



Department
for Environment
Food & Rural Affairs

Rapid Pest Risk Analysis (PRA) for:
Apple dimple fruit viroid

November 2018

DRAFT

Summary and conclusions of the rapid PRA

Apple dimple fruit viroid (ADFVd) has the potential to make any infected apple crop unmarketable. However, since it was first identified in 1996, the viroid has only been identified in 5 different countries, and has a European presence in Italy only. The viroid does not appear to be spreading rapidly through trade or through natural means, and there is no evidence of a vector. As *Malus* species are widely distributed throughout the UK, it is thought that the viroid has the potential to establish in the UK. As the symptoms are very noticeable, it is thought that an outbreak would be detected rapidly with opportunities for containment and eradication. However, rapid detection could be confounded by some apple cultivars that are known to remain asymptomatic. This PRA shows that ADFVd is not a significant threat to apple production in the UK.

Risk of entry

The pathway of entry considered most likely is plants for planting of *Malus* species from Europe. Intra-EU trade of *Malus* species does exist, although there is no available data on the number of plants being traded. It is assumed that trade between Italy, where the viroid is present in the Campania region, and the UK does exist, and no mitigations to prevent entry of infected material are in place. As symptoms of ADFVd are only discernible on the fruit, it is impossible to detect the viroid in young plants without a molecular test. Plants for planting as a route for entry has been assessed as moderately likely with medium confidence. There is also a risk of entry through the import of infected fruit or through seed transmission, but these pathways are considered very unlikely with medium confidence. Entry through mechanical transfer has been rated as unlikely with medium confidence. There are currently no reports of a vector for ADFVd.

Risk of establishment

As *Malus* species are widely grown in the wider environment, and with some grafted maiden and grafted family trees being grown under protected cultivation in the UK, the risk of establishment both outdoors and in protected cultivation was determined to be likely, with high confidence.

Economic, environmental and social impact

ADFVd symptoms include shrivelled fruit with characteristic pitted spots and discolouration on the skin. The fruits infected with ADFVd have little to no commercial value. No reports of other symptoms on any other parts of the tree have been reported. There is no data on the impact of ADFVd infection in the regions where it is currently present, but it is not thought to be causing a significant impact as isolated outbreaks have been identified in individual orchards and on small numbers of trees. Economic impacts to the UK have been assessed as small with low confidence. Environmental impacts have been assessed as small, with low confidence. Social impacts have been assessed as small, with medium confidence.

Endangered area

All *Malus* species growing under protected cultivation and outdoors are at risk of ADFVd infection, although there are no reports of the viroid being able to infect *Malus sylvestris* (crab apple).

Risk management options

Exclusion is the best risk management option for the UK top fruit sector and regulation of ADFVd could be considered, with appropriate phytosanitary measures on the import of *Malus* plants for planting from the countries where the viroid is present. This could include sourcing plants only from pest free areas or certain pest free places of production if criteria that would help ensure disease freedom can be achieved. However, as ADFVd is not thought to represent a significant risk to apple production in the UK, so statutory measures may not represent the most proportionate approach, an alternative option for exclusion of the viroid could involve the top fruit industry voluntarily introducing a DNA-based test for ADFVd as part of the Fruit Propagation Certification Scheme (FPCS).

Under either a statutory or voluntary approach, containment and eradication should be possible in isolated outbreaks of the viroid, both outdoors and under protection. As there are no identified vectors of the viroid, any plants found to be infected could be removed and destroyed, thereby removing the pathogen from the environment. Biosecurity best practice should be put in place to avoid spread of the viroid, and an appropriate surveillance and testing regime should be put in place in order to identify latent infections in asymptomatic hosts at the outbreak site and in the wider environment around the site.

Key uncertainties and topics that would benefit from further investigation

- The full distribution of ADFVd in Europe, as the scattered global distribution could be indicative of the viroid being present, but as yet undetected in other countries.
- A full list of symptomatic and asymptomatic hosts commonly grown in the UK.
- There are no reports of a vector for the viroid, and as there is no evidence that other viroids are directly transmitted by insect vectors it seems likely that the main mode of transmission will be through contact transmission and vegetative propagation.

Images of the pest

	
<p>Several colour variations (green or yellow spots) observed on 'Starking delicious' fruits harvested from infected plants grown in the same field. Image courtesy of Dr Francesco Di Serio, Plantwise.org.</p>	<p>Symptomatic fruits in the field on a cultivar of 'Starking Delicious'. Image courtesy of Dr Francesco Di Serio, Plantwise.org.</p>

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	

All of the available published literature was assessed as part of this PRA, and to the author's knowledge there are no other information sources available for the purposes of a more detailed PRA.

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

Statutory action will not be taken against findings, as this pest could be managed through industry certification schemes.

Yes
Statutory action

No
Statutory action

DRAFT

Stage 1: Initiation

1. What is the name of the pest?

Apple dimple fruit viroid (ADFVd). The abbreviation ADFVd will be used throughout this PRA. ADFVd is a member of the genus *Apscaviroid*, family *Pospiviroidae*, and is in the same genus as *Apple scar skin viroid* (ASSVd).

2. What initiated this rapid PRA?

The first record of this viroid's presence in Iran was published in the British Society for Plant Pathology's (BSPP) New Disease Report in January 2017, which brought it to the attention of Defra's Risk and Horizon scanning team (Roumi *et al.*, 2017). Infection renders the fruit of some apple cultivars unmarketable. As it is also present in Italy, a country from which the UK imports many plants for planting, there is a concern that infected trees could enter the UK. A request for a PRA on ADFVd was made at a Defra Plant Health Risk Group meeting.

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/EC¹) and in the lists of EPPO²?

ADFVd is not listed in the EC Plant Health Directive and to date, has not appeared on the EPPO Alert list.

ADFVd has been listed as a quarantine pest in Israel since 2009.

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

² https://www.eppo.int/ACTIVITIES/quarantine_activities (accessed 1 August 2018)

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

ADFVd has been found in 4 countries in Asia, and in Italy in Europe. The scattered distribution of the viroid is suggestive of it being present in more countries, but no formal reports exist yet.

Table : Distribution of <i>pest name</i>	
North America:	No records
Central America:	No records
South America:	No records
Europe:	Italy (Di Serio <i>et al.</i> , 1996)
Africa:	No records
Asia:	China (Ye <i>et al.</i> , 2013), Iran (Roumi <i>et al.</i> , 2017), Japan (He <i>et al.</i> , 2010), Lebanon (Choueiri <i>et al.</i> , 2007)
Oceania:	No records

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

ADFVd is not known to occur in the UK, and has not been intercepted at the border.

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?

ADFVd has been isolated from *Malus domestica* (apple). Under experimental conditions, the viroid also successfully replicated on *Pyrus communis*, in this case on Fieud 37, a pear seedling developed as a rapid laboratory bioassay to act as a biological indicator for *Pear blister canker viroid* (PBCVd) (Desvignes *et al.*, 1999). However, the fruit of these trees showed no symptoms two years post inoculation (Di Serio *et al.*, 2001). As the viroid has only been shown to infect *Pyrus communis* under experimental conditions, this species has not been considered further as part of this PRA. However, this will be reviewed if reports emerge of natural ADFVd infections in *Pyrus* species. There is no evidence for ADFVd infecting *Malus sylvestris* (crab apple).

Under either field or experimental conditions, fruits of the following apple cultivars appear to be asymptomatic: Baujade, Delbarestivale, Golden Delicious, Granny Smith, Reinette Grise du Canada and Smoothie (all Di Serio *et al.*, 2001). The fruits of cultivars Annurca (Di Serio *et al.*, 2000), Braeburn (Di Serio *et al.*, 2001), Gala (Ye *et al.*, 2013), Fuji (Ye *et al.*, 2013), Jonagold, (Kasai *et al.*, 2017), Pink Lady, (Di Serio *et al.*, 2001), Red Delicious (Di Serio *et al.*, 1996), Starking Delicious (Di Serio *et al.*, 1996) and Stakrimson (Di Serio *et al.*, 2001) have exhibited symptoms.

Most commercial apple cultivars are grafted onto root stocks, as growing apples trees from seed results in unproductive trees that do not grow true to type. It is currently unknown if the differences in asymptomatic and symptomatic *Malus* varieties is related to the root stocks used during grafting.

In 2017, 170.5 thousand tonnes of dessert apples and 71.1 thousand tonnes of culinary apples were produced in the UK. The total estimated value of this market was worth approximately £141.1 million (provisional Defra statistics). Apple production was slightly higher in 2016, with 180.7 thousand tonnes of dessert apples and 80.7 tonnes of culinary apples produced in the UK. However, the general trend has been for increased production of dessert apples over the last 10 years, although culinary apple production has declined in the same time period. In 2017, the UK exported 26 thousand tonnes of apples in 2017, worth approximately £15 million (provisional Defra statistics). Exports of apples have fluctuated during the last decade, ranging from 14 thousand tonnes in 2008, to 26 thousand tonnes in both 2011 and 2017 (Defra statistics).

8. Summary of pest biology and/or lifecycle

ADFVd is a noncoding, single-stranded, circular RNA viroid (Kasai *et al.*, 2017). Different isolates are between 300–307 nucleotides (nt) long. The ADFVd variant identified in Japan is distinct to the variants identified in Italy and China, but is more closely related to the Italian variants than the Chinese variants (Kasai *et al.*, 2017). Although overall sequence homology is relatively low (~85%) between the isolates from Japan and those from Italy, this figure is within the species demarcation of 85-90%. The authors of the study on the molecular characterisation on ADFVd deemed it appropriate to treat these isolates as the same species as they were detected on the same natural host and caused similar characteristic symptoms on certain apple varieties (Kasai *et al.*, 2017).

This viroid causes similar symptoms to those caused by *Apple scar skin viroid* (ASSVd) which is also absent from the UK. ADFVd induces severe to mild diseases in susceptible hosts and symptoms can take two to three years to develop post inoculation. Symptoms appear on the fruit only, with no other symptoms being reported on other parts of the tree and affects on yield are currently unknown. Roughly spherical, depressed green or yellow spots are visible on red skin. Spots are 3 to 4 mm in diameter, but can also coalesce to create large areas of discoloured skin predominantly around the calyx end. Fruits showing symptoms have little to no commercial value.

In the cultivar Jonagold, there is evidence that symptoms are more clearly visible in the early stages of apple growth and become inconspicuous as the pericarp ripens and turns red (Kasai *et al.*, 2017). Additionally, it has been observed that symptoms may be enhanced when fruits are wrapped in a protective paper bag during cultivation (Kasai *et al.*, 2017). Although this report of enhanced symptoms was based on anecdotal evidence, there are reports that the speed and severity of pospiviroid infections are linked to environmental conditions. Symptoms of *Potato spindle tuber viroid* (PSTVd) are more severe following hotter temperatures and this viroid shows greater transmission rates at 25 °C than at 18 °C, and temperatures below 15 °C are inhibitory to viroid transmission (Verhoeven *et al.*, 2010).

There is no evidence of a vector for ADFVd, but the main mode of transmission is thought to be through the propagation of infected vegetative material (Malfitano *et al.*, 20004). Chiumenti *et al.*, (2014) speculate that the viroid has a wider host range than previously thought. Transmission by water has been shown experimentally for PSTVd (Mehle *et al.*, 2014), but evidence to support this under normal growing conditions is limited and there is no evidence to support this in the case of ADFVd.

A distinct, but related viroid species to ADFVd was detected at low levels in *Ficus carica* (fig) in Italy (Chiumenti *et al.*, 2014). This variant is 310 nucleotides long and shares between 81.6-82.8% and 84.2-84.7% homology with the variants of ADFVd from Japan and from Italy and China, respectively (Kasai *et al.*, 2017).

9. 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?

Plants for planting:

The most likely route of entry for ADFVd will be the transfer of infected hosts to the UK. The import of *Malus* plants intended for planting from non-European countries is prohibited, except from Mediterranean countries, Australia, New Zealand, Canada and the continental states of the USA, and these must be dormant plants free from leaves, flowers and fruit. Therefore imports of *Malus* plants for planting from China, Iran and Japan, where ADFVd is present, will not have occurred. There have been no records of *Malus* plants for planting arriving from Lebanon, which is classed as a Mediterranean country under the Plant Health (England) Order, therefore imports are permitted. As the viroid is present in Lebanon, there is a risk of future consignments of infected *Malus* plants for planting arriving from this country. The only available records of *Malus* plants for planting entering England from third countries since 2016 (when recording of the genera of imported plants for planting began) are 12,000 plants from Serbia in 2017, and 10 plants from Switzerland (origin Bosnia and Herzegovina) in 2016.

Commercial apple orchards in the UK are known to import certified rootstocks from Europe, particularly from the Netherlands, Germany and Belgium, although un-certified Conformitas Agraria Communitatis (CAC)-grade stocks may be sourced on occasion (PHSI, pers. coms). Currently, it is rare for UK growers to source planting material for propagation purposes from Europe, but on the rare occurrence that this does happen then the material would be sourced as bud wood (in the summer) or graft wood (in the winter). Although *Malus* plants for planting are covered by the EU Plant Passporting scheme, data on numbers of plants entering the UK is not available. However, as import pathways can be complex, it is possible that *Malus* trees from Italy have entered the UK. Although many companies purport to supply UK-grown apple trees online, it is possible that some of these trees originate from other European countries, as there is no requirement that trees are germinated or grafted in the UK.

The likelihood of the viroid entering the UK on plants for planting and then transferring to a suitable host has been ranked as moderately likely, with medium confidence.

<i>Plants for planting</i>	Very unlikely <input type="checkbox"/>	Unlikely <input type="checkbox"/>	Moderately likely <input checked="" type="checkbox"/>	Likely <input type="checkbox"/>	Very likely <input type="checkbox"/>
<i>Confidence</i>	High Confidence <input type="checkbox"/>	Medium Confidence <input checked="" type="checkbox"/>	Low Confidence <input type="checkbox"/>		

Fruits:

Apple fruit is unlikely to represent the main pathway for entry of ADFVd as, even if contact transmission is possible, infected fruit would need to come into close contact with growing apple trees for successful transmission to occur. Alternatively, if seed transmission is possible (see below), the seeds of the fruit would need to find their way to a suitable substrate before successfully germinating. The likelihood of the viroid entering the UK on apple fruits and then transferring to a suitable host has been ranked as very unlikely, with medium confidence.

<i>Fruits</i>	Very unlikely <input checked="" type="checkbox"/>	Unlikely <input type="checkbox"/>	Moderately likely <input type="checkbox"/>	Likely <input type="checkbox"/>	Very likely <input type="checkbox"/>
<i>Confidence</i>	High Confidence <input type="checkbox"/>	Medium Confidence <input checked="" type="checkbox"/>	Low Confidence <input type="checkbox"/>		

Seed transmission:

There is limited published evidence about whether ADFVd is seed transmitted. ADFVd RNA was not detected in a small scale study that examined 50 seedlings grown from the seeds of symptomatic fruits from an ADFVd infected 'Starking Delicious', suggesting that seed transmission does not occur or is rare (Malfitano *et al.*, 2004). This is in common with what has previously been reported for the closely related viroid *Apple scar skin viroid* (Howell *et al.*, 1998; Desvignes *et al.*, 1999). However, there have been no studies published on seed transmission in asymptomatic varieties. It is uncommon for producers

or gardeners to grow apple trees from seed as they do not grow true to type and rarely produce fruit. Therefore seeds were not considered the main pathway. The likelihood of the viroid entering the UK in apple seed and then transferring to a suitable host has been ranked as very unlikely, with medium confidence.

Seed transmission Very unlikely Unlikely Moderately likely Likely Very likely
 Confidence High Confidence Medium Confidence Low Confidence

Mechanical transmission:

The viroid has been successfully mechanically transmitted to previously viroid-free plants by stem slashing and grafting in experiments (Di Serio *et al.*, 2001). There is, therefore, the possibility of the viroid being present on unsanitized tools and this could therefore be a pathway, but it is thought unlikely that many tools are moved between the UK and those countries where the viroid is present. Therefore this pathway is unlikely to be the main mode of entry for the viroid. However, as the viroid is present in Italy, and could potentially have a wider distribution in Europe than reported, the likelihood of ADFVd entering the UK on unsanitized tools or equipment and then transferring to a suitable host has been ranked as unlikely with medium confidence.

Mechanical transmission Very unlikely Unlikely Moderately likely Likely Very likely
 Confidence High Confidence Medium Confidence Low Confidence

Natural spread:

Natural spread in this case refers to the movement of an infected hypothetical vector, and whether any such infected vector is likely to move from outside of the PRA area into it and onto a suitable host. A three year study of orchards in the Italian Campania region using dot-blot analysis to detect for the presence of viroid RNA, found only one previously unidentified asymptomatic tree in addition to the original symptomatic trees reported prior to the beginning of the study (Malfitano *et al.*, 2004). Findings in Iran and China have also been limited to a few trees and there are no reports of a vector.

Pospiviroids are not thought to have invertebrate vectors, although it is known that *Potato spindle tuber viroid* (PSTVd) is readily transmitted by the aphid species *Myzus persicae* in the presence of the Polerovirus *Potato leafroll virus* (PLRV) in potato (Salazar *et al.*, 1995). There is evidence that PSTVd is transencapsidated by PLRV, meaning that it is enclosed within the protein shell of the virus rather than being attached to the outside of the shell (Syller *et al.*, 1997). Although there are no recognised Poleroviruses which infect *Malus* or *Pyrus* species, transencapsidation of ADFVd cannot be ruled out given the recent findings of previously unknown poleriviruses in *Prunus* species (A. Fox, pers. com). Other studies

have demonstrated that both *Tomato apical stunt viroid* (TASVd) and *Tomato chlorotic dwarf viroid* (TCDVd) can be transmitted by bumblebees (Antignus *et al.*, 2007, and Matsuura *et al.*, 2010, respectively). However it has not yet been demonstrated whether transmission of these viroids is through the pollen or through the bee carrying the viroid and mechanically transmitting it to a host.

The viroid appears unable to spread by natural means. As there is no evidence of a vector of ADFVd, and as the only European record of the viroid is from Italy, natural spread is considered to be unlikely with low confidence.

<i>Natural spread</i>	Very unlikely <input type="checkbox"/>	Unlikely <input checked="" type="checkbox"/>	Moderately likely <input type="checkbox"/>	Likely <input type="checkbox"/>	Very likely <input type="checkbox"/>
<i>Confidence</i>	High Confidence <input type="checkbox"/>	Medium Confidence <input type="checkbox"/>	Low Confidence <input checked="" type="checkbox"/>		

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

There are no reports of a vector of ADFVd in the available literature as discussed above, in Section 9: Natural spread.

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

Outdoor establishment:

Apples are widespread in the UK, with Worcestershire, Herefordshire, Gloucestershire, Somerset and Kent having particularly large apple growing industries. As the pest being considered is a viroid that is intrinsically linked to its host, it is unlikely that the climate of the UK will be a limiting factor in the establishment of ADFVd. There are currently no records of a vector for ADFVd as discussed previously, although if a vector was identified, the climate of the UK could become a limiting factor for the establishment and spread of the vector. The risk of establishment outdoors has been assessed as likely with high confidence.

<i>Outdoors</i>	Very unlikely <input type="checkbox"/>	Unlikely <input type="checkbox"/>	Moderately likely <input type="checkbox"/>	Likely <input checked="" type="checkbox"/>	Very likely <input type="checkbox"/>
<i>Confidence</i>	High Confidence <input checked="" type="checkbox"/>	Medium Confidence <input type="checkbox"/>	Low Confidence <input type="checkbox"/>		

Under protection:

Although it is unlikely that many commercial apples are grown under protection in the UK, some specialist companies keep their grafted maiden and grafted family trees under

protection. As with establishment outdoors, there appear to be no obstacles to the viroid establishing under protection. As the viroid is intrinsically linked to its host and there are no identified vectors, the risk rating for establishment under protection has been assessed as likely with high confidence.

<i>Under Protection</i>	Very unlikely <input type="checkbox"/>	Unlikely <input type="checkbox"/>	Moderately likely <input type="checkbox"/>	Likely <input checked="" type="checkbox"/>	Very likely <input type="checkbox"/>
<i>Confidence</i>	High Confidence <input checked="" type="checkbox"/>	Medium Confidence <input type="checkbox"/>	Low Confidence <input type="checkbox"/>		

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

The viroid is not thought to spread easily by natural means as discussed in Section 9, therefore the rating for Natural Spread is set to very slowly with medium confidence, as there is the possibility that there is an, as yet unidentified, direct or indirect vector.

<i>Natural Spread</i>	Very slowly <input checked="" type="checkbox"/>	Slowly <input type="checkbox"/>	Moderate pace <input type="checkbox"/>	Quickly <input type="checkbox"/>	Very quickly <input type="checkbox"/>
<i>Confidence</i>	High Confidence <input type="checkbox"/>	Medium Confidence <input checked="" type="checkbox"/>	Low Confidence <input type="checkbox"/>		

The viroid does not appear to be spreading rapidly through trade, as it has only been detected in 5 different countries since it was first identified in 1996 (Di Serio *et al.*, 1996). ADFVd appears to be present in only one region of Italy, with the rest of Europe having no records of the disease. However, as some apple cultivars are asymptomatic when harbouring the viroid, it is possible that ADFVd has remained undetected in some countries and has a wider distribution than previously thought. Human activities such as grafting, pruning and handling of infected trees represent the most likely pathway of spread in the UK, and the presence of infected, asymptomatic plants could drive the spread of the viroid. Mechanical transfer of the viroid could occur between asymptomatic plants and varieties that are known to be symptomatic. The rating for spread with trade has been to moderate pace with low confidence.

<i>With trade</i>	Very slowly <input type="checkbox"/>	Slowly <input type="checkbox"/>	Moderate pace <input checked="" type="checkbox"/>	Quickly <input type="checkbox"/>	Very quickly <input type="checkbox"/>
<i>Confidence</i>	High Confidence <input type="checkbox"/>	Medium Confidence <input type="checkbox"/>	Low Confidence <input checked="" type="checkbox"/>		

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

It is not known how much of an impact ADFVd infection is having in the regions where it is currently present. Although the symptoms of the viroid can render infected fruit unmarketable, findings have so far appeared to be isolated to individual orchards and on

relatively few trees (Chiumenti *et al.*, 2014, Kasai *et al.*, 2017). The effect of ADFVd infection on the yield of asymptomatic varieties remains unknown. The rating for impacts in the areas where the viroid is currently distributed has been set at small with low confidence.

<i>Impacts</i>	Very small <input type="checkbox"/>	Small <input checked="" type="checkbox"/>	Medium <input type="checkbox"/>	Large <input type="checkbox"/>	Very large <input type="checkbox"/>
<i>Confidence</i>	High Confidence <input type="checkbox"/>	Medium Confidence <input type="checkbox"/>	Low Confidence <input checked="" type="checkbox"/>		

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

Malus species are an important crop in the UK (see Section 7) and the viroid has the potential to make an apple crop unmarketable if it was to establish in orchards in the UK. Impacts are likely to be significantly higher if a vector of the viroid was identified, although there is no evidence of a direct vector for ADFVd (see Section 9). As ADFVd seems unable to spread naturally, the largest risk of spread is associated with human activities. There is evidence that some viroids are more transmissible and produce more severe symptoms in warmer temperatures (Verhoeven *et al.*, 2010), giving rise to the possibility that UK field conditions could favour the slow spread of the viroid spread and asymptomatic infections. Although it is noted that an outbreak of ADFVd in a single orchard in a symptomatic *Malus* variety has the potential to be very economically damaging for a grower, the infection is unlikely to spread from the original site and have a large scale impact as symptoms will be identified relatively rapidly. However, this would not apply to asymptomatic infections, where the viroid could persist undetected.

The rating for Economic Impacts has been set at small with low confidence.

<i>Economic Impacts</i>	Very small <input type="checkbox"/>	Small <input checked="" type="checkbox"/>	Medium <input type="checkbox"/>	Large <input type="checkbox"/>	Very large <input type="checkbox"/>
<i>Confidence</i>	High Confidence <input type="checkbox"/>	Medium Confidence <input type="checkbox"/>	Low Confidence <input checked="" type="checkbox"/>		

There are no reports of the viroid infecting *Malus sylvestris* (crab apple). If the viroid is subsequently found to infect *M. sylvestris*, this PRA would have to be reviewed as there could be wider environmental impacts. If the viroid was potentially able to infect this wild species, symptoms are thought likely to only affect the fruit, as other parts of the tree are unaffected in cultivated hosts. However, decisions on felling infected trees would have to be carefully considered, as this could cause greater damage to the surrounding environment than the effects of the viroid itself. The rating for Environmental Impacts has been set at small with low confidence.

<i>Environmental Impacts</i>	Very small <input type="checkbox"/>	Small <input checked="" type="checkbox"/>	Medium <input type="checkbox"/>	Large <input type="checkbox"/>	Very large <input type="checkbox"/>
------------------------------	-------------------------------------	---	---------------------------------	--------------------------------	-------------------------------------

Confidence High Medium Low

Apple trees are popular with many gardeners and smallholders. However, any introduction of the viroid into the UK is likely to be on only a few trees. As natural spread is thought to be unlikely, the occurrence of ADFVd in private gardens is quite likely to be a dead end for the viroid. The rating for Social Impacts has been set at small with medium confidence.

Social Impacts Very small Small Medium Large Very large
Confidence High Medium Low

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

ADFVd is not a vector of plant pathogens.

16. What is the area endangered by the pest?

All apple growing areas of the UK are endangered, in particular the south-east, south-west and eastern regions of England, and the counties of Worcester and Hereford are at risk due to the large numbers of orchards in these areas.

Stage 3: Pest Risk Management

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

Exclusion:

As the main pathway for ADFVd is through infected planting material, it is currently not possible to prevent entry of the viroid into the UK. Intra-EU trade of *Malus* plants involves plant passporting and monitoring agreements, but freedom from ADFVd is not currently a requirement for plant movements. Detection of symptomatic plants for planting would be possible on mature plants with symptomatic fruit attached, but plants are not commonly moved at this life stage. Plants are usually traded as young plants without fruit. As there are no characteristic ADFVd symptoms visible on other parts of the plant, it is not possible to detect ADFVd without carrying out a molecular test. Currently, there is no commercially available test for ADFVd. However, it would be possible to develop a PCR-based test for the viroid, but this is dependent on being able to obtain ADFVd-infected material to validate any test against.

Exclusion is the best risk management option for the UK top fruit sector and regulation of ADFVd could be considered, with appropriate phytosanitary measures on the import of *Malus* plants for planting from the countries where the viroid is present. This could include sourcing plants only from pest free areas or certain pest free places of production if criteria that would help ensure disease freedom can be achieved. Another option for exclusion of the viroid could involve requirements for post-entry quarantine, pre-entry quarantine or complete physical isolation of *Malus* plants for planting could be considered by industry.

Alternatively, as this PRA has shown that ADFVd is not a significant threat to apple production in the UK, so statutory measures may not represent the most proportionate approach, an alternative option for exclusion of the viroid could involve the top fruit industry voluntarily introducing a DNA-based test for ADFVd as part of the Fruit Propagation Certification Scheme (FPCS). This would not prevent ADFVd-infected material from entering the UK, as any *Malus* plants for planting from the EU would not have been subjected to the same test for ADFVd, however it would help mitigate the risk of infected propagation material being used by the apple industry.

Eradication & Containment:

Under either a statutory or voluntary approach, containment and eradication may be possible in isolated outbreaks of the viroid, both outdoors and under protection. As there are no identified vectors of the viroid, any plants found to be infected could be removed and destroyed, thereby removing the pathogen from the environment. Hygiene measures would have to be put in place to disinfect any tools, equipment, clothing and vehicles used on outbreak sites, thereby preventing the spread of the viroid to uninfected trees.

Appropriate surveillance and testing would have to be carried out in the wider environment surrounding an outbreak site in order to identify latent infections in asymptomatic hosts. There is evidence to suggest that the viroid goes systemic in inoculated seedlings, and viroid proteins and RNA can be detected in larger trees for both symptomatic and asymptomatic varieties (He *et al.*, 2010, Malfitano *et al.*, 2014, Kasai *et al.*, 2017), suggesting that latent testing could reliably identify asymptomatic plants. This will have to be reviewed in the event that a vector of the viroid is identified, as this will complicate eradication and containment measures.

Malus plants must be accompanied by a plant passport and a supplier document at all stages of movement in the EU, including up until the plants arrive at the final retailer. Under a statutory approach, this means that trace back is possible during an outbreak, and other potentially infected plants that arrived with the initial shipment can be identified and tested.

Non-statutory controls:

Currently, there are no treatments for ADFVd and removal and destruction of infected material is the only option for removing the pathogen from an outbreak site. Regular inspection and latent testing of apple crops throughout the growing season should help

with early identification of infected, symptomatic and asymptomatic trees. As discussed previously, good hygiene measures should be put in place to prevent the spread of the viroid if it is present at a site. Hygiene best practice includes training staff to identify disease symptoms and implement good hygiene measures, using clothing, gloves and overshoes that can be destroyed or washed between orchards. Other hygiene measures include restricting the use of tools and machinery to one location, or chemically disinfecting tools and machinery between orchards. Additionally, detailed records should be kept about the cleaning and movement of machinery. Restricting the number of people working at a particular site can minimise spread of disease and, wherever possible, employees should work in the same area each day and avoid moving between sites.

18. References

- Antignus Y, Lachman O & Pearlsman M (2007): Spread of tomato apical stunt viroid (TASVd) in greenhouse tomato crops is associated with seed transmission and bumble bee activity. *Plant Disease* **91**, 47-50.
- Chiumenti M, Torchetti EM, Di Serio F & Minafra A (2014): Identification and characterization of a viroid resembling apple dimple fruit viroid in fig (*Ficus carica* L.) by next generation sequencing of small RNAs. *Virus Research* **188**, 54-59.
- Choueiri E, Zammar SE, Jreijiri F, Hobeika C, Myrta A & Di Serio F (2007): First report of Apple dimple fruit viroid in Lebanon. *Journal of Plant Pathology* **89**, 301-304.
- Defra (2018): Horticulture statistics - dataset (Excel). Published 31st May 2018. Accessed in October 2018: <https://www.gov.uk/government/statistics/latest-horticulture-statistics>
- Desvignes JC, Cornaggia D, Grasseau N, Ambrós S & Flores R (1999): Pear blister canker viroid: Host range and improved bioassay with two new pear indicators, Fieud 37 and Fieud 110. *Plant Disease* **83**, 419-422.
- Di Serio F, Aparicio F, Alioto D, Ragozzino A and Flores R (1996): Identification and molecular properties of a 306 nucleotide viroid associated with apple dimple fruit disease. *Journal of General Virology* **77**, 2833-2837.
- Di Serio F, Alioto D & Ragozzino A (2000): Apple dimple fruit viroid infections in cvs Annurca and Starking Delicious in Campania (Southern Italy) [*Malus pumila* Mill.]. *Informatore Fitopatologico* **50**, 53-56.
- Di Serio F, Malfitano M, Alioto D, Ragozzino A, Desvignes JC & Flores R (2001): Apple dimple fruit viroid: fulfillment of Koch's postulates and symptom characteristics. *Plant Disease* **85**, 179-182.
- He Y-H, Isono S, Kawaguchi-Ito Y, Taneda A, K, Iijima A, Tanaka K & Sano T (2010): Characterization of a new Apple dimple fruit viroid variant that causes yellow dimple fruit formation in 'Fuji' apple trees. *Journal of General Plant Pathology* **76**, 324-330.
- Howell WE, Skrzeczkowski LJ, Mink GI, Nunez A & Wesselz T (1998): Nontransmission

of apple scar skin viroid and peach latent mosaic viroid through seed. *Acta Horticulturae* **472**, 635-639.

Kasai H, Ito T & Sano T (2017): Symptoms and molecular characterization of apple dimple fruit viroid isolates from apples in Japan. *Journal of General Plant Pathology* **83**, 268-272.

Malfitano M, Alioto D, Ragozzino A, Flores R & Di Serio F (2004): Experimental evidence that Apple dimple fruit viroid does not spread naturally. *Proceedings of the XIXth International Symposium on Virus and Virus-like Diseases of Temperate Fruit Crops: Fruit Tree Diseases*. *ISHS Acta Horticulturae* **657**, 357-360.

Matsuura S, Matshushita Y, Kozuka R, Shimizu S & Tsuda S (2010): Transmission of tomato chlorotic dwarf viroid by bumblebees (*Bombus ignitus*) in tomato plants. *European Journal of Plant Pathology* **126**, 111-115.

Mehle N, Gutiérrez-Aguirre I, Prezelj N, Delić D, Vidic U & Ravnikar M (2014): Survival and transmission of Potato virus Y, Pepino mosaic virus, and Potato spindle tuber viroid in water. *Applied and Environmental Microbiology* **80**, 1455-1462.

Roumi V, Gazel M & Caglayan K (2017): First report of Apple dimple fruit viroid in apple trees in Iran. *BSP New Disease Reports* **35**, 3.

Salazar LR, Querci M, Bartolini I & Lazarte V (1995): Aphid transmission of potato spindle tuber viroid assisted by potato leafroll virus. *Fitopatología* **30**, 56-58.

Syller J, Marczewski W & Pawlowicz J (1997): Transmission by aphids of potato spindle tuber viroid encapsidated by potato leafroll luteovirus particles. *European Journal of Plant Pathology* **103**, 285-289.

Verhoeven JTJ, Hüner L, Marn MV, Plesko IM, Roenhorst JW (2010): Mechanical transmission of Potato spindle tuber viroid between plants of *Brugmansia suaveoles*, *Solanum jasminoides* and potatoes and tomatoes. *European Journal of Plant Pathology* **128**, 417-421.

Ye T, Chen SY, Wang R, Hao L, Chen H, Wang N, Guo LY, Fan ZF, Li SF & Zhou T (2013): Identification and molecular characterization of Apple dimple fruit viroid in China. *Journal of Plant Pathology* **95**, 637-641.

Name of Pest Risk Analysts(s)

Laura Stevens



© Crown copyright 2018

You may re-use this information (excluding logos) free of charge in any format or medium, under the terms of the Open Government Licence v.2. To view this licence visit www.nationalarchives.gov.uk/doc/open-government-licence/version/2/ or email PSI@nationalarchives.gsi.gov.uk

This publication is available via the UK Plant Health Information portal <https://planthealthportal.defra.gov.uk/>

Any enquiries regarding this publication should be sent to us at

The Chief Plant Health Officer

Department for Environment, Food and Rural Affairs

Room 11G32

Sand Hutton

York

YO41 1LZ

Email: plantpestrisks@defra.gsi.gov.uk