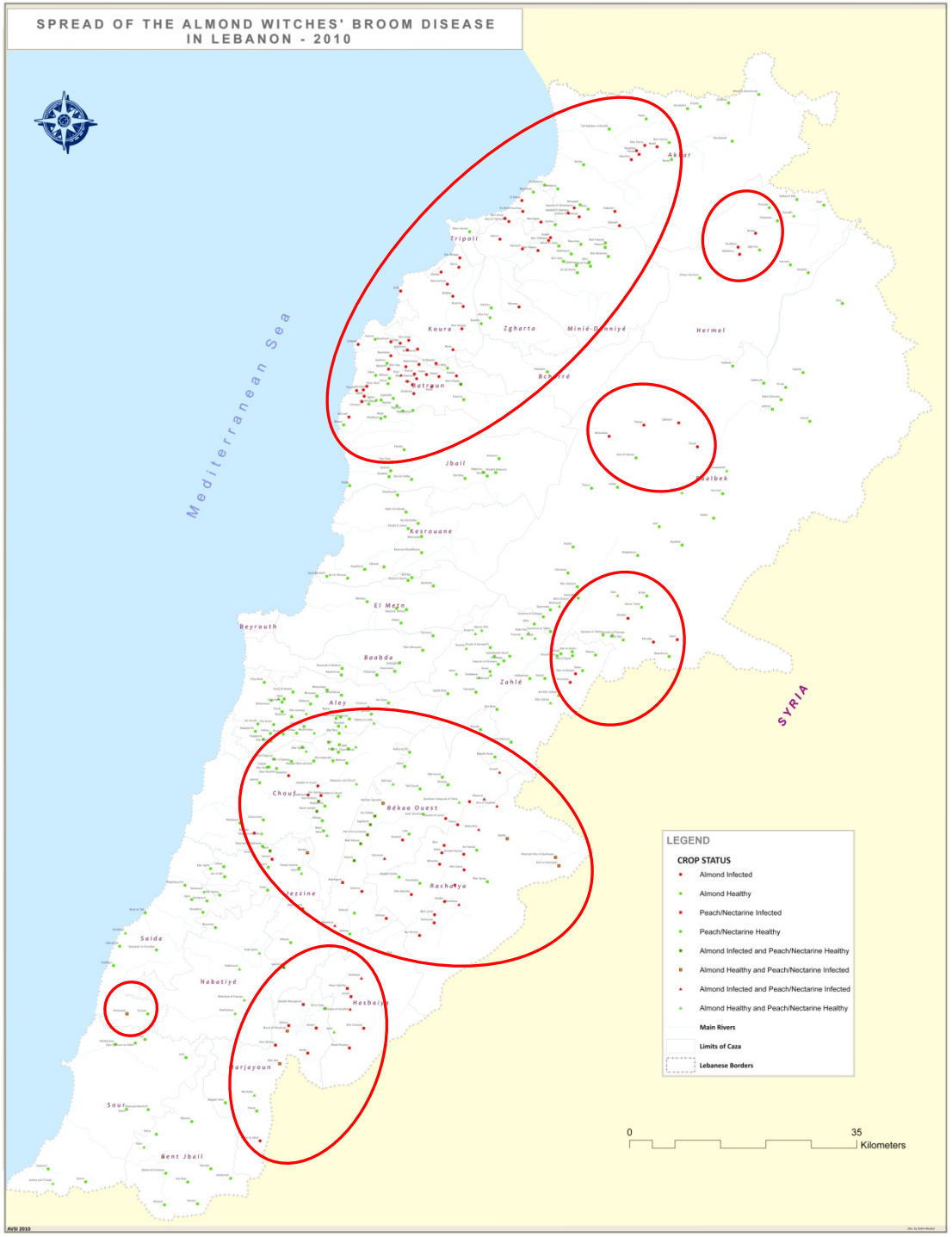
RESULTS

The disease was found in

117 out of 495 visited villages.

Every orchard was :

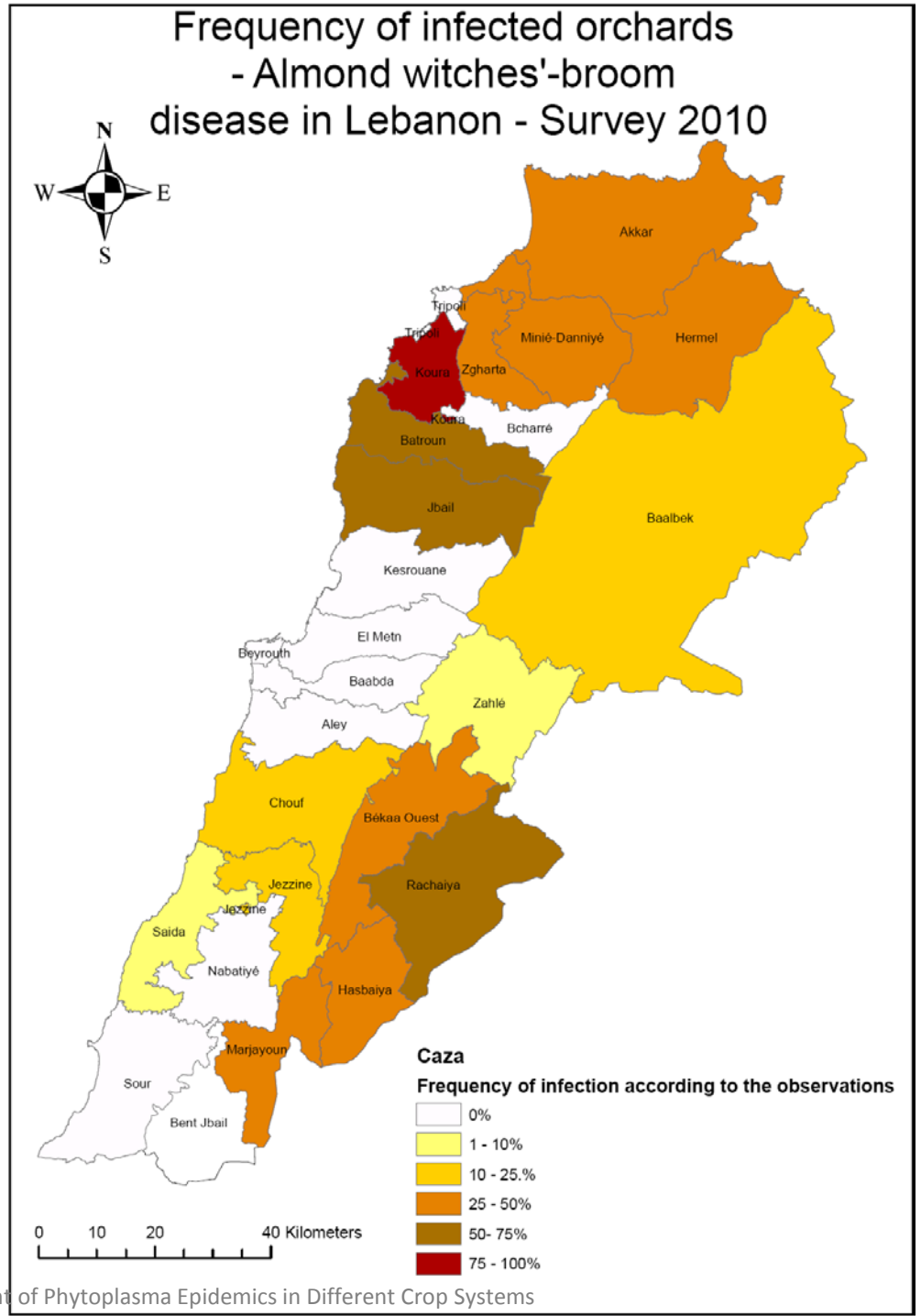
- ✓ localized by GPS (Global Positioning System) position;
- ✓ plotted in the GIS (Geographic Information Systems) database;
- ✓ represented in the national map of the disease spread in Lebanon.



Frequency of the infected orchards on the total visited orchards.

Six Classes:

- 0: 0%
- 1: 0-10%
- 2: 10-25%
- 3: 25-50%
- 4: 50-75%
- 5: 75-100%



RESULTS

In each District:

The percentage of the infected orchards:

Class 0: **8** districts

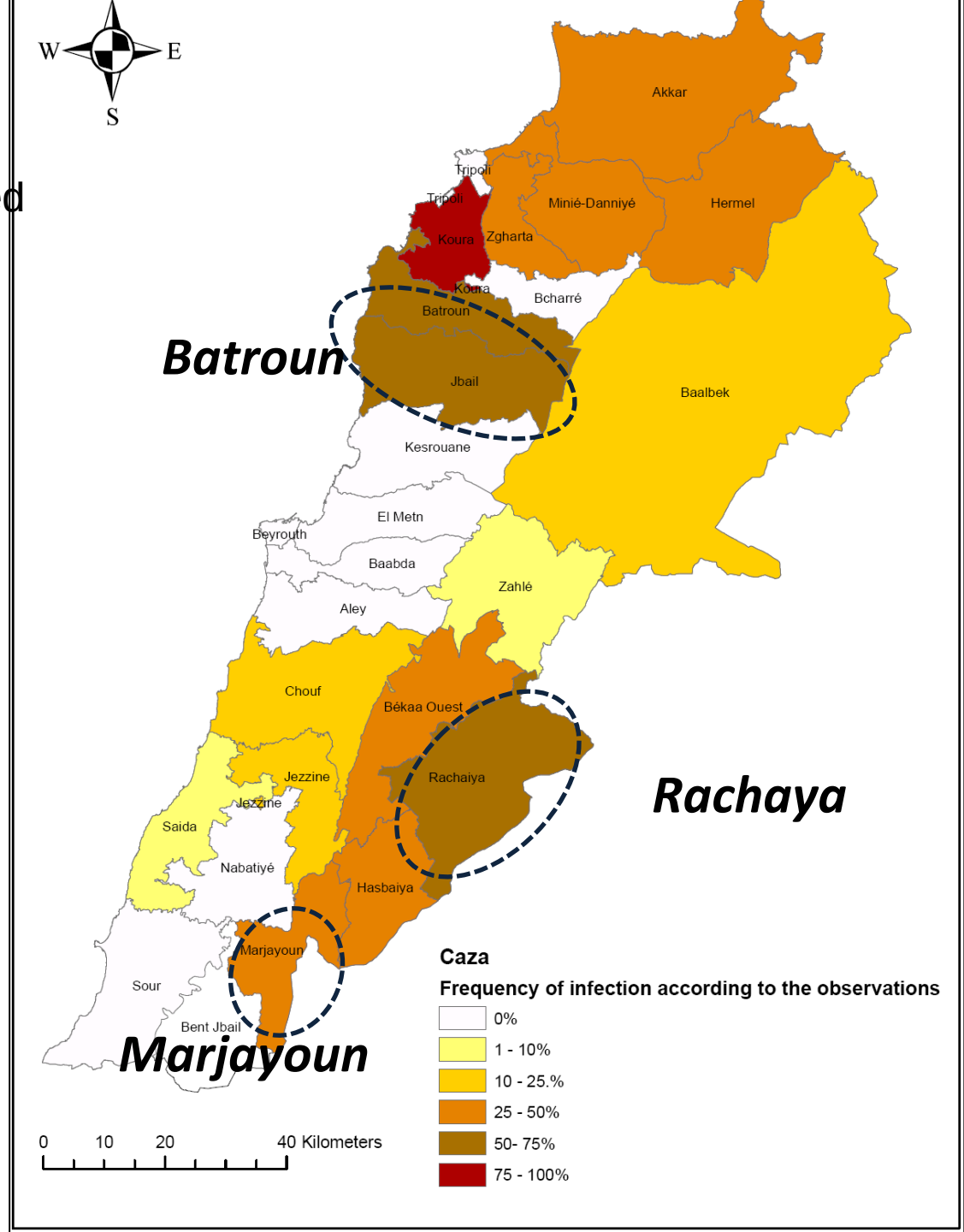
Class 1: **2** districts

Class 2: **3** districts

Class 3: **7** districts

Class 4: **3** districts

Class 5: **1** district



DATA COLLECTION ABOUT THE AlmWB SPREAD IN LEBANON

- The disease is present in 16 out of 24 Districts
- The percentage of infected orchards

Caza (District)	Number of visited orchards	number of infected orchards	Percentage of infected orchards (%)
Baalbeck	72	15	20.83
Bekaa West	77	30	38.96
Hermel	14	5	35.71
Rachaya	61	42	68.85
Zahle	99	4	4.04
Akkar	30	13	43.33
Batroun	41	27	65.85
Donniye	35	13	37.14
Jbeil	69	48	69.57
Koura	24	23	95.83
Zgharta	9	4	44.44
Chouf	95	10	10.53
Hasbaya	29	10	34.48
Jezzine	23	5	21.74
Marjayoun	33	10	30.30
Saida	34	1	2.94

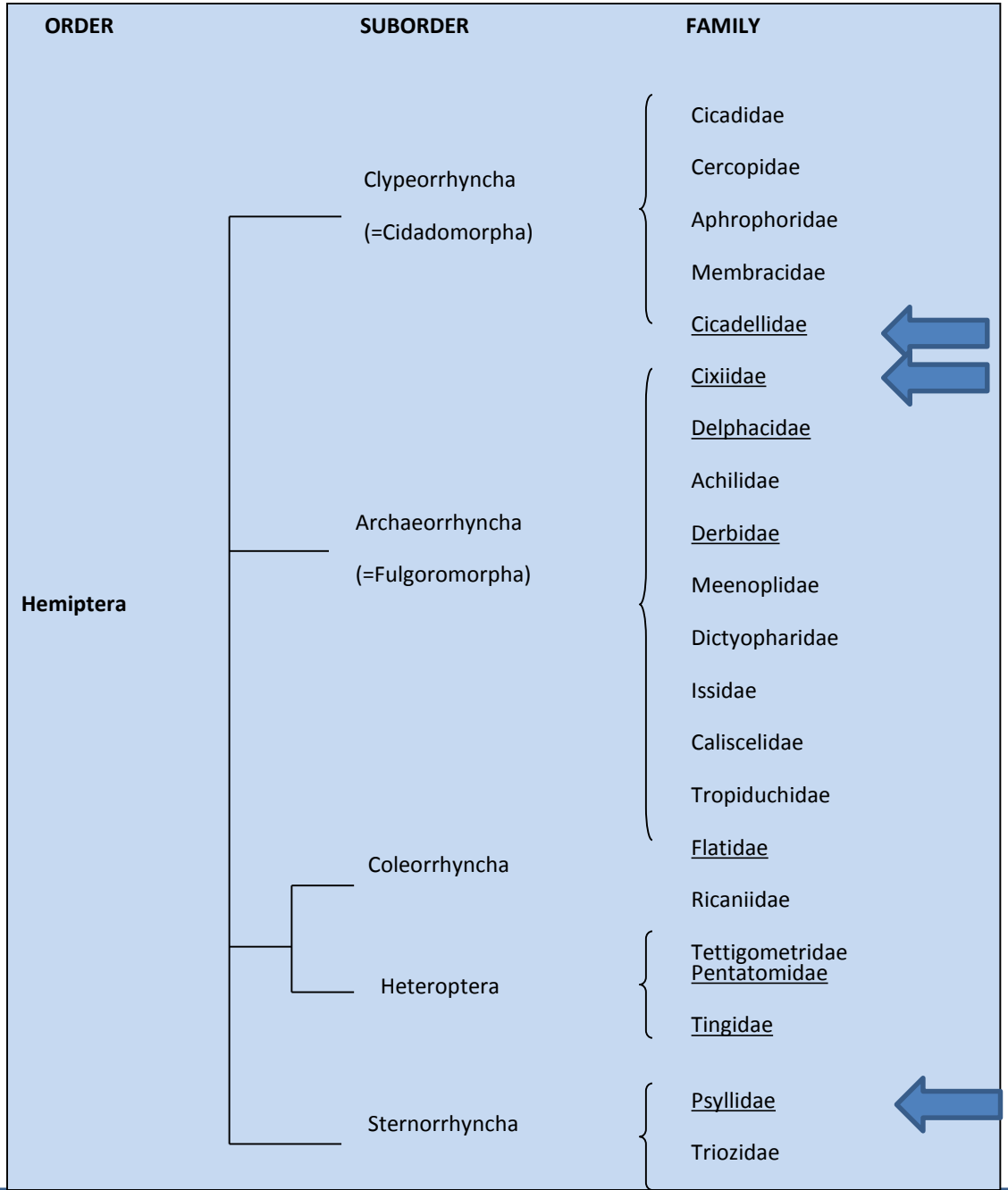
A SCREENING OF THE **INSECT(S) CANDIDATE VECTOR(S) OF THE DISEASE**

Family known as phytoplasma vectors: Cicadellidae, Cixiidae and Psyllidae.

❖ Preliminary studies already carried out on the Cicadellidae species as putative vectors of the phytoplasma (Dakhil *et al.*, 2011)

❖ No information is available on Psyllidae and Cixiidae specimens

❖Molecular analysis for phytoplasma identification were performed on these three groups.



Potential AlmWB insect vectors

Leafhopper species	15 -30 Nov.	December	January	February	March	April	May
Aphrodinae							
Aphrodes makarovi							1
Deltocephalinae							
Allygus theryi						1	
Anoplotettix eckerleini						9	61
Cicadulina bipunctella	3	2					
Euscelidius mundus							5
Fieberiella macchiae		10			1		
Synophropsis lauri		2		1		2	
Thamnotettix seclusus						2	
Thamnotettix wittmeri					2	9	13
Megophthalminae							
Megophthalmus scabripennis						1	
Typhlocybinae							
Arboridia spp.						5	
Asymetrasca decedens	34	53	30	97	544	2760	3901
Edwardsiana rosae					30	9	
Edwardsiana tshinari		7	1		18	46	1
Emelyanoviana naylae						8	7
Empoasca decipiens	2	13		10	68	58	62
Empoasca spp.			202				
Eupteryx nemoricola							1
Eupteryx stachydearum				3		1	2
Ficocyba ficaria	7	5	3		5		
Frutioidia bisignata		30	6	2	1	6	2
Hauptidia ecbalii				1			
Imbecilla imbecilla		1					
Jacobiasca lybica	2	3					
Zygina cf flammigera	10	14	26	9	8	23	26
Zygina rhamni						146	10
Zygina spp.		69					
Zyginella pulchra		1			6		
Zyginidia alexandrina						1	
Unidentified	2	464	13	1	29	49	42

Asymetrasca decedens was the most diffused species, and selected for phytoplasma transmission trials

LEBANON

of Agricultural and Food Sciences

IDENTIFICATION OF CIXIID SPECIES AND INFECTIVITY

1 species for *Cixius*
-> positive to AlmWB phytoplasma

8 species for *Tachycixius*
-> 6/8 positive to AlmWB phytoplasma

6 species for *Setapius*
-> positive to AlmWB phytoplasma

Hyalesthes obsoletus
-> positive to AlmWB phytoplasma

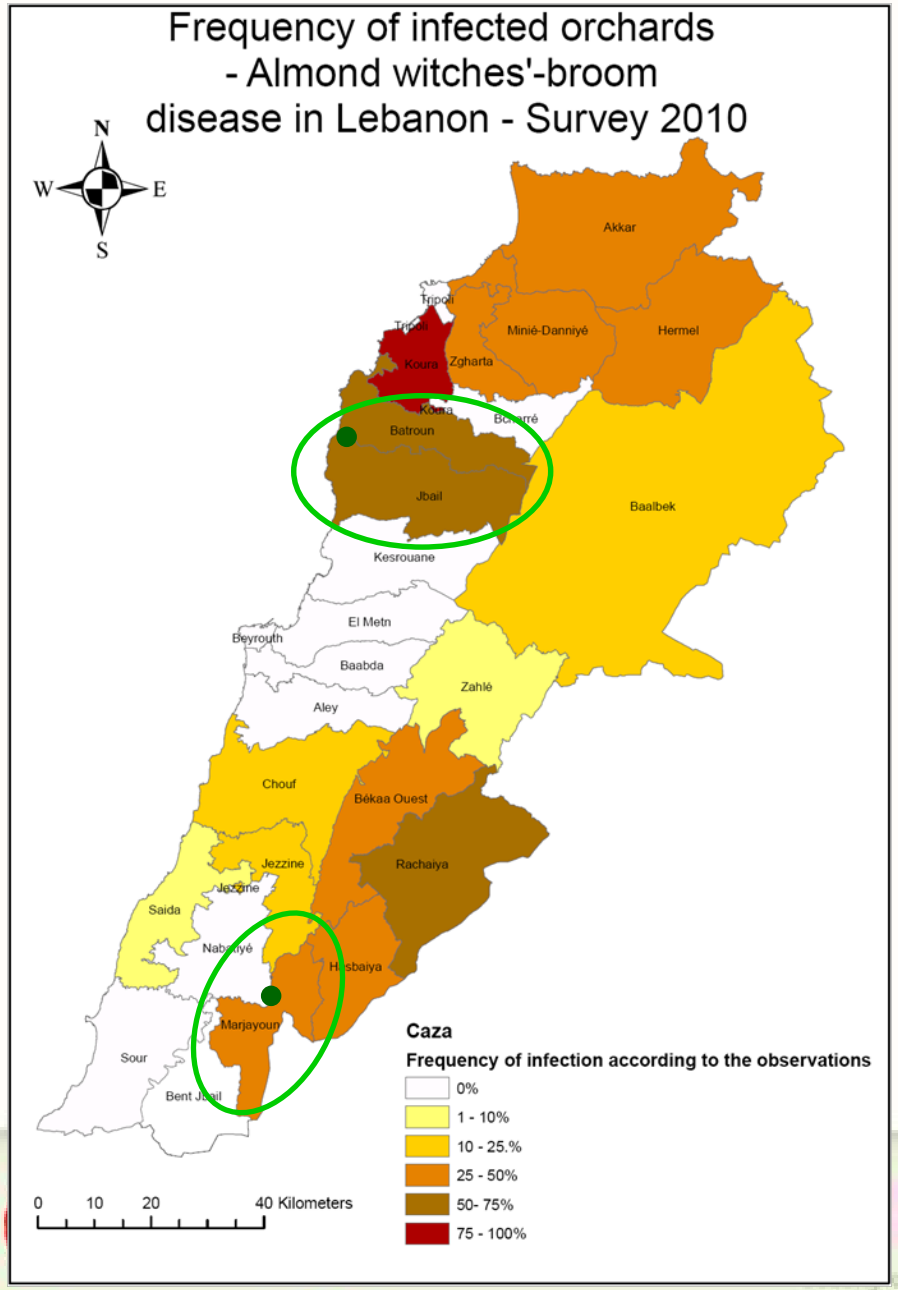
2 species for *Pentastira*
-> no positive to AlmWB phytoplasma

Survey on spontaneous plants

Autumn 2011
Spring 2012

521 samples
76 species

Diverse distribution of the species in North and South



spontaneous weeds in orchard agrosystem and identification of their phytoplasmas

Origin	Collection season	Species	Sample No.	16S rDNA analyses (RFLP - sequencing)
Feghal	Autumn	*Quercus* sp.	2	16SrIX-C
	Spring	***Smilax aspera****	4	**16SrIX-D**
	Spring	*Solanum nigrum*	2	16SrIX-C
	Spring	*Quercus* sp.	3	16SrIX-C
	Spring	*Allium* sp.	1	16SrIX-C
	Spring	*Polypodiales* sp.	1	16SrIX-C
	Spring	*Geranium purpureum*	2	16SrIX-C
Sarada	Spring	*Sinapis arvensis*	1	16SrIX-C
	Spring	*Malus domestica*	1	16SrIX-C
	Spring	*Onobrychis* sp.	1	16SrIX-C
	Autumn	*Inula viscosa*	1	16SrIX-C
	Autumn	*Euphorbia* sp.	1	16SrIX-C
	Autumn	*Scolymus maculatus*	1	16SrIX-C

'*Ca.* P. phoenicium': experimental transmission

Asymmetrasca decedens

Annals of Applied Biology ISSN 0003-4746

RESEARCH ARTICLE

Asymmetrasca decedens (Cicadellidae, Typhlocybinae), a natural vector of '_Candidatus_ Phytoplasma phoenicium'

Y. Abou-Jawdah[1], A. Abdel Sater[1], M. Jawhari[1], H. Sobh[1], H. Abdul-Nour[2], P.A. Bianco[3], M. Molino Lova[3] & A. Alma[4]

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4 DISAFA – Dipartimento di Scienze Agrarie Forestali e Alimentari, Università di Torino, Grugliasco (TO), Italy

Tachycixius spp.

Anthemis sp.

Smilax aspera

Annals of Applied Biology ISSN 0003-4746

RESEARCH ARTICLE

A cixiid survey for natural potential vectors of '_Candidatus_ Phytoplasma phoenicium' in Lebanon and preliminary transmission trials

R. Tedeschi[1,†], L. Picciau[1,†], F. Quaglino[2], Y. Abou-Jawdah[3], M. Molino Lova[4], M. Jawhari[3], P. Casati[2], A. Cominetti[2], E. Choueiri[5], H. Abdul-Nour[6], P.A. Bianco[2] & A. Alma[1]

1 DISAFA, Università degli Studi di Torino, Grugliasco (TO), Italy
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3 Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon
4 AVSI Lebanon, Centre Jean Paul II, Ghadir, Lebanon
5 Department of Plant Protection, Lebanese Agricultural Research Institute, Zahlé, Lebanon
6 Faculty of Sciences, Lebanese University, Beirut, Lebanon

"OPEN CYCLE" FOR *CIXIUS* SP.

Natural host plant of
Cixius sp. and
'Ca. P. phoenicium'

***Cixius* sp.**

Spring and autumn

MORE THAN ONE VECTOR: OPEN CYCLES

Natural host plant of
Cixius sp. and
'Ca. P. phoenicium'

Cixius sp.

A. decedens

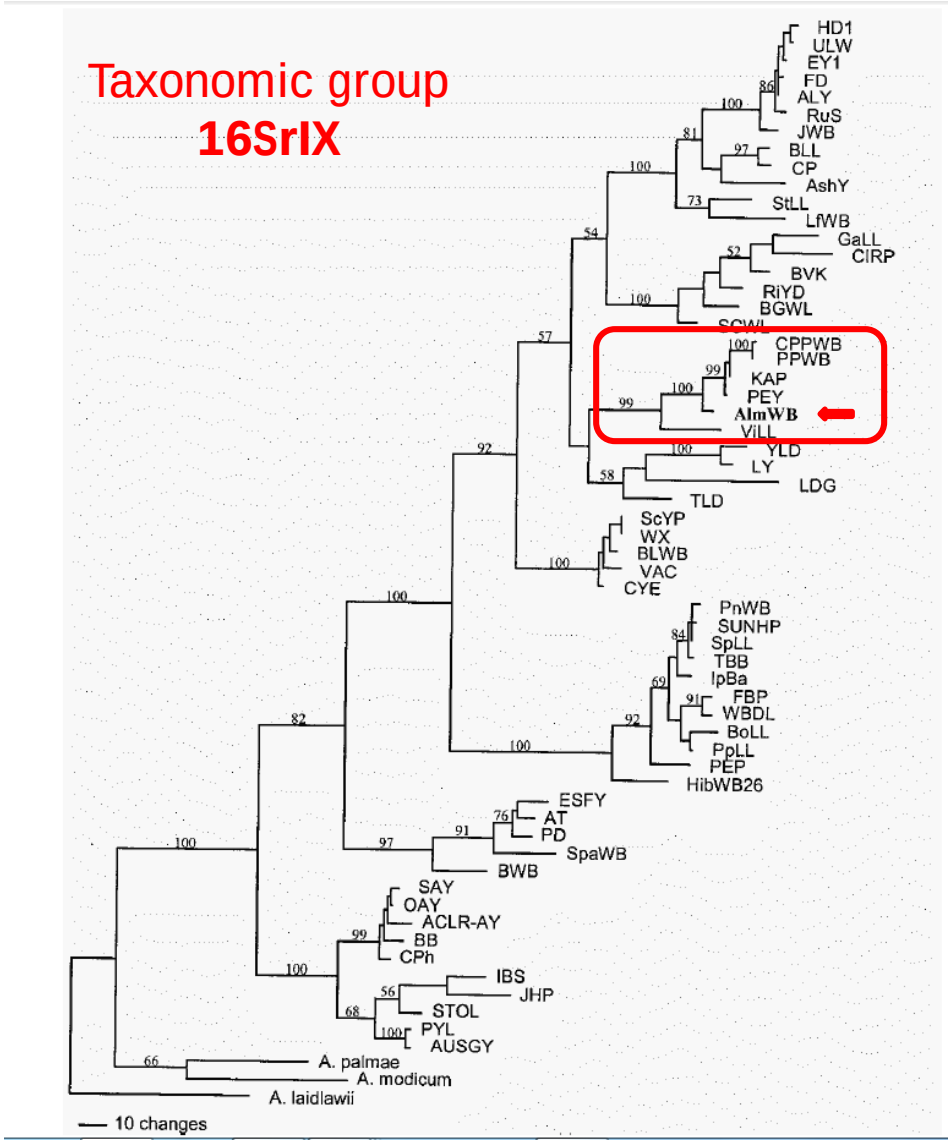
Spring and autumn

"*Candidatus* Phytoplasma phoenicium" in Lebanon and Iran

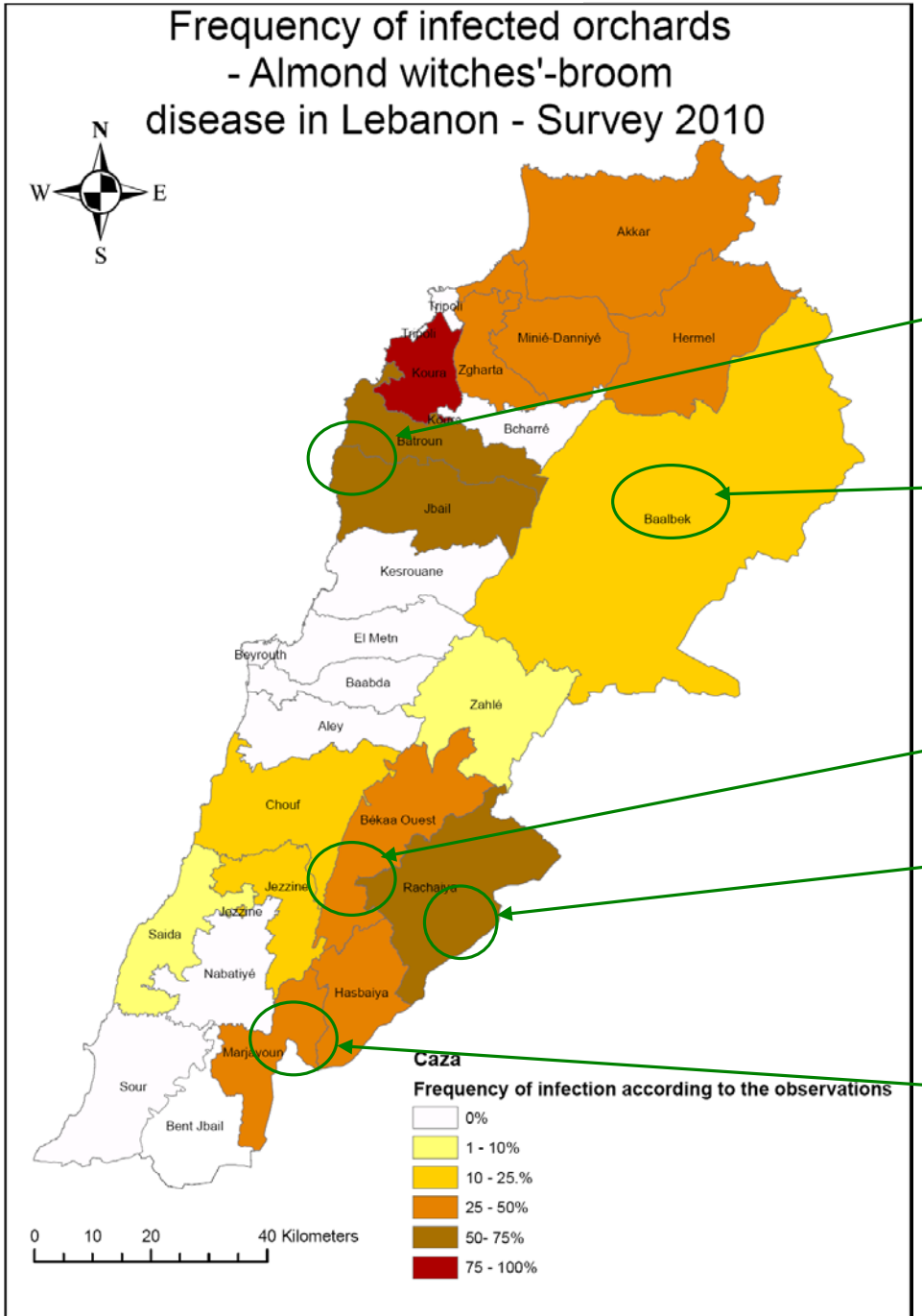
Abou-Jawdah et al., 2002. An epidemic of almond witches'-broom in Lebanon: classification and phylogenetic relationship of the associated phytoplasma. Plant Disease, 86: 477-484.

Verdin et al., 2003. '*Candidatus* phytoplasma phoenicium' sp. nov., a novel phytoplasma associated with an emerging lethal disease of almond trees in Lebanon and Iran. International Journal of Systematic and Evolutionary Microbiology, 53: 833-838.

Salehi et al., 2006. Characterization of a new almond witches' broom phytoplasma in Iran. *Journal of Phytopathology* 154, 386–391



The subgroup distribution



Caza	Origin	Host	Sample	Subgroup
Jbeil	Feghal	almond	Leaf	IX-G
	Feghal	almond	Leaf	IX-D
	Feghal	almond	Leaf	IX-G
	Feghal	almond	Leaf	IX-D
	Feghal	almond	Leaf	IX-G
	Feghal	peach	Leaf	IX-D
	Feghal	peach	Flower	IX-D
	Feghal	peach	Leaf	IX-G
	Feghal	almond	Leaf	IX-D
	Feghal	almond	Leaf	IX-G
	Feghal	almond	Leaf	IX-G
Baalbeck	Mchaytiye	nectarine	Leaf	IX-D
Bekaa West	Kerbet Kanafar	nectarine	Leaf	IX-D
	Kerbet Kanafar	nectarine	Leaf	IX-D
	Kerbet Kanafar	nectarine	Leaf	IX-D
	Qaraoun	peach	Leaf	IX-D
	Qaraoun	peach	Leaf	IX-D
	Sahbine-Akabe	peach	Leaf	IX-D
	Sahbine-Akabe	peach	Leaf	IX-D
	Ayn el Jawzi	peach	Leaf	IX-D
Rachaya	Bakka	peach	Leaf	IX-D
	Mazraait deir el aachayer	peach	Leaf	IX-D
	Mazraait deir el aachayer	nectarine	Leaf	IX-D
	Deir el aachayer	nectarine	Leaf	IX-D
	Deir el aachayer	nectarine	Leaf	IX-D
	Mdoukha	nectarine	Leaf	IX-D
	Rachaya	peach	Leaf	IX-D
Marjayoun	Sarada	nectarine	Flower	IX-G
	Sarada	nectarine	Leaf	IX-F
	Sarada	nectarine	Leaf	IX-D
	Sarada	nectarine	Flower	IX-D
	Sarada	nectarine	Flower	IX-D
	Sarada	peach	Leaf	IX-D
	Marjayoun	nectarine	Leaf	IX-D
	Marjayoun	nectarine	Leaf	IX-D
	Marjayoun	nectarine	Leaf	IX-F
	Marjayoun	nectarine	Leaf	IX-D

Could 16SrIX phytoplasmas associated with almond witches'-broom disease represent an actual risk for Euro-Mediterranean Countries?

1) *Ca.* Phitoplasma phoenicium: not reported in Europe

2) Vectors: *Asymetrasca decedens* is present in Europe.
Cixidae *sspp* from Lebanon: their classification is in progress. No information, so far, for EU

3) Additional/Alternative hosts (wild plants): their presence in Europe and Mediterranean Countries should be evaluated.

Journal of Plant Pathology (2015), **97** (2), 99-103 *Edizioni ETS Pisa, 2015*

SHORT COMMUNICATION

WILD ALMOND (*PRUNUS SCOPARIA*), A POTENTIAL SOURCE OF ALMOND WITCHES' BROOM PHYTOPLASMA IN IRAN

M. Salehi[1], E. Salehi[1], M. Abbasian[1] and K. Izadpanah[2]

[1]*Fars Agricultural and Natural Resources Research Center, Fars, Iran*
[2]*Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz, Iran*

Journal of Geochemical Exploration 123 (2012) 41–44

Contents lists available at SciVerse ScienceDirect

Journal of Geochemical Exploration

journal homepage: www.elsevier.com/locate/jgeoexp

Smilax aspera L. an evergreen Mediterranean climber for phytoremediation

C. Poschenrieder [a,*], M. Llugany [a], A. Lombini [a], E. Dinelli [b], J. Bech [c], J. Barceló [a]

[a] *Plant Physiology Lab., Facultat Biociencies, Universitat Autònoma de Barcelona, 08193 Bellaterra Spain*

The risk and its possible pattern

The phytoplasma can move trough regions, countries, continents

AlmWB: funded projects 2009-2013

- “Integrated management of AlmWB phytoplasma in Lebanon” (L09 A0500), Italian Cooperation (Minister of Foreign Affaires).
- “Milan to the defense, improvement and valorization of biodiversity 2009–2010”, Milan City Hall Administration.
- “National Program for the Improvement of Olive Oil’s Quality and Actions against the Diffusion of Stone Fruit Phytoplasma” (Project No. AID 9627), Lebanese Ministry of Agriculture.

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Papers on Almond witches'-broom (2009-2015)

- Tedeschi R., Picciau L., Quaglino F., Abou-Jawdah Y., Molino Lova M., Jawhari M., Casati P., Cominetti A., Choueiri E., Abdul-Nour N., Bianco P.A., Alma A. (2015). A cixiid survey for natural potential vectors of '*Candidatus* Phytoplasma phoenicium' in Lebanon and preliminary transmission trials. *Annals of Applied Biology* 166, 372-388.
- Abou-Jawdah Y., Abdel Sater A., Jawhari M., Sobh H., Abdul-Nour H., Bianco P.A., Molino Lova M., Alma A. (2014). *Asymmetrasca decedens* (Cicadellidae, Typhlocybi-nae), a natural vector of '*Candidatus* Phytoplasma phoenicium'. *Annals of Applied Biology* 165, 395-403.
- Molino Lova M., Quaglino F., Abou-Jawdah Y., Choueiri E., Sobh H., Casati P., Tedeschi R., Alma A., Bianco P.A. (2011). Identification of new 16SrIX subgroups, -F and -G, among '*Candidatus* Phytoplasma phoenicium' strains infecting almond, peach and nectarine in Lebanon. *Phytopathologia Mediterranea* 50, 273-282.
- Quaglino F., Kube M., Jawhari M., Abou-Jawdah Y., Siewert C., Choueiri E., Sobh H., Casati P., Tedeschi R., Molino Lova M., Alma A., Bianco P.A. (2015). '*Candidatus* Phytoplasma phoenicium' associated with almond witches'-broom disease: from draft genome to genetic diversity among strain populations. *BMC Microbiology,* DOI 10.1186/s12866-015-0487-4n (15:148)
- Casati P., Quaglino F., Abou-Jawdah Y., Picciau L., Cominetti A., Tedeschi R., Jawhari M., Choueiri E., Sobh H., Molino Lova M., Beyrouthy M., Alma A., Bianco P.A. (2015). Wild plants could play a role in the diffusion of diseases associated with phytoplasmas of pigeon pea witches'-broom group (16SrIX). *Accepted on Journal of Plant Pathology*.

- *…….and more 12 presentations at scientific meetings*

Thank you