Department for Environment Food & Rural Affairs

Rapid Pest Risk Analysis (PRA) for:

'Candidatus Phytoplasma fraxini'

January 2016

Summary and conclusions of the rapid PRA

This rapid PRA shows that *Ca.* Phytoplasma fraxini is a damaging pest of *Fraxinus* (ash) and *Syringa* (lilac) in North America, and an emerging problem on a range of tree species in Columbia. Since no insect vector has been identified, the assessment of potential impacts in the UK is subject to considerable levels of uncertainty. Relevant sections of this PRA (establishment outdoors, spread and potential impacts in the UK) have been rated for two scenarios: the absence of an efficient vector in UK, and the presence of an efficient and polyphagous vector. An additional appendix provides a brief overview of the recent findings of phytoplasmas in the the same group of *Ca.* Phytoplasma fraxini in South America that represent a lower risk to the UK. .

Risk of entry

The relatively small volume of trade in host planting material between the UK and North and South America means that entry on plants for planting, the only pathway assessed, is unlikely. However, there are reports of phytoplasmas belonging to the same group as *Ca.* Phytoplasma fraxini in Italy, but these have not been classified to *Candidatus* species. If it is present in Italy, the risk of entry would be considerably higher.

Risk of establishment

Establishment relies on the presence of an efficient vector in the UK or, though this is more unlikely, the disease becoming widespread in clonally propagated material. There is a moderate risk that a vector may be present in the UK hemipteran fauna, and *Ca.* Phytoplasma fraxini appears to be able to adapt to new vectors. *Philaenus spumarius,* the meadow spittlebug, has been implicated in one study as a vector of *Ca.* Phytoplasma fraxini and is present in the UK.

Economic, environmental and social impact

Impacts in the current range of the pest are rated as large, with medium confidence. It has long been associated with decline and dieback of stands of *Fraxinus* in North America, in some areas causing high mortality. It is also present in a number of urban tree species in Columbia, including species of *Fraxinus* and *Populus*, and has caused widespread decline of these trees in Bogotá. Impacts in the UK will be dependent on the susceptibility of UK tree species and capacity of UK insect species to vector the disease: without a suitable vector, economic impacts would be small and social and environmental impacts very small, with the disease essentially being self-limiting. But large economic, environmental and social impacts could be caused in the presence of a polyphagous and efficient vector.

Endangered area

In a reasonable worst case scenario, *Ca.* Phytoplasma fraxini could display a similar epidemiology as seen in Bogotá in Columbia, and spread to a number of broadleaved trees. Those in urban locations, where decline of trees will reduce aesthetic value and may present safety hazards leading to their removal, are particularly endangered in this scenario.

Risk management options

A precautionary approach is recommended. Exclusion would be the best risk management option for the UK, and specific listing of the pest in the annexes of the EU plant health legislation, in addition to requirements on planting material originating from countries were the pest is known to occur, should be considered. Eradication through destruction of infected material may be possible if the outbreak is detected early and vector activity at the outbreak site is limited or absent.

Key uncertainties and topics that would benefit from further investigation

The major uncertainties are:

- The vector of *Ca.* Phytoplasma fraxini – if further North or South American vectors of *Ca.* Phytoplasma fraxini are identified, the risk of introduction of these species should also be assessed and the PRA for *Ca.* Phytoplasma fraxini revised. Further knowledge of the vector(s) in the Americas could help identify potential UK vectors.
- A survey of phloem feeding leafhopper, cixid and psyllid species that feed on particularly vulnerable tree species in the UK, such as *Fraxinus*, could also help identify potential vectors.
- Since there is some evidence that *Philaenus spumarius*, a species of spittlebig present in the UK, can transmit *Ca.* Phytoplasma fraxini more knowledge on the host range of this spittlebug in the UK could resolve its potential to be a significant vector of the disease in the UK.
- Susceptibility of UK native trees (and other plants) to the disease requires clarification – in the USA disease severity varies depending on the species/cultivar of *Fraxinus* or *Syringa* infected. No definitive information could be obtained on the susceptibility of *F. excelsior*, except for the fact that it is a symptomatic host. Reports of any cases of *Ca.* Phytoplasma fraxini on this, or other UK native species, could help resolve this issue.
- A phytoplasma belonging to 16SrVII, the same taxonomic group as *Ca.* Phytoplasma fraxini, has been recorded infecting plants in Italy, but diagnosis was not taken down to *Candidatus* species level – thus it is not known if this finding is *Ca.* Phytoplasma fraxini or another member of the group. If *Ca.* Phytoplasma fraxini is present in Italy, this risk analysis would require review as the risks would be considerably different.

Images of the pest

Witches' Broom on *Fraxinus pennsylvanica*. Image by William Jacobi, Colorado State University.

Is there a need for a detailed PRA or for a more detailed analysis of particular sections of the PRA? If yes, select the PRA area (UK or EU) and the PRA scheme (UK or EPPO) to be used.

No	✓				
Yes		PRA area: UK or EU		PRA scheme: UK or EPPO	

Given the information assembled within the time scale required, is statutory action considered appropriate / justified?

As a non-European phytoplasma, *Ca.* Phytoplasma fraxini is already regulated on certain hosts (*Vitis, Prunus*) and because of the potential impacts on additional hosts, statutory action would be appropriate for any findings of this pathogen.

Yes Statutory action	✓	No Statutory action	

Stage 1: Initiation

1. What is the name of the pest?

'Candidatus Phytoplasma fraxini'

Synonyms: ash yellows phytoplasma, lilac witches'-broom phytoplasma.

Special notes on taxonomy and the scope of the PRA

Phytoplasmas cannot be cultured, and as a consequence their taxonomic names include "*Candidatus*" to indicate it is a well characterized organism, but uncultured. They were previously referred to as mycoplasma-like organisms. Much of phytoplasma taxonomy is based on analysis of the 16Sr gene – this analysis splits phytoplasmas into groups, and further analysis by molecular methods leads to classification into sub-groups.

Ca. Phytoplasma fraxini belongs to Group VII – which is also known as the ash yellows group, with some publications then classifying it further to be within subgroup A (16SrVII-A) (Fránová *et al.* 2014, Griffiths *et al.* 1999). Taxonomically, a phytoplasma can only be referred to as *Ca.* Phytoplasma fraxini if it shows 100% sequence identity to the type strain, otherwise it is called a *Ca.* Phytoplasma fraxini related strain. The main section of this PRA will largely refer to reports of 16SrVII-A phytoplasmas, but may also refer to reports of those that have not been classified to subgroup. Because *Ca.* Phytoplasma fraxini was not formally described until 1999, references to ash yellows phytoplasma/lilac witches'-broom phytoplasma before this date cannot be guaranteed to refer to *Ca.* Phytoplasma fraxini or its related strains, but since they are very likely to, they are included within the main body of the PRA.

The Group VII phytoplasmas include several other subgroups, many of them recently described and still showing high levels of homology to the *Ca.* Phytoplasma fraxini type strain. For these reasons, a brief summary of these phytoplasmas is included within an appendix to this PRA, but the risk they pose is not considered further.

2. What initiated this rapid PRA?

Candidatus Phytoplasma fraxini was identified as a potential threat to European ash (*Fraxinus excelsior*) and added to the UK Plant Health Risk Register in March 2015, from which it was then given a priority for PRA to see if statutory action and regulation are justified.

3. What is the PRA area?

The PRA area is the United Kingdom of Great Britain and Northern Ireland.

Stage 2: Risk Assessment

4. What is the pest's status in the EC Plant Health Directive (Council Directive 2000/29/EC[1]) and in the lists of EPPO[2]?

Ca. Phytoplasma fraxini is not listed by name in the EC Plant Health Directive; however, as a non-European phytoplasma whose hosts include *Vitis* and *Prunus*, it is covered by the entry in Annex IAI for "Non-European viruses and virus-like organisms of *Cydonia* Mill., *Fragaria* L., *Malus* Mill., *Prunus* L., *Pyrus* L., *Ribes* L., *Rubus* L., and *Vitis* L.".

It is not recommended for regulation as a quarantine pest by EPPO, nor is it on the EPPO Alert List.

5. What is the pest's current geographical distribution?

The known distribution of *Ca.* Phytoplasma fraxini is summarised in Table 1. *Ca.* Phytoplasma fraxini is largely found in the Central and North East of the USA and Central and Eastern Canada (CABI, 2015). Reports of disease of *Fraxinus* and *Syringa* that are typical of those caused by *Ca.* Phytoplasma fraxini have been known since the 1920s in the Northeast of the USA (Carr & Tattar 1989), and it would seem to be an endemic pathogen in at least some parts of North America.

Reports of *Ca.* Phytoplasma fraxini and its related strains in South America are more recent. *Ca.* Phytoplasma fraxini related strains were found in Chilean vineyards in samples collected between 2003-2005, and showed between 97-99% sequence identity of the 16Sr gene to North American strains of *Ca.* Phytoplasma fraxini (Fiore *et al.* 2007). There have been further findings since in other hosts in Chile (Arismendi *et al.* 2011) (see section 7). In Columbia, dieback of *Fraxinus uhdei* was described in

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[1] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2000L0029:20100113:EN:PDF

[2] https://www.eppo.int/QUARANTINE/quarantine.htm

2001 and is associated with *Ca.* Phytoplasma fraxini strains with 99% identity to North American strains (Franco-Lara & Henao 2014).

It is not clear if *Ca.* Phytoplasma fraxini is endemic to some parts South America.

Table 1: Distribution of *Ca.* Phytoplasma fraxini	
North America:	Canada (Alberta, Manitoba, Ontario, Quebec, Saskatchewan), USA (Colorado, Connecticut, Illinois, Indiana, Iowa, Kansas, Kentucky, Massachusetts, Michigan, Minnesota, Missouri, Montana, Nebraska, Nevada, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Pennsylvania, South Dakota, Utah, Vermont, West Virginia, Wisconsin, Wyoming) (CABI 2015)
Central America:	No records
South America:	Chile (Arismendi *et al.* 2011, Fiore *et al.* 2007), Columbia (Franco-Lara & Henao 2014)
Europe:	No records
Africa:	No records
Asia:	No records
Oceania:	No records

Not included in Table 1, there are some reports of phytoplasmas belonging to the 16SrVII group elsewhere globally. These have not, however, been classified to *Candidatus* species level – as a consequence this adds an inherent uncertainty about the distribution of *Ca.* Phytoplasma fraxini globally. Such reports are summarised below.

In the first report of *Ca.* Phytoplasma fraxini infecting *Prunus persica* in Canada, Zunnoon-Khan *et al*., 2011, referenced an “incidental report in peach in Southern Italy” of a 16SrVII-related strain. In addition, an experimental crop of *Hypericum perforatum* was reported to be infected with a 16SrVII phytoplasma in Bologna, Italy (Bruni *et al.* 2005). Some literature also references possible findings in *Prunus* in China (Gao *et al.* 2011, Sinclair & Griffiths 2000). It has also been reported that phytoplasmas belonging to group 16SrVII have been found in ornamentals in India (Singh *et al.* 2011).

6. Is the pest established or transient, or suspected to be established/transient in the UK/PRA Area?

Ca. Phytoplasma fraxini is not known to be present in the UK, and nor has it ever been intercepted. There are also no records of other group 16SrVII phytoplasma in the UK.

7. What are the pest's natural and experimental host plants; of these, which are of economic and/or environmental importance in the UK/PRA area?

Candidatus Phytoplasma fraxini was first reported on various species of *Fraxinus* (ash) and *Syringa* (lilac) (Griffiths *et al.* 1999). Sinclair *et al.* 1996 listed the following *Fraxinus* and *Syringa* species as known hosts of ash yellows/lilac witches' broom phytoplasma: *Fraxinus americana* (white ash), *F. angustifolia* (narrow leaved ash), *F. bungeana, F. excelsior* (European ash), *F. latifolia* (Oregon ash), *F. nigra* (black ash), *F. ornus* (manna ash), *F. pennsylvanica* (green ash), *F. profunda* (pumpkin ash), *F. quadrangulata* (blue ash), *F. sogdiana* (Tianshan ash) *F. velutina* (velvet ash), *Syringa x diversifolia, S. x henryi, S. x josiflexa, S. josikaea* (Hungarian lilac), *S. kamarowii* (nodding lilac), *Sx laciniata* (cut-leaf lilac), *S. meyeri* (Korean lilac), *S. x nanceiana, S. oblata, S. x persica* (Persian lilac), *S. x prestoniae, S. pubescens* subsp. *microphylla, S. pubescens* subsp. *patula, S. sweginzowii* (Chengtu lilac), *S. tomentella, S. villosa* (late lilac), *S. vulgaris* (common lilac) and *S. yunnanensis* (Yunnan lilac). In addition, in Columbia *F. uhdei* (Urapan) suffers from ash yellows caused by *Ca.* Phytoplasma fraxini (Filgueira *et al.* 2004, Franco-Lara & Henao 2014).

Given the significant number of *Fraxinus* and *Syringa* species known to be hosts, it is likely that other members of these genera are susceptible to *Ca.* Phytoplasma fraxini.

In Canada, *Ca.* Phytoplasma fraxini strain (in group VII-A) has been reported from *Prunus persica* (peach) and from Pembina plum (Arocha-Rosete *et al.* 2011, Zunnoon-Khan *et al.* 2010), a hybrid of *Prunus salicina* (Japanese plum) and *Prunus nigra* (Canadian plum). It is not known if other species of *Prunus* may be susceptible to *Ca.* Phytoplasma fraxini, but it is likely.

In South America, several other hosts of *Ca.* Phytoplasma fraxini and its related strains have been identified. A molecular study of phytoplasmas present in *Vitis vinifera* (grapevine) showing grapevine yellows symptoms in Chilean vineyards detected 16SrVII-A in 8 samples (out of 90), which showed varying degrees of sequence identity with North American strains of *Ca.* Phytoplasma fraxini (Fiore *et al.* 2007). Further work was then undertaken to see if *Ca.* Phytoplasma fraxini could be identified in any weeds within the vicinity of the vineyard, and a positive result was obtained for *Convolvulus arvensis* (field bindweed), *Galega officinalis* (goat's rue) and *Polygonum avicular* (knotgrass) (Longone *et al.* 2011). Other hosts identified in Chile include *Ugni molinae* (murta), a native bushy plant species, *Paeonia lactiflora* (peony) (Arismendi *et al.* 2011) and *Gaultheria phillyreifolia (*Arismendi et al. 2010)*,* another native Chilean plant.

In Columbia *Ca.* Phytoplasma fraxini was first identified in *F. uhdei* in Bogotá. It was then noted that additional tree species were showing symptoms typical of phytoplasma disease, and *Ca.* Phytoplasma fraxini was additionally identified in the following species: *Acacia malanoxylon* (Australian blackwood), *Croton* spp. (rushfoil), *Eugeia myrtifolia* (brush cherry), *Liquidambar styraciflua* (sweet gum), *Magnolia grandiflora* (bull bay), *Pittosporum undulatum* (sweet pittosporum), *Populus nigra* (black poplar) and *Quercus humboldtii* (Columbian oak). Given the high degree of genetic similarity between the phytoplasma strains isolated (99-100% sequence identity with North America *Ca.* Phytoplasma fraxini and strains from *F. uhdei*), it was theorised that the pest was introduced to Columbia on plants of *F. uhdei* and has since spread to new hosts (Franco-Lara & Henao 2014).

It is very unlikely the full host range of *Ca.* Phytoplasma fraxini has been elucidated.

Hosts of importance to the UK include *Fraxinus excelsior*, a widespread woodland tree currently under threat from the ash dieback fungus *Hymenoscyphus fraxineus.* In addition, *Populus nigra* is a rare and native tree in the UK, with ongoing conservation efforts to preserve the remaining population (Cottrell 2004). Various *Syringa* species are widely grown as ornamentals. Wine production is a growing industry for the UK with some 1884 hectares of *Vitis vinifera* now cultivated for this purpose (Anon 2015). Many additional species may be susceptible to *Ca.* Phytoplasma fraxini if it was transmitted by a polyphagous vector.

8. What pathways provide opportunities for the pest to enter and transfer to a suitable host and what is the likelihood of entering the UK/PRA area?

Phytoplasmas are obligate parasites, and require a living plant or insect vector in order to survive. They are not transmitted by true seed. The only pathways of entry for phytoplasmas are plants for planting or hitchhiking of already infectious vectors. Since the vector for *Ca.* Phytoplasma fraxini has not been identified, this pathway cannot be assessed.

Plants for Planting

Entry on plants for planting is unlikely.

As of December 2015, the current requirements for the import of *Fraxinus* into the UK are that it must originate from a pest free area (PFA) for *Hymenoscyphus fraxineus.* Since no country has declared a PFA, no *Fraxinus* can be imported. If these regulations change, current measures for the plant pest *Agrilus plannipennis* (emerald ash borer) will continue to prevent the import of *Fraxinus* from much of the range of *Ca.* Phytoplasma fraxini in North America, as there are a limited number states/provinces that can be called free of *A. plannipennis* and are thus able to export *Fraxinus* plants for planting. In addition, *Vitis vinifera* cannot be imported from outside of Europe under current EU plant regulations.

Open pathways of entry on plants for planting do exist. *Prunus* plants can be imported dormant from Canada and the continental USA. As mentioned in section 4, *Ca.* Phytoplasma fraxini is regulated as a non-European virus-like organism of *Prunus*. Current requirements state that imports should be accompanied by an 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”. This provides some mitigation to the risk of entry on *Prunus* plants, but it should be noted that phytoplasmas often have a period of latency in woody hosts where symptoms may not be apparent. There are no specific import requirements for the other hosts of *Ca.* Phytoplasma fraxini. There are also no specific commodity codes for *Syringa* or the other unrelated hosts identified in South America, so it is not possible to elucidate current levels of import precisely.

Data on import volume from countries in Table 1, between 2010-2014, was extracted from Eurostat using commodity codes with the following descriptions: Trees, shrubs and bushes, grafted or not, of kinds which bear edible fruits or nuts (excluding vine slips); Outdoor rooted cuttings of young plants of trees, shrubs and bushes (excluding fruit, nut and forest trees); Live forest trees; Outdoor trees, shrubs and bushes, including their roots (excluding cuttings, slips and young plants, and fruit, nut and forest trees). All of these codes will include a considerable proportion of non-hosts.

There were no imports from the South American countries. Imports from North American countries were very low, with the largest amount imported during the five years being 38.5 tonnes from the USA in 2013. Some material may initially be imported to other EU member states, and then sold on to the UK (thus becoming EU in origin), but import is low for this sort of plant material across the EU. For example, the mean import quantity from the USA across the EU and all the listed commodity codes for the last five years is approximately 207 tonnes a year (Eurostat data extracted 30.10.2015). Other EU MS do import planting material directly from Columbia and Chile, albeit at low levels.

There are very few reports of *Ca.* Phytoplasma fraxini in commercial production systems. In North America, it has been theorised that this is due to the lack of a vector in nurseries, and it has been reported that the one case in a shade tree nursery was related to the grafting of the plants on to diseased material (Sinclair *et al.* 1996). However, it has been theorised that *Ca.* Phytoplasma fraxini could have been introduced to Columbia on young *F. uhdei* plantlets (Franco-Lara & Henao 2014). It has also been noted that lilac witches'-broom has been moved between botanical collections on infected plants (Hibben & Franzen 1989).

Taking the above into account, entry on plants for planting is rated as unlikely, with low confidence. Three factors contribute to the low confidence: the lack of specific data on imports from the countries where *Ca.* Phytoplasma fraxini is known the occur, the increasing host range of *Ca.* Phytoplasma fraxini that has been reported in recent years and the uncertainty about the possible presence of the pest in Italy, where a 16SrVII phytoplasma has been detected but not diagnosed to *Candidatus* species. There is considerably more movement of material from Italy into the UK than from the Americas.

Plants for Planting	Very unlikely ☐	Unlikely ☑	Moderately likely ☐	Likely ☐	Very likely ☐
Confidence	High Confidence ☐	Medium Confidence ☐	Low Confidence ☑		

9. If the pest needs a vector, is it present in the UK/PRA area?

Phytoplasmas require an insect vector, but the vector of *Ca.* Phytoplasma fraxini remains unidentified in North America. Perilla-Henao *et al.* 2015 recently published a paper identifying two leafhoppers able to transmit *Ca.* Phytoplasma fraxini strains under experimental conditions.

Phytoplasmas are usually transmitted by phloem feeding Hemiptera including leafhoppers (Cicadellidae), planthoppers (Fulgoromorpha) and psyllids (Psyllidae) – the largest number of vector species belong to the leafhopper subfamily Deltocephalinae (Wilson & Weintraub 2007). In recent years, reports have emerged of xylem-feeding leafhoppers transmitting phytoplasmas, including *P. spumarius* (Rosa et al. 2014).

The phytoplasma-vector relationship is not well understood. Phytoplasmas are transmitted in a circulative (meaning they replicate within the vector), and persistent (meaning the vector is infectious for life) manner by vectors. In order to be vectored the phytoplasma must cross the gut wall of the insect and enter the circulatory system. The phytoplasma then moves to the salivary glands, and will replicate there.

Once in the salivary glands, the phytoplasma can be transmitted to new hosts when the insect feeds.

There have been some studies on movement of phytoplasmas within leafhoppers, and evidence currently suggests that interaction is required between proteins on the outside of the phytoplasma and those in the insect gut to enable to phytoplasma to cross the gut wall (Galetto *et al.* 2011, Siampour *et al.* 2011, Suzuki *et al.* 2006). This interaction does not occur in non-vectors; a phytoplasma species cannot be transmitted by all phloem feeding Hemiptera. It is more likely that each phytoplasma species is only transmitted by a limited number of Hemiptera.

Despite this, many phytoplasmas still have multiple vectors and these can be in different hempiteran groups. For example, *Ca.* Phytoplasma solani is vectored by the cixid species *Reptalus panzeri* (Cvrković *et al.* 2014) and *Hyalesthes obsoletus* (Bressan et al. 2007) but in laboratory studies the leafhopper *Anaceratagallia ribauti* was also able to transmit *Ca.* Phytoplasma solani (Riedle-Bauer *et al.* 2008). The main vector of *Ca.* Phytoplasma mali in some areas of Europe is the psyllid *Cacopsylla melanoneura* (Tedeschi & Alma 2004), though in Germany it is *C. picta* (Mayer et al. 2009), and it can also be transmitted by the leafhopper species *Fieberiella florii* (Tedeschi & Alma 2006).

Current Knowledge on Vectors in North and South America

The epidemiology of *Ca.* Phytoplasma fraxini in North America, where until 2010 all known cases were limited to *Fraxinus* and *Syringa*, strongly suggests that the main vector of ash yellows/lilac witches'-broom has with a strong preference for *Fraxinus* and *Syringa,* or perhaps the family Oleaceae more widely. Potential herbaceous hosts which can often act as a reservoir of phytoplasma disease have been surveyed, but no phytoplasma was detected (Griffiths *et al.* 1994). Furthermore it has been noted that the incidence of *Ca.* Phytoplasma fraxini is often worse when *Fraxinus* and *Syringa* are grown in close proximity, such as in botanical gardens or public plantings (Sinclair & Griffiths 1994).

Vector habitat preference has been proposed as the reason that certain stands of ash or lilac have a higher incidence of *Ca.* Phytoplasma fraxini than others: in general the disease is more prevalent in regions where wooded and open areas are intermixed as opposed to heavily forested areas (Sinclair *et al.* 1990).

Some species in North America have been implicated as vectors of *Ca.* Phytoplasma fraxini, though none have been shown to be vectors under experimental conditions – they merely had the phytoplasma present in the gut during molecular detection, which is usually considered not to be conclusive of vectoring capability. Leafhopper species that have tested positive for the presence of *Ca.* Phytoplasma fraxini are: *Graminella nigrifrons* (Arocha-Rosete et al. 2011); *Scaphoideus titanus* (Olivier et al.

2014) and *Colladonus clitellarius, Scaphoideus intricatus* and other *Scaphoideus* spp. (Hill & Sinclair 2000).

In caged tests, *Philaenus spumarius* and *Paraphlepsius irroratus* transmitted the “yellows agent” (not confirmed as *Ca.* Phytoplasma fraxini) to ash seedlings (Hiruki 1988). The status of *P. spurmarius* as an efficient vector of *Ca.* Phytoplasma fraxini is still questionable, as results of the initial study could not be replicated (Sinclair & Griffiths 1994). *Philaenus spumarius* collected from a site in New York where *Ca.* Phytoplasma fraxini was prevalent did not test positive for the pest (Hill & Sinclair 2000), however *P. spunarius* has been shown to transmit another phytoplasma affecting trees, *Ca.* Phytoplasma ulmi (Rosa *et al.* 2014). This finding is unusual, as *P. spunarius* is a xylem feeding hemiptera and phytoplasmas are limited to the phloem.

None of these species are likely candidates for the main vector of *Ca.* Phytoplasma fraxini in North America as they do not show a strong preference for *Fraxinus* or *Syringa.* With the exception of the common froghopper, *P. spumarius,* none of the implicated leafhopper taxa are present in the UK.

In Columbia, because all isolates of *Ca.* Phytoplasma fraxini are very similar to North American isolates, it suggests the disease is exotic. It is likely it was introduced on plants for planting from North America (*F. uhdei*), and then spread to new hosts (Franco-Lara & Henao 2014). It is very likely that *Ca.* Phytoplasma fraxini has a different vector species (or possibly multiple vectors) than in North America, which feed on a wide range of broadleaved trees. In South America, *Carelmapu ramosi* has been reported as a potential vector (Arismendi *et al.* 2010).

Leafhopper species were collected from grass around trees and a limited number from the canopy of symptomatic trees in Bogata, Columbia. A number of species (not all identified to species level) were positive by molecular methods for the presence of the phytoplasma. The two most abundant species, *Exitianus atratus* and *Amplicephalus funzaensis* were tested for their ability to transmit *Ca.* Phytoplasma fraxini and *Ca.* Phytoplasma asteris to common bean (*Phaseolus vulgaris*) in separate caged tests. Both leafhoppers were reported to be able to vector the two phytoplasmas, and this is the first report of a vector of *Ca.* Phytoplasma fraxini (Perilla-Henao *et al.* 2015). Neither of these species is recorded in the UK.

It is very unlikely the main vectors of *Ca.* Phytoplasma fraxini in North America, or any additional unidentified vectors that may be present in South America, are present in the UK. There have been very few introductions of North American species of Auchenorrhyncha (which contains the leafhoppers and planthoppers) to the UK, and no apparent introductions from South America (Smith *et al.* 2016, *in production,* Smith *et al.* 2007), though this assumes the vector is not a more cosmopolitan species that is native to the UK.

Potential UK Vectors and Likelihood of a Vector being Present in the UK

The presence or absence of a vector will directly influence the ability of *Ca.* Phytoplasma fraxini to establish, spread and cause impacts.

It is likely, with low confidence, that species present in the UK will be capable of vectoring *Ca.* Phytoplasma fraxini.

There are approximately 190 species of phloem feeding leafhoppers in the UK, and for many of these species their host range is poorly understood making it very difficult to judge how many polyphagous species there may be within this number (Dr Chris Malumphy, Fera, *pers. comm.*). Both vectors identified in South America belong to the subfamily Deltocephalinae, and there are over 100 species in this subfamily recorded in the UK (Bantock & Botting 2013). Many are considered oliphagous on grass, though for others the host range is poorly understood, there are examples of UK Deltocephalinae species that are polyphagous on woody plants (Chris Malumphy, Fera, *pers. comm*). This sub-family in the UK includes known vectors of other phytoplasma diseases including *Oncopsis alni* which transmits alder yellows phytoplasma (16SrV) (Maixner & Reinert 1999) and *Macropsis* species which spread phytoplasma diseases in elm (Carraro *et al.* 2004) and bramble (Davies 2000).

There are over 77 psyllid species present in the UK – the nymphal stages are very host specific, but adults can occasionally feed on species not suitable for nymphal development and some species will overwinter on “shelter-plants”(Hodkinson & White 1979), which they may occasionally feed on. As an example, there are four *Psyllopsis* species that feed on Fraxinus in Britain: *Psyllopsis discrepans*, *P. distinguenda*, *P. fraxini* and *P. fraxinicola* (Foerster) (Dr Chris Malumphy, Fera, *pers. comm.*).

The Cixiidae in the UK have been summarised in a previous PRA (Tuffen 2015). There are 12 species recorded in the UK, of which 4 are known to be polyphagous on deciduous trees and shrubs. For some species, the life history and host range is poorly understood.

The suspected xylem-feeding vector *P. spumarius* (Aphrophoridae) is present and very widespread in the UK, and could act as a vector of *Ca.* Phytoplasma fraxini. This species is considered highly polyphagous, though associated with herbaceous hosts more than woody species (Stewart & Bantock 2015). However it has been collected from trees and woody plant species in the UK, for example from the canopy of apple trees (Bleicher *et al.* 2010). It is not clear if other UK Aphrophoridae may be able to act as a vector of *Ca.* Phytoplasma fraxini: species of the *Aphrophora* genus are widespread on trees and shrubs across the UK (Stewart & Bantock 2015).

Because *Ca.* Phytoplasma fraxini has demonstrated the ability to adapt to new hosts, the suspected vector *P. spumarius* is widespread in the UK and there are a range of other phloem feeding species which may be able to act as vectors, it is likely that a vector or vectors for *Ca.* Phytoplasma fraxini will be present in the UK Hemipteran fauna. However a judgement cannot be made about the polyphagy of such a vector or vectors, their abundance, efficiency or mobility – which would all contribute to the establishment or impact potential of *Ca.* Phytoplasma fraxini in the UK. As a consequence, confidence in this rating is low.

Given the uncertainty surrounding the vector of *Ca.* Phytoplasma fraxini, in appropriate sections (establishment outdoors, spread and potential impacts in the UK) two scenarios have been rated. The first is that there is no vector present in the UK. Because there are several examples of potential vector species that are widespread and polyphagous on woody hosts in the UK hemipteran fauna, the second scenario is a reasonable worst case scenario: that the UK vector will fit these criteria and thus endanger a number of tree or other woody hosts.

These are not the only two possible scenarios. Other scenarios include several inefficient vectors, the introduction of one of the North or South American vectors with the pest, an efficient vector with a limited host range or an efficient vector with a limited UK distribution. However, it is not possible to rate all of these scenarios within the PRA.

10. How likely is the pest to establish outdoors or under protection in the UK/PRA area?

(*The likelihood rating should be based on the area of potential establishment, e.g. where hosts are present and the climate is suitable, within the UK/PRA area and take into account the answers provided in section 9.)*

Phytoplasmas can spread through clonal propagation and between plants connected by natural root grafts, but the most significant spread is usually via an insect vector. Thus, in order to establish, there would need to be a vector present in the UK or the pathogen would have to become widespread in clonal propagation. Otherwise, disease in any imported infected plants would be self-limiting.

Two scenarios for outdoor establishment are considered. In the first it is assumed that an efficient vector, polyphagous on a range of woody hosts, is present in the UK fauna. If this was the case, establishment would be very likely, with high confidence.

In the second scenario, no vector is present in the UK. In this scenario, establishment would be unlikely, with medium confidence. It is not rated as very unlikely, because of the possibility for limited establishment via transmission through natural root grafts (see section 11 for more details of spread via this pathway) and because of the potential for accidental propagation via clonal methods. It is common for trees of a great number of species in ornamental nurseries to be propagated clonally from mother trees (macropropagation), and micropropagation techniques, such as tissue culture, are also commercially used (Ahuja 2013), this process is generally not used in forestry nurseries. Confidence is medium. Transmission via natural root grafts has not been shown conclusively for *Ca.* Phytoplasma fraxini. It is also unclear how long an infected mother tree may be clonally propagated from before symptom expression occurs, and the mother plant is rouged out.

Establishment under protection is rated as very unlikely, with medium confidence. This is because the insect vectors of phytoplasmas are not usually found under protection (and thus why the two scenarios used for outdoor establishment are not considered), and many of the recorded hosts are also not grown in protection in the UK. Confidence is medium, as it is very likely that *Ca.* Phytoplasma fraxini could adapt to new hosts and these could potentially be species widely grown under protection, some of which may be propagated clonally leading to the establishment of the disease in that production system.

Outdoors (efficient vector present)	Very unlikely	☐	Unlikely	☐	Moderately likely	☐	Likely	☐	Very likely	✓
Confidence	High Confidence	✓	Medium Confidence	☐	Low Confidence	☐				
Outdoors (vector absent)	Very unlikely	☐	Unlikely	✓	Moderately likely	☐	Likely	☐	Very likely	☐
Confidence	High Confidence	☐	Medium Confidence	✓	Low Confidence	☐				

Under Protection	Very unlikely	✓	Unlikely	☐	Moderately likely	☐	Likely	☐	Very likely	☐
Confidence	High Confidence	☐	Medium Confidence	✓	Low Confidence	☐				

11. How quickly could the pest spread in the UK/PRA area?

Natural Spread

Natural spread will be strongly influenced by a suitable vector being present in the UK, and thus natural spread is rated under two scenarios as described in section 9. Spread rate may be limited by the host preferences or scarcity of any vector(s).

Natural root grafts were noted between *Fraxinus* that grew in close proximity at diseased sites in the USA (Carr & Tattar 1989), and since other phytoplasmas have been shown to be transmitted via such root grafts (Bragagna *et al.* 2006, Johson *et al.* 2000), there is a risk of spread via this pathway, though it has not been conclusively proven to be a pathway of spread for *Ca.* Phytoplasma fraxini. Any spread very natural root grafts would be very slow.

Incidence of *Ca.* Phytoplasma fraxini has been studied over time at several ash stands in North America and increases in incidence of up to 9% per year have been observed at some sites. (Sinclair & Griffiths 1995, Sinclair & Griffiths 1994).

In general, the natural dispersal capacity of leafhoppers and other potential vectors of *Ca.* Phytoplasma fraxini is low, and movement occurs on a local scale. Some leafhopper species have migratory morphs, and will travel considerable distances. For example the aster leafhopper (*Macrosteles quadrilineatus*) vectors *Ca.* Phytoplasma asteris and is migratory in North America (Hoy *et al.* 1992). Should any vector of *Ca.* Phytoplasma fraxini be a migratory species natural spread could occur quickly.

Natural spread in the presence of an efficient vector is rated as slowly, with low confidence, since the dispersal habits of any potential vector are unknown. Natural spread in the absence of an efficient vector is very slowly, with high confidence.

Spread with Trade

If *Ca.* Phytoplasma fraxini were to enter with propagation material, it could spread quickly in trade. This may occur unwittingly for some hosts, as tolerance occurs in some species and infection can be asymptomatic. For example, *S. vulgaris* was thought to potentially be resistant to *Ca.* Phytoplasma fraxini, as at an arboretum with a high incidence of disease there were no symptomatic *S. vulgaris* plants but testing showed they were infected and just apparently tolerant of the disease (Hibben & Franzen 1989). Presence in *U. molinae* and *Paeonia* in Chile was thought to be both due to vegetative propagation and the feeding of an insect vector (Arismendi *et al.* 2010). Thus spread in trade is rated as quickly, with high confidence, irrespective of insect vectors.

Natural Spread (efficient vector present)	Very slowly	☐	Slowly	✓	Moderate pace	☐	Quickly	☐	Very quickly	☐
Confidence	High Confidence	☐	Medium Confidence	☐	Low Confidence	✓				
Natural Spread (vector absent)	Very slowly	✓	Slowly	☐	Moderate pace	☐	Quickly	☐	Very quickly	☐

Confidence	High Confidence ✓	Medium Confidence ☐	Low Confidence ☐		
With trade	Very slowly ☐	Slowly ☐	Moderate pace ☐	Quickly ✓	Very quickly ☐
Confidence	High Confidence ✓	Medium Confidence ☐	Low Confidence ☐		

12. What is the pest's economic, environmental and social impact within its existing distribution?

Incidents of "ash decline" and "ash dieback", later attributed to *Ca.* Phytoplasma fraxini, have been reported over a number of decades in North America. Symptoms include witches-broom, dieback of branches, reduced growth rates, dwarfed shoots and leaves and mortality in more susceptible trees (Sinclair & Griffiths 1994). Similar symptoms are seen in *Syringa* (Hibben & Franzen 1989).

Disease in *Fraxinus* has been described in some instances as "progressing rapidly": in one field study over a period of forty months, over half of the infected trees studied died (Cha & Tattar 1991). In Phoenix, Arizona, an 8 year study of *Fraxinus* urban shade trees observed a greatly increased disease severity over the period, leading to 30% mortality of the trees studied (Bricker & Stutz 2004). This shows that *Ca.* Phytoplasma fraxini can have serious impacts in urban shade trees, as well as in more open wooded areas where it had been largely studied.

There are fewer reports of impacts on *Syringa* than *Fraxinus*, though it has been noted that some collections in arboreta have been severely affected, and disease reduces the amenity value of the plants (Sinclair *et al.* 1996). At the Arnold Arboretum, for example, the disease was heavily damaging in *Syringa* in the 1980s and though the incidence is now lower, *Syringa* plants still require removal due to the disease (Michael S. Dossmann, Arnold Arboretum, *pers. comm.* 27.10.2015).

It is important to note that disease severity varies depending on species or cultivar of *Fraxinus* or *Syringa* that are infected. It has been noted that late blooming lilacs, including hybrids with *josikaea* or *villosa* lineage, are particularly susceptible (Hibben & Franzen 1989). Sinclair *et al.* (2000) tested 11 cultivars of *Fraxinus* across two sites. One of the sets of inoculated cultivars, *F. pennsylvanica* cv. Urabanite, were all killed by the infection over the three year trial period (one control tree also died) (Sinclair *et al.* 2000). In contrast, some infected *F. americana* have been observed to maintain moderate growth and normal branching habit for up to 15 years after infection was first detected (Sinclair & Griffiths 1994), but it is more usual for significant reductions in growth to be seen in infected trees (Sinclair *et al.* 1993).

Infection by *Ca.* Phytoplasma fraxini has also been found to make *Fraxinus* and *Syringa* more susceptible to cold damage (Sinclair *et al.* 1996).

In Bogotá, Columbia, *Ca.* Phytoplasma fraxini has been a particular problem in urban tree populations. The predominant urban tree in Bogotá in the 1990s was *F. uhdei*, which had been introduced from the USA in the 1950s (Franco-Lara & Henao 2014). Decline of trees was noted in the 1990s and reported to be caused by *Ca.* Phytoplasma fraxini in 2004 (Filgueira *et al.* 2004). In 2000, disease incidence on *F. uhdei* was very high. Of the 600 trees examined, all were symptomatic, with 92% being moderately or severely affected, and by 2014, many of the trees had been removed and those that were still present were "barely surviving"(Franco-Lara & Henao 2014).

Later, disease was observed in a number of other street trees in Bogotá, including *Liquidambar styraciflua* planted to replace *F. uhdei*, with symptoms typical of phytoplasma infection including tufted foliage, epicormic shoots, witches'-broom and little leaves. Symptoms develop throughout the year and can leave some species "almost unrecognisable" (Franco-Lara & Henao 2014). Molecular analysis indicated that nine species of tree were infected with *Ca.* Phytoplasma fraxini, *Ca.* Phytoplasma asteris or had a mixed infection of *Ca.* Phytoplasma fraxini and *Ca.* Phytoplasma asteris, though only *Ca.* Phytoplasma fraxini was detected in diseased *Acacia melanoxylon* (Franco-Lara & Henao 2014). Thus decline of Bogotá street trees is attributable to two major phytoplasma species often present in mixed infections, *Ca.* Phytoplasma fraxini appears to be playing a significant role but the impacts may be compounded by the presence of *Ca.* Phytoplasma asteris.

Overall, impacts of *Ca.* Phytoplasma fraxini in its current range are rated as large, with medium confidence.

Impacts	Very small	☐	Small	☐	Medium	☐	Large	✓	Very large	☐
Confidence	High Confidence	☐	Medium Confidence	✓	Low Confidence	☐				

13. What is the pest's potential to cause economic, environmental and social impacts in the UK/PRA area?

Potential impact in the UK is subject to considerable uncertainty due a lack of knowledge concerning potential vectors in the UK. As a consequence, potential impacts are rated using the two scenarios as in sections 10 and 11.

The significant decline in street trees in Bogotá is, as described in section 12, associated with both *Ca.* Phytoplasma fraxini and *Ca.* Phytoplasma asteris, and the two species were often found in a mixed infection. It is important to note that some strains of *Ca.* Phytoplasma asteris are present in the UK, though their distribution and hosts are largely unknown. Thus as seen in Bogotá, there is potential for accumulative impacts if both species infect trees.

The susceptibility of UK tree species is an additional source of uncertainty. *Fraxinus excelsior* is known to be a symptomatic host from studies in arboreta in Massachusetts (Hibben *et al.* 1991), but there no detailed descriptions of the symptoms were supplied. One of the authors of the study indicated that it was unlikely that *F. excelsior* would have shown prominent symptoms, or this would have been noted (Prof. Wayne Sinclair, *pers. comm.,* 07.10.2015). The Arnold Arboretum (where the initial study took place) confirmed that some accessions of *Fraxinus excelsior* that were infected with *Ca.* Phytoplasma fraxini have been removed. However, there are also specimens of *F. excelsior* infected with the disease that are still living (Michael S. Dosmann, *pers. comm.* 30.10.2015).

The native UK species *Populous nigra* is susceptible to *Ca.* Phytoplasma fraxini, symptoms in trees included deformation of the crown, yellowing, atypical elongation of apical shoots and tufted foliage (Franco-Lara & Henao 2014).

In the presence of an efficient and polyphagous vector, economic, environmental and social impacts have been rated large, with low confidence. Confidence is low for all impact ratings because the susceptibility of many native or widely planted UK species, with the exception of *P. nigra*, remains unknown and some widely planted tree species may actually be tolerant to *Ca.* Phytoplasma fraxini infection and show few, if any, symptoms.

In this reasonable worst case scenario, if susceptible tree species are present than significant decline of these species could occur, including mortality. This would lead to economic impacts by reducing timber yield in species used for timber production, and possible decline and death of nursery trees.

Though there are comparatively few reports of environmental impacts of *Ca.* Phytoplasma fraxini, it has been recorded as causing decline and death of trees in the wider environment, though some level of tolerance always seems to be present in populations, preventing their local extinction. It is not known if natural resistance may occur in those *F. excelsior* grown in the UK. In addition, *P. nigra* is a comparatively rare species in the UK under conservation – no mortality caused by *Ca.* Phytoplasma fraxini has been recorded to date, but widespread phytoplasma infection would still interfere in the conservation of this species.

Social impacts could be expected where disease occurs in urban plantings; this would reduce the aesthetic value of the trees. Trees may also have to be removed if they decline to the point where they become a safety hazard.

In the absence of an efficient vector, impacts would be restricted by the inability of the pest to spread. Economic impacts have been rated as small with medium confidence, rather than very small, as situations may occur in which *Ca.* Phytoplasma fraxini is present in asymptomatic mother plants used for clonal propagation, and decline is not noted for a number of years. Social and environmental impacts are rated as very small, with high confidence, as outbreaks in urban plantings or the wider environment would be self-limiting without a vector to transmit the disease to new hosts.

In the presence of an efficient vector

Economic Impacts	Very small ☐	Small ☐	Medium ☐	Large ✓	Very large ☐
Confidence	High Confidence ☐	Medium Confidence ☐	Low Confidence ✓		
Environ - mental Impacts	Very small ☐	Small ☐	Medium ☐	Large ✓	Very large ☐
Confidence	High Confidence ☐	Medium Confidence ☐	Low Confidence ✓		
Social Impacts	Very small ☐	Small ☐	Medium ☐	Large ✓	Very large ☐
Confidence	High Confidence ☐	Medium Confidence ☐	Low Confidence ✓		

In the absence of an efficient vector

Economic Impacts	Very small ☐	Small ✓	Medium ☐	Large ☐	Very large ☐
Confidence	High Confidence ☐	Medium Confidence ✓	Low Confidence ☐		
Environ - mental Impacts	Very small ✓	Small ☐	Medium ☐	Large ☐	Very large ☐
Confidence	High Confidence ✓	Medium Confidence ☐	Low Confidence ☐		
Social Impacts	Very small ✓	Small ☐	Medium ☐	Large ☐	Very large ☐
Confidence	High Confidence ✓	Medium Confidence ☐	Low Confidence ☐		

14. What is the pest's potential as a vector of plant pathogens?

Ca. Phytoplasma fraxini cannot act as a vector.

15. What is the area endangered by the pest?

The endangered area is dependent on the presence of a suitable vector in the UK. In a reasonable worst case scenario, *Ca.* Phytoplasma fraxini could spread to a number of broadleaved tree species. Those in urban locations, where decline of trees will reduce aesthetic value and may present safety hazard which leads to their removal, are particularly endangered in this scenario.

Stage 3: Pest Risk Management

16. What are the risk management options for the UK/PRA area?

Exclusion

Though *Ca.* Phytoplasma fraxini is technically a regulated organism because of its status as a non-European virus-like pest that infects *Prunus* and *Vitis*, to avoid potential introduction on other hosts listing in the annexes of the EU plant health regulations by name could be considered. In a reasonable worst case scenario, the pathogen has the potential to be a destructive pest not only in the UK but across Europe should it be introduced. Which annex of the legislation the pest is listed in depends on its status in Italy – further clarification of findings of 16SrVII phytoplasmas in this EU country is required.

If *Ca.* Phytoplasma fraxini is absent from the EU, listing in Annex IAI should be considered. In addition, requirements could be included in Annex IV either on all hosts, or on certain key hosts such as *Prunus*, *Fraxinus, Syringa* and *Populas nigra* which requires planting material to be sourced from a pest free area.

Eradication and Containment

If an outbreak is detected at recently planted sites, where there is sufficient evidence to suggest that the disease was introduced on the planting material, eradication efforts would most likely be warranted. Because of the difficulties associated with testing of phytoplasmas, all material in an infected lot should be destroyed by incineration or deep burial. Because phytoplasmas are able to survive in the roots, if the outbreak is on trees or other woody hosts the roots would need to be destroyed to ensure infected trees do not regenerate from stumps. Surveys should then be carried out at the site in the subsequent year to see if any spread to new hosts has occurred. Surveys would be best conducted in late summer, when phytoplasma titre is at its highest and thus most readily detectable (Cha & Tattar 1991).

Herbaceous hosts can act as a reservoir of phytoplasma disease, but this would depend on the feeding activity of the vector. This has been noted in Chile for *Ca.* Phytoplasma fraxini, it has been observed in other members of the 16SrVII ash yellows phytoplasmas (Meneguzzi *et al.* 2008) and more widely in a range of phytoplasma pathogens (Alhudaib *et al.* 2009, Berger *et al.* 2015, un Nabi *et al.*). Thus any herbaceous plant showing possible phytoplasma symptoms (such as yellowing or reddening of leaves, phyllody –where flowers are converted to leaves - or proliferation of shoots) should be tested, as well as a more limited number of asymptomatic herbaceous hosts in the immediate vicinity of infected plants.

If any samples unrelated to the planted material are positive, a survey to identify potential vectors would be advised, and the risk management strategy then reviewed. If evidence of spread is found, containment may be a more viable option, depending on the extent of the spread and the number of alternative hosts identified. Containment measures would be prevention of propagation from any host material on site, or removal of plants of hosts from the demarcated area. Chemical treatments to control any vector populations may be advisable in some cases, though in many instances this would be prohibitively costly for trees or where vectors may be using alternative hosts.

Non-Statutory Controls

Various cultural controls have been proposed for control of the pest in *Fraxinus* in North America. These controls are based on studies that show *Ca.* Phytoplasma fraxini is significantly more prevalent at certain site types, which is believed to be related to the preferences of the vector. Thus these measures may not be suitable in the UK, as it is likely the pest would have a different vector species. Use of certified, disease free propagation material will reduce spread and impacts. If there are sites with a high incidence of *Ca.* Phytoplasma fraxini, then thinning and removal of diseased trees may also reduce the spread.

17. References

Ahuja MR (2013) *Micropropagation of Woody Plants*. Springer Netherlands.

Alhudaib K, Arocha Y, Wilson M & Jones P (2009): Molecular identification, potential vectors and alternative hosts of the phytoplasma associated with a lime decline disease in Saudi Arabia. *Crop Protection* **28**, 13-18.

Anon (2015) A few facts and figures about English and Welsh wines and vineyards. English Wine Producers, Market Harborough (accessed 12/10/2015.

Arismendi N, Andrade N, Riegel R & Carrillo R (2010): Presence of a Phytoplasma associated with Witches' Broom Disease in Ugni molinae Turcz. and Gaultheria phillyreifolia (Pers.) Sleumer determined by DAPI, PCR And DNA sequencing. *Chilean Journal of Agricultural Research* **70**, 26-33.

Arismendi N, Gonzalez F, Zamorano A, Andrade N, Pino AM & Fiore N (2011) Molecular identification of 'Candidatus Phytoplasma fraxini'in murta and peony in Chile. In *Bulletin of Insectology*. Department of Agroenvironmental Sciences and Technologies, pp. S95-S96.

Arocha-Rosete Y, Kent P, Agrawal V, Hunt D, Hamilton A, Bertaccini A, Scott J, Crosby W, Michelutti R & Maini S (2011) Preliminary investigations on Graminella nigrifrons as a potential vector for phytoplasmas identified at the Canadian Clonal Genebank. In *Bulletin of Insectology*. Department of Agroenvironmental Sciences and Technologies, pp. S133-S134.

Bantock T & Botting J (2013) British Bugs. British Bugs UK. Available at: http://www.britishbugs.org.uk/index.html (accessed 03.08.2015.

Barros TS, Davis RE, Resende RO & Dally EL (2002): Erigeron witches'-broom phytoplasma in Brazil represents new subgroup VII-B in 16S rRNA gene group VII, the ash yellows phytoplasma group. *Plant disease* **86**, 1142-1148.

Berger J, Schweigkofler W, Kerschbamer C, Roschatt C, Dalla Vía J & Baric S (2015): Occurrence of Stolbur phytoplasma in the vector Hyalesthes obsoletus, herbaceous host plants and grapevine in South Tyrol (Northern Italy). *VITIS-Journal of Grapevine Research* **48**, 185.

Bleicher K, Orosz A, Cross J & Markó V (2010): Survey of leafhoppers, planthoppers and froghoppers (Auchenorrhyncha) in apple orchards in South-East England. *Acta Phytopathologica et Entomologica Hungarica* **45**, 93-105.

Bragagna P, Deromedi M, Filippi M, Forno F, Mattedi L, Ciccotti A & Bianchedi P (2006) Natural and experimental transmission of Candidatus Phytoplasma mali by root bridges. In *XX International Symposium on Virus and Virus-Like Diseases of Temperate Fruit Crops-Fruit Tree Diseases 781*, pp. 459-464.

Bressan A, Turata R, Maixner M, Spiazzi S, Boudon-Padieu E & Girolami V (2007): Vector activity of Hyalesthes obsoletus living on nettles and transmitting a stolbur phytoplasma to grapevines: a case study. *Annals of applied biology* **150**, 331-339.

Bricker JS & Stutz JC (2004): Phytoplasmas associated with ash decline. *Journal of Arboriculture*, 193-199.

Bruni R, Pellati F, Bellardi MG, Benvenuti S, Paltrinieri S, Bertaccini A & Bianchi A (2005): Herbal drug quality and phytochemical composition of Hypericum perforatum L. affected by ash yellows phytoplasma infection. *Journal of agricultural and food chemistry* **53**, 964-968.

CABI (2015) Phytoplasma fraxini (ash yellows). CABI, Wallingford, UK. Available at: http://www.cabi.org/cpc/datasheet/3876 (accessed 30.10.2015.

Carr K & Tattar T (1989): Symptoms and distribution of ash yellows in Massachusetts. *Arboricultural Journal* **13**, 97-111.

Carraro L, Ferrini F, Ermacora P, Loi N, Martini M & Osler R (2004): Macropsis mendax as a vector of elm yellows phytoplasma of Ulmus species. *Plant Pathology* **53**, 90-95.

Cha B & Tattar TA (1991): Symptom development of ash yellows and fluctuation of mycoplasma-like organism population in white ash (Fraxinus americana L.). *Arboricultural Journal* **15**, 323-343.

Conci L, Meneguzzi N, Galdeano E, Torres L, Nome C & Nome S (2005): Detection and molecular characterisation of an alfalfa phytoplasma in Argentina that represents a new subgroup in the 16S rDNA ash yellows group ('Candidatus Phytoplasma fraxini'). *European journal of plant pathology* **113**, 255-265.

Cottrell J (2004) Conservation of Black Poplar (*Populus nigra* L.). Forestry Commission, Edinburgh, UK. Available at: http://www.forestry.gov.uk/pdf/FCIN057.pdf/$FILE/FCIN057.pdf (accessed 12/10/2015.

Cvrković T, Jović J, Mitrović M, Krstić O & Toševski I (2014): Experimental and molecular evidence of Reptalus panzeri as a natural vector of bois noir. *Plant Pathology* **63**, 42-53.

Davies D (2000): The occurrence of two phytoplasmas associated with stunted Rubus species in the UK. *Plant Pathology* **49**, 86-88.

Fernández FD, Conci VC, Kirschbaum DS & Conci LR (2013): Molecular characterization of a phytoplasma of the ash yellows group occurring in strawberry (Fragaria x ananassa Duch.) plants in Argentina. *European journal of plant pathology* **135**, 1-4.

Filgueira J, Franco-Lara L, Salcedo J, Gaitan S & Boa E (2004): Urapan (Fraxinus udhei) dieback, a new disease associated with a phytoplasma in Colombia. *Plant Pathology* **53**, 520-520.

Fiore N, Prodan S, Paltrinieri S, Gajardo A, Botti S, Pino AM, Montealegre J & Bertaccini A (2007): Molecular characterization of phytoplasmas in Chilean grapevines. *Bulletin of Insectology* **60**, 331.

Flôres D, Mello AA, Junior NM & Bedendo I (2013): First Report of a Group 16SrVII-C Phytoplasma Associated with Shoot Proliferation of Sunn Hemp (Crotalaria juncea) in Brazil. *Plant disease* **97**, 1652-1652.

Flôres D, Mello APdOA, Pereira TBC, Rezende JAM & Bedendo IP (2015): A new subgroup 16SrVII-D phytoplasma identified in association with erigeron witches' broom. *International journal of systematic and evolutionary microbiology*, ijs. 0.000274.

Franco-Lara L & Henao LMP (2014): Phytoplasma diseases in trees of Bogotá, Colombia: a serious risk for urban trees and crops. *Phytoplasmas and phytoplasma disease management: how to reduce their economic impact*, 90.

Fránová J, Bertaccini A & Duduk B (2014) Molecular tools in COST FA0807 Action. In *Phytoplasmas and phytoplasma disease management: how to reduce their economic impact*. International Phytoplasmologist Working Group, Assunta Beraccini, pp. 179-194.

Galetto L, Bosco D, Balestrini R, Genre A, Fletcher J & Marzachì C (2011): The major antigenic membrane protein of “Candidatus Phytoplasma asteris” selectively interacts with ATP synthase and actin of leafhopper vectors. *PLoS One* **6**, e22571.

Gao R, Wang J, Zhao W, Li X-D, Zhu S-F & Hao Y-J (2011): Identification of a phytoplasma associated with cherry virescence in China. *Journal of Plant pathology*, 465-469.

Griffiths H, Sinclair W, Davis R, Lee I, Dally E, Guo Y, Chen T & Hibben C (1994): Characterization of mycoplasmalike organisms from Fraxinus, Syringa, and associated plants from geographically diverse sites. *Abstracts describing parts of the work have appeared* **14**, 48.

Griffiths HM, Sinclair WA, Smart CD & Davis RE (1999): The phytoplasma associated with ash yellows and lilac witches'-broom:‘Candidatus Phytoplasma fraxini’. *International Journal of Systematic and Evolutionary Microbiology* **49**, 1605-1614.

Hibben C & Franzen L (1989): Susceptibility of lilacs to mycoplasmalike organisms. *J. Environ. Hortic* **7**, 163-167.

Hibben C, Sinclair W, Davis R & Alexander III J (1991): Relatedness of mycoplasmalike organisms associated with ash yellows and lilac witches'-broom. *Plant disease* **75**, 1227-1230.

Hill G & Sinclair W (2000): Taxa of leafhoppers carrying phytoplasmas at sites of ash yellows occurrence in New York State. *Plant disease* **84**, 134-138.

Hiruki C (1988) *Tree Mycoplasmas and Mycoplasma Diseases*. University of Alberta Press.

Hodkinson I & White I (1979) *Homoptera: Psylloidea. Handbooks for the Identification of British Insects, vol. II, part 5 (a)*. Royal Entomological Society of London, London.

Hoy CW, Heady SE & Koch TA (1992): Species composition, phenology, and possible origins of leafhoppers (Cicadellidae) in Ohio vegetable crops. *Journal of economic entomology* **85**, 2336-2343.

Johson K, Maas J, Davis R & Postman J (2000) The ‘Oregon hazelnut stunt syndrome’and phytoplasma associations. In *V International Congress on Hazelnut 556*, pp. 407-410.

Longone V, Gonzáles F, Zamorano A, Pino AM, Araya J, Díaz V, Paltrinieri S, Calari A, Bertaccini A & Picciau L (2011) Epidemiological aspects of phytoplasmas in Chilean grapevines. In *Bulletin of Insectology*. Department of Agroenvironmental Sciences and Technologies, pp. S91-S92.

Maixner M & Reinert W (1999): Oncopsis alni (Schrank)(Auchenorrhyncha: Cicadellidae) as a vector of the alder yellows phytoplasma of Alnus glutinosa (L.) Gaertn. *European Journal of Plant Pathology* **105**, 87-94.

Mayer CJ, Jarausch B, Jarausch W, Jelkmann W, Vilcinskas A & Gross J (2009): Cacopsylla melanoneura has no relevance as vector of apple proliferation in Germany. *Phytopathology* **99**, 729-738.

Meneguzzi N, Torres L, Galdeano E, Guzmán F, Nome S & Conci L (2008): Molecular characterization of a phytoplasma of the ash yellows group (16Sr VII-B) occurring in *Artemisia annua* and *Conyza bonariensis* weeds. *Agriscientia* **25**, 7-15.

Olivier C, Saguez J, Stobbs L, Lowery T, Galka B, Whybourne K, Bittner L, Chen X & Vincent C (2014): Occurrence of phytoplasmas in leafhoppers and cultivated grapevines in Canada. *Agriculture, Ecosystems & Environment* **195**, 91-97.

Pereira TB, Dally EL, Davis R, Banzato T & Bedendo IP (2015): Ming aralia [Polyscias fruticosa (L.) Harms.], a new host of a phytoplasma subgroup 16SrVII-B,'Candidatus Phytoplasma fraxini'-related, strain in Brazil. *Plant disease*.

Perilla-Henao L, Wilson M & Franco-Lara L (2015): Leafhoppers Exitianus atratus and Amplicephalus funzaesis transmit phytoplasmas of groups 16SrI and 16SrVII in Colombia. *Plant Pathology*.

Riedle-Bauer M, Sára A & Regner F (2008): Transmission of a stolbur phytoplasma by the Agalliinae leafhopper Anaceratagallia ribauti (Hemiptera, Auchenorrhyncha, Cicadellidae). *Journal of Phytopathology* **156**, 687-690.

Rosa C, McCarthy E, Duong K, Hoover G & Moorman G (2014): First report of the spittlebug Lepyronia quadrangularis and the leafhopper Latalus sp. As vectors of the Elm Yellows Associated Phytoplasma, Candidatus Phytoplasma ulmi in North America. *Plant disease* **98**, 154-154.

Siampour M, Galetto L, Bosco D, Izadpanah K, Marzachì C, Bertaccini A & Maini S (2011) In vitro interactions between immunodominant membrane protein of lime witches’ broom phytoplasma and leafhopper vector proteins. In *Bulletin of*

Insectology. Department of Agroenvironmental Sciences and Technologies, pp. S149-S150.

Sinclair W, Gleason M, Griffiths H, Iles J, Zriba N, Charlson D, Batzer J & Whitlow T (2000): Responses of 11 Fraxinus cultivars to ash yellows phytoplasma strains of differing aggressiveness. *Plant disease* **84**, 725-730.

Sinclair W & Griffiths H (1995): Epidemiology of a slow-decline phytoplasmal disease: ash yellows on old-field sites in New York State. *Phytopathology* **85**, 123-128.

Sinclair W & Griffiths H (2000): Variation in aggressiveness of ash yellows phytoplasmas. *Plant disease* **84**, 282-288.

Sinclair W, Griffiths H & Davis R (1996): Ash yellows and lilac witches'-broom: phytoplasmal diseases of concern in forestry and horticulture. *Plant disease* **80**, 5.

Sinclair W, Iuli R, Dyer A, Marshall P, Matteoni J, Hibben C, Stanosz G & Burns B (1990): Ash yellows: geographic range and association with decline of white ash. *Plant disease* **74**, 604-607.

Sinclair WA & Griffiths HM (1994): Ash yellows and its relationship to dieback and decline of ash. *Annual Review of Phytopathology* **32**, 49-60.

Sinclair WA, Griffiths HM & Treshow M (1993): Impact of ash yellows mycoplasmalike organisms on radial growth of naturally infected white, green, and velvet ash. *Canadian journal of forest research* **23**, 2467-2472.

Singh M, Chaturvedi Y, Tewari AK, Rao GP, Snehi SK, Raj SK & Khan MS (2011) Diversity among phytoplasmas infecting ornamental plants grown in India. In *Bulletin of Insectology*. Department of Agroenvironmental Sciences and Technologies, pp. S69-S70.

Smith RM, Baker RH, Malumphy CP, Hockland S, Hammon RP, Ostojá-Starzewski JC & Collins DW (2007): Recent non-native invertebrate plant pest establishments in Great Britain: origins, pathways, and trends. *Agricultural and Forest Entomology* **9**, 307-326.

Stewart A & Bantock T (2015) Introduction to the Auchenorrhyncha, UK. Available at: http://www.ledra.co.uk/introduction.html (accessed 30.12.2015.

Suzuki S, Oshima K, Kakizawa S, Arashida R, Jung H-Y, Yamaji Y, Nishigawa H, Ugaki M & Namba S (2006): Interaction between the membrane protein of a pathogen and insect microfilament complex determines insect-vector specificity. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 4252-4257.

Tedeschi R & Alma A (2004): Transmission of apple proliferation phytoplasma by Cacopsylla melanoneura (Homoptera: Psyllidae). *Journal of Economic Entomology* **97**, 8-13.

Tedeschi R & Alma A (2006): Fieberiella florii (Homoptera: Auchenorrhyncha) as a vector of “Candidatus Phytoplasma mali”. *Plant disease* **90**, 284-290.

Tuffen, MG (2015) Rapid Pest Risk Analysis for *Candidatus* Phytoplasma fragariae.

un Nabi S, Dubey DK, Rao G, Baranwal V & Sharma P Molecular characterization of ‘Candidatus Phytoplasma asteris’ subgroup IB associated with sesame phyllody disease and identification of its natural vector and weed reservoir in India. *Australasian Plant Pathology*, 1-9.

Wilson MR & Weintraub PG (2007): An introduction to Auchenorrhyncha phytoplasma vectors. *Bulletin of Insectology* **60**, 177.

Zunnoon-Khan S, Arocha-Rosete Y, Scott J, Crosby W, Bertaccini A & Michelutti R (2010): First report of ‘Candidatus Phytoplasma fraxini’(group 16SrVII phytoplasma) associated with a peach disease in Canada. *Plant Pathology* **59**, 1162-1162.

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Appendix – Other 16SrVII Phytoplasmas

Introduction

Apart from 16SrVII-A, the subgroup to which *Ca.* Phytoplasma fraxini belongs, three other 16SrVII subgroups have been described to date. Members of these subgroups are currently restricted to South America, and appear to be more strongly associated with herbaceous hosts rather than woody tree or shrub species. None have been found to be associated with *Fraxinus* or *Syringa.* Each subgroup is briefly outlined below. Because in general import levels of the identified hosts (some of which are ornamentals occasionally grown in the UK) is very low from South America it is thought to be very unlikely that these group VII phytoplasma will enter the UK. However the risk could increase if the pathogen spreads to other ornamental production systems or current trade patterns change.

16SrVII-B

Erigeron Witches'-Broom Phytoplasma

The first phytoplasma strain to be classified into 16SrVII-B causes a disease known as Erigeron Witches'-Broom. It was first described in Brazil in 2002 infecting both *Erigeron* (a genus in the daisy family including ornamentals) and *Catharanthus roseus* (Madagascan periwinkle) (Barros *et al.* 2002). Symptoms included little-leaf, chlorosis and witches'-broom. Erigeron Witches'-Broom Phytoplasma is the type strain for the 16SrVII-B subgroup.

Ming aralia Little Leaf

This disease of the ornamental *Polyscias fruticosa* was first observed in Brazil in 2013, causing yellowing and abnormally small leaves. Sequencing showed 100% identity to the type strain of Erigeron Witches'-Broom Phytoplasma (Pereira *et al.* 2015), indicating this disease is caused by the same pathogen.

Artemisia witches'-broom Phytoplasma

Two weed species in Argentina, *Erigeron* (*Conyza*) *bonariensis* (fleabane) and *Artemisia annua* (sweet wormwood) displaying yellows and witches'-broom symptoms were found to be infected with a 16SrVII-B phytoplasma (Meneguzzi *et al.* 2008). This represented the first finding of sub-group 16SrVII-B in Argentina.

16SrVII-C

Argentinean Alfalfa Witches'-Broom Phytoplasma

The first member of the subgroup 16SrVII-C was described from *Medicago sativa* (Alfalfa) in 2005, though a disease causing a severe witches'-broom of alfalfa had been present in the Cuyo region of Argentina for the ten years previous (Conci *et al.* 2005). In 2010, phyllody was noted in strawberry crops, a symptom typical of phytoplasma disease and was shown to also be in 16SrVII-C (Fernández *et al.* 2013).

Crotalaria shoot proliferation

16SrVII-C subgroup phytoplasmas have also been detected in Brazil, causing a disease of *Crotalaria juncea*, or sunn hemp, a crop grown as green manure in tropical and subtropical regions. Plants showed shoot proliferation, leaf malformation and yellowing, but levels of disease incidence were low: 1 – 2 %. Molecular analysis showed 100% sequence identity to Argentinean Alfalfa Witches'-Broom Phytoplasma (Flôres *et al.* 2013). This was the first report of a 16SrVII-C phytoplasma in Brazil.

16SrVII-D

Erigeron bonariensis Witches' Broom

Erigeron does not just suffer from 16SrVII-B phytoplasmas, a new subgroup was described from *E. bonariensis* in Brazil in 2015 (Flôres *et al.* 2015). This disease caused similar symptoms of witches' broom and stunting.

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