Almond witches' broom phytoplasma: disease monitoring and preliminary control measures in Lebanon

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Abstract

A national survey was conducted in Lebanon in 2009/2010 and updated in 2012 in order to determine the extent of spread of almond witches' broom disease associated with '*Candidatus* Phytoplasma phoenicium' in the country, as well as to conduct preliminary research about the disease epidemiology and further molecular characterization of the phytoplasma. Over four years of continuous work, Lebanese and Italian efforts were joined to map the endemic areas as opposed to focus areas, to discover the phytoplasma vector(s) and to elaborate preliminary management plans.

Key words: phytoplasma, stone fruits, nursery, control measures.

Introduction

'Candidatus Phytoplasma phoenicium', a member of the 16S rDNA phytoplasma group IX, is considered the aetiological agent of almond witches' broom (AlmWB) disease, which caused in Lebanon the death of more than 100,000 almond trees in the last decade (Abou-Jawdah *et al.,* 2002). In the last few years, severe infections, frequently associated with a noticeable yield reduction, have also been observed on peach and nectarine trees (Abou-Jawdah *et al*., 2009). Almond witches' broom determines on almonds the death of the trees in a few years, causing impressive economical losses for the farmers, drastically reducing the production. Moreover, since the disease is spreading

also in nectarine and peach orchards, it represents a very dangerous threat not only for the Lebanese, but also for all the Mediterranean cultivations of these stone fruits.
A national survey was conducted in Lebanon in 2009/2010 and updated in 2012 in order to determine the extent of spread of almond witches' broom disease (AlmWB) associated with '*Candidatus* Phytoplasma phoenicium' presence in the country, divided in 26 districts, where local offices of the "Ministry of Agriculture extension services" are located. Training sessions for sixty technicians have been conducted in order to train the extension service personnel about the disease symptoms and the sample collection methods.

The national survey of orchards and nurseries

During 2012, according to the national census, all 561 stone fruit cultivated regions have been visited, in order to collect leaf samples for the detection of phytoplasma presence. During the survey, 1,419 orchards have been monitored; each orchard was located by GPS, in order to record its position and to draw a regional map on the spread of the disease in the area using the GIS (Geographic Information System) software. During the visits all the trees present in each orchard were monitored for almond witches' broom symptom presence. In order to confirm the infection of symptomatic plants or to verify the presence of the pathogen in trees showing doubtful symptoms, an average of 15 leaf samples were collected in each district and processed for '*Ca.* P. phoenicium' identification. A common protocol of detection has been established and shared among three laboratory units at AUB, LARI and USEK departments, by using the semi-specific primer pair AlWF2/AlWR2 (Abou-Jawdah *et al*., 2003). A total of 231 samples were collected and processed.
A complete survey of 279 mother plants at the LARI-Tal Amara station (used for the Lebanese certified seedling production), as well as of 136 registered and non-registered nurseries has been implemented, in order to check the phytosanitary status of the nursery sector in Lebanon. A scientific committee has been created to discuss and share, every 6 months, the partial achieved results and the strategies to be implemented among all the involved partners and subjects.
The research about the phytoplasma vector(s) is still on going and in order to reduce the *foci* of infection in the country, an eradication plan has been first implemented in a pilot area (Zahle district) in 2012 and then extended to seven districts in 2013.

Results

Almond, peach and nectarine orchards were severely affected and the number of infected trees was increasing. Out of 231 samples, 158 tested positive to the analyses (Table 1). The presence of the disease, detected in 16 out of 26 districts in 2010, was also confirmed in two new districts in 2012 (Bent Jbeil and Bcharre); positive samples have been detected in stone fruit orchards located in 221 out of 561 monitored villages (Figure 1).

Figure 1. Map of the almond witches' broom spread in the Lebanese regions.

Table 1. Results of the PCR analyses conducted in 2012 on stone fruits.

	Location	Number of collected samples	Positive samples (16SrIX specific PCR)
North Lebanon	Akkar	12	9
	Donniyé	13	7
	Koura	9	8
	Tripoli	1	1
	Batroun	20	17
	Bcharré	3	2
	Zgharta	9	9
	Jbeil	9	5
	Kesrouane	5	1
	Metn	3	0
	Baabda	2	0
South Lebanon	Aley	4	2
	Chouf	12	8
	Jezzine	18	13
	Saida	3	1
	Sour	5	2
	Marjeyoun	20	15
	Hasbaya	17	11
	Bent Jbeil	6	3
	Nabatiyé	4	2
Bekaa Valley	Hermel	6	5
	Baalback	15	7
	Zahlé	7	3
	West Bekaa	13	13
	Rachaya	15	14
Total		231	158

Two additional 16SrIX subgroups were detected (-F and -G) in addition to the prevalent subgroup -D (Molino Lova *et al.*, 2011).

Concerning the nursery sector, 282 samples were collected and analyzed from registered and non-registered nurseries as well as 279 samples from mother trees in Tal Amara station. All the mother plants were negative for AlmWB phytoplasma presence. However, five out of 136 visited nurseries were found selling seedlings that tested positive for AlmWB; a prompt decision from the Lebanese Ministry of Agriculture was adopted to destroy all the seedlings.

Extension workshops have been organized for 593 farmers and 47 nurserymen. In 2012, pilot areas were selected, elimination of 182 AlmWB-infected trees and their replacement have been implemented in 14 villages. Again during 2013, a plan for tree

elimination and crop replacement has been implemented by the Ministry of Agriculture to reduce the impact of the disease and to support the rural affected areas in Baalback, West Bekaa, Rachaya, Hasbaya, Marjayoun, Bent Jbeil and Jezzine districts. A total of 6,206 infected trees have been eliminated until the end of October, 2013 in 91 villages. The 674 involved farmers will receive from the Ministry of Agriculture new fruit tree seedlings, adapted to the different regions, in compensation for their economic losses.

Conclusions

The complex epidemiology of phytoplasma diseases, mainly of '*Ca*. P. phenicium' necessitates cooperation of researchers in different disciplines in order to understand the disease epidemiology and to develop appropriate disease containment and management strategies. Legislation and control measures are urgently necessary to limit the diffusion of almond witches' broom in Lebanon but also to avoid its spread to other Middle East countries and to Europe.

References

Abou-Jawdah, Y., Karakashian, A., Sobh, H., Martini, M., Lee, I-M. 2002. An epidemic of almond witches' broom in Lebanon: classification and phylogenetic relationship of the associated phytoplasma. *Plant Disease* 86: 477-484.

Abou-Jawdah, Y., Dakhil, H., El-Mehtar, S., Lee, I-M. 2003. Almond witches' broom phytoplasma, a potential threat to almond, peach and nectarine. *Canadian Journal of Plant Pathology* 25: 28-32.

Abou-Jawdah, Y., Sobh, H., Akkary, M. 2009. First report of almond witches' broom phytoplasma ('*Candidatus* Phytoplasma phoenicium') causing a severe disease on nectarine and peach trees in Lebanon. *EPPO Bulletin* 39: 94-98.

Molino Lova, M., Quaglino, F., Abou-Jawdah, Y., Choueiri, E., Sobh, H., Alma, A., Tedeschi, R., Casati, P., Bianco, P.A. 2011. Identification of new 16SrIX subgroups, rIX-F and rIX-G, among '*Candidatus* Phytoplasma phoenicium' strains infecting almond, peach and nectarine in Lebanon. *Phytopathologia mediterranea* 50: 273-282.