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Pest Risk Analysis for

'*Candidatus* Phytoplasma phoenicium'
(Bacteria: Acholeplasmataceae) causing almond witches' broom

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EPPO
21 Boulevard Richard Lenoir
75011 Paris
www.eppo.int
hq@eppo.int

This risk assessment follows the EPPO Standard PM 5/5(1) *Decision-Support Scheme for an Express Pest Risk Analysis* (available at http://archives.eppo.int/EPPOStandards/pra.htm) and uses the terminology defined in ISPM 5 *Glossary of Phytosanitary Terms* (available at https://www.ippc.int/index.php). This document was first elaborated by an Expert Working Group and then reviewed by the Panel on Phytosanitary Measures and if relevant other EPPO bodies.

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Photo: *Witches' Broom* on almond. Courtesy: Marina Molino Lova (AVSI-Lebanon)

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Based on this PRA, '*Candidatus* Phytoplasma phoenicium' was added to the A1 Lists of pests recommended for regulation as quarantine pests in 2017.

Pest Risk Analysis for '*Candidatus* Phytoplasma phoenicium' (Bacteria: Acholeplasmataceae) causing almond witches' broom

PRA area: EPPO region
Prepared by: EWG on '*Candidatus* Phytoplasma phoenicium'
Date: 6-9 December 2016 (the PRA was further reviewed and amended by other EPPO bodies, see below)

Composition of the Expert Working Group (EWG)

ABOU-JAWDAH Yusuf (Prof.)	Agriculture Department, Faculty of Agricultural and Food Sciences, American University of Beirut, Bliss Street, 11-0236, 1107-2020 Riad El-Solh, Lebanon Tel: +961-1343002 - abujawyf@aub.edu.lb
AVENDANO GARCIA Nuria (Ms) (core member)	TRAGSATEC, C/Julian Camarillo, 6a. Sector 4a., 28037 Madrid, Spain Tel: +34-607736171 - nag@tragsa.es
BIANCO Piero Attilio (Mr)	Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi di Milano, via Celoria 2, 20133 Milano, Italy Tel: +39-250316794 - piero.bianco@unimi.it
KARBOUA Samira (Ms)	Institut National de la Protection des Végétaux, N°1 BT F59 CITE 1016 LOGTS El Achour Alger, 16000 El- Achour, Algeria Tel: +213-555101132 - lettifikarbouainpv@yahoo.com
LHEUREUX Fabrice (Mr)	Ctifl, centre de Lanxade, 28 route des Nébouts, 24130 Prigonrieux, France Tel: +33-553580005 - lheureux@ctifl.fr
LOISEAU Marianne (Ms)	Laboratoire de la santé des végétaux-ANSES, 7 rue Jean Dixméras, 49044 Angers Cedex 01, France Tel: +33-241207459 - marianne.loiseau@anses.fr
MOLINO LOVA Marina (Ms)	AVSI Foundation - Lebanon, St. Fawqa Street, 1200 Jounieh Ghadir, Lebanon Tel: +961-3451883 - marina.molinolova@avsi.org
SAGLAM Asuman (Ms) (core member)	Izmir Zirai Karantina Müdürlügü, No. 11 Liman Caddesi -Alsancak, 35220 Konak-Izmir, Turkey Tel: +90-2324634911 - asumanergun07@gmail.com
SIAMPOUR ASHKAVANDI Majid (Mr)	Department of Plant Protection, College of Agriculture, Shahrekord University, 88186-34141 Shahrekord, Iran (Islamic Republic Of) Tel: +98-3832324428 - siampour@agr.sku.ac.ir
TUFFEN Melanie (Ms)	Department for Environment, Food & Rural Affairs, Room 02FA01/05, NAFIC, YO41 1LZ Sand Huton, United Kingdom Tel: +44-2080262514 - melanie.tuffen@defra.gsi.gov.uk
Observer	
RICCIONI Luca (Mr)	Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, I-00156 Roma, Italy Tel: +39-0682070329 - luca.riccioni@crea.gov.it
EPPO	
GROUSSET Fabienne (Ms) SUFFERT Muriel (Ms)	OEPP/EPPO, 21 boulevard Richard Lenoir, 75011 Paris, France Tel: +33-145207794 - fg@eppo.int; ms@eppo.int

This PRA follows EPPO Standard PM 5/5 *Decision-Support Scheme for an Express Pest Risk Analysis*, but using a 5-level scale for likelihoods and magnitudes. For the determination of ratings of likelihoods and uncertainties, experts were asked to provide a rating and level of uncertainty individually during the meeting, based on the evidence provided in the PRA and on the discussions in the group. Each EWG member provided anonymously a rating and level of uncertainty, and proposals were then discussed together in order to reach a final decision.

All personal communications in this PRA were obtained in 2016. Prior to the EWG, comments on specific issues were obtained from Rosemarie Tedeschi and Luca Picciau (Università di Torino, Italy), Fabio Quaglino (Università degli Studi di Milano), J.Th.J. Verhoeven (Dutch NPPO).

Following the EWG, the PRA was further reviewed by correspondance in January-February 2017 by the following PRA core members: Roel Potting (The Netherlands), Arild Sletten (Norway), Robert Steffek (Austria), Nursen Urstun (Turkey), Dirk Jan van der Gaag (The Netherlands), as well as by EPPO Staff (Camille Picard and Françoise Petter).
The Pest Risk Management was reviewed by the EPPO Panel on Phytosanitary Measures in March 2017. EPPO Working Party on Phytosanitary Regulation and Council agreed that '*Candidatus* Phytoplasma phoenicium' should be added to the A1 Lists of pests recommended for regulation as quarantine pests in 2017.

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Summary of the Pest Risk Analysis for '*Candidatus* Phytoplasma phoenicium' (Bacteria: Acholeplasmataceae) causing almond witches' broom			
PRA area: *EPPO region*			
Describe the endangered area: where almond, peach, nectarine and apricot are cultivated and the known vectors (*Asymmetrasca decedens* and two *Tachycixius* species) occur, i.e. the Mediterranean Basin and Portugal, north to the southern part of Germany (Baden-Württemberg) and East towards the West of Russia, as well as the Near East and Central Asia. In North Africa, *A. decedens* is currently recorded only in Tunisia. There is an uncertainty for the rest of the EPPO region, where the presence of known vectors is not known and where there is not a dense presence of *Prunus* hosts. The occurrence of other potential vectors is suspected; therefore impacts may occur if other effective vectors are present, and new vectors may also allow the phytoplasma to spread to new hosts.			
Main conclusions *Overall assessment of risk:* '*Ca.* P. phoenicium', causal agent of almond witches' broom, is currently reported only in Iran and Lebanon. Vectors (*Asymmetrasca decedens* and two *Tachycixius* species) have been identified and other potential vectors are suspected. *A. decedens* is polyphagous and present across large parts of the EPPO region. The two *Tachycixius* vectors are not well known (distribution, hosts). '*Ca.* P. phoenicium' has had devastating effects on the production of almond, peach and nectarine in Iran and Lebanon with corresponding social and economic impacts. It led to the death and removal of large numbers of trees. Reports also suggest apricot as a host. There are no curative treatments. The likelihood of entry on pathways was assessed as being: - *high (with a moderate uncertainty)* for host plants for planting (except seeds) - *high (with a moderate uncertainty)* for natural spread to neighbouring countries without natural obstacles/borders, within 20 years (i.e. Lebanon to Israel or Syria; Iran to Iraq, Turkey or Azerbaijan) [*low with a low uncertainty* for the rest of the EPPO region] - *low (with a moderate uncertainty)* for plants for planting of non-hosts [hosts of the vectors] - *low* (*with a low uncertainty)* for host cut plant parts (cut branches) The likelihood of entry on all other pathways assessed was rated as very low. The phytoplasma has a high likelihood of establishing, spreading and causing impacts especially where the known vectors are known to occur. EPPO countries include some of the largest producers of almonds, peaches, nectarines and apricots globally and the pest could also have similar devastating effects in the EPPO region as in Iran and Lebanon. The EWG determined the need to implement phytosanitary measures on host plants for planting (excluding seeds) to prevent introduction into the PRA area. Based on the experience in Lebanon, it is considered that if the phytoplasma was introduced, early control measures (monitoring, detection and eradication) would significantly reduce the impact of disease. However, early detection is not guaranteed and it is desirable to avoid introduction. *Phytosanitary Measures to reduce the probability of entry:* Risk management options were studied for host plants for planting (except seeds)			
Phytosanitary risk for the *endangered area* (*Individual ratings for likelihood of entry and establishment, and for magnitude of spread and impact are provided in the document*)	**High** ☒	Moderate ☐	Low ☐
Level of uncertainty of assessment *(see Q 17 for the justification of the rating. Individual ratings of uncertainty of entry, establishment, spread and impact are provided in the document)*	High ☐	**Moderate** ☒	Low ☐
Other recommendations: The EWG made recommendations (detailed in section 18) relating to the need for including '*Ca.* P. phoenicium' in certification schemes, and for further research.			

Stage 1. Initiation

Reason for performing the PRA: almond witches' broom was brought to the attention of the EPPO Secretariat in 2001 by scientists who observed a new phytoplasma disease causing extensive mortality on almond trees in Lebanon. This almond disease was added to the EPPO Alert List in 2001 and deleted in 2006, as no particular international action was requested by EPPO member countries. Since then the disease has spread within Lebanon, the new phytoplasma species '*Candidatus* Phytoplasma phoenicium' was found to be associated with the disease in Lebanon and Iran, the host range has extended to peach and nectarine, and epidemiological studies have identified insect vectors and wild host plants. Considering the severity of this disease on several major stone fruit trees, the EPPO Secretariat decided that '*Ca*. P. phoenicium' should be added again to the EPPO Alert List (EPPO, 2015). The Panel on Phytosanitary Measures suggested '*Ca.* P. phoenicium' as a priority for PRA, which was confirmed by the Working Party on Phytosanitary Measures in June 2016.

Coverage of the PRA. This PRA covers '*Ca.* P. phoenicium'. '*Ca.* P. phoenicium' is the only species described to date in the pigeon pea witches' broom phytoplasma group (16SrIX) and corresponds to subgroup 16SrIX-B of that group (and also to subgroups 16SrIX-D, -F, -G if referring to specific sources, as explained under *Taxonomy*).

Phytoplasma strains that are not '*Ca.* P. phoenicium' are not covered in this PRA. This includes several strains found causing almond witches' broom in Iran:

- Some strains of subgroup 16SrIX-C, sometimes referred to in the literature as KAlmWB or 'Iranian almond witches' broom phytoplasma'. KAlmWB was found associated with almond witches' broom in Khafr by Salehi et al. (2006) (in addition to strains of subgroup 16SrIX-B). Many other strains in subgroup 16SrIX-C have been reported in different continents on other hosts (but not on *Prunus*, except in Iran): Italy (Ferretti et al., 2014; Martini et al., 2012 etc.), Colombia (Bertaccini and Duduk, 2009), India (Azadvar and Baranwal, 2010). Interestingly, strains of subgroup 16SrIX-C have also been found in wild plants in almond orchards in Lebanon, but not on almond (Casati et al., 2016) (nor other *Prunus*).
- Some isolates in other 16Sr groups were associated with symptoms of almond witches' broom in Central Iran (Isfahan province) (groups 16SrII and 16SrVI, Zirak et al., 2009), but these records need to be reconfirmed.

It is considered that measures taken against '*Ca.* P. phoenicium' would also cover the risk of introduction of these strains with Prunus from Iran.

The EPPO standard PM 5/5 *Decision-Support Scheme for an Express Pest Risk Analysis* was used, as recommended by the Panel on Phytosanitary Measures. Pest risk management was conducted according to the EPPO Decision-support scheme for quarantine pests PM 5/3(5) (detailed in Annex 1).

PRA area: EPPO region (map at www.eppo.org).

Stage 2. Pest risk assessment

1. Taxonomy

Taxonomic classification. Kingdom: Bacteria / Phylum: Tenericutes / Class: Mollicutes / Order: Acholeplasmatales / Family: Acholeplasmataceae / Genus: Phytoplasma / Species: *Candidatus* Phytoplasma phoenicium Verdin, Salar, Danet, Choueiri, Jreijiri, El Zammar, Gélie, Bové & Garnier, 2003 (Verdin et al., 2003).

Disease. AlmWB (*used from here onwards to refer to the disease*); English: almond witches' broom (name used in publications in English relating to Lebanon, later also to Iran); almond brooming (previous name of the disease in English publications from Iran); French: maladie des balais de sorcière de l'amandier; Persian: Jarook badam. Arabic: miknasat al-sahirat.
Various other names are used in publications to differentiate between strains or hosts, such as *Prunus scoparia* witches' broom (PSWB-P) (Salehi et al, 2015), Lebanese almond witches' broom (LAlmWB) (Salehi et al., 2006).

‘AlmWB’ is used by some authors in relation to strains in subgroup 16SrIX-C (i.e. for KAlmWB), which are not covered in this PRA (see *Coverage of the PRA*).

Taxonomic background. Some taxonomic background is useful to understand the literature on AlmWB. The taxonomy of phytoplasma is complex[1] and changes have occurred, leading to some confusion. Zhao and Davis (2016) recently suggested that a new clear phytoplasma classification system be implemented in order to prevent further confusion.

The literature on AlmWB refers to two different classifications of subgroups within the 16SrIX group (based on 16S rRNA gene sequences Abou-Jawdah et al. (2002), Wei et al. (2007) and Molino Lova et al. (2011), and on ribosomal protein [rp] and secY genes in Lee et al. (2012)). Quaglino et al. (2015) proposed to define ‘*Ca.* P. phoenicium’ strains as members of subgroup 16SrIX-B and its variants [(subgroups 16SrIX-D if per Wei et al. (2007), -F and -G if per Molino Lova et al. (2011)]. The currently accepted classification of group IX phytoplasmas follows this approach (Lee et al., 2012 with new tentative subgroups in Casati et al., 2016), and subgroups 16SrIX-D, -F and -G now refer to phytoplasmas causing other diseases on other hosts (see Table 1).

Reference strains. AF390136 (Abou Jawdah et al., 2002), AF515636, AF 515637 (Verdin et al., 2003). It should be noted that some sequences in some databases may be incorrectly mentioning ‘*Ca*. P. phoenicium’ and are related to other subgroups than 16SrIX-B (see below).

Special notes on taxonomy. In this PRA, records were retained as being ‘*Ca.* P. phoenicium’ (e.g. in Section 6 on distribution, and Section 7 on hosts) only if they unambiguously relate to subgroup 16SrIX-B (incl. -D, -F, -G for publications using the ‘older’ classification – see above). All other records of ‘*Ca*. P. phoenicium’ with evidence that they belong to a subgroup other than 16SrIX-B are not ‘*Ca*. P. phoenicium’. It is worth noting that a mention that a strain is “related to *Ca*. P. phoenicium” does not necessarily mean that it is subgroup 16SrIX-B / ‘*Ca*. P. phoenicium’.

In particular the following records should not be considered as ‘*Ca*. P. phoenicium’:

- Marchi et al. (2015) reports finding “a phytoplasma closely related to *Ca.* P. phoenicium” on various wild herbaceous dicotyledonous plants in vineyards in Italy; the strains identified in Italy belong unambiguously to subgroup 16SrIX-C (F. Quaglino, personal communication).
- Rizza et al. (2016) mention the finding of insects “infected by ‘*Ca*. P. phoenicium’” in Sicily, but identify the strains as belonging to subgroup 16SrIX-E.
- Madhupriya et al. (2013, India) report ‘99% identity with '*Ca* P. phoenicium' on *Phlox drummondi*’, but the profiles shown associate the strain to subgroup 16SrIX-C.
- Barbosa et al. (2012, Brazil) characterize a phytoplasma on *Catharanthus roseus* (periwinkle) as belonging to subgroup 16SrIX-A, but mention that it “belongs” to “the *Ca*. P. phoenicium species”.
- Bayat et al. (2013) mentions finding of subgroup 16SrIX-D on *Chrysanthemum morifolium* in Iran, without details as to which classification they refer to. This finding relates to the classification in Lee et al. (2012) (J.T.J. Verhoeven, personal communication), and is therefore not ‘*Ca.* P. phoenicium’. The same publication mentions that several strains of ‘*Ca.* P. phoenicium’ have been identified from diverse host species like *Lactuca serriola, L. sativa, Solanum lycopersicon, Sonchus* sp., but refers to the subgroup 16SrIX-E. It also mentions *Carthamus tinctorius* as a host of subgroup 16SrIX-B, but the source referred to does not mention this host.
- Records on *Lactuca serriola* and *Catharanthus roseus* in Verdin et al. (2003) were later shown to refer to another subgroup (Verdin et al., 2004).

[1] According to international guidelines of the International Phytoplasma Working Group (IPWG) in relation to ‘Candidatus Phytoplasma’, species designation has been based on the 16S rRNA gene sequence. A strain can become a new species if the similarity of 16S rRNA is below 97.5%. If the similarity is above 97.5%, a new species may be designated only if it has different insect vectors, different natural hosts and significant molecular differences. Lee et al. (2012) note that this potentially exclude many strains to become new species because their vectors or comprehensive host range are not known.

Table 1. Sub-groups of 16SrIX (pigeon pea witches' broom) (from Lee et al., 2012; Casati et al., 2016)

IX-A	Pigeon pea witches' broom
IX-B	Almond witches' broom incl. 16SrIX-B variants (formerly described as subgroups 16SrIX-D, -F, -G as per Wei et al. (2007) and Molino Lova et al. (2011)) / '*Ca*. P. phoenicium'
IX-C	Knautia arvensis phyllody
IX-D	Echinops witches'-broom
IX-E	Juniper witches'-broom
IX-F	Honduran Gliricidia little leaf
IX-G	Tomato big bud
IX-H	*Argyranthemum frutescens* witches' broom

2. Pest overview

2.1 Biology

Phytoplasmas live in the phloem of their host plants. They can be transmitted by grafting and insect vectors. They can also spread to neighbouring hosts via natural root grafts, even in the absence of a vector. Elements of the biology of '*Ca.* P. phoenicium' are given below, its vectors are dealt with in section 4, and natural and experimental hosts in section 7.

Presence in the host. In Lebanon on almond, '*Ca.* P. phoenicium' was found to be present in the phloem tissue of the stems and the roots throughout the year (Jawhari et al., 2015). It is not known if this would be the same under different climatic or environmental conditions.

The incubation period of 'Ca. P. phoenicium' in stone fruits varies and may be more than one year. In graft inoculation trials, symptoms were expressed in 1 month in Verdin et al. (2003), and 4-6 months in Abou Jawdah et al. (2003). In insect transmission trials in Lebanon, '*Ca.* P. phoenicium' was detected in almond and peach seedlings that did not show characteristic symptoms for one year after inoculation, suggesting that the incubation period may be longer (Mackesy and Sullivan, 2016, citing Abou-Jawdah et al., 2014). In natural conditions, there are no data on the latency period, which seems to vary depending on varieties and conditions. However, symptoms appeared later on *Prunus orientalis* (wild almond) than on cultivated almond (Abou-Jawdah et al., 2002).

'Ca. P. phoenicium' may not cause symptoms. It was detected in asymptomatic almond trees in the field (Abou-Jawdah et al., 2002). However, it is not known if this is due to a long latency period (see above). In Lebanon, it seems to rarely be completely asymptomatic on almond, peach or nectarine in the field. In a survey on 368 samples in Lebanon (Molino Lova et al., ND), 99.1% of asymptomatic almond and 100% of asymptomatic peach/nectarine tested negative, i.e. only a small proportion of asymptomatic almond tested positive.

The susceptibility of cultivars varies. In a survey in Lebanon in 2000-2002, some almond cultivars were highly susceptible (severe proliferation and witches' broom leading to rapid death, all trees of the variety dying), while others were less susceptible (limited parts of the canopy, or only few trees in an orchard) (Verdin et al., 2003). However, such differences may also be due to differences in latency in different cultivars. No specific study on susceptibility has been carried out to date. For the purpose of this PRA, it is important to note that there is no completely resistant cultivar of almond, peach and nectarine at the moment.

2.2 Symptoms

Other phytoplasmas (e.g. '*Ca.* P. prunorum') may cause similar symptoms, and identification to the group and the subgroup level is necessary to correctly identify '*Ca.* P. phoenicium'. Photos of symptoms on almond, peach and nectarine are given in Annex 2 and additional photos can be found in Molino Lova et al. (2011, p. 57-67).

Almonds
On cultivated almond (*Prunus dulcis*) (from Molino Lova et al., 2011; Abou-Jawdah et al., 2002; Molino Lova, 2011):

- shoot proliferation at several points on the main trunks of affected trees, or from the roots, with an occasional appearance of witches' broom. Proliferation symptoms are always observed, but witches' broom symptoms may appear only on some trees. Proliferation and witches' broom are also observed on branches
- perpendicular development of many axillary buds on the branches, with small and yellowing leaves (pale green), and shoots becoming stunted with short internodes (rosetting)
- early flowering (20-30 days earlier than normal); the peduncle of flowers was generally longer than in healthy trees
- general decline of affected trees
- severe dieback
- off-season growth
- in dry weather, the leaves may appear brownish-red.

In the first symptomatic year, only some branches show symptoms, while the entire canopy is affected from the second year. Trees decline rapidly and some die within 3-4 years following appearance of the first symptoms, while others may survive several years thereafter. The yield of infected trees is reduced. In the first symptomatic year, fruits are few, small and dark, with shriveled or sour almonds. Total loss of production happens in the year following appearance of symptoms.

On wild almonds, symptoms on *P. orientalis* are similar, but their appearance is delayed (Abou-Jawdah et al., 2002); on *P. scoparia* (Salehi et al., 2015), '*Ca*. P. phoenicium' caused yellowing, witches' broom on different parts of the tree, decline, dieback and death.

Peach and nectarine (from Molino Lova et al., 2011; Abou-Jawdah et al., 2010; Molino Lova, 2011)
The main difference between symptoms on almond and peach/nectarine is the development of phyllodies on peach/nectarine. Symptoms are:

- proliferation of shoots from the collar of the trunk and occasionally witches' broom on branches (Molino Lova, 2011)
- early flowering (15-20 days earlier than normal) and development of buds
- some months after the normal flowering period, abnormal flowers (phyllody) and smaller light green leaves
- early senescence of trees (reddening of leaves and fall).

Symptoms initially affect only some branches, and in subsequent years all branches. The disease does not lead to dieback as fast as in almond (Molino Lova, 2011). On both peach and nectarine, in the year of appearance of symptoms, most infected trees do not set any fruits, but some trees bear a limited number of deformed fruits, generally elongated and curved. The year following the appearance of symptoms, fruit production stops.
In Lebanon, in trial fields with infested peach and nectarine trees, no mortality was observed after 3 years. In the field, no mortality has been observed to date, but it is noted that growers destroy infested trees rapidly (M. Molino Lova, personal communication).

On almond, peach and nectarine in Lebanon, infected trees were observed to be more susceptible to infection by powdery mildew (Abou-Jawdah et al., 2003).

Apricot. On naturally-infected apricot trees in Iran, '*Ca*. P. phoenicium' causes leaf roll and proliferation (M. Siampour, personal communication). In Lebanon, a recovery phenomenon (remission of symptoms) was observed in apricot grafted on infested almond (Y. Abou-Jawdah, personal communication).

GF-677 (*Prunus amygdalus* x *Prunus persica*, used as rootstock): internode shortening, chlorosis, reduced size of leaves especially in the broom, proliferation of slender upright shoots, witches' broom, stunting and dieback (Salehi et al., 2011).

2.3 Detection and identification

'*Ca.* P. phoenicium' is generally symptomatic, but it may be present in the absence of symptoms because of its long incubation period (see 2.1). This should be taken into account for sampling.

Sampling and extraction techniques are important as phytoplasmas may be distributed unevenly in the plant. Therefore it is recommended to take representative samples, e.g 3-4 samples per tree, with composite sampling. For phytoplasmas in general, testing of asymptomatic plants may not be completely reliable because they would not detect very low titres, which would result in false negatives. On symptomatic trees, samples should be taken on symptomatic branches. '*Ca*. P. phoenicium' could be found in petals, leaf petioles, but the highest concentration was in the phloem tissue of stems and roots (Y. Abou-Jawdah, personal communication).

Detection is based on PCR, and identification on sequencing of PCR amplicons and/or RFLP with 17 restriction enzymes (Lee et al., 1998). Biological indexing may also be used for phytoplasma detection in certification or quarantine programmes (Marcone et al., 2014). International standards are available for the detection of phytoplasmas (IPPC, 2016 – diagnostic protocol on phytoplasmas), and for their identification (EPPO, 2016 –Standard PM 7/129 on DNA barcoding); sequences are available in NCBI for the 16S rRNA and elongation factor Tu (tufB) genes of '*Ca*. P. phoenicium', although there is only one sequence for the elongation factor Tu (tufB) gene so far. The amplification of tufB seems to be not possible with the primers set of the PM7/129 but this part of the genome can be amplified by the designed primers in Quaglino et al. (2015) (Loiseau, pers. comm. 2017). The process to add sequences to Q-bank has been initiated following this EWG. Recently, PCR and qPCR protocols have been developed for the specific detection of '*Ca*. P. phoenicium (i.e. specific to the subgroup 16SrIX-B) (Jawhari et al., 2015) that could be used on plants and insect vectors. Previous molecular methods were later shown to be only semi-specific.

3. Is the pest a vector?

Yes ☐ **No** ✓

4. Is a vector needed for pest entry or spread?

Yes ✓ **No** ☐

The involvement of one or several vectors was suspected because of the pattern of spread of AlmWB in Lebanon, affecting young and old trees in well-managed and neglected orchards, as well as isolated wild trees (Abou-Jawdah et al., 2002, 2014). *Asymmetrasca decedens* (Abou-Jawdah et al., 2014) and certain *Tachycixius* species are vectors (Tedeschi et al., 2015). Other species have been identified as potential vectors (Annex 3). All currently known or potential vectors are hemipteran leafhoppers (Cicadellidae) or planthoppers (Cixiidae). Species in other families were tested but not found carrying '*Ca*. P. phoenicium' (e.g. Psyllidae in Molino Lova, 2011). Vectors in Iran have not been confirmed.

Phytoplasma normally persist for the whole life of their insect vector once infested. There is currently no information on modalities of transmission of AlmWB (efficiency of different life stages, latency period, existence of transovarial transmission). However, transovarial transmission has rarely been studied/reported for phytoplasmas. For this reason, the EWG considered that eggs are not infested by '*Ca*. P. phoenicium', with some uncertainty.

***Asymmetrasca decedens* (synonym *Empoasca decedens*) (leafhopper; Hemiptera: Cicadellidae: Typhlocibinae)** was shown to transmit '*Ca*. P. phoenicium' to almond and peach (Abou-Jawdah et al., 2014). It is polyphagous on weeds and economic crops (see section 7 on *Hosts*), and has a wide distribution (see section 6 on *Distribution*) including part of the EPPO region. Eggs are laid in the inner part of midribs, petioles and young shoots. Nymphs are located on shoots and usually feed on the underside of the leaf (Alvarado et al. 1994; MAGRAMA 2015). In laboratory experiments, the life cycle was completed in about 56 days at 12ºC, and in about 11 days at 27ºC (Torres et al., 2002). The adult (about 4 mm long) may survive for several weeks. The nymphs jump, and adults fly and jump. In the Middle East, *A. decedens* is active throughout the year and has 4-5 generations per year. In citrus groves, the pest often occurs in large numbers on the weed undergrowth, from which it moves onto the fruits, its numbers peaking in autumn and winter (Gerson 2016). In stone fruits in Lebanon, populations peak in spring and summer (Dakhil et al., 2011).

A. decedens is a pest causing direct damage by feeding on various crops. Most publications mention damage to foliage; it has also been reported to cause cosmetic damage on *Citrus* fruit (orange and mandarin in Velimirovic, 1980; *Citrus* fruit in Vacante and Gerson, 2012 reporting damage in Turkey – see also under

Potential impact). Damage to fruit has not been recorded on other species (incl. peach and nectarine; e.g. Chaieb and Bouhachem Boukhris, 2012). It is expected that such damage would have been reported if it occurred, because *A. decedens* is a widely-studied species and considered as a pest of economic crops. In field surveys in Lebanon in AlmWB-infested almond orchards, *A. decedens* was the most abundant Hemiptera and represented over 82% of leafhoppers trapped (Dakhil et al., 2011). In an infested orchard, there would be a large number of *A. decedens* carrying the pathogen. *A. decedens* has not been confirmed as a vector in Iran; it is considered as a potential vector in Salehi et al. (2015) and it is also abundant in stone fruit orchards in Iran.

***Tachycixius* cf. *cypricus* and *Tachycixius viperinus* (planthoppers; Hemiptera: Cixiidae)** transmitted '*Ca*. P. phoenicium' to peach in experiments in Lebanon (based on one specimen of each species). The authors noted that the result (identity of the species) should be verified, because the two specimens were part of batches containing individuals of different species (Tedeschi et al., 2015). Infested *Tachycixius* cf. *cypricus* and *Tachycixius viperinus* were also both collected from weed hosts *Smilax aspera* and *Anthemis* spp., both found infected by '*Ca*. P. phoenicium' (Tedeschi et al., 2015). Mackesy and Sullivan (2016) suggest that such weeds may act as reservoir for the spread of AlmWB to stone fruit hosts, but this has not been demonstrated to date. There is little information on the two *Tachycixius* species, whose taxonomic position within the genus still need to be clarified.

Abou-Jawdah et al. (2014) make the hypothesis that vectors other than *A. decedens* may not be common pests of stone fruits, but live on wild plants or other hosts and infest stone fruits during part of their life cycles or when their natural hosts are not available (as in the case of *Tachycixius*). Cixiidae may play a role in transmission from 'wild' or alternative hosts to stone fruits, while *A. decedens* probably plays a major role in spreading the disease within or to nearby stone fruit orchards (Abou-Jawdah et al., 2014; Tedeschi et al., 2015).

The movement of *A. decedens* and *Tachycixius* between their various host plants is illustrated in Annex 4.

This PRA considers the risk of introducing '*Ca.* P. phoenicium' with individuals of *A. decedens* and *Tachycixius*, but does not provide a complete pest risk analysis of each species.

Potential vectors identified in Lebanon and Iran are listed in Annex 3. All these insects were shown to carry the phytoplasma in their body, but transmission has not been proven to date. It should be noted that these publications used earlier detection methods that were later shown to be only semi-specific to subgroup 16SrIX-B, i.e. results may indicate the presence of phytoplasmas of the group 16SrIX other than '*Ca*. P. phoenicium'. Some of the potential vectors (such as *Frutioidea bisignata, Zygina flammigera, Empoasca decipiens*) are present in the EPPO region. Several authors note taxonomic difficulties within the Cixiidae family, the fact that many species that have been collected are undescribed, and that the biology of the species is not well known (hosts, population dynamics, etc.). Other leafhoppers or planthoppers present in other parts of the EPPO region may act as vectors if '*Ca.* P. phoenicium' was introduced.

Information on the vectors is provided in different sections. Potential vectors are not covered further.

5. Regulatory status of the pest

'*Ca.* P. phoenicium' is not listed as a quarantine pest by EPPO countries according to the EPPO Global Database (EPPO GD, 2016). It was added to the EPPO Alert List in 2015 (EPPO, 2015). The EU Directive 2000/29 regulates the broad category 'virus and virus-like organisms of *Prunus*' (I.A1, d, 5) under which it names some phytoplasmas [mycoplasma] e.g. peach rosette, peach X-disease, peach yellows, as well as 'non-European virus and virus-like organisms of *Prunus*'. As organisms listed are given as examples (the list of organisms starts with 'such as', '*Ca.* P. phoenicium' can be covered under this category, although not specifically named.

Regarding non-EPPO countries, '*Ca*. P. phoenicium' is regulated in Lebanon since 2011. It is a pest of concern for the USA (Mackesy and Sullivan, 2016). Phytoplasmas are listed as harmful organisms in Australia and Nauru (according to Mackesy and Sullivan, 2016). A quick search in regulations available to the EPPO Secretariat found only phytoplasmas of other groups than 16SrIX. No further information was sought.

6. Distribution

6.1 Distribution of '*Ca* P. phoenicium'

'Ca P. phoenicium' is reported only from Lebanon and Iran, and is widespread where *Prunus* hosts are grown.

Details on the situation of *Ca.* P. phoenicium in Lebanon and Iran

Lebanon. A disease of almond trees causing severe mortality was reported for the first time at the beginning of the 1990s in Southern Lebanon, then in 1995 in Northern Lebanon (Abou-Jawdah et al., 2002, 2014). The disease was called almond witches' broom and the fact that a phytoplasma was associated with it was reported in 2001 (Choueiri et al., 2001). '*Ca*. P. phoenicium' had been found for the first time in 1999 on three nectarine trees present in an almond orchard; however, a major spread on peach and nectarine was not reported until 2008. AlmWB has spread rapidly and is present from coastal areas to high mountainous areas (>1200 m), in several ecological niches (Abou-Jawdah et al., 2002, 2014). In national surveys in areas where stone fruits (almond, peach and nectarine) are cultivated, the disease was detected in 18 (out of 26) districts of Lebanon (Molino Lova et al., ND, Molino Lova et al., 2014). Measures to eradicate or contain the phytoplasma were taken in the infested areas (see section 12). In addition a survey was performed on all 279 mother plants (279 samples) at the LARI-Tal Amara station (used for the Lebanese certified seedling production) and in 136 registered and non-registered nurseries (282 samples). All 279 mother plants tested negative. 5 (out of 136) nurseries were found selling seedlings that tested positive (Molino Lova et al., 2014), and strict measures were implemented (see Section 12).
'*Ca.* P. phoenicium' is still spreading in Lebanon. A distribution map based on the results of the 2012 survey is given below.

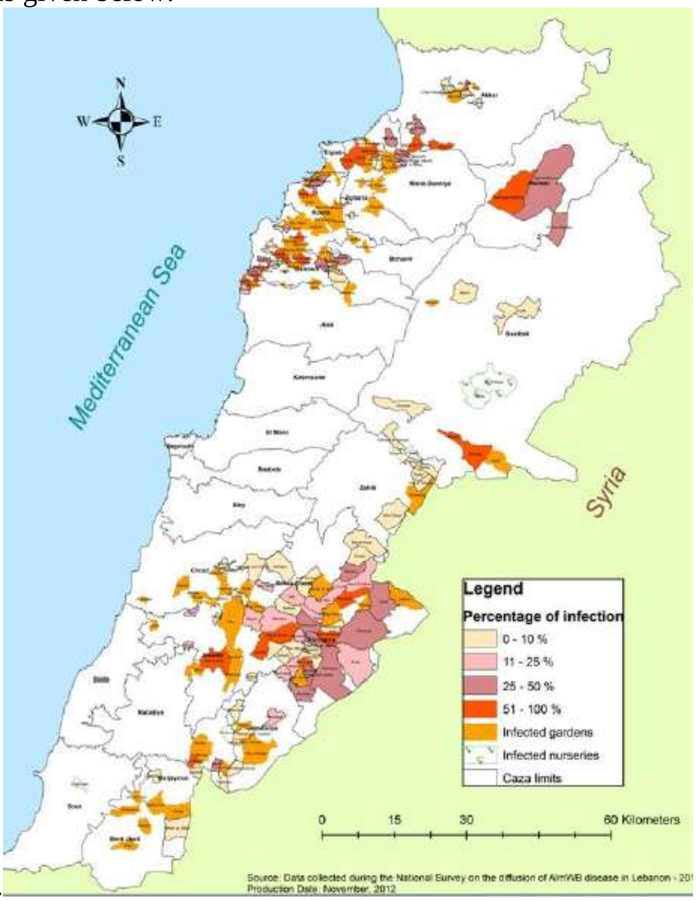


Fig. 1. '*Ca.* P. phoenicium' in Lebanon (from Al Achi and Choueiri, 2015 - powerpoint presentation)

Iran. Severe losses to almond trees by a disease initially named almond brooming have been observed in Iran since 1995 (Salehi and Izadpanah, 1995; Molino Lova, 2011). An uncharacterized phytoplasma was detected in the 1990s in an almond tree affected by the disease (Verdin et al., 2003, citing Bové et al., 1999; Salehi et al., 2006 citing Salehi and Izadpanah, 1995). It was later shown that both '*Ca.* P. phoenicium' and some strains in the subgroup 16SrIX-C caused almond witches' broom/almond brooming in Iran (Salehi et al., 2006). Almond witches' broom phytoplasma occurs in all important almond-growing areas in Iran according to Ghayeb Zamharir (2011). It is not always clear if the records in the literature relate to '*Ca*. P. phoenicium' or strains in the subgroup 16SrIX-C. Peach witches' broom was reported in Fars province in 2001 (Salehi and Izadpanah [title only], 2001). On apricot, '*Ca*. P. phoenicium' has been found so far in a very limited area (M. Siampour, personal communication).
The provinces indicated in various publications are circled on the map below: Fars, Kohgiluyeh-Boyer Ahmad, Kerman (*Ca.* P. phoenicium; Salehi et al., 2015), Kurdistan (Pourali and Salehi, 2012). '*Ca*. P. phoenicium has also been found in Razavi Khorasan, and is possibly present in other almond-growing areas of Iran (M. Siampour, personal communication). Other records of AlmWB in Iran in the literature refer to subgroup 16SrIX-C.

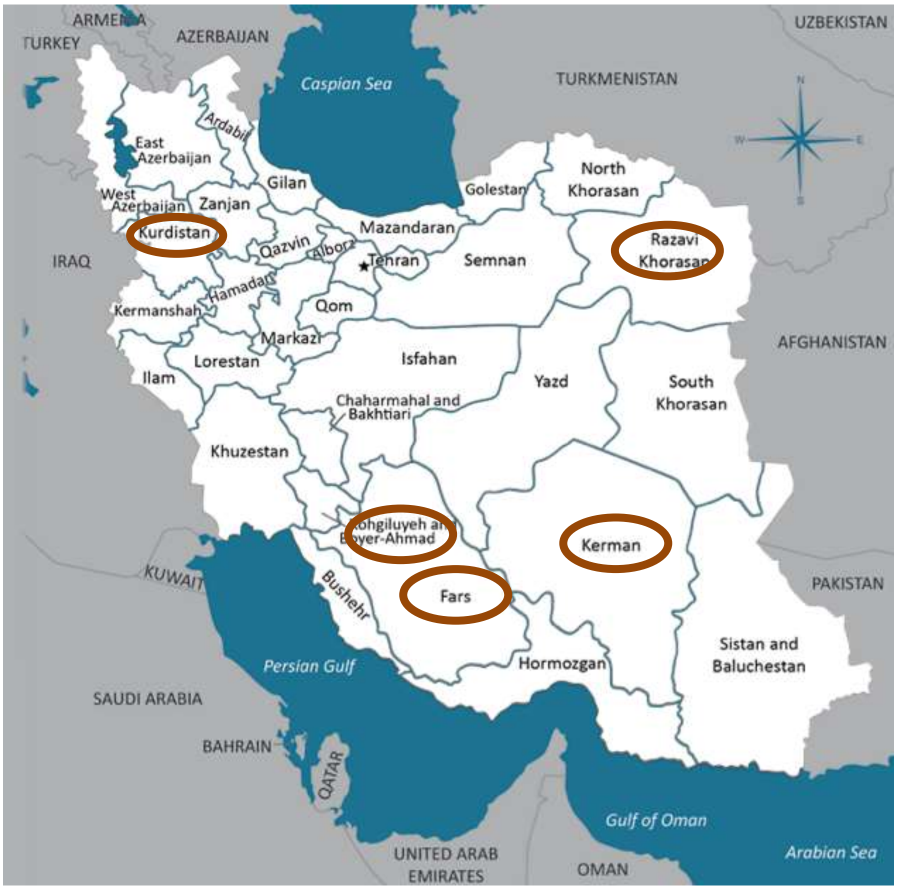


Fig. 2. '*Ca.* P. phoenicium'in Iran

It is worth noting that other subgroups of 16SrIX are present in Lebanon and Iran. For example 16SrIX-C was widespread on wild plants in Lebanon but never found on *Prunus* (Casati et al., 2016) and 16Sr IX-E is present in Iran (Jamshidi et al., 2014).

__Additional information on the distribution of '*Ca.* P. phoenicium' in other countries of the Middle East__
The pest has not been reported from other countries in the Middle East.

- **Israel.** '*Ca*. P. phoenicium' appears to be present in Lebanon at the border with Israel (Upper Galilea and Golan). The plain of Marjayoun (where '*Ca*. P. phoenicium' was found on peach and nectarine) is directly adjacent to Israel where stone fruits are also cultivated (Molino Lova, 2011).
- **Syria, Iraq and Afghanistan.** 'Ca. P. phoenicium' is present in Lebanon close to the Syrian border. In Iran, it is present close to the borders with Iraq and Afghanistan. Molino Lova (2011), based on Verdin et al. (2003) noted that the phytoplasma may be present and spreading in the Middle East (Syria and Iraq are

situated between Iran and Lebanon). **Jordan**: no symptomatic plants were observed during visits in some almond and stone fruit orchards in 2012-2013; official surveillance for AlmWB is being conducted (P. Bianco, personal communication).**Turkey**. A study was carried out in 2007-2008 in private and commercial almond orchards of Kahramanmaras villages, and in the almond, plum and peach plantations of the Nuts Research and Experimental Orchards (SEKAMER) at Kahramanmaras Sutcu Imam University in order to detect AlmWB, using PCR. No phytoplasma was found (Yüzer, 2008). '*Ca*. P. phoenicium' is also not mentioned in a review of phytoplasma diseases in the Eastern Mediterranean region of Turkey (Çarpar and Sertkaya, 2015).

- **Azerbaijan.** A survey was carried out at the end of 2016 in areas close to Iran and no symptoms were observed. New surveys will be carried out in 2017. It should be noted that the main crops in southern Azerbaijan are citrus and tea **(**J. Guliyev, Head the State Phytosanitary Control Service of Azerbaijan, pers. comm. 2017-01).

Doubtful record

- **Cuba**. Perez-Lopez (2014) is entitled 'Identification of *'Candidatus* Phytoplasma phoenicium' in periwinkle from Cuba' (XVI Congreso Internacional y XLI Congreso Nacional de la Sociedad Mexicana de Fitopatología, Mexico). However, the same author in 2016 (Perez-Lopez et al., 2016) does not refer to this in a review of phytoplasma in South America (covering Cuba). No more information was found and this record is considered doubtful.

6.2 Distribution of insect vectors

Asymmetrasca decedens: Cyprus, Egypt, France, Germany, Georgia (Russia), Greece, Iraq, Italy, Jordan, Libya, Madeira, Montenegro, Slovakia, Slovenia, Turkey, Spain, Switzerland (Nickel, 2010; new record for Germany, giving references for other countries), Iran, Israel, Lebanon, Portugal (mainland), Tunisia (Coutinho et al., 2015; for mainland Portugal - first record in 2013, symptoms observed in previous years, and giving references for other countries), Pakistan (Lodos and Kalkandelen, 1983), China (Chou and Ma, 1981; Liu et al., 2014; both as first records), India (Khan and Nighat, 1990 for Jammu and Kashmir), Turkmenistan (refers to Murgab oasis, 'Turkmenia, USSR'; Alekseev et al., 1976).
Nickel (2010) mentions that findings at the beginning of the 2000s in Switzerland and 2010 in Germany probably results from recent spread.
Uncertain records (mentioned in some country lists but without references, no specific record was found): Korea Rep. (Haghighian and Sadeghi, 2001); Korea Dem. Rep. (Tahriri Adabi et al., 2013). Dmitriev (2016) also refers to Korea with reference to Dworakowska (1968) (not available to the assessor) and North Korea (with reference to Liu et al., 2014; which mentions Korea, but without details).

Tachycixius* cf. *cypricus: Lebanon (Tedeschi et al., 2015), Turkey, Cyprus (Demirel and Hasbenli, 2015).
Tachycixius viperinus Dlabola, 1965: Lebanon (Tedeschi et al., 2015). Also reported from Bulgaria (Bourgoin, 2016 – Flow database).
No other record was found, but these species are not well documented.

7. Host plants and their distribution in the PRA area

7.1 Hosts of *Ca.* P. phoenicium

'*Ca*. P. phoenicium' has been found mostly on almond, peach and nectarine, and also on several other cultivated or wild *Prunus* species. Recently, Tedeschi et al. (2015) detected it in two wild herbaceous plants in almond orchards. It is likely that the host list is incomplete because some host plants are asymptomatic. Table 1 lists natural and experimental hosts of '*Ca*. P. phoenicium' (with subgroups indicated were relevant, as per Molino Lova et al., 2011). Species are listed as experimental hosts only if they are not natural hosts. Within '*Ca*. P. phoenicium', some strains may have a different host range; Quaglino et al. (2015) showed types associated with almond or with peach.

Table 1. Known hosts of *Ca*. P. phoenicium
Family is mentioned only for non-Rosaceae.
[W] refers to wild hosts in the country of detection

Regarding presence in the PRA area, where 'ornamental' is indicated without a reference, availability was checked in the PPP-Index (http://www.ppp-index.de/)

Species	Common name	Country where recorded as host	Presence (and use) in the EPPO region	Reference
Natural hosts				
Prunus amygdalus	almond	Iran, Lebanon	Cultivated for fruit and wild	Molino Lova et al., 2011
Prunus armeniaca	apricot	Iran	Cultivated for fruit	M. Siampour, personal communication. See Note 1
Prunus orientalis [W]	wild almond	Lebanon	Wild, ornamental	Abou-Jawdah et al., 2002
Prunus persica	peach	Iran, Lebanon	Cultivated for fruit	Verdin et al., 2003; Molino Lova et al., 2011
Prunus persica var. *nucipersica*	nectarine	Iran, Lebanon	Cultivated for fruit	Molino Lova et al., 2011
Prunus scoparia [W]	wild almond	Iran	Not known	Salehi et al., 2015
GF-677 (*Prunus amygdalus* x *Prunus persica*)		Iran	Main rootstock for almond and peach in Europe	Salehi et al., 2011
Anthemis spp. (Asteraceae) [W]	chamomillas	Lebanon	Ornamental, medicinal and wild	Tedeschi et al., 2015
Smilax aspera (Smilacaceae) [W]	rough bindweed, sarsaparilla	Lebanon	Wild, ornamental, medicinal	Tedeschi et al., 2015
Experimental hosts				
Prunus mariana GF8-1 (*P. cerasifera* x *P. munsoniana*)				Verdin et al., 2003

Note 1. *Prunus armeniaca*. References to apricot in the Iranian literature (Salehi et al., 2015 citing others) do not always mention the subgroup ((i.e. '*Ca.* P. phoenicium' or the strains of subgroup 16SrIX-C); However, M. Salehi recently deposited sequences from phytoplasmas isolated from apricot (NCBI Acc. No. KY014991 to KY014993), which show most similarity with '*Ca*. P. phoenicium' (16SrIX-B) with a RFLP similarity coefficient of 0.97. *P. armeniaca* is therefore considered as a host of '*Ca*. P. phoenicium'. In Lebanon, it has not been observed on apricot in infested areas. In graft-inoculation studies on four varieties of apricot, trees did not develop symptoms or symptom remission was observed after two months; low concentration of the phytoplasma was found in some recovered trees (in press; Y. Abou-Jawdah, personal communication).

Note 2. Other *Prunus* are not recorded as hosts. This is especially the case of cherry and plum trees. In the Bekaa region of Lebanon where almond, peach and nectarine are grown, AlmWB has not spread to adjacent cherry trees, while it has spread to peach and nectarine (Abou-Jawdah et al., 2002). Plum and cherry were also not infected in graft inoculation trial (Abou-Jawdah et al., 2003). When grafting plum on infested almond in Lebanon, one of the plum varieties showed only transient mild symptoms (Abou-Jawdah, personal communication). These results may vary with different varieties of the hosts or strains of the phytoplasma.

Note 3. A few plants of *Anthemis* sp. and *Smilax aspera* were found carrying '*Ca*. P. phoenicium' in an almond orchard in Lebanon in a study on vectors. These plants were weeds in the orchard. There is no information on the *Anthemis* species concerned. In addition, there is no information about the presence of the phytoplasma in ornamental varieties.

7.2 Hosts of insect vectors

A. decedens is highly polyphagous on a wide variety of plants. Freitas and Aguin-Pombo (2006) provide a list of 61 host species (cultivated and wild; herbaceous, shrubs and trees) in 50 genera. Hosts include peach, plum, apricot, almond, citrus, grapevine, raspberry, cotton, potato, grain plants (Freitas and Aguin-Pombo, 2006), cherry (MAGRAMA, 2015), beans, beet, lucerne (Abou-Jawdah et al., 2014, citing others), *Salix* (Allegro et al., 2011), *Populus* (Tahriri Adabi et al., 2013). The authors do not make a distinction between breeding hosts and feeding hosts.
A. decedens has attacked new hosts where introduced: in Madeira 6 of 7 hosts recorded were new host records (Freitas and Aguin-Pombo, 2006). In Spain, it was recorded as being the most important leafhopper infesting almond trees in eastern Spain during summer 1996 (Jacas et al., 1997) and has also caused severe damage on peach in nurseries and young trees (Alvarado et al., 1994).

***Tachycixius* cf. *cypricus*, *Tachycixius viperinus*.** These species are not well studied. Hosts include *Smilax aspera* and *Anthemis* spp. (Tedeschi et al., 2015), probably other weeds, and it is found occasionally on peach. The original description of the species (Dlabola, 1965) does not mention host plant species. *Tachycixius* (as many Cixiidae) nymphs live on the roots of their host plants. For *T. pilosus*, present in the UK, nymphs feed on weeds and adults on deciduous woody plants. Similar observations were reported in Lebanon for other *Tachycixius* spp. (Tedeschi et al., 2015).

8. Pathways for entry

Molino Lova et al. (2011) noted that transboundary transportation/trade of seedling by humans probably played a role in the spread of the disease. Host plants imported from countries where *A. decedens* is present were considered as a possible pathway for the introduction of *A. decedens* into mainland Portugal (Coutinho et al., 2015).

To define possible pathways, the following features of the phytoplasmas and the vectors were considered:

- Phytoplasmas are found in the phloem; they may be associated to leaves, stems and roots. They remain viable (and can be transmitted) throughout the life of the infected plants.
- Vectors are thought to be leafhoppers or planthoppers. All stages of leafhoppers (such as the known vector *A. decedens*) feed on leaves or stems. Both nymphs and adults are able to become infected while feeding on an infested plant and remain infective throughout their life but are not reported to transmit the phytoplasmas to eggs. Eggs are laid on leaves or stems. *Tachycixius* (as many Cixiidae) nymphs live on the roots of their host plants. *A. decedens* has also been reported to feed on *Citrus* fruit.

Some commodities may carry only the phytoplasma, some the phytoplasma and infectious vectors, and some only infectious vectors.

The pathways below were considered during the PRA process. **Note**: the term '***host***' below refers to the hosts of the phytoplasma (and not of vectors), unless specified otherwise. The plant species concerned are detailed in the text for each pathway.

- Host plants for planting (except seeds)
- Non-host plants for planting (except seeds) [hosts of the vectors]
- Host cut plant part (cut branches)
- Natural spread via vectors
- Non-host cut plant parts [hosts of the vectors]
- Host fruit
- *Citrus* and other non-host fruit with leaves or stems [hosts of the vectors]
- Seeds and nuts of hosts
- Pollen of hosts
- Wood and wood products of hosts
- Live vectors on their own (e.g. for scientific purposes)

8.1 Consideration of pathways

For all pathways and at the scale of the PRA area, it is considered that the current phytosanitary requirements in place in the PRA area are not sufficient to prevent the introduction of '*Ca*. P. phoenicium'. There are some prohibitions on the movement of *Prunus* plants for planting that may decrease the risk associated with the pathways. Such prohibitions would prevent entry on some commodities into at least part of the PRA area, but not all hosts are prohibited or regulated, and the existing prohibitions do not apply to the entire EPPO region.

Host plants for planting are studied in details in Table 2. Non-host plants for planting (except seeds) [hosts of the vectors], host cut plant parts (cut branches), and natural spread via vectors, are considered after the table. All other pathways were considered very unlikely and are dealt with in section 8.2.

Examples of prohibition or inspection are given in individual pathways for some EPPO countries (it was not possible in this express PRA to fully analyse the regulations of all EPPO countries). Similarly, the current phytosanitary requirements of EPPO countries in place on the different pathways are not detailed in this

PRA(although some were taken into account when looking at management options). EPPO countries would have to check whether their current requirements are appropriate to help prevent the introduction of the pest.

Table 2. Host plants for planting (except seeds)

Pathway	**Host plants for planting (except seeds)**
Coverage	• Commodities such as pot plants, rooted or unrooted cuttings, scions, tissue culture (but not seed and pollen; see section 8.2). Association with tissue culture is common for phytoplasmas (e.g. Jarausch et al. 1996) • Natural hosts of '*Ca.* P. phoenicium' in Table 1. *Anthemis* and *Smilax aspera* were not covered because there is no information about the presence of the phytoplasma in ornamental varieties.
Pathway prohibited in the PRA area?	Partly. For the EU: plants of *Prunus* intended for planting from Iran (as non-Mediterranean). For Israel, all propagative material of *Prunus*
Pathway subject to a plant health inspection at import?	Presumed in most EPPO countries, at least in the EU
Pest already intercepted?	Not known
Most likely stages that may be associated	The phytoplasma may be associated with its host plants. If vectors are associated with the plants, the phytoplasma may be in the vector.
Important factors for association with the pathway	The phytoplasma is present in the phloem tissue of host plant. In Lebanon, it is present throughout the year in the whole plants, including in dormant plants. Infected plants are asymptomatic during the incubation period. '*Ca*. P. phoenicium' has possibly a wider host range than known (more possible pathways). '*Ca*. P. phoenicium' has been found in nursery environment in Lebanon and Iran. In Lebanon '*Ca*. P. phoenicium" is regulated since 2011 (Decree of January 18, 2011), and part of the production is carried out in certified nurseries.
Survival during transport and storage	The phytoplasma would survive during transport.
Trade	No data were found on imports into the EPPO region of *Prunus* material from Lebanon or Iran. There was no import into the EU from Lebanon or Iran under commodity codes covering 'trees bearing edible fruits or nuts' (Eurostat. There were no imports of *Prunus* from Iran or Lebanon in the (incomplete) data received from a few EPPO countries for trade in 2010 during the EPPO Study on Plants for Planting (EPPO, 2012). There was no import of host plants in Azerbaijan from Iran or Lebanon **(**J. Guliyev, pers. comm. 2017-01). It is not known if other countries that are closer to Iran or Lebanon have larger imports from these countries than other parts of the EPPO region. Consequently, it is assumed here that there is no or minimal commercial trade of *Prunus* from these two countries to the PRA area. Due to the large number of serious viruses and pathogens associated with *Prunus*, part of the planting material is probably exchanged in the form of *in vitro* cultures or has been regenerated. It is not known if rootstocks are traded. In addition to trade, plants for planting may be moved by travellers, but volumes are not known. It was noted that the tourism statistics of Turkey show large numbers of persons entering from Iran (over 10 000 by rail and over 140 000 by road in 2015), and this may also be a source of introduction of plant material.
Transfer to a host	Transfer would always occur with infected plants for planting as they carry the phytoplasma. Therefore import of onlyone infected plant would be sufficient for the phytoplasma to enter.
Likelihood of entry	*High with a moderate uncertainty* (existence of trade). Potentially very high but without trade very low (no data have been found that plants for planting of host plants are actually

Pathway	Host plants for planting (except seeds)
and uncertainty	traded from countries where the pest is present to EPPO countries).

Non-host plants for planting (except seeds) [hosts of the vectors]. The vectors may occur on fresh plants for planting of their hosts (excluding tissue cultures and cuttings, due to the small size of material which would not support their survival). Adult vectors are considered unlikely to remain associated with the plants during processing and packing. .For infectious vectors to be associated with consignments of non-hosts, they should first have fed on infested host plants, and then pass from hosts to non-hosts. This is considered possible, especially if crops are close to each other, although information is lacking on the biology of vectors and how much they change hosts during their lifetime. Adults may carry the phytoplasma. Nymphs are unlikely to have acquired the phytoplasma from an infested plant as they are not very mobile. The phytoplasma does not occur in non-host plants. Some life stages of vectors may be in soil (*Tachycixius*) and weeds (alternative hosts). Infectious vectors are very unlikely to be associated with dormant plants for planting.

Data is lacking on vectors, and this pathway is based on *A. decedens* and its hosts that may be traded as plants for planting, such as *Catharanthus roseus*, *Dahlia*, *Tagetes minuscula*, *Quercus pubescens*, *Carya*, *Mentha*, *Ocimum basilicum*, *Morus alba*, *Passiflora edulis*, *Malus domestica*, *Prunus*, *Rubus*, *Populus alba* and *Populus nigra*, *Ulmus*, *Vitis vinifera*. Import of some plants for planting is prohibited in the EPPO region, but not all. Requirements are in place in many EPPO countries in relation to plants for planting (including the requirements that they are not imported with soil and weeds), and plants may be subject to inspection at import.

There was limited data on trade, only for Lebanon. During the EPPO Study on Plants for Planting (EPPO, 2012), partial data on imports of plants for planting were received from a few EPPO countries. Genera imported as plants for planting from Lebanon included a few hosts of *A. decedens* such as *Rubus* (2500 plants), *Mentha* (60 plants), *Capsicum annuum* (1 plant). The main exports from Lebanon to the EPPO countries concerned were *Cotoneaster* and *Fragaria* (25.000 each) (not hosts of *A. decedens*). The data appears to indicate a small trade, but it covered only a few EU countries and not EPPO countries that are closer to Lebanon.

For the phytoplasma to successfully transfer, infectious vectors present in consignments would have to move to host plants of the phytoplasma. It is not excluded that there are hosts of the phytoplasma close to where the plants for planting are established. However, data are not available on the modalities of transmission of this phytoplasma, how easy or difficult/successful this is, nor on the spread capacity of known vectors. .

Likelihood of entry: Low with a moderate uncertainty (trade, presence of infectious vectors on non-hosts, host range of vectors)

Host cut plant parts (cut branches). [note: scions are covered under plants for planting]. '*Ca.* P. phoenicium' may be present in cut branches of *Prunus* hosts. It is not known whether cut branches of *Prunus* are used for decoration or other purposes. The phytoplasma would remain viable only as long as the phloem remains viable. If cut branches carry only the phytoplasma, it is unlikely that a vector species already present in the country feeds on the branches (before or after they are discarded) and further acquires and transmit the phytoplasma to host plants in the country of import. If vectors are also present on the plant at harvest, they are likely to leave plant parts during processing or transport. If some life stages of vectors were nevertheless still associated with the material, the material would need to be in a state of freshness allowing survival of the vector. It is assumed that cut plant parts are likely to be used indoors or processed. For the vector to find hosts in the country of import and transmit the phytoplasma, the material would have to be discarded in the vicinity of hosts of '*Ca.* P. phoenicium'. It is not known if cut branches of *Prunus* are traded at all. No information was found on trade of the relevant commodities from Iran and Lebanon. In Israel import of cut branches of *Prunus* is prohibited. It is not known how much cross-border movement of such material would take place in the area where '*Ca*. P. phoenicium' occurs; consequently the risk of regional spread may vary from continental spread.

Likelihood of entry: low with a low uncertainty.

Natural spread via vectors. Natural spread of '*Ca*. P. phoenicium' depends on its vectors. The known vectors are present in part of the EPPO region. They are endemic where the phytoplasma currently occurs, and there is no indication that they spread rapidly. *A. decedens* is known to be carried by wind (Molino Lova,

2011). Spread will be faster if there is a continuum of cultivated or wild hosts, but there is an uncertainty on whether this exists between Lebanon/Iran and their neighbours. The phytoplasma has spread about 50 km in the past 20 years in Lebanon (Abou-Jawdah et al., 2014); this results from a combination of human-assisted and natural spread.
In Lebanon, '*Ca.* P. phoenicium' is reported adjacent or close to the border between the South of Lebanon and Israel, as well as with Syria. In Iran, Kurdistan is the northernmost province reported infested and its Northern border is at about 250 km from the nearest EPPO country (Turkey). The phytoplasma is also present close to the borders with Iraq and Afghanistan. Both from Lebanon and Iran, there is no natural obstacle to spread to neighbouring countries. The phytoplasma may enter other countries through dispersal of its vectors, but it will be slow.

<u>Spread to neighbouring countries without natural obstacles/borders, within 20 years (i.e. Lebanon to Israel and Syria; Iran to Iraq and Afghanistan, Turkey and Azerbaijan)</u> - *Likelihood of entry: high with a moderate uncertainty (continuous presence of hosts).*
<u>Other EPPO countries</u> - *Likelihood of entry: very low with a low uncertainty.*

Overall rating of the likelihood of entry (natural + human-assisted)

Rating of the likelihood of entry	*Very low* ☐	*Low* ☐	*Moderate* ☐	*High* ✓	*Very high* ☐
Rating of uncertainty			*Low* ☐	*Moderate* ✓	*High* ☐

8.2 Very unlikely pathways

The following pathways are considered very unlikely:

- **Non-host cut plant parts [hosts of the vectors].** Vectors may occur on fresh plant parts of their hosts with foliage or stems (cut flowers, cut branches, leaf vegetables) and the phytoplasma may remain in its vector for the life time of the insect. Data is lacking on vectors, and this assessment is based only on the known vector *A. decedens* and its hosts. The phytoplasma would not be associated with the plant parts, only infectious vectors. Many hosts of *A. decedens* may be traded as cut flowers, cut branches or leaf vegetables, such as: *Catharanthus roseus*, *Helianthus annuus*, *Dahlia*, *Tagetes minuta*, *Ocimum basilicum*, *Mentha*, *Zea mays*, *Populus alba*, *Populus nigra*, *Foeniculum vulgare*, *Cynara cardunculus*. If vectors are present on the plant at harvest, they are unlikely to remain on the plant material. Such material would degrade over time, but it is intended to be sold fresh and would allow the survival of vectors. According to FAO Stat, in 2013 there were small exports of cut plant parts of *A. decedens* hosts from Lebanon to EPPO countries (ca. 2 t of lettuce and chicory to Jordan; ca. 3 t of cabbage and brassicas to France and the UK; ca. 70 t of fresh vegetables (no details) to Belarus, Denmark, France, Sweden, Switzerland, Tunisia, Jordan (it is not known if this includes some leaf vegetables or only fruit)). No data was found for Iran. Cut plant parts are more likely to be used indoors or processed. Both in case of processing and indoor use, infectious vectors may be associated to discarded material. However, for the vector to transfer to hosts and transmit the phytoplasma, this material should be discarded in the vicinity of phytoplasma hosts, and still be in a state of freshness that allow survival of the vector. Data are not available on the modalities of transmission of this phytoplasma, how easy or difficult/successful this is, nor on the spread capacity of known vectors.
  Likelihood of entry: very low with a low uncertainty.
- **Host fruit.** Specific botanic structures may also carry the phytoplasmas. For example, the peduncles of cherry fruit and the bottom-pointed portion of the shell of almond while it is still soft can be used for diagnostic of X-disease phytoplasma (Australian Government, 2016). '*Ca.* P. phoenicium' may occur in fresh almond with green shell, which may be a traded commodity. Infested trees of other hosts rapidly produce deformed fruit or stop producing fruit. In all cases, vectors are unlikely to remain associated with fruit at harvest (and they probably do not feed on these plant parts), and would not be associated with consignments in trade. If a phytoplasma is present in the fruit, transfer to another host at destination is unlikely as vectors are very unlikely to feed on the fruit.
  Likelihood of entry: very low with a low uncertainty.
- ***Citrus* fruit and other non-host fruit with leaves or stems.** *A. decedens* was found associated with *Citrus* fruit; for other hosts, it is not associated with the fruit itself and may be associated with the fruit

only if leaves/stems are present Even if infectious vectors are present in *Citrus* or other non-host fruit crops at harvest, they are unlikely to remain associated with fruit at harvest (as they are mobile) and remain in fruit consignments through processing procedures, packing and transport. If infectious individuals were nevertheless associated with consignments, it is not known whether they would survive in consignments and how long they would remain infectious. Transfer to hosts at destination would require that the vectors leave fruit consignments and find hosts of '*Ca.* P. phoenicium'.

Likelihood of entry: very low with a low uncertainty.

- **Seeds and nuts.** The phytoplasma is not associated with seeds or nuts of its hosts (except as described above under 'host fruit'). The vectors are not associated with seeds or nuts of their hosts.

  Likelihood of entry: very low with a low uncertainty.
- **Pollen of hosts.** Phytoplasmas are not known to be pollen transmitted (EFSA, 2013 citing Card et al., 2007).

  Likelihood of entry: very low with a low uncertainty.
- **Wood and wood products of hosts**. The phytoplasma is in the phloem of plants, but will die when the wood dries. Transfer to other hosts from such commodities is very unlikely (no vector would feed on those).

  Likelihood of entry: very low with a low uncertainty.
- **Vectors on their own (e.g. for scientific purposes)**. Many countries regulate the import of live animals for scientific purposes. In the EU, some countries require living vectors to be kept under quarantine conditions.

  Likelihood of entry: very low with a low uncertainty.

9. Likelihood of establishment outdoors in the PRA area

9.1 Climatic suitability

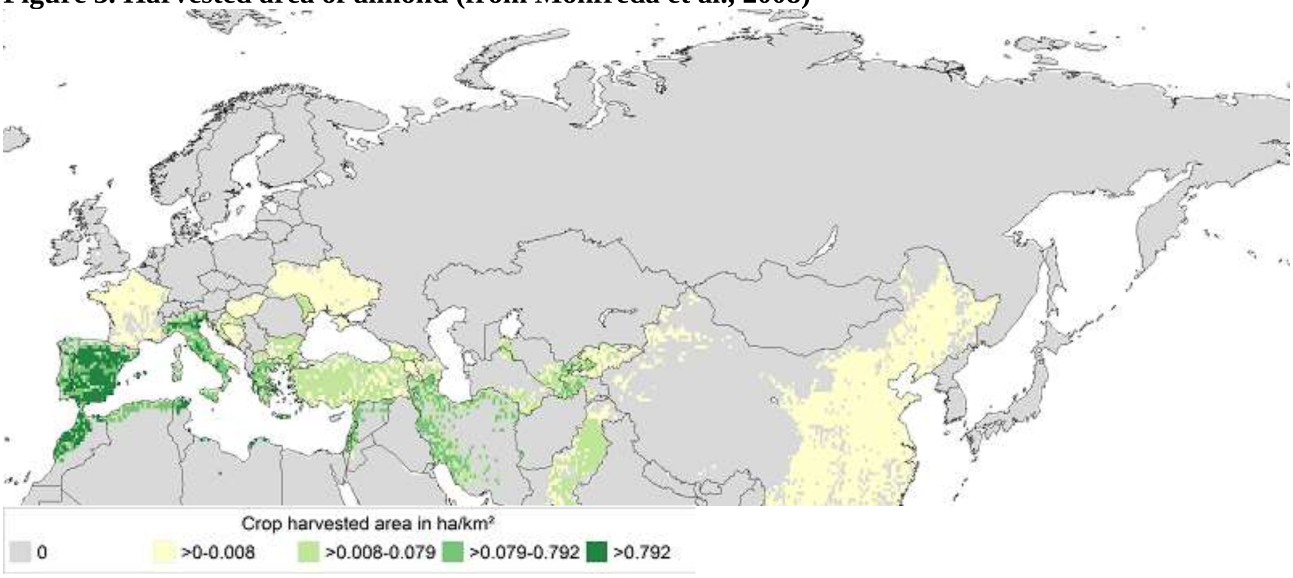
'*Ca.* P. phoenicium' is present in a wide range of altitudes in Lebanon, from the sea level to 1200 m, which also reflect a range of climatic conditions. The climatic conditions would have an impact on the insect vector more than on the phytoplasma, which would be limited only by the presence of its hosts. *A. decedens* occurs in the southern part of the EPPO region, and in the north up to Southern Germany (Baden-Württemberg) and has been spreading. In Iran, the disease is recorded in areas where minimum average temperatures in winter are below zero. There may also be other potential vectors with an even more northernly distribution. Climatic conditions are suitable for the vectors at least in the southern part of the EPPO region.

9.2 Host plants

Almond, peach, nectarine and apricot are grown commercially mostly in the southern part of the region, but are also present as ornamentals or garden plants throughout the region, including in the North. In 2013, in the EPPO region according to FAOStat:

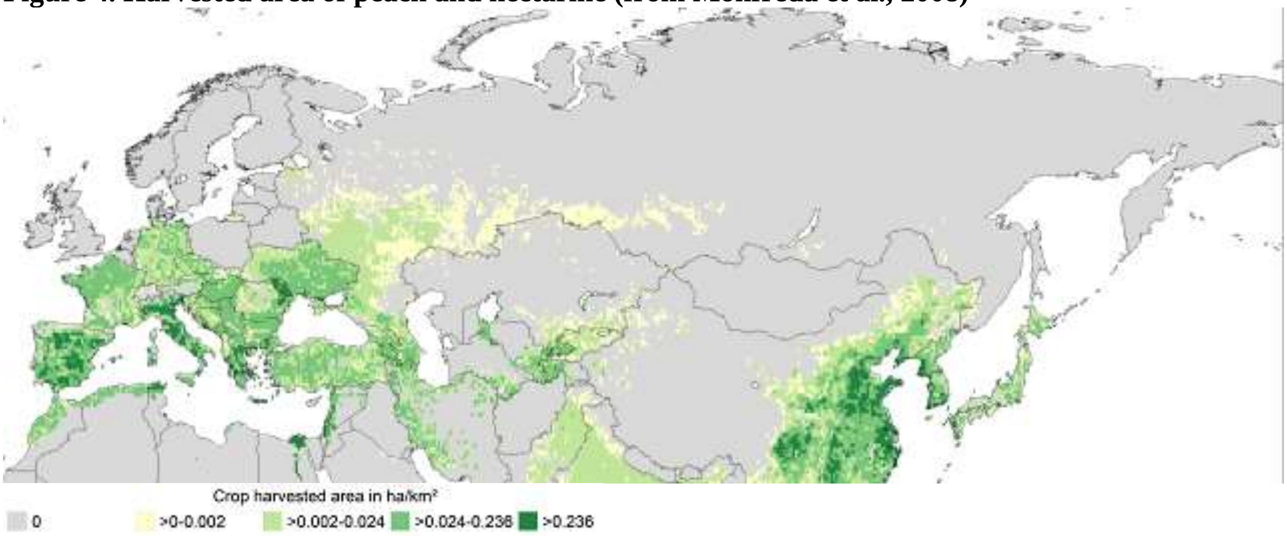
- Almonds were cultivated in 24 EPPO countries on over 1.058.000 ha. In terms of area, the top 5 EPPO countries were Spain, Tunisia, Morocco, Italy and Algeria (with ca. 534.000, 191.000, 153.000, 56.000 and 40.000 ha). Spain was the 3rd producer worldwide (149.000 t), and Morocco, Turkey, Italy, Tunisia, Algeria, Greece, Uzbekistan and Israel were amongst the 20 biggest producers worldwide.

Figure 3. Harvested area of almond (from Monfreda et al., 2008)



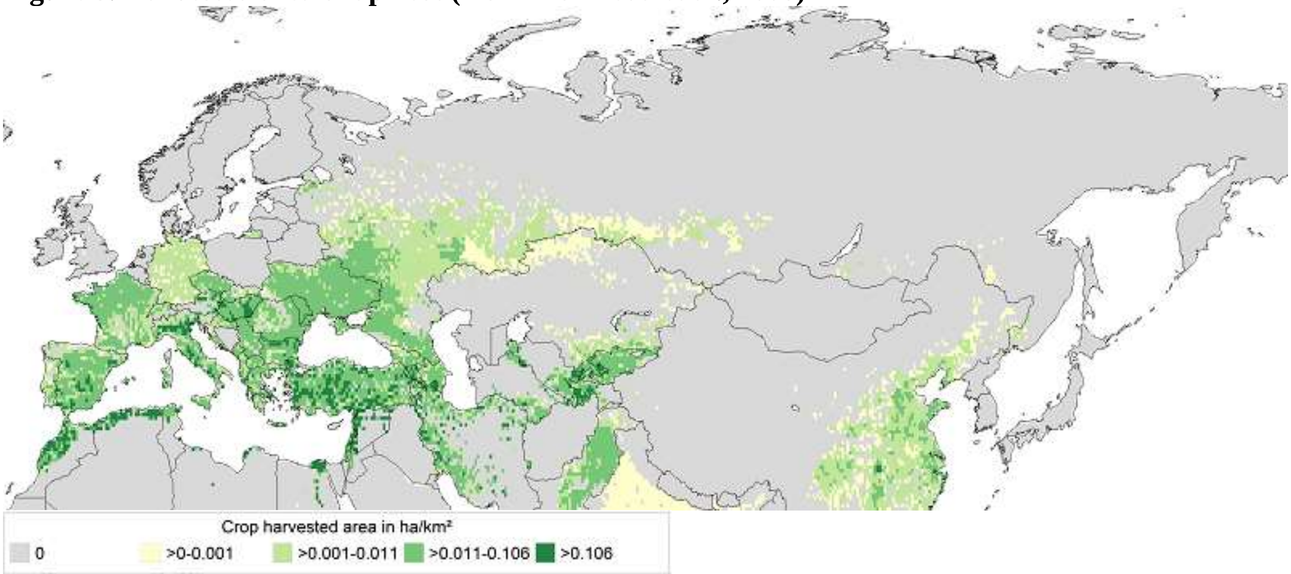
- Peach and nectarine were cultivated in 36 EPPO countries on over 370.000 ha. In terms of area, the top 5 EPPO countries were Spain, Italy, Greece, Turkey and Algeria (with ca. 84.000, 76.000, 43.000, 29.000 and 19.000 ha). Italy and Spain were the 2nd and 3rd producers worldwide (1.402.000 t and 1.330.000 t) and Greece, Turkey, France, Algeria, Tunisia and Uzbekistan were amongst the 20 biggest producers worldwide.

Figure 4. Harvested area of peach and nectarine (from Monfreda et al., 2008)



- Apricot was cultivated in 35 EPPO countries on over 350.000 ha. In terms of area, the top 5 EPPO countries were Turkey, Algeria, Uzbekistan, Spain and Italy (with ca. 245.000 ha). Turkey, Algeria and Uzbekistan were the 3 largest producers worldwide (812.000 t, 480.000 t and 320.000 t) and Italy, Ukraine, France, Spain, Greece, Morocco and the Russian Federation were amongst the 20 biggest producers worldwide.

Figure 5. Harvested area of apricot (from Monfreda et al., 2008)



The rootstock GF677 is the most used rootstock for almond and peach in Europe. *Prunus scoparia* is native in Iran and has been used as rootstock for drought resistance; it is not known if it is used in the EPPO region. *Prunus orientalis* is available in the EU as an ornamental.

The wild *Prunus* species and other host plants of '*Ca*. P. phoenicium' (*Smilax aspera*, *Anthemis* spp.) are widespread in the EPPO region. Wild plants may serve as reservoir for the phytoplasmas. This was a hypothesis made for *Smilax aspera* and *Anthemis* spp. carrying '*Ca*. P. phoenicium' in Lebanon (Tedeschi et al., 2015). *Smilax aspera* and some *Anthemis* species are also grown as ornamentals in the EPPO region.

The host range of '*Ca*. P. phoenicium' is probably wider than currently known (as not all host plants are symptomatic). In addition, if the phytoplasma passes onto new vectors in other areas of the EPPO region, there is a risk of transferring it to the hosts of this new vector.

9.3 Other elements relevant for establishment

The vector *A. decedens* is present in the EPPO region where almond, peach and nectarine are widely grown (see Distribution) and will allow establishment to occur. Where *A. decedens* does not occur, other vectors may be present and help establishment, but may be less efficient. In addition, there are less crops of hosts in these areas. There is limited data on the distribution of the known *Tachycixius* vectors. Some potential vectors have been identified (Annex 3), and there may be other vectors in the EPPO region (see section 4).

Conclusion on the probability of establishment of '*Ca*. P. phoenicium' outdoors in the PRA area

<u>Where *A. decedens* is known to occur:</u>

Rating of the likelihood of establishment outdoors	*Very low* ☐	*Low* ☐	*Moderate* ☐	*High* ☐	*Very high* ✓
Rating of uncertainty			*Low* ✓	*Moderate* ☐	*High* ☐

<u>Where *A. decedens* do not occur:</u>

Rating of the likelihood of establishment outdoors	*Very low* ☐	*Low* ☐	*Moderate* ✓	*High* ☐	*Very high* ☐
Rating of uncertainty			*Low* ☐	*Moderate* ☐	*High* ✓

Uncertainty: distribution of Tachycixius vectors, presence of other potential vectors

10. Likelihood of establishment in protected conditions in the PRA area

Major host plants (*Prunus*) are generally not grown in protected conditions. However, for early fruit production, peach trees are grown commercially under tunnels in Jordan (M. Molino Lova, personal communication), and apricot trees in Turkey. In Lebanon and Iran, almond, peach and nectarines are not grown under protected conditions.
Prunus hosts may be grown under protected conditions in nurseries. If plants of *Prunus* are brought into glasshouses, their presence in glasshouses would probably be temporary. A population may be present in the glasshouse in the infected plants, and spread to neighbouring plants if a vector is present.
Production of *Prunus* under protected conditions is likely to be subject to some controls that may help early detection.
Establishment in protected conditions is considered possible but is not critical for the establishment of this pest in the PRA area. There may be individual transient outbreaks that would eventually be eradicated.

11. Spread in the PRA area

'Ca. P. phoenicium' can spread naturally with its vector(s) and through human-assisted pathways. Planting of infected material in Lebanon and Iran is thought to be one of the most important spread pathways between regions. The presence of a vector is necessary for the further local spread of the phytoplasma. Host trees in gardens and abandoned orchards may serve as reservoirs for the disease.

A. decedens is known to be carried by wind (there is no information on the spread of *Tachycixius* vectors). Natural spread will be faster if there is a continuum of cultivated or wild hosts. In countries where *A. decedens* is present in high populations, it may be controlled due to direct damage from feeding to crops; this would decrease the rate of spread. Where control measures are implemented against *A. decedens*, natural spread is likely to be relatively local and play a minor role in the overall spread. Spread was observed within Lebanon, but it is not possible to determine how fast natural spread was with its vector(s). Spread has been slow in Lebanon in the past 20 years. The long incubation period (similar to other phytoplasma diseases) complicates early visual detection of the disease, and may have played a role in its spread to distantly isolated regions in Lebanon with infected asymptomatic seedlings (Abou-Jawdah et al., 2014). Neither the disease nor the phytoplasmas have yet been reported in neighbouring countries, which may indicate a slow spread (because it is likely that an epidemic at the same scale as in Lebanon or Iran would have been reported). Molino Lova (2011) mentions that AlmWB appeared in Lebanon and Iran at the same period, and it is unlikely that this was due to spread by insect vectors (due to the distance). There is no trade of plants or seedlings of almond from Lebanon and Iran according to Molino Lova (2011), which may indicate that the pest has spread nationally through human-assisted pathways, but that natural spread has not allowed it to reach other countries to date.

There is a large trade of *Prunus* plants for planting within the EPPO region that may facilitate spread if the phytoplasma were introduced, considering also that the known vector *A. decedens* occurs in part of the EPPO region and that other potential vectors may be present. Vegetative propagation is common for *Prunus*, which would increase the risk of spread from one infested mother plant. In the EU, there are requirements regarding absence of symptoms of '*Candidatus* Phytoplasma prunorum' during one complete cycle of vegetation for plant passporting. This may reduce the spread for the EU. Certification schemes may also be in place in countries for fruit production. However most of the *Prunus* fruit plants for planting are produced under a *Conformitas Agraria Communitatis* (CAC) quality level. As such plants for planting shall only, by visual inspection carried out by the supplier, be found practically free from the listed pests, this lower the benefit of certification schemes in decreasing the magnitude of spread for the EU.

Rating of the magnitude of spread	*Very low* ☐	*Low* ☐	*Moderate* ✓	*High* ☐	*Very high* ☐
Rating of uncertainty			*Low* ☐	*Moderate* ✓	*High* ☐

Uncertainty: patterns of spread in some environments, length of incubation period

12. Impact in the current area of distribution

Nature of the damage

'*Ca.* P. phoenicium' causes yield reduction on almond, peach and nectarine, with either unmarketable fruits or no fruit. On infected almond, peach and nectarine, total loss of production happens 1 year after appearance of the first symptoms. Where trees still produce fruits, these are fewer and deformed, resulting in practically 100% marketable yield loss (Abou-Jawdah *et al.*, 2002).

'*Ca.* P. phoenicium' causes the death of almond trees. This may occur already within 3-4 years, but some trees survive much longer. In highly susceptible cultivars, dieback may occur in all trees of an affected orchard (Verdin et al., 2003). Mortality has not been observed to date on peach and nectarine (but infested trees are eliminated rapidly – see section 2.2).

Impact in countries where it occurs

Iran. Surveys have shown the considerable distribution of AlmWB in Iran. Many infected trees have been destroyed and replaced with non-host trees (trees other than almonds and peach; M. Siampour, personal observations). However, better designed trials are needed to evaluate the impact of AlmWB. This may not be an easy task as almond trees in Iran are suspected to be simultaneously infected by AlmWB and other pathogens. Observations also showed that the trees are also suffering from the drought under climate change (M. Siampour, personal observations). Note that two different phytoplasma strains belonging to subgroups 16SrIX-C and 16SrIX-B have been involved in the occurence of AlmWB. Recent analysis have confirmed that phytoplasma strain of 16SrIX-B is more prevalent on *Prunus* (Salehi, personal communication). Molino Lova (2011) mentions that AlmWB in Iran has caused severe losses on almond trees since 1995, i.e. at the same time as in Lebanon, and that the production has been seriously affected in some provinces. The disease has had very big economic impact on almond farmers. In some regions, almond trees were replaced with pomegranate. The disease has generated many publications.

Lebanon. '*Ca*. P. phoenicium' has killed over 150 000 trees within two decades (Abou-Jawdah et al., 2014). In the regions of Akkar, Koura and Tripoli, most almond trees have died or are dying (over 65.000 trees). In the major stone fruit production area of the Bekaa, the disease is still restricted and has been contained with official phytosanitary measures and great cooperation of farmers. The disease has not spread to adjacent cherry trees in the same region, while it has spread to peach and nectarine (Abou-Jawdah et al., 2002; 2008).
Almond witches' broom has been a regulated pest since 2011, and a national plan has been implemented in 2012-2014 to manage the disease (destruction of infected trees and use of healthy planting material). Extensive awareness campaigns have been organised for farmers and nurserymen, mainly in areas of nursery production, and awareness material has been produced and circulated (booklets, posters, etc.). Control programmes have extensively involved farmers. In 2012, pilot areas were selected, and eradication campaign involving the elimination of 182 AlmWB-infected trees and their replacement have been implemented in 14 villages. Even before the pest became regulated, some farmers destroyed infected trees in an attempt to stop the disease. Between April and October 2013, a total of 6206 stone fruit trees have been eradicated by MoA and AVSI personnel (4155 in Bekaa valley and 2051 in Southern Lebanon). From 2014 onwards, the management has relied on growers to destroy infected trees; the area where the certified nurseries are located is considered disease-free.

In Northern Lebanon, where almond was extensively cultivated in dry areas and could not easily be replaced by other crops with sufficient economic return, '*Ca*. P. phoenicium' had important social impacts. The supplementary income provided by almond production to some families disappeared, and some farmers were also forced out of agriculture.

Control

Control of phytoplasma diseases is difficult in the field, and in the case of epidemics usually relies on the removal of infected plants, the control of insect vectors, the use of phytoplasma-free planting material and the use of resistant cultivars. There are no curative treatments against phytoplasmas. Early detection and eradication of phytoplasma sources are important for successful control.

The following control measures were found effective when combined against '*Ca.* P. phoenicium':

- use of certified plants from tested mother plants and healthy buds.
- avoiding grafting (taking scions) from infected trees

- weed control
- monitoring of orchards and destruction of infected trees, including the roots to avoid sprouting
- vector management. Management of vectors has relied on chemical control, but Molino Lova (2011) note that this is slowly shifting to integrate habitat management to reduce the pest incidence. However, the knowledge of vectors is still very incomplete.
- replacing infected trees by non-hosts.

There are no known cultivars that are fully resistant to the disease. It is noted that breeding of resistant cultivars may be the only way to control the disease (Ghayeb Zamharir, 2011). Grafting almond scions on tolerant/resistant crops may represent another option (Y. Abou-Jawdah, personal communication).

Preliminary results in South Lebanon were very promising, whereby monitoring, elimination/burning of all infected plants in integration with vector control and weed management, over three consecutive years eliminated the disease in two infested nectarine orchards where the disease was identified early following its introduction (Y. Abou-Jawdah and M. Molino Lova, personal communication).

Rating of the magnitude of impact in the current area of distribution	*Very low* ☐	*Low* ☐	*Moderate* ☐	*High* ☐	*Very high* ✓
Rating of uncertainty			*Low* ✓	*Moderate* ☐	*High* ☐

13. Potential impact in the PRA area

Will impacts be largely the same as in the current area of distribution? **Yes** **/No**

‘*Ca*. P. phoenicium’ is considered as a threat for the production of almond, peach and nectarines in the Mediterranean area (Molino Lova et al., 2014) and AlmWB may prove to be more devastating to stone fruit production than *Plum pox virus* (Abou-Jawdah et al., 2010). Apricot has also been identified as a host.

Almond is less intensively managed than peach, nectarine or apricot in the Mediterranean region (especially because it can be grown without irrigation and it has fewer pests; in some countries almond may be a secondary production in some farms, and receive less attention). Almond and apricot have a longer productive life than peach and nectarine, which can reach several decades. Host trees in gardens and abandoned orchards would remain for many years and are not managed.

In the EPPO region there are already several diseases associated to plant pathogenic phytoplasmas in stone fruits. According to Abou-Jawdah et al. (2003), the introduction of AlmWB may lead to mixed infections that would further aggravate the situation. However, in commercial production, it may be that control methods against other phytoplasma of *Prunus*, e.g. European stone fruit yellows, may help reduce the impact of ‘*Ca*. P. phoenicium’. In the production of propagation material, certification schemes that cover the annual testing of mother plants for the presence of phytoplasmas would ensure the production of healthy planting material and help early detection.

In addition, measures in place against *A. decedens* in *Prunus* (e.g. in Spain in commercial production, MAGRAMA, 2015) would decrease populations and therefore may also have a positive effect, but will not be sufficient to avoid further transmission. In Turkey, control measures (weed control, lime sprays) are taken against *A. decedens* in fields where summer hosts are grown, to avoid that populations migrating to citrus orchards reach high levels in the autumn (Plant Protection Technical Instructions).

Control of phytoplasma diseases rely on the use of healthy propagating material, vector control, elimination of infected plants and use of resistant cultivars. Of those, in the case of ‘*Ca*. P. phoenicium’, it is possible to ensure that propagating material is healthy and to eliminate infected plants, but there is insufficient knowledge on vectors and resistant cultivars.

‘*Ca*. P. phoenicium’ may have devastating impacts if eradication is not implemented rapidly.

If '*Ca.* P. phoenicium' was introduced into the EPPO region, considerable yield loss and tree mortality can be foreseen in countries with extensive production of almond and other hosts and where *A. decedens* is present. In areas where the known vectors are not present, or host plant production is low, impacts will be lower, unless there are other potential vectors or alternative hosts. In addition, the host range of the phytoplasmas is limited by the vectors' feeding choices, and the phytoplasma may have a broader host range if other vectors can transmit it. The potential damage in the rest of the EPPO region is less certain where the presence of *A. decedens* is not known and where there is not a dense presence of *Prunus* hosts.

The magnitude of potential impact is considered to be a high (and not very high as in the current area of distribution) because the epidemiology of the disease is now better known which could help eradication/containment to be successful, and current pest management practice may limit pest pressure.

Rating of the magnitude of potential impact	*Very low* ☐	*Low* ☐	*Moderate* ☐	*High* ✓	*Very high* ☐
Rating of uncertainty			*Low* ☐	*Moderate* ✓	*High* ☐

Uncertainties: effect of current measures (e.g. certification schemes, control against A. decedens *as a pest), whether such measures exist throughout the EPPO region, impact on varieties used in EPPO region, other potential vectors in the EPPO region, presence of neglected host plantations.*

14. Identification of the endangered area

The endangered area covers areas where almond, peach, nectarine and apricot are cultivated and known vectors occur. This corresponds to the Mediterranean Basin and Portugal, north to the southern part Germany (Baden-Württemberg) and East towards the West of Russia, as well as the Near East and Central Asia. In North Africa, *A. decedens* is currently recorded only in Tunisia, but it is not known if its distribution has been studied in Morocco and Algeria.

It is noted that *A. decedens* is probably still spreading; at least northwards, it has reached Southern Germany (See section 6 on *Distribution*), and the endangered area will expand as the vector's range expands.

There is an uncertainty for the rest of the EPPO region, where the presence of known vectors is not known and where there is not a dense presence of *Prunus* hosts. Impacts may occur if other effective vectors are present, and new vectors may also allow the phytoplasma to spread to new hosts.

15. Overall assessment of risk

'*Ca.* P. phoenicium' (belonging to the subgroup 16SrIX-B) is the causal agent of almond witches' broom. It is currently reported only in Iran and Lebanon. The cycle of the disease is complex and includes weeds as alternate hosts, and insect vectors. Vectors (*A. decedens* and two *Tachycixius* sp.) have been identified and other potential vectors are suspected. *A. decedens* is polyphagous and present across large parts of the EPPO region. The two *Tachycixius* vectors are not well known (distribution, hosts).

'*Ca.* P. phoenicium' has had devastating effects on the production of almond, peach and nectarine in Iran and Lebanon with corresponding social and economic impacts. It led to the death and removal of large numbers of trees in Iran and Lebanon. Reports also suggest apricot as a host. There are no curative treatments.

Introduction by natural spread through vectors in the EPPO region is unlikely except to nearby neighbouring countries and at a slow rate. However, vegetatively propagated material of hosts could introduce the pest to other parts of the EPPO region.

The phytoplasma has a high likelihood of establishing, spreading and causing impacts especially where the known vectors are known to occur.

EPPO countries include some of the largest producers of almonds, peaches, nectarines and apricots globally and the pest could also have similar devastating effects in the EPPO region as in Iran and Lebanon.

The EWG determined the need to implement phytosanitary measures on host plants for planting (excluding seeds) to prevent introduction into the PRA area. Based on the experience in Lebanon, it is considered that if the phytoplasma was introduced, early control measures (monitoring, detection and eradication) would significantly reduce the impact of disease. However, early detection is not guaranteed and it is desirable to avoid introduction.

Stage 3. Pest risk management

16. Phytosanitary measures

16.1 Measures on individual pathways

The EWG considered that phytosanitary measures are necessary for *Prunus* host plants for planting except seeds. Measures for *Anthemis* species and *Smilax aspera* were not considered because there is no information about the presence of the phytoplasma in ornamental varieties that could be traded. However, similar measures as for Prunus could be recommended if commercial varieties were proved to be hosts.
The EWG considered that measures were not technically justified for other pathways and such measures are therefore not studied here. Measures for plants for planting are studied in Annex 1.

The measures aim at preventing the introduction of '*Ca*. P. phoenicium' on plant material. Some of the measures identified may also prevent the introduction of the strains of subgroup 16SrIX-C that cause almond witches' broom in Iran.

There is a lack of knowledge on vectors. *A. decedens* and the two *Tachycixius* species identified as vectors are probably not the only possible vectors of '*Ca*. P. phoenicium'. Where 'vectors' are mentioned in the measures, they are intended to cover all potential vectors, which, according to current knowledge belong to the families Cicadellidae and Cixiidae.

Possible pathway	Measures identified (see details in Annex 1)
Prunus host plants for planting (except seed)	PFA (incl. measures to prevent infestation by vectors during storage/transport for plants with foliage) Plants grown under complete physical isolation (including measures preventing infestation by vectors during storage/transport for plants with foliage) In-vitro plants tested for absence of *Ca.* P. phoenicium

16.2 Eradication and containment

Phytoplasma are difficult to control and eradicate. Several features would complicate eradication or containment:
- early detection is critical to the success of eradication. However, it is complicated by the fact that '*Ca.* P. phoenicium' may remain latent for several months and even more than a year.
- leafhopper or planthopper vectors are generally polyphagous, which may favour the establishment of foci on wild plants. Vector species are already present in the PRA area.
- the phytoplasma may have other vectors once introduced in the EPPO region, which would facilitate spread and complicate containment and eradication.

In Lebanon, in the northern part of the country, due to late action, eradication was not possible and thus containment was applied However, eradication was applied in the southern part where the pest was detected early, based on the experience in the northern part.

Eradication may be more feasible in some environments than in others. In nurseries, trees may be isolated/destroyed, and vector access prevented.

An eradication programme should include:
- *delimitation and measures in the infested area*: When an infected plant has been found, the infested area is

delimited to 100 m around the infected plant, and different measures are applied in areas of 15 m and 100 m around the infected plant. The infested area includes infected plants, any plant showing symptoms, and plants that may be infected (close to infected plants or coming from a common source of production with infected plants, or grown from infected plants).

- Any infected plant should be destroyed (including their roots to avoid suckers).
- Host plants in a radius of 15 m around an infected tree (to cover for the possibility of low undetected infestations on neighbouring hosts) should also be destroyed (including their roots to avoid suckers). Asymptomatic hosts located at the edge of the 15 m-perimeter from the infected plant should be sampled and tested prior to destruction and, if positive the area should be re-delimitated.
- Host plants in a radius of 100 m around the infected plant [this distance takes account of the flight of vectors] should be inspected, and symptomatic plants should be sampled and tested. An intensive survey should be conducted on host plants and suspected weed hosts, as well as any plant showing typical phytoplasma symptoms (due to uncertainties in the host range). In the following 3 years, trees previously asymptomatic should be observed early in the season for the presence of symptoms.

Above a certain level of infection in an orchard (e.g. 25%), the whole orchard should be destroyed.
Prior to the destruction of any tree in the infested area, treatments should be applied against vectors to prevent their spread from infected plants to other plants. There should also be restrictions on the movement of any host plant material out of the infested area. Trace-back should be carried out to identify the source of the planting material and possible other contaminated stock. Finally control should be carried out in the infested area against vectors and against plants that may host those vectors, including weeds.

- *delimitation and measures in the buffer zone*. Information is lacking on the insect vectors and their biology (including spread) to allow determination of the size of the buffer zone. In Lebanon, a buffer zone of 5 km around the certified nurseries was implemented and found appropriate to protect nurseries from infestation. The EWG considered that a buffer zone of 5 km from the edge of the infested area would be appropriate. In the buffer zone, visual inspection should be conducted. Populations of potential vectors should be maintained at low levels. There should be restrictions and controls on movement of all host plants for planting out of the area.

The eradication programme should be maintained for at least 3 years after the last detection.

In addition, investigations on potential vectors in the infested area may be considered (collection, identification and test), in order to determine which species are vectors in the area.

For containment, only symptomatic plants should be removed and host plants in a radius of 100 m should be visually inspected, a buffer zone of 5 km should be delimited, and measures should be applied (vector control and restriction on the movement of hosts out of the area).

For both eradication and containment, awareness actions are essential.

As for the emergency measures defined against *Xylella fastidiosa* (EU, 2015), delimitation of an infested area may not be necessary if: (a) there is evidence that the phytoplasma was introduced recently into the area with the plants on which it was found; (b) there is an indication that those plants were infected before their introduction into the area concerned; (c) no vectors carrying the specified organism have been detected, on the basis of tests carried out in accordance with internationally validated testing methods, in the vicinity of those plants.

17. Uncertainty

- A lot of information is lacking on the epidemiology of the disease including:
  - the host range of '*Ca.* P. phoenicium', including alternative hosts and the role of weeds as reservoir, other *Prunus*;
  - whether ornamental *Anthemis* species and ornamental *Smilax aspera* are hosts;
  whether the phytoplasma is present in the phloem tissue of the stems and the roots throughout the year in all climates of the PRA area

whether finding on apricot in Iran (limited distribution) is due to a new strain of the phytoplasma in Iran, or to differences in apricot varieties or in vector species;
- variability of susceptibility among cultivars, and whether resistant cultivars can be developed;
- vectors in Lebanon and Iran (including clarification of the vector status of species shown to carry the phytoplasma but for which transmission has not been clarified; identification of other vector species), and potential vectors in the EPPO region, spread distance of vectors, transovarial transmission, efficiency of vectors.
- modalities of transmission of AlmWB from vector to host (efficiency of different life stages, latency period, existence of transovarial transmission).

- Reliability of testing on asymptomatic plants (timing of the sampling, incubation period and suitable tissue)
- Distribution of '*Ca*. P. phoenicium' in the Middle East.
- Trade of host plants from areas where the phytoplasma occurs, whether *Smilax aspera* is traded.

18. Remarks

'*Ca.* P. phoenicium' should be integrated into certification schemes for almond, as well as for peach, nectarine and apricot, in the EPPO region. The relevant EPPO Standard (PM4/30(1) *Certification scheme for almond, apricot, peach and plum*) should be revised.

There is a need for reliable sequences in Q-bank and NCBI.

There is a need for collaborative research in particular on:
- the etiology and epidemiology of the disease, in Lebanon and Iran. Since phytoplasma are not to be proved as agent of their attributed diseases on the basis of the whole fulfilment of the Koch' postulates, a robust set of data on their molecular characters (sequences) is required as well as a reliable taxonomic classification (draft and/or full genome, MLSA etc). Then, the frequent association of the phytoplasma (16SrIX-B in Lebanon and related phytoplasma strains in Iran? or 16SrIX-C in Iran) should be demonstrated.
- Vectors: data available concerning vectoring are to be confirmed in Iran. In particular for the insect species that has been discovered to be a new species (*Tachycixius*). Further field samplings are required to improve the knowledge on the vectors (new species, biology, host plant range) followed by transmission trials to confirm the vector ability.
- tolerant and resistant varieties: information about the response of the different varieties should be proved where the disease is present (endemic areas).

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Annex 1. Consideration of pest risk management options

The table below summarizes the consideration of possible measures for host plants for planting (based on EPPO Standard PM 5/3). When a measure is considered appropriate, it is noted "yes", or "yes, in combination" if it should be combined with other measures in a systems approach. "No" indicates that a measure is not considered appropriate. A short justification is included.

There is a lack of knowledge on vectors. Where 'vectors' are mentioned in the measures, they are intended to cover all potential vectors, which, according to current knowledge of '*Ca.* P. phoenicium', belong to the families Cicadellidae and Cixiidae.

Option	**Host plants for planting**
Existing measures in EPPO countries	The measures in place are not sufficient to prevent the risk of entry at the scale of the whole EPPO region (*limitations are noted in italics below*). For EU countries, the EU Directive 2000/29: - Prohibits the import of 'plants of *Prunus* intended for planting, if dormant plants free from leaves, flowers and fruit' from non-Mediterranean countries (*this covers Iran but not Lebanon*) - Regulates the broad category 'virus and virus-like organisms of *Prunus*' (I.A1, d, 5) under which it names some phytoplasma [mycoplasma] such as peach rosette, peach X-disease, peach yellows, as well as 'non-European virus and virus-like organisms of *Prunus*'. *Consequently 'Ca. P. phoenicium' can be covered under this category, although not specifically named. This may lead to different interpretations by different countries*. - Requires for Plants of […] Prunus L., [..] intended for planting, other than seeds, originating in countries where the relevant harmful organisms are known to occur on the genera concerned [for " non-European viruses and viruslike organisms, "official statement that no symptoms of diseases caused by the relevant harmful organisms have been observed on the plants at the place of production since the beginning of the last complete cycle of vegetation (IV, 19.2). *One year is not enough to detect asymptomatic plants during the incubation period.* Similar requirements will be implemented in EPPO countries applying regulations aligned to the EU. For Israel, the import of all propagative material of *Prunus* is prohibited. The current import requirements of Algeria and Turkey would not prevent the introduction of '*Ca*. P. phoenicium'. The situation was not checked for other EPPO countries.
Options at the place of production	
Visual inspection at place of production	Yes, in combination. Visual inspections at appropriate time during the growing season (e.g. at flowering) may detect symptoms but '*Ca*. P. phoenicium' is asymptomatic during the incubation period which may last for several months or even longer. Visual inspections of plants and monitoring for vectors (with yellow sticky traps) may help detect the vectors but some life stages may be difficult to see, and vectors may be on weeds at some periods of the year.
Testing at place of production	Yes, in combination. PCR may be used to detect phytoplasmas, incl. '*Ca*. P. phoenicium' (see section 2). If needed, specific PCR methods may be used to identify '*Ca*. P. phoenicium' (e.g. Jawhari et al., 2015). Composite samples (up to 10 plants) may be used to monitor plants. Tests may be part of a certification scheme or part of requirements to establish a pest-free area. In vitro plants produced from tested mother plants are a suitable option. In addition, for plants for planting with foliage, measures to prevent infestation by vectors during storage/transport should be implemented (e.g. covering with an

Option	Host plants for planting
	appropriate net).
Treatment of crop	No treatment is available against phytoplasmas. Shoot tip and stem cutting cultures associated to heat treatment were considered suitable for phytoplasma elimination from regenerated shootlets (Chalak and Choueiri 2015) but they are not currently used in the production of propagating material. Tetracycline antibiotics cause a temporary remission of symptoms (Ghayeb Zamharir 2011), but do not kill phytoplasma. They are not allowed in crop production in most EPPO countries. Insecticide treatments may be applied against the vectors. They may help lower populations but would not ensure complete elimination of the vector and therefore would not prevent transmission of the phytoplasma.
Resistant cultivars	No. There are currently no resistant cultivar known. However, grafting on other non-host *Prunus* species (e.g. plum) to provide resistance/tolerance are under investigation.
Specified age of plant, growth stage or time of year of harvest	No. Plants at any age or size may carry the phytoplasma.
Produced in a certification scheme	Not alone. A certification scheme similar to EPPO Standard PM 4/30 *Certification scheme for almond, apricot, peach and plum* but ensuring testing for '*Ca.* P. phoenicium' and covering measures against possible vectors should be implemented. Mother plants should be tested every year for phytoplasmas including '*Ca.* P. phoenicium'. In vitro plants produced from tested mother plants are a suitable option. In addition, for plants for planting with foliage, measures to prevent infestation by vectors during storage/transport should be implemented (e.g. covering with an appropriate net). *The Panel on Phytosanitary Measures considered that this option does not provide a sufficient level of assurance because vectors are difficult to control and latent infections of mother plants are difficult to detect. Plants should be grown under physical isolation.*
Growing under complete physical isolation	Yes (in combination with the use of pest-free plants as starting material). Phytoplasma-free plants may be grown in conditions preventing infestation by vectors as described in EPPO Standard on isolation PM 5/8 *Guidelines on the phytosanitary measure 'plants grown under complete physical isolation'*. Requirements include: • Windows and doors locked shut when not in use, and when open, windows fitted with appropriate screens • Double doors (with traps between the 2 doors) • Use of a net of a suitable mesh size to exclude the relevant vector(s). In addition it is recommended to control host weeds in the vicinity of the structure. This is currently recommended for mother plants supplying scions to certified nurseries in Lebanon. In addition, for plants for planting with foliage, measures to prevent infestation by vectors during storage/transport should be implemented (e.g. covering with an appropriate net)
Possibility for pest-free production site, place of production, area?	Yes (see detailed consideration for pest-free site and Pest-free area below). The vectors have a limited capacity for natural spread.

Option	Host plants for planting
Pest free production site and pest free place of production	Yes, only under complete physical isolation (see above) It is not considered possible to maintain a pest-free place of production or a pest-free production site in the open in an infested area, because vectors are widespread there and there is not enough information on their mode of spread.
Pest-free area	Yes, following ISPM 4. The pest free area established on the basis of surveillance. The exporting country should provide surveillance data to demonstrate that the pest is absent from all or part of its territory and information on how pest freedom is maintained. For a country where the pest is present in part of the country, measures should be in place to prevent that infested plants are moved to the pest free area. Delimiting surveys should be conducted to determine the exact pest distribution. Populations of the vectors should be controlled and maintained at a low level in the nurseries producing host plants for export. In addition, for plants for planting with foliage, measures to prevent infestation by vectors during storage/transport should be implemented (e.g. covering with an appropriate net and treatment with appropriate insecticides). To provide a buffer against the introduction of the disease by vectors, the PFA should be at a distance of at least 5 km from the nearest known infestation. This distance is based on the requirements for certified nurseries applied in South Lebanon, which were successful to contain the disease.
Options after harvest, at pre-clearance or during transport	
Visual inspection of consignment	No. Plants may be asymptomatic. Usually plants for planting are traded dormant without leaves, which makes the detection of symptoms difficult. Visual inspection may detect the presence of leafhoppers and planthoppers (but not all life stages).
Testing of commodity	No. The phytoplasma may be detected in dormant stem and root tissues by PCR. However, phytoplasmas are not distributed evenly in the plants, which may make the test unreliable for large plants. Recent infections at low titer may also not be detected. The vectors are widespread in countries where the phytoplasma occurs, so plants are likely to have been exposed to them in infested areas. The level of sampling and testing needed to guarantee pest freedom of the consignement may not be feasible in some cases. Tests are expensive compared to the cost of plants for planting.
Treatment of the consignment	No. No treatment is currently available to eliminate the phytoplasma Hot water treatment (dipping dormant woody grafts over a specified time period in hot water of specified temperature) was successfully tested for European stone fruit yellows phytoplasma (Krizan et al, 2008). However, the method is not common nursery practice, it needs further testing to be widely applied. This method was not tested for '*Ca* P. phoenicium'
Pest only on certain parts of plant/plant product, which can be removed	No. The phytoplasma is present in the entire plant.
Prevention of infestation by packing/handling method	Yes, in combination. Healthy plants (produced under physical isolation, or in a PFA, or in a certification scheme) should be stored or transported in conditions preventing their infestation by infectious vectors (e.g. covered by an appropriate net). Weeds should be removed to avoid introduction of reservoirs of the phytoplasma
Options that can be implemented after entry of consignments	
Post-entry quarantine	Yes, in combination. Post-entry quarantine would allow detecting the presence of the phytoplasma, but the

Option	Host plants for planting
	disease can be latent for several months (over one year – see section 2). There is uncertainty on the length of the appropriate period. Targeted tests may detect latent infections after one growing season. As the incubation period is considered to be up to 18 months, a quarantine of 24 months is recommended. Inspections should be carried out during the same period against vectors. The material should be tested during post-entry quarantine. This option is only relevant for small consignments of high value material in the framework of bilateral agreements.
Limited distribution of consignments in time and/or space or limited use	No. Not relevant for plants for planting.
No requirements at import but only Surveillance and eradication in the importing country	No. Plants for planting are likely to be planted in orchards. Infestations by '*Ca*. P. phoenicium' would be difficult to detect early, except if inspections are carried out regularly after importation and planting at the place of production. Vectors are present in the EPPO region and may spread the phytoplasmas even if plants are asymptomatic. Phytoplasmas are difficult to eradicate.

Annex 2. Symptoms of '*Ca*. P. phoenicium' - Courtesy: M. Molino Lova

Symptoms on Almonds

 Smaller leaves and sterile flowers	 Witches' Broom
 Lateral proliferation	 Die-Back of the tree

Symptoms on Peach and Nectarine


Lateral proliferation


Flower Phyllody


Witches' Broom


Abnormal fruits


Yellow leaves

Annex 3. Potential vectors of '*Ca.* P. phoenicium'

Tests showed that the species below carried '*Ca.* P. phoenicium', but transmission has not been confirmed to date. It should be noted that some publications used earlier detection methods that were later shown to be only semi-specific to subgroup 16SrIX-B, i.e. the results may indicate the presence of phytoplasmas of the group 16SrIX other than '*Ca*. P. phoenicium'. Where methods are known to be specific (i.e. relating to '*Ca.* P. phoenicium' only), this is indicated below.

- *Eumecurus* sp., *Cixius* sp., *Tachycixius bidentifer, Tachycixius* cf. *creticus* (Lebanon; Tedeschi et al., 2015). [specific methods were used]
- *Tachycixius* spp., *Hyalesthes obsoletus* and *Pentastira* spp. (genus level was used when the species found was undescribed species; Tedeschi et al., 2013), but these preliminary results concerning *Pentastira* spp. have not been confirmed in further researches (Tedeschi et al., 2015).
- Transmission trials have showed that *Circulifer haematoceps* transmits 16SrIX-C phytoplasma (Salehi et al., 2016), but it was not yet tested for transmission of subgroup 16SrIX-B (Y. Abou-Jawdah, personal communication).
- *Allygus* sp., *Annoplotettix danutae*, *Balclutha* sp., *Empoasca decipiens*, *Euscelidius mundus*, *Fieberiella macchiae*, *Lylatina inexpectata*, *Thamnottetix seclusis* (Lebanon; Dakhil et al., 2011).
- *Cixius bifidispinus* (Picciau et al., 2016 – also describing this new species)
- *Frutioidea bisignata*, *Zigina discolor*, *Psamotettix striants* and an unidentified planthopper species captured infrequently (In Iran, Fars province, Ghayeb Zamharir, 2011 citing Siampour et al., 2004).

Some of the species above, such as *Empoasca decipiens* and *Hyalesthes obsoletus* are known to transmit other phytoplasmas.

In addition, it is not excluded that leafhoppers or planthoppers that are not present in Lebanon or Iran may serve as vectors if the phytoplasmas were introduced into the EPPO region. For example:

- *Osbornellus horvathi* (Deltocephalinae) tested positive for 16SrIX phytoplasmas (possibly undescribed subgroups) in Sicily, Italy (Rizza et al., 2013). *O. horvathi* is recorded to occur in Sicily and North Africa (Fauna Europaea - De Jong et al., 2014).
- *Neoaliturus fenestratus* (Cicadellidae) was able to transmit phytoplasmas of the subgroup 16SrIX-C in experiments on *Cichorium* (Ermacora et al., 2013). *N. fenestratus* is widespread in the EPPO region (Europe to Russia, North Africa and Near East) (Fauna Europaea - De Jong et al., 2014).
- *Tachycixius pilosus* (two other *Tachycixius* species were identified as vectors in Lebanon – see section 4) is present throughout the EPPO region, from Europe to Russia and in the Near East and North Africa (Fauna Europaea - De Jong et al., 2014).

It is not possible to give a complete list of potential insect vectors in the EPPO region.

Annex 4. Epidemiology of AlmWB: movement of the vectors between their hosts

(adapted from a diagramme by Y. Abou-Jawdah and A. Alma; some pictures from EPPO Global Database [other hosts of *A. decedens*], Molino Lova, 2011 [proliferation], DISAFA, University of Turin [*Tachycixius* sp.])