

Department for Environment Food & Rural Affairs

# Pest specific plant health response plan:

Outbreaks of 'Candidatus Phytoplasma mali'



**Figure 1.** *Malus domestica* infected with '*Ca.* Phytoplasma mali' © Paul Martens via EPPO Global database (<u>https://gd.eppo.int/taxon/PHYPMA/photos</u>)

We are the Department for Environment, Food and Rural Affairs. We're responsible for improving and protecting the environment, growing the green economy, sustaining thriving rural communities and supporting our world-class food, farming and fishing industries.

We work closely with our 33 agencies and arm's length bodies on our ambition to make our air purer, our water cleaner, our land greener and our food more sustainable. Our mission is to restore and enhance the environment for the next generation, and to leave the environment in a better state than we found it.

## OGL

#### © Crown copyright 2024

This information is licensed under the Open Government Licence v3.0. To view this licence, visit www.nationalarchives.gov.uk/doc/open-government-licence/

This publication is available at

https://planthealthportal.defra.gov.uk/pests-and-diseases/contingency-planning/

This contingency plan has been undertaken taking into account the environmental principles laid out in the <u>Environment Act 2021</u>. Of particular relevance are:

**The prevention principle**, which means that any policy on action taken, or not taken should aim to prevent environmental harm.

The precautionary principle, which assists the decision-making process where there is a lack of scientific certainty.

Any enquiries regarding this document should be sent to us at:

The UK Chief Plant Health Officer

Department for Environment, Food and Rural Affairs

Room 11G32, York Biotech Campus

Sand Hutton

York

YO41 1LZ

Email: plantpestsrisks@defra.gov.uk Website: www.gov.uk/defra

## Executive summary

Background		
Regulation	GB quarantine pest	
Key Hosts	Apples (Malus domestica and Malus pumila)	
Distribution	Albania, Austria, Belarus, Belgium, Bosnia-Herzegovina, Bulgaria, Canada, Croatia, Czechia, Finland, France, Germany, Greece, Hungary, Italy, Lithuania, Moldova, Netherlands, Norway, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Switzerland, Syria, Tunisia, Türkiye, and Ukraine	
Key pathways	Plants for planting	
Industries at risk	Apple orchards	
Symptoms	<ul> <li>small, irregularly serrated leaves</li> </ul>	
(2.4*)	<ul> <li>excessive proliferation and witches broom symptoms</li> </ul>	
	<ul> <li>stunting and reduced vigour</li> </ul>	
	reductions in fruit size	
	Surveillance	
Demarcated zones (5.31-5.35)	Infected zone = Infected site Buffer zone ≤ 500 m	
Surveillance activities (5.18-5.24)	Visual surveillance for symptoms, sweep netting or tray beating for the vector	
	Response measures	
Interceptions (5.1-5.7)	Destruction is via deep burial or incineration. Tracing exercises are carried out where required and an UKPHINS notification should be made. Further surveillance of the area for inland findings.	
Outbreaks	movement restrictions on host material	
(5.36-5.45)	foliar insecticide treatments for psyllid vectors	
	• removal and destruction of infected and neighbouring trees	
	monitoring for further signs of infection	
	Key control measures	
Biological	N/A	
Chemical	Foliar insecticide treatments for the vector	
Cultural	<ul> <li>removal of infected hosts</li> </ul>	
(5.14-5.16)	pruning of suckers	
	cutting of scions in winter	
	Declaration of eradication	
The outbreak can be two growing seasons Phytoplasma mali'.	e declared eradicated (by the Chief Plant Health Officer) after at least s in which no hosts have been found to be infected with ' <i>Candidatus</i>	

\*Numbers refer to relevant points in the plan

#### Contents

Exe	cutive summary	3
1.	Introduction and scope	5
2.	Summary of the threat	5
3.	Risk assessments	6
4.	Actions to prevent outbreaks	7
5.	Response activities	7
	Official action to be taken following the suspicion or confirmation of an interception of <i>Ca</i> . Phytoplasma mali'	of 7
	Official action to be taken following the suspicion of an ' <i>Ca.</i> Phytoplasma mali' outbreak	8
	Confirming a new outbreak	.10
	Criteria for determining an outbreak	.12
	Official Action to be taken following the confirmation of an outbreak	.12
6.	Criteria for declaring eradication / change of policy	.15
7.	Evaluation and review of the contingency plan	.15
8.	Appendix A	.16
	Data sheet for 'Candidatus Phytoplasma mali'	.16
9.	References	.28
10.	Authors and reviewers	.31
	Authors:	.31
	Reviewers:	.31

## 1. Introduction and scope

- 1.1. This pest specific response plan has been prepared by the Defra Risk and Horizon Scanning team. It describes how the Plant Health Service for England will respond if an infection of *'Candidatus* Phytoplasma mali' (Apple proliferation (AP) is discovered.
- 1.2. The plant health authorities in Northern Ireland, Scotland, Wales and the Crown Dependencies have been consulted on this plan and will use it as the basis for the action they will take in the event of '*Ca*. Phytoplasma mali' being detected in their territories.
- 1.3. This document will be used in conjunction with the Defra Generic Contingency Plan for Plant Health in England (https://planthealthportal.defra.gov.uk/assets/uploads/Generic-Contingency-Plan-for-Plant-Health-in-England-FINAL-2.pdf), which gives details of the teams and organisations involved in pest response in England, and their responsibilities and governance. It also describes how these teams and organisations work together in the event of an outbreak of a plant health pest.
- 1.4. The aims of this response plan are to facilitate the containment and eradication of *Ca.* Phytoplasma mali' and to make stakeholders aware of the planned actions.

## 2. Summary of the threat

- 2.1. 'Candidatus Phytoplasma mali' is an obligate bacterium which resides in the phloem of infected hosts and is the causal agent of apple proliferation disease (AP). It is one of three phytoplasmas belonging to the economically significant apple proliferation clade, which also includes pear decline (PD) and European stone fruit yellows (ESFY)
- 2.2. The pathogen is widespread in Europe, particularly in the south, and is sporadically found in Africa, Asia and North America. There are some further unreliable records of '*Ca.* Phytoplasma mali' in Cyprus, India, Russia and South Africa.
- 2.3. The major hosts of 'Ca. Phytoplasma mali' are apples (Malus domestica and Malus pumila). The cultivars Belle de Boskoop, Gravenstein, Starking, Golden Delicious and Winter Banana are noted as the most sensitive to infection. 'Candidatus Phytoplasma mali' has been identified in other hosts and has shown impacts on Prunus spp., Lilium spp. (lily) and Dahlia spp. However, due to the limited reports of impacts on these hosts, Malus spp. are considered to be the key hosts.
- 2.4. Symptoms of infection with '*Ca.* Phytoplasma mali' include excessive proliferation and witches broom symptoms; internode elongation; and stunting. Infection in apple

crops leads to reductions in fruit size and quality, making the fruit unmarketable. Symptoms are more pronounced in young trees which can experience low vigour and decline.

- 2.5. 'Candidatus Phytoplasma mali' can be introduced via infected propagating material, such as infected trees, scions and rootstocks. Once introduced it is primarily spread via the psyllid vectors *Cacopsylla melanoneura* and *C. picta* and leafhoppers, although it is also spread via natural root fusions and grafts; parasitic dodder plants such as *Cuscuta subinclusa*; and grafting infected scions or rootstocks to healthy material.
- 2.6. *Cacopsylla melanoneura* is widespread in the UK and feeds primarily on hawthorn but also feeds on apples, medlar and pear, overwintering on conifers. *Cacopsylla picta* is not present in the UK.
- 2.7. '*Candidatus* Phytoplasma mali' was found in the UK in 1978 in a single Cox's Orange Pippin tree in Essex, planted from English stock in 1962. The tree was left in the orchard until 1985 when it was destroyed. No other adjacent trees showed signs of symptoms. As of February 2023, there have been no further interceptions or outbreaks of '*Ca.* Phytoplasma mali' in the UK.

## 3. Risk assessments

- 3.1. 'Candidatus Phytoplasma mali' has an unmitigated and mitigated UK Plant Health Risk Register score of 64 and 48, respectively. Overall, scores on the risk register can range from 1 (very low risk) to 125 (very high risk). These scores are reviewed as and when new information becomes available (<u>https://planthealthportal.defra.gov.uk/pests-and-diseases/uk-plant-health-riskregister/viewPestRisks.cfm?cslref=99</u>).
- 3.2. Pest Risk Analyses or assessments have been performed by France (ANSES, 2011), EFSA (MacLeod *et al.*, 2012), Norway (Sletten *et al.*, 2012) and EPPO (2018). Three of these are publicly available on the EPPO PRA platform: <u>https://pra.eppo.int/organism/PHYPMA</u>
- 3.3. The French PRA concluded that, despite being widespread in France, the economic impact on fruit production was limited; EFSA concluded that the potential impact was low but with high uncertainty due to expected variation caused by local conditions; and the Norwegian PRA concluded that the introduction and establishment of the pathogen was likely and there was a high probability of extensive economic damage. EPPO concluded the pathogen met Regulated Non-Quarantine Pest (RNQP) criteria and recommended its listing in legislation. As '*Ca.* Phytoplasma mali' is present in GB, it cannot meet GB RNQP criteria.

## 4. Actions to prevent outbreaks

- 4.1. *'Candidatus* Phytoplasma mali' is a GB Quarantine Pest (<u>Schedule 1</u> of <u>The Plant</u> <u>Health (Phytosanitary Conditions) (Amendment) (EU Exit) Regulations 2020</u>) and is therefore prohibited from being introduced into, or spread within, GB.
- 4.2. 'Candidatus Phytoplasma mali' is an EU Union RNQP. An RNQP is a non-quarantine pest or disease whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and is therefore regulated. RNQPs are only regulated when identified in association with specific plants for planting.
- 4.3. The Plant Health Service for England (including the Animal and Plant Health Agency (APHA), Defra and Fera Science Ltd.) should be aware of the measures described in this plan and be trained in responding to an outbreak of '*Ca.* Phytoplasma mali'. It is important that capabilities in detection, diagnosis, and risk management are available.

## 5. Response activities

# Official action to be taken following the suspicion or confirmation of an interception of '*Ca.* Phytoplasma mali'

- 5.1. If 'Ca. Phytoplasma mali' is suspected by the Plant Health and Seeds Inspectorate (PHSI) to be present in a consignment moving in trade, the PHSI must hold the consignment until a diagnosis is made. Ideally, the consignment should be placed in a sealed cold store and any opened containers should be resealed (which could be via wrapping in plastic if this facility is available). Other consignments of host plants of significance that are at risk of cross-contamination should also be held pending a risk assessment on whether cross-contamination has or could have potentially occurred. Samples should be sent to Fera Science Ltd., Plant Clinic, York Biotech Campus, Sand Hutton, York, YO41 1LZ (01904 462000), in a sealed bag or container, within at least two other layers of containment, which are not liable to be crushed during transit.
- 5.2. When a finding of '*Ca.* Phytoplasma mali' is confirmed, the PHSI should advise the client of the action that needs to be taken by way of an official notice. The consignment should be destroyed by either incineration or deep burial, or another approved method, or re-exported in a sealed container. The method of destruction/re-export will be chosen on a case-by-case basis.

- 5.3. Where there is a high risk of escape of any potential vectors before destruction or reexport, fumigation and/or insecticides may be used under guidance from the Defra Risk and Horizon Scanning team.
  - Prior to any insecticides being used, the risk posed by the insecticides to people and the environment will be assessed.
  - Any applications should be made following the advice on the product label and be in accordance with HSE guidance. In some cases, there may be a requirement to carry out a Local Environment Risk Assessment for Pesticides (LERAP) depending on the product used and the situation of the finding.
- 5.4. An UKPHINS (UK Plant Health Interception Notification System) notification should be made upon confirmation of an interception of '*Ca.* Phytoplasma mali'. UKPHINS is the IT system for recording findings and non-compliance in order to maintain records and notify other National Plant Protection Organisations (NPPOs) of plant health issues.
- 5.5. If all or part of the consignment has been distributed to other premises prior to diagnosis, trace forward and trace back inspections should take place upon suspicion or confirmation of '*Ca.* Phytoplasma mali'. Details of recent past and future consignments from the same grower/supplier should also be obtained.
- 5.6. A pest alert to raise awareness of '*Ca.* Phytoplasma mali' and its symptoms should be distributed to growers and importers where '*Ca.* Phytoplasma mali' has been found, and to those in the local area and those associated with the infested premises.
- 5.7. If intercepted inland, and there is the potential for spread from the imported consignment, any host plants should be inspected on the site (and released if found free) and, if deemed necessary by the IMT, again in the following season for signs of presence of the disease or vector. These surveys could require a number of visits and the installation and monitoring of sticky traps, dependent on the situation.

## Official action to be taken following the suspicion of a '*Ca.* Phytoplasma mali' outbreak

5.8. Suspected outbreaks will be assessed on a case-by-case basis. An Outbreak Triage Group (OTG), chaired by the Chief Plant Health Officer (CPHO) or their deputy and including specialists from APHA, Defra and other organisations, should be set up to assess the risk and decide on a suitable response. Where appropriate, the OTG will also decide who will be the control authority, and the control authority will then nominate an Incident Controller. An Incident Management Team (IMT) meeting, chaired by the Incident Controller, will subsequently convene to produce an Incident

Action Plan (IAP). See the *Defra Generic Contingency Plan for Plant Health in England* for full details.

5.9. The OTG will determine the alert status, which will consider the specific nature of the outbreak. These alert levels, in order of increasing severity, are white, black, amber and red (more details on these levels can be found in table 2 of the *Defra Generic Contingency Plan for Plant Health in England*). Under most scenarios, an infection of '*Ca.* Phytoplasma mali' in an orchard is likely to be given a black alert status. A black alert status refers to a plant pest with potential for limited geographical spread leading to moderate economic, environmental or social impacts.

#### **Restrictions on movement of material**

- 5.10. '*Candidatus* Phytoplasma mali' is associated with infected plants for planting and propagating material. Therefore, plants for planting, scions and grafts should be prevented from leaving the affected area (and wider site if considered a risk), other than under a statutory plant health notice for destruction by deep burial, incineration or another approved method.
- 5.11. Movement of material, equipment and machinery, which may result in the movement of life stages of psyllid vectors between infested and non-infested sites, should also be restricted. However, if movement is necessary, the material, equipment and machinery should be thoroughly cleaned at the designated outbreak site to remove any potential vectors.
- 5.12. The movement of personnel into an infested area should be restricted, especially during the early investigation phase and/or if the vector or disease is detected. Personnel should follow good hygiene practice to reduce the risk of carrying the pest to other areas of the site.

#### Preliminary trace forward / trace backward

5.13. If an infested consignment or batch is considered to be the source of the suspect outbreak, investigations regarding the origins of the infested consignment will be undertaken to locate other related and therefore potentially infested consignments moving to and from the site. If applicable the relevant NPPO should be contacted.

#### General biosecurity advice and advisory measures for growers

5.14. Staff should be trained to monitor apple and crab apple trees for symptoms of '*Ca.* Phytoplasma mali' infection.

- 5.15. The phytoplasma concentrates in the roots of trees during the winter, so any cutting of scions should be carried out before spring to reduce the likelihood of transfer. This material would still be restricted from moving from the site.
- 5.16. The pruning of suckers, which could provide a pathway for psyllids living on weeds moving back onto the tree, is recommended. Any waste should be destroyed by incineration or deep burial.
- 5.17. Foliar insecticide treatments for potential vectors could reduce further spread of the pathogen and should be carried out as in 5.3.

### Confirming a new outbreak

#### How to survey to determine whether there is an outbreak

- 5.18. Information to be gathered by the PHSI on the suspicion of an infection of '*Ca*. Phytoplasma mali', in accordance with ISPM 6; guidelines for surveillance (<u>https://www.ippc.int/en/publications/615/</u>):
  - The origin of the host plants and associated pathways and dates of planting
  - Details of other premises or destinations where the potentially infected host plants have been sent
  - Details of how waste material is disposed of
  - The layout of the premises and surrounding area, including a map of the fields/cropping, at risk growers, and details of neighbouring crops, especially any commercial or non-commercial hosts in glasshouses and in fields
  - Details of the host grown including cultivar or variety, planting date, growth stage, likely harvest date and any other relevant information
  - Description of surrounding habitat, including all potential hosts and weeds
  - Area and level of infection, including a description of the symptoms seen (photos should be taken) and the location within the affected premise e.g. whether it is widespread across the planting, clustered in hotspots, or whether it is related to specific operations
  - The date and time the sample was taken
  - Details on any current treatments/controls in place
  - Details of the movement of people, equipment, machinery etc. to and from the infected area
  - Cultural, biosecurity and working practices
  - The name, address, email and telephone number of the person who found the pest and/or its symptoms, and the business owner
  - Presence of shelter plants e.g. Picea abies

This information should be included on the plant disease investigation template.

- 5.19. Further to information gathering, surveys of other host plants should be carried out to confirm the extent of the infection e.g. in surrounding fields and orchards growing hosts of '*Ca*. Phytoplasma mali'. This should include samples and photographs of suspect plants where possible. This initial survey will be used to determine if it is an isolated finding or an established outbreak.
- 5.20. Finance for the surveys will depend on the individual circumstances of the outbreak, and will be subject to discussion, usually between Defra policy and the PHSI.

#### Sampling

- 5.21. *Malus* spp. trees can be visually examined for symptoms of '*Ca.* Phytoplasma mali' which are described in more detail in Appendix A but may include:
  - small, irregularly serrated leaves
  - excessive proliferation and witches broom symptoms
  - stunting and reduced vigour
  - reductions in fruit size
- 5.22. Following the putative identification of '*Ca.* Phytoplasma mali', symptomatic samples of above ground host material should be sent for confirmatory diagnosis as in point 5.1. Each sample should be labelled with full details of the sample number, location (grid reference), plant variety and suspect pest. Care should be taken to avoid cross contamination between samples, for example samples in separate bags and the changing of disposable gloves and disinfection of equipment between sampling.
- 5.23. 'Candidatus Phytoplasma mali' is present in the roots of infected trees year round, concentrating there in the winter. Root sampling is a means of asymptomatic sampling year round but is also where sampling should focus in the winter. Samples should be sent for confirmatory diagnosis as in 5.22.
- 5.24. Sampling for vectors can be done if deemed necessary via sweep netting or using white beating trays. For *C. melanoneura* this is more successful if carried out in the morning and lower down in the canopy.

#### **Diagnostic procedures**

5.25. A range of diagnostic techniques are available for '*Ca.* Phytoplasma mali' (see Appendix A for details). Of these, PCR assays are considered to be the most efficient means of diagnosis. Universal and specific primers (to the 16SrX-group) are available for the amplification of phytoplasma DNA, and therefore these assays can be used for detection and identification. There is an EPPO protocol available for this (<u>https://www.eppo.int/RESOURCES/eppo\_standards/pm7\_diagnostics</u>). Fera Science Ltd. Will use real-time PCR for detection and PCR and sequencing for identification.

### Criteria for determining an outbreak

- 5.26. An outbreak will be declared if there is evidence showing that '*Ca.* Phytoplasma mali' has established and can spread in the wider environment. For example:
  - If a tree in an orchard is found to be infected with 'Ca. Phytoplasma mali'
  - If an apple tree is found to be infected with '*Ca*. Phytoplasma mali' in the wider environment.
  - If an apple tree in a public or private garden is found to be infected and there is a risk of spread
- 5.27. If '*Ca.* Phytoplasma mali' is detected at a port or confined to a particular consignment with no risk of spread, then an outbreak should not be declared.

## Official Action to be taken following the confirmation of an outbreak

5.28. The scale of the outbreak will determine the size and nature of the IMT and action.

#### Communication

- 5.29. The IMT will assess the risks and communicate details to the IPPC and EPPO in accordance with ISPM 17: pest reporting (<u>https://www.ippc.int/en/publications/606/</u>), as well as within Government to Ministers, senior officials and other government departments, devolved administrations, and agencies (e.g. the Environment Agency) on a regular basis as appropriate; and to stakeholders.
- 5.30. A generic communications plan is available for use across all plant health outbreaks. This will be owned by APHA and FC communications teams and is intended to provide consistency across outbreaks. This plan aligns with the Plant Biosecurity strategy and can be tailored to the outbreak, using pest and outbreak specific information. It includes a list of key stakeholders and templates for:
  - Core narratives
  - Press releases

- Reactive lines
- Frequently Asked Questions

#### **Demarcated zones**

- 5.31. Once an outbreak has been confirmed, a demarcated area must be established around known infected plants. This will include two zones:
  - A defined **infected zone** (i.e. the infected site(s)).
  - A **buffer zone**, which will initially be at least 500 m from the infected zone, but as there is limited capacity for spread this could be reduced dependent on the situation. The buffer zone should include other premises in which stock has been sent or received, and/or any other premises where there is a perceived risk. This will include other orchards, protected horticulture sites or gardens/allotments growing hosts of '*Ca.* Phytoplasma mali'.
- 5.32. Initial maps of outbreak sites should be produced by officials.
- 5.33. All host plants in the infected and buffer zones should be visually inspected where feasible, and suspect samples should be sent for diagnosis. Surveying rates should be determined by the IMT and may include symptomatic above ground material or asymptomatic root samples.
- 5.34. The demarcated area should be adjusted in response to further findings. If '*Ca*. Phytoplasma mali' is found within an area outside the infected zone, this should subsequently be designated as infected.
- 5.35. Surveys will be carried out annually for at least two years after the year of the outbreak. This should be done in summer when the plants are in active growth.

#### Pest management procedures

- 5.36. Host plants should not be moved out of the demarcated area, with the exception of plants being moved for destruction under statutory plant health notice.
- 5.37. Movement of material, equipment and machinery, which may result in the movement of life stages of psyllid vectors between infected and non-infected areas, should also be restricted. However, if movement is necessary, the material, equipment and machinery should be thoroughly cleaned at the designated outbreak site to remove any potential vectors.
- 5.38. '*Candidatus* Phytoplasma mali' is spread by vectors, such as *C. melanoneura*, and therefore the control of potential psyllid and leafhopper vectors is important to

prevent spread to nearby healthy crops. Therefore, all host plants in the infected zone should be treated as soon as possible with a foliar insecticide. Recommendations will be made on an appropriate insecticide treatment regime in consultation with the Defra Risk and Horizon Scanning team, and any applications should be made in line with 5.3. In addition:

- If the situation demands it, it may be necessary to require the use of insecticides even for growers where only biological control agents are being used.
- Growers will be placed under notice to apply the recommended insecticides and make the applications using their own or contractor's equipment. Records of applications will be kept, including details of the amount of product and water used.
- Sticky traps should be monitored to determine the efficacy of the treatments.
- If a risk to bees is identified, bee advisors and local beekeepers should be contacted to inform them of any insecticide applications and their timing. Bee inspectors should be able to provide contact details.
- 5.39. As there are no effective chemical methods for controlling '*Ca.* Phytoplasma mali' and it can spread via natural root grafts, the only effective method of eradication is destruction. Following treatment with a foliar insecticide, any infected hosts, and neighbouring trees should be uprooted and destroyed. Any remaining plant material from this process, such as leaf debris from the crop, volunteers and weeds, should also be destroyed, because of the potential for the debris to act as a source of inoculum for vectors.
- 5.40. Fruits may be marketed if deemed appropriate, although infected fruit is unlikely to be marketable.
- 5.41. Monitoring of host plants in the infected and buffer zone for symptoms of '*Ca*. Phytoplasma mali' should be carried out, until the outbreak has been declared eradicated, on a frequency to be determined by the IMT.
- 5.42. If new hosts are planted, they should come from disease free stock and, after planting, regular monitoring should be carried out to ensure there are no hosts infected with '*Ca.* Phytoplasma mali'.
- 5.43. Official inspections, with the frequency determined by the IMT, should be carried out over the following two growing seasons.

#### **Disposal plan**

- 5.44. The primary means of disposing of infected material and plants is by incineration (licensed) or deep burial. Deep burial may be done at an approved landfill site, or on the site or nearby farm, if practical and in agreement with the local Environment Agency. Incineration must comply with appropriate waste management regulations, Environment Agency in England, Scottish Environment Protection Agency and Natural Resources Wales. If the material has to be moved off the premises, it should be contained within at least two sealed layers, if possible (e.g. small plant within two plastic bags).
- 5.45. Any disposal of waste material must be done in accordance with the relevant legislation. Growers need to obtain permission for exemptions from the Agricultural Waste Regulations from the Environment Agency. No charges are made for these exemptions. Further information on activities that require a permit and those which require the registration of an exemption can be found on the EA website at: <a href="https://www.gov.uk/topic/environmental-management/environmental-permits">https://www.gov.uk/topic/environmental-management/environmental-permits</a>.

## 6. Criteria for declaring eradication / change of policy

6.1. The outbreak can be declared eradicated (by the Chief Plant Health Officer) after at least two years in which no symptoms of '*Ca*. Phytoplasma mali' have been found in the demarcated area.

## 7. Evaluation and review of the contingency plan

- 7.1. This pest specific contingency plan should be reviewed regularly to consider changes in legislation, control procedures, pesticides, sampling and diagnosis methods, and any other relevant amendments.
- 7.2. Lessons should be identified during and after any outbreak of '*Ca*. Phytoplasma mali' or any other pests, including what went well and what did not. These should be included in any review of the contingency plan leading to continuous improvement of the plan and response to outbreaks.

## 8. Appendix A

### Data sheet for 'Candidatus Phytoplasma mali'

#### Identity

PREFERR	ED SCIENTIFIC NAME	AUTHOR (taxonomic authority)	
'Candidatus	s Phytoplasma mali'	Seemüller and Schneider 2004	
'Candidatus	s Phytoplasma mali'		
CLASS:	Mollicutes		
ORDER:	Acholeplasmatales		
FAMILY:	Acholeplasmataceae		
GENUS:	Phytoplasma		
SPECIES:	' <i>Candidatus</i> Phytoplasma mali'		
SYNONYMS	8		
Apple proliferation phytoplasma (Seemüller <i>et al.</i> , 1994)			
Phytoplasma mali		(Seemüller and Schneider 2004)	

COMMON NAMES Apple proliferation AP Witches' broom of apple

(Source: CABI, 2019; EPPO, 2023, NCBI, 2023)

#### Notes on taxonomy and nomenclature

'*Candidatus* Phytoplasma mali' is one of six phytoplasmas belonging to the economically significant apple proliferation clade, which also includes pear decline (PD) and European stone fruit yellows (ESFY) (Seemüller and Schneider, 2004; EFSA, 2012; CABI, 2019). In 2004 it was proposed to move these phytoplasmas into the *Candidatus* Phytoplasma genus by Seemüller and Schneider (2004). Whilst analysis of the 16S rDNA sequences of the three pathogens revealed the pathogens to be nearly identical, further analysis of other regions increased the level of difference to above the 2.5% threshold for assigning phytoplasmas to a species rank under the provisional status of *Candidatus* (EFSA, 2012). *Candidatus* is used to denote that the species, whilst being well characterised, cannot be cultured (Sullivan, 2016). Following this, it was proposed to rename apple proliferation to 'Ca. Phytoplasma mali'.

#### **Biology and ecology**

#### Lifecycle of 'Ca. Phytoplasma mali'

Phytoplasmas are obligate bacteria which reside in the phloem of infected hosts, in the sieve tubes of the current season's growth. They are transmitted primarily by phloem feeding insect vectors such as leafhoppers, planthoppers and psyllids (Zimmerman *et al.*, 2015).

Once infected, the distribution of the pathogen in the host varies throughout the year. Due to phloem sieve tube degradation in the winter, the phytoplasma is forced into the roots. It then reinvades the stems during the spring and its concentration peaks in late summer to early autumn (EFSA, 2012; EPPO, 2023a). As a result, infected trees may lack infected buds and appear asymptomatic at certain times of year. The concentration in the roots, meanwhile, is consistently high throughout the season (Sullivan, 2016).

Movement of '*Ca.* Phytoplasma mali' to the stems as the temperature increases coincides with overwintering adult vectors migrating from shelter plants (usually conifers) into apple orchards where they feed, mate and oviposit (Tedeschi *et al.*, 2012; Sullivan, 2016). This leads to further transmission of the phytoplasma to new hosts. In terms of spread capacity, Schmid (1975) (via CABI, 2019) reported an average increase of infected hosts at 18% per year in an infected Swiss apple orchard, with 73% of trees becoming infected over a 12-year period.

#### Lifecycle of vectors

One means of spread of '*Ca.* Phytoplasma mali' at a local level is via phloem feeding vectors such as leafhoppers, planthoppers and psyllids which can transmit the phytoplasma both as adults and nymphs (Sletten *et al.*, 2012; Zimmerman *et al.*, 2015).

Reported vectors of 'Ca. Phytoplasma mali' include psyllids such as *Cacopsylla mali, C. melanoneura* and *C. picta* (syn. *C. costalis*) (EFSA, 2012; Tedeschi *et al.*, 2012; Lemmetty *et al.*, 2013; Sullivan, 2016; CABI, 2019); and leafhoppers such as *Philaenus spumarius* and *Fiebierella florii* (CABI, 2019). However, the psyllids *C. picta* and *C. melanonerua*, are considered to be the primary vectors for the disease (Miñarro *et al.*, 2016; Sullivan, 2016). The latter appears to show regional differences in its transmission efficiency (Fischnaller *et al.*, 2020), with Tedeschi *et al.* (2013) reporting that in Italy it is the most important vector, whilst in Germany it is not of concern (Tedeschi *et al.*, 2013; Miñarro *et al.*, 2016; Sullivan, 2016; Fischnaller *et al.*, 2020).

*Cacopsylla melanoneura* and *C. picta,* which are univoltine in the north temperate zone and move between food host plants (such as apple and hawthorn) to shelter plants (such as *Picea abies*) during their life cycle (EFSA, 2012; Barthel *et al.*, 2020). Conifers act as shelter plants, where the adult psyllids overwinter, migrating back to the food host plants to feed, reproduce and develop during the spring and summer. In Italy, *C. melanoneura* 

oviposition occurs from March to April, with nymphs feeding and developing until May. Once the adults have developed, they remain on the food hosts until mid-June, when the adults migrate to the conifers to overwinter. The lifecycle of *C. picta* is similar but begins roughly two months later (Barthel *et al.*, 2020).

The pathogen is acquired by these vectors during feeding and is transmitted in a persistent-propagative manner (Sullivan, 2016; Görg *et al.*, 2021). This means '*Ca.* Phytoplasma mali' can replicate within, and be transmitted for the entire lifetime of, the vector, including after the vector has overwintered (Tedeschi *et al.*, 2012; CABI, 2019). The latency period of the vector is variable, temperature dependent and is reported to range between a few days and 80 days (Sletten *et al.*, 2012). This has been shown to be more efficient in *C. picta* than in *C. melanoneura* (EFSA,2012).

The vectors *C. mali, C. melanoneura* and *F. florii* are present in the UK. *Cacopsylla mali* and *C. melanoneura* are relatively widespread in the UK (British Bugs, 2023, 2023a), whilst *F. florii* is a relatively recent introduction to the UK and more limited in distribution (British Bugs, 2023b). While '*Ca.* Phytoplasma mali' has been found in *C. mali* (Miñarro *et al.*, 2016), it is assumed that in the UK *C. melanoneura* would represent the primary vector. Italy is the only region where *C. melanoneura* is reported as a more efficient vector than *C. picta* (EFSA, 2012), which could indicate transmission will be reduced in the UK compared to most other countries.

#### Hosts/crops affected

The major hosts of '*Ca.* Phytoplasma mali' are apples (*Malus domestica* and *M. pumila*) (Sullivan, 2016). There is variation in the susceptibility of apple cultivars, with Belle de Boskoop, Gravenstein, Starking, Golden Delicious and Winter Banana considered to be the most sensitive to infection (EPPO, 2023a). Using AP-group specific primers, the pathogen has also been observed in naturally infected trees of a number of other *Malus* spp. and hybrids, of which many showed specific symptoms or growth suppression (Sletten *et al.*, 2012).

AP-type phytoplasmas have been identified in *Prunus* species and *Crataegus monogyna* (hawthorn) in Italy, but it has not been determined whether these isolates are pathogenic to apple (Sletten *et al.*, 2012).

Other hosts as reported by Sullivan (2016) include *Catharanthus roseus* (Madagascan periwinkle), *Convolvulus arvensis* (field bindweed), *Corylus avellana* (hazel), *Cynodon dactylon* (Bermuda grass), *Dhalia cultorum* (dahlia), *Lilium* spp. (lily), *Pyrus communis* (pear) and *Vitis vinifera* (grapevine). A literature search on these hosts has found some impacts on *Prunus* spp. (Seljakb & Ravnikara, 2007), lily (Kamińska & Śliwa, 2008) and dahlia (Kamińska & Śliwa, 2008a), whilst *C. roseus* appears to be an experimental host.

Due to the limited reports of impacts on these other hosts, *Malus* spp. are considered to be the key hosts.

#### Plant stage affected

All growth stages are affected (CABI, 2019)

#### Plant parts affected

Shoots, leaves, fruits and roots (Sletten et al., 2012).

#### Symptoms/signs

*Candidatus* Phytoplasma mali' infected hosts can exhibit a variety of symptoms, which are usually a result of disturbances in the balance of host growth regulators. This can lead to symptoms including excessive proliferation and witches broom symptoms; internode elongation; and stunting (Bertaccini, 2007).

Infection in apple leads to reductions in fruit size and quality, resulting in unmarketable fruits (Sletten *et al.*, 2012). Symptoms are more pronounced in young trees which can experience low vigour and decline (Zimmerman *et al.*, 2015).

Infected hosts can experience recovery, with a lack of symptoms in the above ground tissues whilst high populations of the pathogen reside in the roots (Sullivan, 2016). Musetti *et al.* (2004) found that hydrogen peroxide ( $H_2O_2$ ) produced by the host appeared to counteract the virulence of the pathogen, whilst calcium dependent callose synthesis and phloem-protein plugging of the sieve tubes can physically prevent the pathogen recolonising the crown in the following year. Due to the inability of the pathogen to reinfect the scion, the host will show only mild symptoms or appear asymptomatic.

#### Similarities to other species/diseases/plant damages

*'Candidatus* Phytoplasma mali' is phylogenetically similar to other phytoplasmas such as PD and ESFY which also belong to the 16SrX group of phytoplasmas (Seemüller and Schneider, 2004). Peach yellow leaf roll (PYLR) phytoplasma has also been found to be closely related but can be distinguished by Southern Blot hybridization (Sullivan, 2016). Differences in 16S rDNA sequences, 16S-23S rDNA spacer regions, protein encoding genes, vector transmission and host range can be used to distinguish the more closely related AP, PD and ESFY (Seemüller and Schneider, 2004).

On infected hosts there are a number of non-specific symptoms such as discolouration and distortion which could be confused with abiotic factors or other pathogens (EFSA, 2012).

#### Morphology

Phytoplasmas, formerly known as mycoplasma-like organisms (MLO) are self-replicating bacteria which possess small genomes, lack cell wall components and are obligate pathogens, dependent on their hosts for nutrients and refuge (EFSA, 2012; Sullivan, 2016; CABI, 2019). They also have a low GC content (percentage of guanine and cytosine in their genome), which is used in bacterial systematics. They are unable to be cultured *in vitro* and cannot be morphologically distinguished using microscopy (Sullivan, 2016), relying on molecular techniques for diagnosis.

*Candidatus* Phytoplasma mali' is highly pleomorphic (able to morphologically adapt in response to environmental conditions), and is 200-800 nm in diameter with a trilaminar cytoplasmic membrane (EFSA, 2012).

#### **Detection and inspection methods**

Detection is based on visual inspections and follow up samples. Sampling during the winter should focus on root sampling due to the pathogen relocating to this part of the host. Surveying for vectors can be done by beating trays and sweep nets, but optimal surveying for *C. melanoneura* differs to that for *C. picta*, with the former being caught more successful in the morning and lower in the canopy (Barthel *et al.*, 2020).

Host symptoms to look for, based on the EPPO datasheet, are listed below (EPPO, 2023a).

#### Whole trees

Trees experience a lack of vigour. Shoots develop reddish-brown bark showing necrotic lesions. Branches can wither and diseased young trees may die in more severe infections.

#### Buds

The first noticeable symptom is the late growth of terminal buds in autumn, which may be followed by rosetting of terminal leaves. These are susceptible to powdery mildew infections. The most noticeable symptom is the premature development of axillary buds which lead to a witches broom appearance across a number of branches (Figures 2 and 3).

#### Leaves

Early leaf emergence of small, irregularly serrated leaves is symptomatic of infection (Figure 4). These may become chlorotic or reddened, and infection often leads to defoliation. Stipules may be abnormally long and up to four per leaf.

#### **Flowers and Fruits**

Whilst blossoms are generally unaffected, their emergence can be delayed. Fruits are reduced in size (up to 75%) (Figure 5), can appear flattened and show reductions in quality with poor flavour due to reduced sugar and acidity.

#### **Diagnostics**

Whilst '*Ca.* Phytoplasma mali' can be detected by visual inspections, as it causes some quite generic symptoms, and some symptoms could be confused with other abiotic or biotic factors, confirmation of the presence of '*Ca.* Phytoplasma mali' should be done via diagnostic tests. Some tests listed by EFSA (2012) are given below.

#### Grafting onto woody indicators

Presence can be detected by chip-budding or grafting cuttings onto *Malus domestica* cv. Golden Delicious and observing for five years. This is used for nuclear stock plants, given the duration of the test and low levels of specificity and sensitivity.

#### **DAPI** staining

This involves the staining of thin sections of young tissues with 4'6 diamidino-2phenylindole which can then be analysed under a florescence microscope. A blue fluorescence (at 460 nm) in the sieve tubes will indicate the presence of phytoplasma infection.

#### ELISA

ELISA using monocolonal specific antibodies allows for the detection and identification of *'Ca.* Phytoplasma mali'. This is reliable when using leaf midribs or stems from late spring to summer, due to the variable distribution of the phytoplasma in the plant throughout the year.

#### PCR assays

Universal and specific primers (to the 16SrX-group) are available for the amplification of '*Ca.* Phytoplasma mali' DNA, and therefore these assays can be used for detection and identification. There is an EPPO protocol available for this available here - <u>https://www.eppo.int/RESOURCES/eppo\_standards/pm7\_diagnostics</u>.



**Figure 2.** Shoot proliferation on apple tree infected with '*Ca.* Phytoplasma mali' © Paul Martens via EPPO Global Database



**Figure 3.** Healthy apple tree branch (right) and branch infected with '*Ca.* Phytoplasma mali' (left) © Paul Martens via EPPO GD



**Figure 4.** Healthy apple fruit (left) and fruits reduced in size due to '*Ca.* Phytoplasma mali' infection (right) © Dr. Federico Bondaz, Plant Protection Unit of Val d'Aosta region (IT) via EPPO GD



**Figure 5.** Healthy apple leaf (left) and '*Ca.* Phytoplasma mali' infected leaf showing reduced lamina size and enlarged stipules © Institut für Pflanzenschutz im Obstbau, Dossenheim (DE) via EPPO Global Database

#### Distribution



**Figure 6.** *Candidatus* Phytoplasma mali' distribution as of March 2023. (Source: EPPO, 2023). The link below provides up to date distribution data. <u>https://gd.eppo.int/taxon/PHYPMA/distribution</u>

#### History of introduction/spread

The disease is native to Europe and was first reported in Italy in the 1950s (EFSA, 2012). It is now widespread in Europe, particularly in the south, and can be found sporadically in Africa, Asia and North America. There are some further unreliable records of the pathogen in Cyprus, India, Russia and South Africa (EFSA, 2012; Sullivan, 2016; EPPO, 2023a).

It was found in the UK in 1978 in a single Cox's Orange Pippin tree in Essex, planted in 1962. Sample wood was taken and grafted onto four rootstocks in an isolated glasshouse, and within two years all inoculated hosts showed symptoms of '*Ca.* Phytoplasma mali'. The tree was left in the orchard until 1985 when it was destroyed. No other adjacent trees showed signs of symptoms (Davies *et al.*, 1986).

#### **Phytosanitary status**

**Table 1.** Global phytosanitary categorisation of '*Ca.* Phytoplasma mali' (Adapted from EPPO, 2023).

Country/NPPO/RPPO	List	Year of addition	
AFRICA			
Egypt	A1 list	2018	
Morocco	Quarantine pest	2018	
Tunisia	Quarantine pest	2012	
AMERICA			
Argentina	A1 list	2019	
Brazil	A1 list	2018	
Canada	Quarantine pest	2019	
Chile	A1 list	2019	
Mexico	Quarantine pest	2018	
Paraguay	A1 list	1992	
USA	Quarantine pest	1989	
Uruguay	A1 list	1992	

Country/NPPO/RPPO	List	Year of addition
ASIA		
Bahrain	A1 list	2003
China	Quarantine pest	2021
Israel	Quarantine pest	2009
Joran	A1 list	2013
Kazakhstan	A1 list	2017
EUROPE		
Georgia	A1 list	2018
Moldova	Quarantine pest	2017
Norway	Quarantine pest	2012
Switzerland	Regulated non-quarantine pest	2019
Türkiye	A2 list	2016
UK	A1 list	2020
RPPO/EU		
COSAVE	A2 list	2018
EAEU	A2 list	2016
EPPO	A2 list	1975
EU	Regulated non-quarantine pest	2019

#### Means of movement and dispersal into the UK

Infected propagating material is the main means of introduction. The risk of this is relatively high due to the possibility of importing or moving asymptomatic material. Infected trees, scions and rootstocks are all possible pathways of introduction (EPPO, 2023a), and introduction via infected vectors is also theoretically possible due to their persistent transmission (EFSA, 2012).

Local spread is most commonly via the psyllid vectors *C. melanoneura* and *C. picta.* The pathogen can also spread locally via natural root fusions and grafts (Sullivan, 2016); parasitic dodder plants such as *Cuscuta subinclusa* (Sullivan, 2016; Görg *et al.*, 2021); and grafting infected scions or rootstocks to healthy material (Sullivan, 2016; CABI, 2019; Görg *et al.*, 2021).

It is not known to be seed or pollen transmitted (Tedeschi *et al.,* 2012; Lemmetty *et al.,* 2013; Sullivan, 2016; CABI, 2019; Görg *et al.,* 2021).

#### Control

In terms of prevention, the sourcing of healthy propagation material is key (EFSA, 2012), and work on the breeding of resistant cultivars and rootstocks is therefore important (CABI, 2021; EPPO, 2023a). Phytoplasma free bud and graftwood can be achieved by utilising hot water or air treatments, which can then be grafted onto '*Ca.* Phytoplasma mali' free rootstocks (EFSA, 2012). There is some evidence of cross-protection, using intentionally inoculated mild strains as suppressors of aggressive strains of '*Ca.* Phytoplasma mali', which could be utilised in the future following further research (Schneider *et al.*, 2014). This would also require legislative changes to allow for the release of a GB QP.

There are no effective curative control measures for the disease and, once infected, trees may become a permanent source of inoculum (EFSA, 2012; Fischnaller *et al.*, 2020). Injecting trees with antibiotics such as tetracycline and oxytetracycline have been applied in the past, but whilst some success has been shown, there are issues with repeat treatments, residues and environmental exposure (EFSA, 2012). As such, control of the pathogen once introduced focuses on the removal of infected trees (Fischnaller *et al.*, 2020), the use of healthy propagation material for any new introductions (Sletten *et al.*, 2012; CABI, 2021), cutting scions before spring to reduce the likelihood of transfer of '*Ca.* Phytoplasma mali' (Sullivan, 2016), and the pruning of suckers (CABI, 2021).

Control of psyllids and other potential vectors is a means of limiting the spread of existing infections (Sletten *et al.*, 2012; Šafářová *et al.*, 2016; Fischnaller *et al.*, 2020). Šafářová *et al.* (2016) compared levels of '*Ca.* Phytoplasma mali' in Czech commercial apple orchards under an IPM strategy with those under an organic strategy between 2013-2015. It was found that the number and presence of the vectors *Cacopsylla picta* and *C. melanoneura* were reduced in the IPM orchards. These orchards had undergone insecticide treatments of Calypso 480 SC (thiacloprid) and Reldan 22EC (chlorpyrifos), neither of which are approved in the UK. Treatments led to a lower occurrence of diseased trees in the integrated orchard (5.8-7.7%) when compared to the organic orchard (19.6%) over a two-year period.

EFSA (2012) notes the following as natural enemies of psyllids – *Anthocoris nemorum, Orius minitus, Chrysopa carnea* and *Forficula auricularia*. Of these *A. nemorum* and *C. carnea* are available for use as biological control agents in England and Wales without a

licence, whilst *F. auricularia*, the common earwig, is widespread and may keep numbers of psyllid vectors in check if present in outbreak areas.

#### Impacts

#### Economic impact

Apple proliferation is one of the most economically important diseases in European apple cultivation (Sullivan, 2016; CABI, 2021; Fischnaller *et al.*, 2020). CABI (2021) suggests it is more of an issue in the northern areas of southern Europe, as the temperatures are the most conducive to symptom expression, with impacts in cooler or warmer regions being reduced, and Sullivan (2016) notes that it has caused serious damage to several traditional apple growing areas of Italy and Germany.

In Italy, it is a legal requirement to uproot infected trees to try and eradicate outbreaks. Severe outbreaks in the north have occurred since the 1990s and resulted in economic losses of over €100 million in 2001 (Barthel *et al.*, 2020), and the removal of over a million trees in the South Tyrol region between 2006 and 2010 (Fischnaller *et al.*, 2020).

Commercial value of the fruit is reduced by 30-100% (Sullivan, 2016), and Miñarro *et al.* (2016) note that annual losses in both Italy and Germany have been estimated to be in excess of €125 million.

#### **Environmental impact**

Increased spraying for the vector could lead to environmental impacts on native species. Infection may also leave trees sensitive to infection by the powdery mildew *Podosphaera leucotricha* which could lead to increased fungicide usage (Sullivan, 2016).

#### Social impact

Social impacts of an outbreak of '*Ca.* Phytoplasma mali' would be related to businesses or gardeners who need to remove infected hosts and replant with healthy stock.

## 9. References

**ANSES** (2012). Avis de l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environment et du travail relatif à la réalisation d'une analyse de risqué phytosanitaire sur les phytoplasmes des arbres fruitiers. [Online]. Available from: <u>https://pra.eppo.int/pra/a8de2ea2-27fa-488b-8f8b-f48a7226d7ea</u>. (Accessed: 28/03/2023).

**Barthel, D., Kerschbamer, C., Panassiti, B., Malenovský, I., & Janik, K.** (2020). Effect of Daytime and Tree Canopy Height on Sampling of Cacopsylla melanoneura, a 'Candidatus Phytoplasma mali'Vector. *Plants*, *9*(9), 1168.

**Bertaccini, A**. (2007). Phytoplasmas: diversity, taxonomy, and epidemiology. *Frontiers in Bioscience-Landmark*, *12*(2), 673-689.

British Bugs (2023). *Cacopsylla mali.* [Online]. Available from: <u>https://www.britishbugs.org.uk/homoptera/Psylloidea/Psylla\_mali.html</u>. (Accessed 28/03/2023).

British Bugs (2023a). *Cacopsylla melanoneura*. [Online]. Available from: <u>https://www.britishbugs.org.uk/homoptera/Psylloidea/Cacopsylla\_melanoneura.html</u>. (Accessed 28/03/2023).

British Bugs (2023b). *Fieberiella florii*. [Online]. Available from: <u>https://www.britishbugs.org.uk/homoptera/Cicadellidae/Fieberiella\_florii.html#:~:text=(Cica\_dellidae)%20Fieberiella%20florii&text=A%20recent%20immigrant%20to%20the,to%20rule %20out%20Synophropsis%20lauri. (Accessed 28/03/2023)</u>

**CABI** (2021). *Phytoplasma mali* (apple proliferation). [Online]. Available from: <u>https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.6502</u>. (Accessed 16/01/2023).

**Davies, D. L., Stickels, J. E., & Adams, A. N.** (1986). A single occurrence of apple proliferation disease in England. *Plant pathology*, *35*(3), 400-402.

**EPPO** (2018). *RNQP pest project:* Candidatus *Phytoplasma mali (Apple proliferation mycoplasma) (PHYPMA).* [Online]. Available from: <u>https://rnqp.eppo.int/recommendations/summarysheet\_pest?pest=PHYPMA</u> (Accessed 29/03/2023)

**EPPO** (2023). *'Candidatus* Phytoplasma mali (PHYPMA)'. [Online]. Available from: <u>https://gd.eppo.int/taxon/PHYPMA</u> (Accessed: 16/01/2023).

**EPPO** (2023a). Data sheet on Apple proliferation phytoplasma. [Online]. Available from: <u>https://gd.eppo.int/taxon/PHYPMA/documents</u> (Accessed: 25/01/2023). **Fischnaller, S., Parth, M., Messner, M., Stocker, R., Kerschbamer, C., & Janik, K.** (2020). Surveying Potential Vectors of Apple Proliferation Phytoplasma: Faunistic Analysis and Infection Status of Selected Auchenorrhyncha Species. *Insects*, *12*(1), 12.

**Görg, L. M., Gallinger, J., & Gross, J.** (2021). The phytopathogen 'Candidatus Phytoplasma mali'alters apple tree phloem composition and affects oviposition behavior of its vector Cacopsylla picta. *Chemoecology*, *31*(1), 31-45.

**Kamińska, M., & Śliwa, H.** (2008). Molecular Characterisation of *'Candidatus* Phytoplasma asteris' and *' Candidatus* Phytoplasma mali' associationed with lily leaf scorch and flower bud abscission. In *XII International Symposium on Virus Diseases of Ornamental Plants 901* (pp. 41-48).

**Kamińska, M., & Śliwa, H**. (2008a). Mixed infection of dahlia plants in Poland with apple proliferation and aster yellows phytoplasmas. *Plant Pathology*, *57*(2).

**Lemmetty, A., Soukainen, M., & Tuovinen, T.** (2013). First Report of 'Candidatus Phytoplasma mali,'the Causal Agent of Apple Proliferation Disease, in Apple Trees in Finland. *Plant Disease*, *97*(10), 1376-1376.

MacLeod, A., Anderson, H., Follak, S., Van Der Gaag, D.J., Potting, R., Smith, J., Steffek, R., Vloutoglou, I., Holt, J., Karadjova, O. and Kehlenbeck, H. (2012). Pest risk assessment for the European Community plant health: a comparative approach with case studies. *EFSA Supporting Publications*, *9*(9), p.319E.

Miñarro, M., Somoano, A., Moreno, A., & García, R. R. (2016). Candidate insect vectors of apple proliferation in Northwest Spain. *SpringerPlus*, *5*(1), 1-10.

**Musetti, R., Sanità di Toppi, L., Ermacora, P., & Favali M.A. (2004).** Recovery in apple trees infected with the apple proliferation phytoplasma: an ultrastructural and biochemical study. *Phytopathology*, 94(2), 203-208.

**NCBI** (National Center of Biotechnology Information) (2023). *Candidatus* Phytoplasma mali. [Online]. Available from: <u>https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=37692&lvl=3</u> <u>&lin=f&keep=1&srchmode=1&unlock</u>. (Accessed 16/01/2023).

Šafářová, D., Starý, M., Válová, P., Opatíková, M., Bílková, L., & Navrátil, M. (2016). Impact of insecticides treatment on phytoplasma infection risk in apple orchards. *Horticultural Science*, *43*(3), 112-116.

**Schmid, G. (1973).** Prolonged observations on spread and behaviour of proliferation disease in apple orchards. In *IX International Symposium on Fruit Tree Virus Diseases 44* (pp. 183-192). (abstract only)

Schneider, B., Sule, S., Jelkmann, W., & Seemüller, E. (2014). Suppression of aggressive strains of 'Candidatus Phytoplasma mali'by mild strains in Catharanthus roseus

and Nicotiana occidentalis and indication of similar action in apple trees. *Phytopathology*, *104*(5), 453-461.

**Seemüller, E., & Schneider, B. (2004).** 'Candidatus Phytoplasma mali', 'Candidatus Phytoplasma pyri' and 'Candidatus Phytoplasma prunorum', the causal agents of apple proliferation, pear decline and European stone fruit yellows, respectively. *International Journal of Systematic & Evolutionary Microbiology*, 54(4), 1217-1226.

**Seljakb, G., & Ravnikara, M.** (2007). First report of 'Candidatus Phytoplasma mali'in Prunus avium, P. armeniaca and P. domestica. *Plant Pathology*, *56*, 721.

**Sletten, A., Hofsvang, T., Rafoss, T., & Sundheim, L.** (2012). Pest risk assessment for apple proliferation phytoplasma ("Candidatus Phytoplasma mali"). *Norwegian Scientific Committee for Food Safety (VKM), 11,* 905-907.

**Sullivan, M**. (2016). CPHST Pest Datasheet for '*Candidatus* Phytoplasma mali'. USDA-APHIS-PPQ-CPHST. [Online]. Available from: <u>http://download.ceris.purdue.edu/file/3310</u>

**Tedeschi, R., Baldessari, M., Mazzoni, V., Trona, F., & Angeli, G**. (2012). Population dynamics of Cacopsylla melanoneura (Hemiptera: Psyllidae) in Northeast Italy and its role in the apple proliferation epidemiology in apple orchards. *Journal of economic entomology*, *105*(2), 322-328.

Zimmermann, M. R., Schneider, B., Mithöfer, A., Reichelt, M., Seemüller, E., & Furch, A. C. (2015). Implications of Candidatus Phytoplasma mali infection on phloem function of apple trees. *Endocytobiosis and cell research: Journal of the International Society of Endocytobiology*, 26, 67-75.

## **10. Authors and reviewers**

## Authors:

Simon Honey	(Defra)
-------------	---------

### **Reviewers:**

Jane Barbrook	(APHA)
Matthew Everatt	(Defra)
Anastasia Korycinska	(Defra)
Anna Skelton	(Fera Science Ltd.)
Laura Stevens	(Defra)