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Preliminary survey of potential vectors of '*Candidatus* Phytoplasma phoenicium' in Lebanon and probability of occurrence of apricot chlorotic leaf roll (ACLR) phytoplasma

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Abstract

Malaise traps and sticky yellow traps were placed in two almond orchards infected with almond witches' broom (AlmWB) phytoplasma in 2004 and in 2010. Collected insects were tested by nested PCR using universal primers for detection of phytoplasma. In 2004 *Asymmetrasca decedens, Euscelidius* sp. and *Fieberiella* sp. gave positive results with the universal primers and also with the pigeon pea group specific primers. The mere presence of phytoplasma in an insect is not a proof that it is a vector, but it may help narrowing the choice of insects for conducting actual transmission tests. In 2010, early in the season, in over 20 species surveyed we were not able to detect phytoplasma except in *Psammotettix provincialis*. The PCR amplicon was sequenced (1180 bp) and found to be most closely related to the Aster yellows (AY) group 16SrI, subgroup F which includes apricot chlorotic leaf roll (ACLR) phytoplasma. Late in the season, most PCR tests were negative, the major reason was correlated with DNA degradation which resulted from the hot summer temperatures combined with the method of insect collection. The implications of these findings are discussed.

Key words: almond witches' broom, nested PCR, *Asymmetrasca decedens*, *Euscelidius* sp., *Fieberiella* sp., *Psammotettix provincialis.*

Introduction

Almond witches' broom (AlmWB) phytoplasma was reported as a devastating almond disease that killed over 100,000 almond trees in Lebanon (Abou-Jawdah *et al.*, 2002). AlmWB has been tentatively called '*Candidatus* Phytoplasma phoenicium' and belongs to the pigeon pea phytoplasma group 16Sr IX (Abou-Jawdah *et al.*, 2002; Verdin *et al.*, 2003). Grafting experiments showed that it is transmitted to peach and nectarine but not to apricot (Abou-Jawdah *et al.*, 2003). These results were confirmed recently as AlmWB was reported to cause severe epidemics on peach and nectarine in South Lebanon (Abou-Jawdah *et al.*, 2009). A similar disease was reported in Iran. The rapid and extensive spread of the disease suggests the presence of efficient vector(s) and calls for an integrated disease management approach which would require information about the disease epidemiology. The present work tries to identify potential vectors in order to start transmission tests for identification of the real vector(s).

Materials and methods

Leafhoppers were collected at bi-weekly intervals from Malaise traps and from yellow sticky traps installed in two AlmWB infected orchards. Insects were removed and identified directly or stored in 70% ethanol until identification. In 2004 the insects in the malaise trap were killed by pyrethroid pesticide releasing pellets; while in 2010, the insects were trapped in 70% ethanol. DNA was extracted from individual insects for large-sized species, or from groups of 5 insects in the case of *Asymmetrasca decedens* (Paoli) and similar small-sized species. Phytoplasma was detected either by nested PCRs using universal primer pairs P1/P7 and R16F2n/R16R2 (Gundersen and Lee, 1996), or by PCR using the primer pair **(**AlwF2/R2) for detection of pigeon pea taxonomic group (Abou-Jawdah *et al.*, 2003).

Results and discussion

Among the 10 genera of leafhopper tested in 2004, seven genera gave positive results with the group specific primers and only three with the universal primers (table 1). *A. decedens*, *Euscelidius* sp. and *Fieberiella* sp. gave positive results using both the phytoplasma universal primer pairs and the pigeon pea group specific primers. From our experience using both techniques the group specific primers give more sensitive detection than the universal primers; thus probably the phytoplasma concentration is higher in these three genera and therefore may be given priority in transmission assays. *Euscelidius* and *Fieberiella* genera include known phytoplasma vectors, while relatively a limited number of reports are available on *Empoasca,* a relative to *Asymmetrasca,* as a potential phytoplasma vector (Pastore *et al.*, 2004).

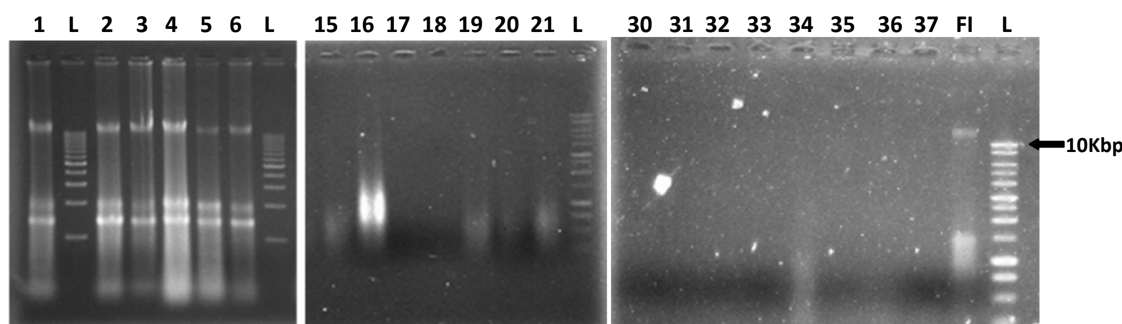

Figure 1. Gel electrophoresis of DNA extracts from different insects collected at three different dates. 1-6: Spring, 15-21: mid Summer, 30-37: late Summer. FI is a freshly collected insect, L= 1kbp ladder.

Table 1. Detection of phytoplasma by PCR and nested PCR in DNA extracts of 10 leafhopper genera collected in 2004 from North Lebanon.

Insect	No. of PCR positive samples/No. of total samples	
	Nested with R16F2/R2n	primers AlwF2/R2
Allygus sp.	0/2	1/2
Asymmetrasca decedens	2/5	5/5
Cicadulina sp.	0/2	0/2
Empoasca decipiens	0/8	3/8
Euscelidius sp.	1/14	10/14
Euscelis sp.	0/1	0/1
Fieberiella sp.	1/1	1/1
Laylatina sp.	0/8	1/8
Thamnotettix sp.	0/3	1/3
Zygina sp.	0/3	0/3

In 2010, Lebanon experienced a very hot summer and the leafhoppers were trapped in glass jars containing 70% ethanol and collected at biweekly intervals. This may have led to DNA degradation in samples collected starting from June (figure 1). Therefore, in hot climates it would be necessary to adapt the trapping method to preserve the insect DNA.

DNA extracts from one leafhopper *Psammotettix provincialis* (Ribaut) collected early in the 2010 season gave positive results in nested PCR tests. The amplicon sequence was most closely related to aster yellows (AY) 16SrI subgroup-F (16SrI-F) which includes apricot chlorotic leaf roll (ACLR) and leafhopper borne, CVB.

In conclusion, field observation suggests the presence of more than one AlmWB vector (primary and secondary), one responsible for long distance migration and feeds only occasionally on stone fruits, and another like *A. decedens* which may be a much less efficient vector but is present in large numbers in stone fruit orchards. These observations coupled with PCR results must be confirmed by transmission assays. Other insects in the Psyllidae or Cixiidae should be also monitored.

In Lebanon, in AlmWB infested areas, apricot is considered as one of the possible replacement crops for almond since it was proven to be resistant to AlmWB. However, the probability of occurrence of ACLR phytoplasma which lead to the decline of apricot trees in some European regions, must be thoroughly investigated. Due to the diversity of AY group, surveys must be conducted using PCR tests based not only on 16Sr DNA sequences but also on secY and rp gene sequences (Lee *et al.*, 2005). The epidemic potential of AlmWB calls for a regional and international cooperation to eradicate it or prevent its further spread.

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