SCIENTIFIC OPINION

Scientific opinion on the risks posed by *Prunus* pollen, as well as pollen from seven additional plant genera, for the introduction of viruses and virus-like organisms into the EU[1]

EFSA Panel on Plant Health (PLH)[2,3]

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ABSTRACT

The Panel on Plant Health assessed the risk of introduction of listed viruses and virus-like agents through the import of pollen for pollination of eight plant genera. Because of the absence of an identified pollen trade for *Fragaria*, *Rubus*, *Ribes*, *Vitis* and *Cydonia*, the risk of introduction of viruses listed in Directive 2000/29/EC was assessed to be negligible, with low uncertainty. For *Prunus*, *Malus* and *Pyrus*, trade was identified and the risk evaluated in detail. For 12 viruses and one viroid the probability of entry was rated unlikely to moderately likely and the probability of establishment very unlikely to unlikely. In the case of the two remaining agents, *Apple latent spherical virus* (ALSV) and *Apple fruit crinkle viroid* (AFCVd), the probability of entry was rated as very unlikely with a moderately likely to likely (ALSV) or very unlikely to unlikely (AFCVd) probability of establishment. Therefore, for all agents, significant limitations to their introduction were identified. Almost all ratings are, however, associated with high uncertainty because of the absence of information on many important aspects. As a consequence, the risk of introduction of listed viruses and viroids by pollen for pollination of the genera *Prunus*, *Malus* and *Pyrus* is considered negligible to low, with high uncertainty. The current legislation provides a substantial level of risk reduction but risks could be further reduced by addressing specifically pollen in Annexes III and IV. The two risk reduction options identified with high or very high effectiveness and feasibility to further reduce the risk of introduction are the extension to all non-EU countries of the existing prohibition on *Prunus*, *Malus* and *Pyrus* pollen imports and a request for the individual testing of all imported pollen shipments. The uncertainty on the effectiveness and feasibility of these measures was assessed as low and low to medium, respectively.

KEY WORDS

Malus, pollen transmission, pollination, *Prunus*, *Pyrus*, viroid, virus

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Plant Health (PLH Panel) was asked to deliver a scientific opinion on the risks posed by the import of pollen from the genera *Prunus* L., *Cydonia* Mill., *Fragaria* L., *Malus* Mill., *Pyrus* L., *Ribes* L., *Rubus* L. and *Vitis* L. for the introduction of viruses and virus-like organisms into the EU. The PLH Panel was also asked to identify risk reduction options in the event that pollen poses a risk for introduction of these organisms.

The Panel conducted the risk assessment following the general principles of the "Guidance on a harmonised framework for pest risk assessment and the identification and evaluation of pest risk management options" (EFSA PLH Panel, 2010), the "Guidance on evaluation of risk reduction options" (EFSA PLH Panel, 2012), as well as the Guidance of the Scientific Committee on Transparency in the Scientific Aspects of Risk Assessments carried out by EFSA (EFSA, 2009). In conducting the assessment, the Panel took into account current EU plant health legislation.

According to Council Directive 2000/29/EC pollen is included in the definition of plants intended for planting. Because of the absence of an identified commercial trade in pollen for pollination from the genera *Fragaria*, *Rubus*, *Ribes*, *Vitis* and *Cydonia*, the risk of introduction of the viruses listed in Council Directive 2000/29/EC through this pathway was assessed by the Panel to be negligible, with low uncertainty, mostly associated with the possibility that pollen from these five genera could be traded for pollination purposes despite the fact that the Panel was unable to identify such trade. However, such a possibility seems unlikely given that the species concerned are self-fertile, which strongly limits the interest of assisted pollination.

It should be stressed, however, that if, for unforeseen reasons, commercial trade in pollen of *Fragaria*, *Rubus*, *Ribes*, *Vitis* and *Cydonia* for pollination purposes were to develop, the above assessment would need to be re-analysed, since some viruses of the species listed in Council Directive 2000/29/EC may have the potential to be pollen-borne.

Concerning pollen of the genera *Prunus*, *Malus* and *Pyrus*, trade was identified and the risk of this pathway was, therefore, evaluated in detail. For 12 viruses and one viroid, the probability of entry was rated as unlikely to moderately likely and the probability of establishment as very unlikely to unlikely. In the case of the two remaining agents, ALSV and AFCVd, the probability of entry is very unlikely and is associated with a moderately likely to likely (ALSV) or very unlikely to unlikely probability of establishment (AFCVd). This indicates that for all 15 agents considered, very significant limitations to their introduction were identified. Almost all of these ratings were, however, associated with high uncertainty because of the absence of relevant information on many important aspects.

As a consequence of the above analysis, the Panel considers that the risk of introduction of listed viruses and viroids by pollen for pollination of the genera *Prunus*, *Malus* and *Pyrus* is negligible to low, with high uncertainty.

As requested by the Commission, the Panel concentrated its analysis on risk reduction options having an impact on the introduction of virus and virus-like agents through the pollen for pollination pathway. Analysis of the current legislation demonstrated that it already provides a substantial level of risk reduction, by banning the importation of pollen (as included in plants for planting) from a range of countries and by requesting that imported pollen is either officially certified under a certification scheme, or derived in a direct line from material that has been maintained under appropriate conditions and subjected to official testing at least once during the last three complete cycles of vegetation. However, risks could be further reduced by addressing pollen specifically in Annexes III and IV of Council Directive 2000/29/EC, in order to facilitate the understanding and the proper implementation of its requirements, and by making clear that visual observation of plants from which pollen is collected is not an appropriate and efficient testing technique.

The Panel identified two risk reduction options with high or very high effectiveness and feasibility to reduce the risk of entry: the extension to all non-EU countries of the existing prohibition on *Prunus*, *Malus* and *Pyrus* pollen imports and a request for an individual testing of all imported pollen shipments. The uncertainties of the effectiveness and feasibility of these measures were assessed as low and low to medium, respectively.

Eradication, the only risk reduction option with impact on establishment, was evaluated as having moderate effectiveness when considering the orchards and their environment, with medium technical feasibility and medium uncertainty.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The current European Union plant health regime is established by Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community (OJ L 169, 10.7.2000, p.1).

The Directive lays down, amongst others, the technical phytosanitary provisions to be met by plants and plant products and the control checks to be carried out at the place of origin on plants and plant products destined for the Union or to be moved within the Union, the list of harmful organisms whose introduction into or spread within the Union is prohibited and the control measures to be carried out at the outer border of the Union on arrival of plants and plant products.

In the EU virus and virus-like organisms of the genera *Prunus* L., *Cydonia* Mill., *Fragaria* L., *Malus* Mill., *Pyrus* L., *Ribes* L., *Rubus* L. and *Vitis* L. are regulated harmful organisms listed in Annex IAI (d)5 of Council Directive 2000/29/EC. Consequently, the introduction of these organisms into, and their spread within all Member States, is banned. This listing covers viruses and virus-like organisms which are nominally mentioned in Annex IAI (d)5 as examples, as well as all non-European viruses and virus-like organisms. The latter refers to species of viruses and virus-like organisms which are not known to occur in Europe.

Additional viruses and virus-like organisms of the genera *Prunus* L., *Cydonia* Mill., *Fragaria* L., *Malus* Mill., *Pyrus* L., *Ribes* L., *Rubus* L. and *Vitis* L. are listed in Annex IAII, IIAI and IIAII of Council Directive 2000/29/EC.

According to Council Directive 2000/29/EC/EU pollen is included in the definition of plants intended for planting. In line with the Annex IIIA points 9 and 18 of that Directive, the import of *Prunus* pollen and pollen of the other seven above-mentioned genera is prohibited from non-European countries, other than Mediterranean countries, Australia, New Zealand, Canada and the Continental States of the USA. Furthermore, pollen from these genera needs to fulfil the special import requirements related to viruses and virus-like organisms specified in Annex IVAI points 19.2, 20, 21.1, 22.1, 22.2, 23.1, 23.2 and 24. Moreover, according to Annex VBI 1 all plants intended for planting originating in third countries must be subject to a plant health inspection in the country of origin and for their import into the EU they must be accompanied by a phytosanitary certificate stating that they are free from harmful organisms regulated in the EU. Consequently, pollen of *Prunus* and the other seven above-mentioned genera require inspection prior to export to the EU as well as a phytosanitary certificate.

Recently two consignments of *Prunus domestica* (plum) pollen and one consignment of *Prunus avium* (sweet cherry) pollen originating in the USA were intercepted in the EU due to the detection at import of *Prunus* viruses. Cherry pollen tested positive for Cherry leaf roll virus (CLRV), *Prunus* necrotic ringspot virus (PNRSV) and Prune dwarf virus (PDV). In plum pollen Cherry leaf roll virus (CLRV), Cherry rasp leaf virus (CRLV), *Prunus* necrotic ringspot virus (PNRSV) and Prune dwarf virus (PDV) were detected.

These interceptions have raised concern about the possibility of the introduction of *Prunus* viruses and virus-like organisms via pollen into the EU. Even though the interceptions clearly show that *Prunus* pollen can be a pathway for entry into the EU territory of *Prunus* viruses, it is not clear whether infected pollen could lead to the establishment of *Prunus* viruses in the EU. The same question arises in connection with pollen from the other seven above-mentioned genera. Therefore the Commission is seeking advice from the European Food Safety Authority (EFSA) on the risks to plant health posed by pollen for the introduction of viruses and virus-like organisms not only of the genus *Prunus* but also of the genera *Cydonia* Mill., *Fragaria* L., *Malus* Mill., *Pyrus* L., *Ribes* L., *Rubus* L. and *Vitis* L..

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested, pursuant to Article 29(1) and Article 22(5) of Regulation (EC) No 178/2002, to provide a scientific opinion on the risks posed by the import of pollen from the genera *Prunus* L., *Cydonia* Mill., *Fragaria* L., *Malus* Mill., *Pyrus* L., *Ribes* L., *Rubus* L. and *Vitis* L. for the introduction of viruses and virus-like organisms into the EU. In case pollen poses a risk for introduction of these organisms, EFSA is requested to identify risk reduction options.

ASSESSMENT

1. Introduction

1.1. Purpose

This document presents an assessment prepared by the Panel on Plant Health (PLH) on the risks posed by the import of pollen from the genera *Prunus* L., *Cydonia* Mill., *Fragaria* L., *Malus* Mill., *Pyrus* L., *Ribes* L., *Rubus* L. and *Vitis* L. for the introduction of viruses and virus-like organisms into the European Union (EU), in response to a request from the European Commission. The risk assessment area is the territory of the European Community (EU-28), and the opinion includes identification and evaluation of risk reduction options in terms of their effectiveness in reducing the risk of introduction of listed agents posed by the trade in pollen of the above genera for pollination purposes.

1.2. Scope

The scope of the opinion is to assess the risk of introduction (entry and establishment) into the EU of viruses and virus-like organisms listed on the annexes of Council Directive 2000/29/EC[4] posed by the trade in pollen of the genera *Prunus* L., *Cydonia* Mill., *Fragaria* L., *Malus* Mill., *Pyrus* L., *Ribes* L., *Rubus* L. and *Vitis* L. for pollination purposes. The opinion focuses on these listed viruses, independently of their significance as pests. The assessment of the risk of (further) spread within the EU is also analysed but the magnitude of impact is not analysed in detail in this opinion.

Whether certain viruses should be listed or should be delisted is not within the remit of this opinion.

The identification and evaluation of risk reduction options is limited to options to reduce the risk of entry and establishment, as requested by the European Commission.

2. Methodology and data

2.1. Methodology

2.1.1. The guidance documents

The assessment was conducted in line with the principles described in the guidance on a harmonised framework for pest risk assessment and the identification and evaluation of pest risk management options (EFSA PLH Panel, 2010), and in the Guidance of the Scientific Committee on Transparency in the Scientific Aspects of Risk Assessments carried out by EFSA (EFSA, 2009).

The evaluation of risk reduction options was conducted in line with the principles described in the above-mentioned guidance documents, as well as with those in the guidance on methodology for evaluation of the effectiveness of options to reduce the risk of introduction and spread of organisms harmful to plant health in the EU territory (EFSA PLH Panel, 2012).

2.1.2. Methods used for conducting the risk assessment

The Panel conducted the assessment with the current EU plant health legislation in place considering that the viruses of concern are regulated in the EU.

In order to evaluate, for each plant genus, the risks posed by pollen import, the Panel first identified the listed agents that might be associated with the pollen of each genus and then, using a scheme derived from the structure of a general pest categorisation, it evaluated the probability of entry, establishment and spread of each virus through the pollen for pollination pathway. Lastly, considering

[4] Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. OJ L 169, 10.7.2000, p. 1–148.

these ratings and the list of viruses that might be associated with a consignment of pollen of each of the plant genera under consideration, the Panel determined the risks posed by pollen import.

The conclusions for entry, establishment and spread are presented separately. The descriptors used to assign qualitative ratings to the probability of entry, establishment and spread, as well as to the risk posed by the import of pollen are provided in Appendix A.

2.1.3. Methods used for evaluating the risk reduction options

The Panel identified potential risk reduction options and evaluated them with respect to their effectiveness and technical feasibility, i.e. consideration of technical aspects that influence their practical application. The evaluation of the efficiency of management options in terms of the potential cost-effectiveness of measures and their implementation is not within the scope of the Panel's evaluation.

The descriptors used to assign qualitative ratings for the evaluation of the effectiveness and technical feasibility of management options are provided in Appendix A.

2.1.4. Level of uncertainty

For the conclusions of the risk assessment on entry, establishment and spread, and for the evaluation of the effectiveness of the management options, the levels of uncertainty have been rated separately.

The descriptors used to assign qualitative ratings to the levels of uncertainty are provided in Appendix A.

2.2. Data

2.2.1. Literature search

The evidence considered by the Panel in its assessment was obtained from:

- Specific literature searches related to pollen transmission, the listed viruses and recently described viral agents of the plant genera under consideration. Searches were performed in June and July 2013 using the CAB International and Web of Science databases to identify relevant scientific papers and the grey literature. Information was extracted from the Plant Quarantine Data Retrieval System of the European and Mediterranean Plant Protection Organization (EPPO PQR, 2012).

- Expert knowledge in the field. When expert judgement and/or personal communication were used, justification and evidence are provided to support the statements. Personal communications have been considered only when in written form and supported by evidence and when other sources of information were not publicly available (see Appendix B).

2.2.2. Data collection

The EUROPHYT database[5] was consulted on 4 July 2013, searching specifically for non-compliance data related to pollen.

[5] EUROPHYT is a web-based network launched by the Directorate General for Health and Consumers, and is a sub-project of PHYSAN (Phyto-Sanitary Controls) specifically concerned with plant health information. The EUROPHYT database manages notifications of interceptions of plants or plant products that do not comply with EU legislation.

3. Pest risk assessment

3.1. Background information on pollen transmission of plant viruses

According to Council Directive 2000/29/EC pollen is included in the definition of plants intended for planting. Pollen, the male gametes of higher plants, is produced in the male organs of flowers, the anthers. Once mature, it is released and can be transferred, in a process called pollination, to the female organs (pistil of the ovary), either passively by wind or through the activity of insects or of other animals. Upon landing on the pistil of a compatible plant, pollen cells will germinate and produce a pollen tube which will then grow to finally meet, in the ovary, the female gamete, the ovule, resulting in fertilisation. The fertilised ovule will then develop into an embryo protected by the maternally derived seed envelopes, while the tissues of the ovary will develop into a fruit containing the seed(s). Germination of pollen grain and fertilisation of the ovule occurs only between sexually compatible plants belonging to the same species or to closely related ones. In addition, in many plant species, genetic mechanisms exist to prevent the pollen of that species from germinating on its own pistil or to prevent further fecundation events. Such species are termed self-incompatible and require pollen from other plants of the same species for a successful fecundation to occur and for seeds and fruits to be produced. In other species, termed self-compatible, the pollen produced by a plant is able to successfully germinate and fertilise the plant ovules to permit seed and fruit development.

Some plant pathogenic agents, including bacteria, viruses and viroids, can be transferred from host plant to host plant through the pollination process. Other so-called ‘virus-like agents’, such as phytoplasmas or spiroplasmas, are not known to be pollen transmitted, probably because of their strict phloem limitation (Card et al., 2007).

Virus or viroid transmission through pollen is not only the result of passive transfer of viral particles contaminating the surface of pollen grains but results from pollen grain infection and requires infected pollen germination and the ensuing fertilisation (Mink, 1993; Card et al., 2007). This results in the production of infected embryos and seeds, which, upon germination, will develop into infected seedlings. This process is referred to as vertical (or to the seed) transmission (Mink, 1993; Card et al., 2007). In this situation, the pollinated plant bearing the infected seeds will remain healthy, the infection remaining limited to the embryo/seed. In rarer cases, the virus present in the infected germinating pollen or in the infected embryo is able to spread to maternal tissues, resulting in an infection of the mother plant itself, in a process referred to as horizontal (or to the mother plant) transmission (Mink, 1993; Card et al., 2007). Because of the mechanisms involved, the few horizontally transmitted viruses are also transmitted vertically, but the converse is not true and the majority of viruses that are transmitted vertically to the seeds are in fact not transmitted horizontally to the pollinated mother plants (Mink, 1993; Card et al., 2007).

It should also be considered that not all seed-transmitted viruses are pollen transmitted because seed infection via contamination of the female gametes is generally much more efficient than infection via the male gametes (Mink, 1993; Card et al., 2007). Therefore, the fact that a virus is seed transmitted can be taken only as an indication that the virus in question might be pollen transmitted but clearly not as a conclusive element of proof.

Similarly, the fact that a virus is observed to be associated with pollen (detection of the virus in pollen samples) is not necessarily an indication that this particular agent is pollen transmitted (Hamilton et al., 1977) since pollen surface contamination or the presence of parts of contaminated anther tissues can often be observed even for viruses that are not pollen transmitted (Mink, 1993; Card et al., 2007).

Since transmission of viruses by pollen requires pollination/fertilisation, virus transmission by pollen is essentially restricted to sexually compatible species. For example, an analysis of the population genetics of *Cherry leafroll virus* demonstrated that the structure of viral populations is strongly influenced by the host plant, suggesting that movement of viral isolates between different host species is very rare for this virus (Rebenstorf et al., 2006). Even within a species, transmission may be impeded between varieties that differ too much in their flowering time. In a few cases, pollen transmission has been shown to be aided by insects such as thrips, probably through their interaction with pollen and through wounds they might make and contaminate on the pollinated plant (Sdoodee and Teakle, 1993; Klose et al., 1996).

For virus transmission through infected pollen in open pollinating plants, virus-infected pollen has to compete with pollen from healthy plants. Because, as shown for *Prunus necrotic ringspot virus* (PNRSV) (Amari et al., 2007a), virus infection generally results in reduced pollen fitness, poor germination and delayed growth, production of seeds from infected pollen can be inefficient. In addition, the rate of germination of seeds developing from virus-infected embryos—arising from virus-infected mother plants or from pollen infections—can often be reduced when compared with healthy seeds (Yang and Hamilton, 1974; Amari et al., 2007a,b). For perennial plants, however, virus spread by pollen plays a significant role in the ecology of the viruses, so that a large proportion of plants may become infected over time (Murant et al., 1974; Bristow and Martin, 1999).

The mechanisms by which viruses are transmitted through pollen are not fully understood and, similarly, the extent to which pollen transmission under field conditions occurs may be difficult to assess. Most of the studies aiming to confirm pollen transmission involve either the caging of naturally infected plants to avoid contamination by pollen from external sources (Murant et al., 1974) or hand pollination using infected pollen (Mircetich et al., 1982). But such delicate and complex experiments are rarely performed. From an analysis of the available literature, it is evident that the host species plays a major role in pollen transmission and hence it cannot be assumed that pollen transmissibility is a general feature of a particular virus that applies in all its hosts. Rather, pollen transmission is a feature shown by a given virus in some hosts but not in others (Mink, 1993; Card et al., 2007), with a general tendency that it is more frequent in herbaceous than in woody perennial hosts. Thus, from pollen transmission in a herbaceous host, e.g. *Tobacco ringspot virus* (TRSV) in *Nicotiana benthamiana* (Zadeh and Foster, 2004), it cannot be concluded that the virus is pollen transmitted in its woody hosts.

Although pollen transmission is more frequently observed in some viral genera, its occurrence strictly depends on the particular virus species/strain involved. Therefore, the fact that a given virus belongs to a genus containing pollen-transmitted agents cannot be used to conclude, by analogy, that pollen transmission also takes place for that particular virus.

As a consequence of these various elements, some particular traits, such as seed transmissibility, presence of virus particles in pollen, or taxonomic affinities with pollen-transmitted agents, can be used only as indications but definitely not as proof that a particular virus will be pollen transmitted.

3.2. Genera of which pollen for pollination is commercially available

Through databases and internet searches, the Panel was unable to identify any evidence indicating that pollen of *Fragaria*, *Rubus*, *Ribes*, *Vitis* and *Cydonia* is sold commercially for assisted pollination purposes. This probably reflects the pollination biology of these species, which are generally self-fertile and for which the advantages of artificial pollination would therefore be extremely limited. However, the Panel identified four US companies[6] commercially offering pollen for pollination of apples, pears and a range of *Prunus* species (cherries, plums, prunes, apricots, peaches and almonds). With the exception of sour cherry and peach, all of these species are generally self-sterile.

The fact that pollen of *Fragaria*, *Rubus*, *Ribes*, *Vitis* and *Cydonia* is not sold commercially for the purpose of assisted pollination was confirmed in communications with the representatives of three of the identified pollen-selling US companies (Neil McClure, Firman Pollen Company Inc., July 2013; Scott Beddard, FirmYield Pollen, August 2013; and Rebb Firman, Pollen Collection and Sales, August 2013, personal communications) (see Appendix B). Furthermore, for *Fragaria* and *Rubus*, absence of

[6] See websites at http://www.firmanpollen.com/index.html, http://www.firmyieldpollen.com/index.html, http://www.pollencollectionandsales.com/, http://www.californiaagsupply.com/pollen.htm

commercial trade of pollen for pollination was also confirmed by experts of a technical hearing (EFSA, in press).

Because of the absence of commercial trade for the purpose of assisted pollination, the risk of introduction of viruses listed in Council Directive 2000/29/EC for *Fragaria*, *Rubus*, *Ribes*, *Vitis* and *Cydonia* through this pathway was assessed by the Panel to be negligible and therefore was not further analysed. This assessment carries low uncertainty, mostly associated with the possibility that pollen of these five genera could be traded for pollination purposes despite the fact that the Panel was unable to identify such trade. However, such a possibility seems unlikely given that the species concerned are self-fertile, which strongly limits the interest of assisted pollination.

It should be stressed, however, that the initiation and development of commercial trade in pollen of *Fragaria*, *Rubus*, *Ribes*, *Vitis* and *Cydonia* species for pollination purposes would necessitate an updated assessment since several of the virus species listed in Council Directive 2000/29/EC for these genera may have the potential to be pollen-borne.

As a consequence of this analysis, the Panel further evaluated the risk of the introduction of viruses with commercial trade in pollen for pollination purposes of the genera *Malus*, *Pyrus* and *Prunus*. Although no trade in *Cydonia* pollen was identified, and no specific analysis performed, the viruses infecting plants of *Malus* and *Pyrus* have generally been found to infect *Cydonia* when experimentally tested (Hadidi et al., 2011, and chapters therein), so that a reasonable evaluation of the risks associated with *Cydonia* pollen could be deduced, with further uncertainties, from the analysis of the risks associated with *Malus* and *Pyrus* pollen (see below).

3.3. Viruses listed in annexes of Council Directive 2000/29/EC known to infect plants of the genera *Malus*, *Pyrus* and *Prunus*

Viral agents listed in Annex I of Council Directive 2000/29/EC and which have natural hosts in the *Malus*, *Pyrus* and *Prunus* genera were identified on the basis of database searches and expert knowledge. A list of these agents is provided in Table 1 together with the virus acronym, the host genera with traded pollen and references relating to recent scientific syntheses on these agents.

Table 1: Agents listed in Annex I of Council Directive 2000/29/EC and which have natural host plants in the *Prunus*, *Pyrus*, *Malus* and *Cydonia* genera

Virus	Acronym	Hosts in	References
Tobacco ringspot virus	TRSV	*Prunus*, *Malus*	Martelli and Uyemoto (2011)
Tomato ringspot virus	ToRSV	*Prunus*, *Malus*	Martelli and Uyemoto (2011)
Cherry rasp leaf virus (American)	CRLV	*Prunus*, *Malus*	Martelli and Uyemoto (2011); James (2011a)
Peach mosaic virus (American)	PcMV	*Prunus*	Larsen and James (2011)
Peach rosette mosaic virus	PRMV	*Prunus*	Martelli and Uyemoto (2011)
Plum line pattern virus (American)	APLPV	*Prunus*	Myrta et al. (2011)

Similarly, viral agents listed for the *Malus*, *Pyrus* and *Prunus* genera in Annex II of Council Directive 2000/29/EC were identified and are listed in Table 2. It should be noted that, at the time of redaction of Council Directive 2000/29/EC, the agent(s) responsible for little cherry disease were unknown, explaining the listing as ‘little cherry pathogen’, while two different viruses, *Little cherry virus 1* and *Little cherry virus 2*, have now independently been associated with this disease (Jelkmann and Eastwell, 2011).

Table 2: Agents listed for plants of the genera *Prunus*, *Pyrus*, *Malus* and *Cydonia* in Annex II of Council Directive 2000/29/EC

Virus	Acronym	Hosts in	References
Little cherry pathogen (non-European isolates)	LChV1 and LChV2	*Prunus*	Jelkmann and Eastwell (2011)
Plum pox virus	PPV	*Prunus*	Barba et al. (2011)

3.4. Identification of viruses listed as 'non-European viruses' in Annex IAI of Council Directive 2000/29/EC and known to infect plants of the genera *Malus*, *Pyrus*, *Prunus*

In addition to the above individually listed viruses, Annex IAI of Council Directive 2000/29/EC collectively lists 'non-European viruses and virus-like organisms' of *Malus* Mill., *Prunus* L. and *Pyrus* L. In order to identify precisely the relevant agents, two strategies were used.

The geographic distribution of all agents naturally infecting these three genera was evaluated on the basis of recent scientific syntheses (Hadidi et al., 2011, and chapters therein), allowing the identification of viruses or viroids never reported or with only limited records in Europe.

In addition, database searches were performed in an effort to identify newly described viral agents not included in the above synthesis. Only a single agent, *Apple green crinkle virus* (James et al., 2013), was identified, but this virus appears to be widely distributed throughout the world, including in the EU-28, and was not therefore considered by the Panel to be a non-European agent.

It should be noted that several closely related agents, Asian prunus viruses 1, 2 and 3, have been described, with their taxonomic status still unclear as to whether these three agents represent three different but related species or three strains of a single species (Candresse et al., 2011). Since there is no indication that they might differ in their biology, they are treated as belonging to one species, named Asian prunus virus, in the rest of the present opinion.

Table 3 provides a tentative list of the non-European agents of the *Malus*, *Prunus* and *Pyrus* genera together with the virus acronym, the host genera with traded pollen, the known distribution of the agent and references relating to recent scientific syntheses.

Table 3: Tentative list of non-European viruses and viroids with natural hosts in the *Prunus*, *Pyrus*, *Malus* and *Cydonia* genera

Virus	Acronym	Hosts in	Distribution	References
Apple latent spherical virus	ALSV	*Malus*	Japan	Koganezawa and Ito (2011a)
Asian prunus virus	APV	*Prunus*	Japan, China	Candresse et al. (2011)
Cherry mottle leaf virus	CMLV	*Prunus*	USA, Canada, reports from Belgium, former Czechoslovakia, Italy, Poland and Romania	James (2011b)
Cherry twisted leaf virus	CTLV	*Prunus*	USA, Canada, reports from Denmark and Romania	James (2011c)
Apple fruit crinkle viroid	AFCVd	*Malus*	Japan	Koganezawa and Ito (2011b)
Apple scar skin viroid	ASSVd	*Malus*, *Pyrus*, *Cydonia*	China, Japan, Korea, India, USA, Canada, reports from the UK, Italy, Greece	Hadidi and Barba (2011)

In conclusion, a total of 13 viruses and two viroids are therefore considered to be relevant for the *Malus*, *Prunus* and *Pyrus* genera and listed in Council Directive 2000/29/EC and are therefore assessed in detail in the second part of the present opinion.

3.5. Assessment of viruses and viroids listed in Council Directive 2000/29/EC for the *Malus*, *Prunus* and *Pyrus* genera

3.5.1. Identity of the pest

3.5.1.1. Viruses of concern and their taxonomy, vectors, host range and detection

For the 13 viruses and the two viroids identified as described above, information was gathered using recent scientific syntheses, expert knowledge and additional references identified during database searches. It should be stressed that while some of these viruses, such as ToRSV or PPV, are well known and have been extensively studied over many years, others have been discovered only very recently, in the past 10–15 years, so that very little information is available for them. Elements of the taxonomy, existence and identity of vectors and natural host range of these agents are provided in Table 4.

Table 4: Taxonomy, vectors and known natural hosts of agents listed in Council Directive 2000/29/EC and which have natural host plants in the *Prunus*, *Pyrus*, *Malus* and *Cydonia* genera

Virus	Acronym	Taxonomy	Vectors	Natural hosts	References
Tobacco ringspot virus	TRSV	Genus *Nepovirus*, Family *Secoviridae*	North American nematode species: *Xiphinema americanum sensu lato*, *X. americanum sensu stricto*, *X. californicum*, *X. rivesi*, *X. intermedium*, *X. tarjanense*	Cherry, grapevine, blueberry, blackberry, apple, many herbaceous crops and ornamentals. Wide experimental host range	Stace-Smith (1985); Martelli and Uyemoto (2011)
Tomato ringspot virus	ToRSV	Genus *Nepovirus*, Family *Secoviridae*	North American nematode species: *X. americanum sensu lato*, *X. americanum sensu stricto*, *X. bricolensis*, *X. californicum*, *X. rivesi*, *X. intermedium*, *X. tarjanense*	*Prunus* species, apple, blueberry, blackberry, grapevine, red and black currants, rose, strawberry, many herbaceous crops and ornamentals. Wide experimental host range	Stace-Smith (1984); Martelli and Uyemoto (2011); Sanfaçon and Fuchs (2011)
Cherry rasp leaf virus (American)	CRLV	Genus *Cheravirus*, Family *Secoviridae*	North American nematode species: *X. americanum sensu lato*, *X. americanum sensu stricto*, *X. californicum*, *X. rivesi*	*Prunus* species, apple, raspberry, potato. Several herbaceous species known as natural or experimental hosts	Stace-Smith and Hansen (1976); James (2011a); Martelli and Uyemoto (2011)
Peach mosaic virus (American)	PcMV	Genus *Trichovirus*, Family *Betaflexiviridae*	Peach bud mite, *Eriophyes insidiosus* (North and Central American distribution)	*Prunus* species are the only known natural hosts but several herbaceous experimental hosts known	Larsen and James (2011); Oldfield and Proeseler (1996)
Peach rosette mosaic virus	PRMV	Genus *Nepovirus*, Family *Secoviridae*	North American nematode species: *X. americanum sensu lato*, *Longidorus diadecturus*, *L. elongates*	Peach, grapevine and blueberry. Several herbaceous species known as natural or experimental hosts	Ramsdell and Gillett (1998); Martelli and Uyemoto (2011)
Plum line pattern virus	APLPV	Genus *Ilarvirus*, Family *Bromoviridae*	No known vector	*Prunus* species are the only known natural hosts but several	Fulton (1984); Myrta et al. (2011)

Virus	Acronym	Taxonomy	Vectors	Natural hosts	References
(American)				herbaceous experimental hosts known	
Little cherry virus 1	LChV1	Unassigned species within the Family *Closteroviridae*	No known vector	*Prunus* species are the only known natural hosts	Jelkmann and Eastwell (2011)
Little cherry virus 2	LChV2	Genus *Ampelovirus*, Family *Closteroviridae*	Apple mealybug *Phenacoccus aceris* (present in many EU countries)	*Prunus* species are the only known natural hosts	Jelkmann and Eastwell (2011)
Plum pox virus	PPV	Genus *Potyvirus*, Family *Potyviridae*	Over 20 common European aphid species	Cultivated, ornamental and wild *Prunus* species	Barba et al. (2011)
Apple latent spherical virus	ALSV	Genus *Cheravirus*, Family *Secoviridae*	No known vector	Apple is the only known natural host but large experimental host range	Koganezawa and Ito (2011a)
Asian prunus virus	APV	Tentative species in the Genus *Foveavirus*, Family *Betaflexiviridae*	No known vector	*Prunus* species are the only known natural hosts	Candresse et al. (2011)
Cherry mottle leaf virus	CMLV	Genus *Trichovirus*, Family *Betaflexiviridae*	Bud mite, *Eriophyes inaequalis* (North and Central American distribution)	*Prunus* species are the only known natural hosts but several herbaceous experimental hosts are known	Oldfield and Proeseler (1996); James (2011b)
Cherry twisted leaf virus	CTLV	Unassigned species within the Family *Betaflexiviridae*	No known vector	*Prunus* species are the only known natural hosts. One herbaceous experimental host is known	James (2011c)
Apple fruit crinkle viroid	AFCVd	Genus *Apscaviroid*, Family *Pospiviroidae*	No known vector	Apple and hop only known natural hosts	Koganezawa and Ito (2011b)
Apple scar skin viroid	ASSVd	Genus *Apscaviroid*, Family *Pospiviroidae*	No known vector	Apple, pear, apricot and cherry. *Cydonia*, *Sorbus*, *Chaenomeles* and *Pyronia* species are experimental hosts	Koganezawa (1989); Hadidi and Barba (2011)

Although some of the viruses and viroids addressed in the present opinion have only recently been described, all are now well characterised and their genomic sequences known for one or more strains/isolates. In the case of the better-known agents (TRSV, ToRSV, PPV) serological detection with commercial or laboratory reagents is available. However, for the more recently described agents, or for those difficult to purify, no serological detection is available (APV, CMLV, CTLV). The same situation applies also to viroids, which do not encode proteins and cannot therefore be detected by serological means. However, for all of these agents, polymerase chain reaction (PCR)-based detection assays and primers have been described and are, in some cases, widely used (Hadidi et al., 2011, and chapters therein). The possibility that these primers may fail to amplify some divergent isolates of the less well-known agents has, however, to be considered. Overall, reasonably good diagnostic assays are available for all the viruses and viroids addressed in the present opinion.

3.5.1.2. Pollen transmission

As indicated in the background information section on pollen transmission, two different situations have to be considered: transmission of the virus to the pollinated plant itself (so-called horizontal transmission or transmission to the mother plant) and transmission of the virus to the seeds produced by the pollinated plant (vertical transmission or transmission to the seeds). It should also be stressed that evidence of seed transmission of a virus does not demonstrate that a virus is pollen transmitted, since transmission through the female gametes is much more frequent than transmission through the pollen.

In order to describe and analyse the information available on the pollen transmission of the viruses and viroids concerned by the present opinion, the Panel separately assessed the evidence for vertical (to the seed) and horizontal (to the mother plant) transmission because these two mechanisms have quite different implications for virus epidemiology and for the assessment of the probability of introduction and spread of a given agent. In view of its potential link to pollen transmission, seed transmission information was also recorded.

As described in Table 5, for the sake of simplification, four groups of agents with increasingly strong evidence for pollen transmissibility were determined, on the basis of available evidence considered in each transmission situation.

Table 5: Transmission groups with increasing evidence for pollen transmissibility considered in the present opinion

Transmission group	Information available on pollen transmission of the agent	Agents of the same genus known to be pollen transmitted
Group 1	No	No
Group 2	No	Yes
Group 3	Yes, in hosts other than *Prunus*, *Malus* or *Pyrus*	Yes
Group 4	Yes in *Prunus*, *Malus* or *Pyrus*	Yes

It should be stressed that the information available on the possibility of pollen transmission of many of the agents analysed in the present opinion is extremely limited, if not altogether absent, in particular when it comes to the agents described recently. As a consequence, any conclusion on the existence (or absence) of pollen-mediated transmission for a given agent is almost always associated with important uncertainties.

It should also be noted that imported pollen for pollination is mixed with unspecified “carrier” agent(s) and is brought in contact with plants either through the action of bees or via the use of mechanical dispensing equipment (blowers). While it can be assumed that pollination by bees, which is similar to natural pollination, does not substantially impact virus transmission, there is currently no information as to whether the more artificial, blower-assisted, pollination might enhance the spread of viruses present in the pollen used. Scenarios in which the high windspeed used would result in mechanical wounding of plant tissues, and in a form of mechanical transmission of the viruses, could be envisaged but there is currently no scientific information to support or dispel such ideas, further creating uncertainties in the present evaluation.

3.5.1.3. Vertical (to the seeds) pollen transmission

As summarised in Table 6, the agents considered in the present opinion can be divided into four vertical transmission groups on the basis of the scientific evidence gathered by the Panel.

Table 6: Transmission groups and relevant evidence identified concerning the vertical (to the seeds) transmission of the viruses considered in the present opinion

Virus	Acronym	Evidence for vertical transmission by pollen	Agents known to be vertically transmitted by pollen in the same genus[(a)]	References
Vertical transmission group 1—No evidence for vertical pollen transmission of the agent and no known pollen transmitted agent in the same genus				
Asian prunus virus	APV	No relevant information identified, not reported to be pollen transmitted in any of the known hosts	No *Foveavirus* known to be pollen transmitted	Candresse et al. (2011)
Little cherry virus 1	LChV1	No relevant information identified, not reported to be pollen transmitted in any of the known hosts	No *Closteroviridae* known to be pollen transmitted	Jelkmann and Eastwell (2011)
Little cherry virus 2	LChV2	No relevant information identified, not reported to be pollen transmitted in any of the known hosts	No *Closteroviridae* known to be pollen transmitted	Jelkmann and Eastwell (2011)
Cherry mottle leaf virus	CMLV	No relevant information identified, not reported to be pollen transmitted in any of the known hosts	No *Trichovirus* known to be pollen transmitted	James (2011b)
Cherry twisted leaf virus	CTLV	No relevant information identified, not reported to be pollen transmitted in any of the known hosts	Closest species in the *Betaflexiviridae* family (CGRMV, CNRMV) not known to be pollen transmitted	James (2011c)
Peach mosaic virus (American)	PcMV	Not pollen transmitted in peach, no relevant information identified for any of the other known hosts	No *Trichovirus* known to be pollen transmitted	Hutchins et al. (1951); Larsen and James (2011)
Apple fruit crinkle viroid	AFCVd	Not reported to be pollen transmitted in its woody hosts and no evidence of natural spread to neighbouring trees	No *Apscaviroid* known to be pollen transmitted	Koganezawa and Ito (2011b)
Apple scar skin viroid	ASSVd	No relevant information identified, not reported to be pollen transmitted in any of the known hosts Despite discrepancies in the literature, ASSVd appears to be seed borne in apple, resulting in a low transmission rate from ASSVd-positive seeds	No *Apscaviroid* known to be pollen transmitted	Koganezawa (1989); Kim et al. (2006); Hadidi and Barba (2011)
Vertical transmission group 2—No evidence for vertical pollen transmission of the agent but known pollen transmitted agent(s) in the same genus				
Cherry rasp leaf virus (American)	CRLV	CRLV can be detected in pollen from infected cherry trees but pollen or seed transmission in cherry was never demonstrated. CRLV is known to be seed transmitted in several herbaceous host species, with no information as to whether pollen-mediated transmission might contribute to this observation	Several nepoviruses, including TRSV and ToRSV known to be pollen transmitted	Wagnon et al. (1968); Stace-Smith and Hansen (1976); James (2011a); Martelli and Uyemoto (2011)
Peach rosette mosaic virus	PRMV	Not reported to be pollen transmitted. Known to be seed transmitted in several herbaceous host species and in grapevine, with no information as to whether pollen-mediated transmission might contribute to this observation	Several nepoviruses, including TRSV and ToRSV known to be pollen transmitted	Ramsdell and Myers (1978); Ramsdell and Gillett (1998); Martelli and Uyemoto (2011)

Virus	Acronym	Evidence for vertical transmission by pollen	Agents known to be vertically transmitted by pollen in the same genus[(a)]	References
Plum line pattern virus (American)	APLPV	No report of natural transmission of APLPV by either seed or pollen, but transmission in *Prunus* has not been investigated Not seed transmitted in the experimental herbaceous hosts *Petunia* and bean	Several ilarviruses, including *Prune dwarf virus* and *Prunus necrotic ringspot virus* known to be pollen transmitted	Fulton (1984); Mink (1995); Myrta et al. (2011); Pallas et al. (2012)
Plum pox virus	PPV	Despite early publications to the contrary, the current scientific consensus is that there is no seed transmission (and therefore no pollen transmission) in any of the woody hosts No report of pollen transmission in any other host	Several potyviruses, including *Soybean mosaic virus*, *Lettuce mosaic virus and Bean common mosaic virus* known to be pollen transmitted	Glasa and Candresse (2005); Barba et al. (2011)
Vertical transmission group 3—Agent known to be vertically transmitted by pollen in hosts other than *Prunus*, *Malus* or *Pyrus* and known pollen-transmitted agent(s) in the same genus				
Tobacco ringspot virus	TRSV	A cherry isolate of TRSV was pollen and seed transmitted in *Nicotiana* spp. TRSV is seed transmitted in several host species but is not reported to be pollen transmitted in its woody hosts	Several nepoviruses, including TRSV and ToRSV known to be pollen transmitted	Stace-Smith (1985); Zadeh and Foster (2004); Martelli and Uyemoto (2011)
Tomato ringspot virus	ToRSV	A ToRSV isolate was pollen and seed transmitted in *Pelargonium*. ToRSV is seed transmitted in several host species but is not reported to be pollen transmitted in its woody hosts	Several nepoviruses, including TRSV and ToRSV known to be pollen transmitted	Stace-Smith (1984); Scarborough and Smith (1977); Martelli and Uyemoto (2011)
Vertical transmission group 4—Agent known to be vertically transmitted by pollen in *Prunus*, *Malus* or *Pyrus* and known pollen-transmitted agent(s) in the same genus				
Apple latent spherical virus	ALSV	Seed and pollen transmitted in apple	ALSV	Nakamura et al. (2011)

(a): As assessed from reviews on pollen transmission of plant viruses and viroids (Mink, 1993; Card et al., 2007).

In summary, of all the viruses and viroids analysed, only ALSV is known to be pollen transmitted vertically (to the seeds) in one of the hosts (apple) analysed in the present opinion. There is no information on pollen transmission in *Prunus*, *Malus* or *Pyrus* of any of the other viruses considered. TRSV and ToRSV have been shown to be pollen transmitted vertically in herbaceous hosts, suggesting that they might also be pollen transmitted in some of their woody hosts. However, the patterns of spread of these viruses in woody hosts are more consistent with their known transmission by soil inhabiting nematodes (Martelli and Taylor, 1989; Martelli and Uyemoto, 2011).

3.5.1.4. Horizontal (to the mother plants) pollen transmission

As summarised in Table 7, the agents considered in the present opinion can be divided into two horizontal transmission groups on the basis of the scientific evidence gathered by the Panel.

Table 7: Transmission group and relevant evidence identified concerning the horizontal (to the mother plant) transmission of the viruses considered in the present opinion

Virus	Acronym	Evidence for horizontal transmission by pollen	Agents known to be horizontally transmitted by pollen in the same genus[(a)]	References
Horizontal transmission group 1—No evidence for horizontal pollen transmission of the agent and no known horizontally pollen-transmitted agent in the same genus				
Asian prunus virus	APV	No relevant information identified, not reported to be pollen transmitted in any of the known hosts	No *Foveavirus* known to be pollen transmitted	Candresse et al. (2011)
Little cherry virus 1	LChV1	No relevant information identified, not reported to be pollen transmitted in any of the known hosts	No *Closteroviridae* known to be pollen transmitted	Jelkmann and Eastwell (2011)
Little cherry virus 2	LChV2	No relevant information identified, not reported to be pollen transmitted in any of the known hosts	No *Closteroviridae* known to be pollen transmitted	Jelkmann and Eastwell (2011)
Cherry mottle leaf virus	CMLV	No relevant information identified, not reported to be pollen transmitted in any of the known hosts	No *Trichovirus* known to be pollen transmitted	James (2011b)
Cherry twisted leaf virus	CTLV	No relevant information identified, not reported to be pollen transmitted in any of the known hosts	Closest species in the *Betaflexiviridae* family (CGRMV, CNRMV) not known to be pollen transmitted	James (2011c)
Peach mosaic virus (American)	PcMV	Not pollen transmitted in peach, no relevant information identified for any of the other known hosts	No *Trichovirus* known to be pollen transmitted	Hutchins et al. (1951); Larsen and James (2011)
Apple fruit crinkle viroid	AFCVd	Not reported to be pollen transmitted in its woody hosts and no evidence of natural spread to neighbouring trees.	No *Apscaviroid* known to be pollen transmitted	Koganezawa and Ito (2011b)
Apple scar skin viroid	ASSVd	No relevant information identified, not reported to be pollen transmitted in any of the known hosts Despite discrepancies in the literature, ASSVd appears to be seed-borne in apple, resulting in a low transmission rate from ASSVd-positive seeds	No *Apscaviroid* known to be pollen transmitted	Koganezawa (1989); Kim et al. (2006); Hadidi and Barba (2011)
Horizontal transmission group 2—No evidence for horizontal pollen transmission of the agent but known horizontally pollen-transmitted agent(s) in the same genus				
Cherry rasp leaf virus (American)	CRLV	No relevant information identified, not known to be horizontally pollen transmitted in any of its hosts	Several nepoviruses, including *Tomato black ring*, *Blueberry leaf mottle virus* and *Cherry leafroll virus* known to be horizontally pollen transmitted	Wagnon et al. (1968); Stace-Smith and Hansen (1976); James (2011a); Martelli and Uyemoto (2011)
Peach rosette mosaic virus	PRMV	No relevant information identified, not known to be horizontally pollen transmitted in any of its hosts.	Several nepoviruses, including *Tomato black ring*, *Blueberry leaf mottle virus* and *Cherry leafroll virus* known to be horizontally pollen transmitted	Ramsdell and Myers (1978); Ramsdell and Gillett (1998); Martelli and Uyemoto (2011)

Virus	Acronym	Evidence for horizontal transmission by pollen	Agents known to be horizontally transmitted by pollen in the same genus[(a)]	References
Plum line pattern virus (American)	APLPV	No relevant information identified, not known to be horizontally pollen transmitted in any of its hosts	Several ilarviruses, including *Prune dwarf virus* and *Prunus necrotic ringspot virus* known to be horizontally pollen transmitted	Fulton (1984); Mink (1995); Myrta et al. (2011); Pallas et al. (2012)
Plum pox virus	PPV	No relevant information identified, not known to be horizontally pollen transmitted in any of its hosts	*Soybean mosaic virus* known to be horizontally pollen transmitted	Glasa and Candresse (2005); Barba et al. (2011)
Tobacco ringspot virus	TRSV	A cherry isolate of TRSV was pollen transmitted in *Nicotiana* spp. but TRSV is not known to be horizontally pollen transmitted in any of its hosts	Several nepoviruses, including *Tomato black ring*, *Blueberry leaf mottle virus* and *Cherry leafroll virus* known to be horizontally pollen transmitted	Stace-Smith (1985); Zadeh and Foster (2004); Martelli and Uyemoto (2011)
Tomato ringspot virus	ToRSV	A ToRSV isolate was pollen and seed transmitted in *Pelargonium* but ToRSV is not known to be horizontally pollen transmitted in any of its hosts	Several nepoviruses, including *Tomato black ring*, *Blueberry leaf mottle virus* and *Cherry leafroll virus* known to be horizontally pollen transmitted	Scarborough and Smith (1977); Stace-Smith (1984); Martelli and Uyemoto (2011)
Apple latent spherical virus	ALSV	Vertically pollen transmitted in apple but not reported to be horizontally transmitted to the mother plant	ALSV is vertically pollen transmitted in apple	Nakamura et al. (2011)

(a): As assessed from reviews on pollen transmission of plant viruses and viroids (Mink, 1993; Card et al., 2007).

In summary, none of the agents analysed is known to be horizontally pollen transmitted (to pollinated mother plants), although some of them belong to plant virus genera containing horizontally transmitted agents.

3.5.1.5. Diseases and symptoms

With two exceptions, the agents considered in the present opinion have been described as responsible for significant diseases and damage in the three genera considered (*Prunus*, *Malus*, *Pyrus*). Some of them, such as TRSV, ToRSV and PRMV, are also of concern in a range of other crops (see Table 4).

The two exceptions to this situation are APV and ALSV. For APV, there are currently no precise data on the symptoms it may cause in its *Prunus* hosts because it is a virus discovered relatively recently that has so far always been observed in mixed infection with other pathogenic viruses or viroids (Candresse et al., 2011). ALSV is reported to cause asymptomatic infections in apple (hence its name) and to be asymptomatic or poorly symptomatic in its experimental hosts (Koganezawa and Ito, 2011a).

3.5.2. Current distribution

Information on distribution was extracted from the EPPO PQR database (EPPO PQR, 2012) and from Hadidi et al. (2011). No specific effort was made to ensure that a more comprehensive picture on the distribution was obtained, as it does not significantly influence the assessment. Similarly, no efforts were made to reconcile conflicting data from the two sources used or to try to pinpoint or update information that was too broad or unclear. It should, however, be stressed that, in the case of many of the viruses concerned (lesser known agents or agents of recent description/discovery), there are very significant uncertainties about their precise geographic distribution (and prevalence) because of the absence of specific surveys targeting them.

The information on distribution is provided in the following two tables, which summarise data on distribution within (Table 8) and outside (Table 9) the risk assessment area.

Table 8: Distribution in the risk assessment area of the viruses and viroids analysed in the present opinion

Virus	Acronym	Distribution according to EPPO PQR (2012)	Distribution according to Hadidi et al. (2011)
Tobacco ringspot virus	TRSV	Hungary, Lithuania, Poland, the UK	The Netherlands, the former Yugoslavia, the former Soviet Union
Tomato ringspot virus	ToRSV	Croatia, France, Italy, Lithuania, Slovakia, Slovenia	The former Soviet Union, the former Yugoslavia, Sweden, the UK, the Netherlands, Denmark
Cherry rasp leaf virus (American)	CRLV	No records in EU	The UK (Scotland)
Peach mosaic virus (American)	PcMV	No records in EU	Greece, Italy
Peach rosette mosaic virus	PRMV	No records in EU	No records in EU
Plum line pattern virus (American)	APLPV	Italy	No information
Little cherry virus 1	LChV1	Belgium, the Czech Republic, Germany, Greece, Italy, Poland, Romania, the UK	Present in Europe but not detailed
Little cherry virus 2	LChV2	Germany, Poland	Present in Europe but not detailed
Plum pox virus	PPV	Austria, Belgium, Bulgaria, Croatia, Cyprus, the Czech Republic, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Luxembourg, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, the UK	Austria, Belgium, Bulgaria, Croatia, Cyprus, the Czech Republic, Estonia, France, Germany, Greece, Hungary, Italy, Lithuania, Luxembourg, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, the UK
Apple latent spherical virus	ALSV	No distribution data in EPPO PQR	No records in EU
Asian prunus virus	APV	No distribution data in EPPO PQR	No records in EU
Cherry mottle leaf virus	CMLV	No distribution data in EPPO PQR	Belgium, former Czechoslovakia, Italy, Poland, Romania, the former Yugoslavia
Cherry twisted leaf virus	CTLV	No distribution data in EPPO PQR	Denmark, Romania
Apple fruit crinkle viroid	AFCVd	No distribution data in EPPO PQR	No records in EU
Apple scar skin viroid	ASSVd	No distribution data in EPPO PQR	Greece, the UK

Table 9: Distribution outside the risk assessment area of the viruses and viroids analysed in the present opinion

Virus	Acronym	Distribution according to EPPO PQR (2012)	Distribution according to Hadidi et al. (2011)
Tobacco ringspot virus	TRSV	**Europe**: Georgia, Russia, Turkey, Ukraine **Africa**: Congo, Egypt, Malawi, Morocco, Nigeria **America**: Brazil, Canada, Chile, Cuba, Dominican Republic, Mexico, USA (in 32 states), Uruguay, Venezuela **Asia**: China, India, Indonesia, Iran, Japan, Kyrgyzstan, Saudi Arabia, Sri Lanka, Taiwan **Oceania**: Australia, New Zealand, Papua New Guinea	North America, Australia, Japan, India, Iran, Nigeria, the former Yugoslavia, the former Soviet Union
Tomato ringspot virus	ToRSV	**Europe**: Belarus, Russia, Serbia, Turkey **Africa**: Egypt, Togo **America**: Canada, Chile, Peru, Puerto Rico, USA (in 30 states), Venezuela **Asia**: China, Iran, Japan, Jordan, Korea, Oman, Pakistan **Oceania**: New Zealand	North America, Chile, Sweden, Japan, Taiwan, New Zealand, the former Yugoslavia, the former Soviet Union
Cherry rasp leaf virus (American)	CRLV	**Europe**: – **Africa**: – **America**: Canada, USA (11 states) **Asia**: – **Oceania**: –	North America
Peach mosaic virus (American)	PcMV	**Europe**: – **Africa**: – **America**: Mexico, USA (eight states) **Asia**: – **Oceania**: –	USA, Mexico, India
Peach rosette mosaic virus	PRMV	**Europe**: Turkey **Africa**: Egypt **America**: Canada, USA (two states) **Asia**: – **Oceania**: –	North America
Plum line pattern virus (American)	APLPV	**Europe**: Albania **Africa**: – **America**: Canada, USA (10 states) **Asia**: Lebanon **Oceania**: New Zealand	No information

Little cherry virus 1	LChV1	**Europe**: Switzerland, Turkey **Africa**: – **America**: Canada, USA (three states) **Asia**: Japan **Oceania**: New Zealand	Canada, North America, New Zealand[(a)]
Little cherry virus 2	LChV2	**Europe**: – **Africa**: – **America**: Canada **Asia**: China, Japan **Oceania**: –	
Plum pox virus	PPV	**Europe**: Albania, Bosnia and Herzegovina, Moldova, Montenegro, Norway, Russia, Serbia, Switzerland, Turkey, Ukraine **Africa**: Egypt, Tunisia **America**: Argentina, Canada, Chile, USA (three states) **Asia**: China, India, Iran, Japan, Jordan, Kazakhstan, Pakistan, Syria **Oceania**: –	Albania, Argentina, Bosnia and Herzegovina, Canada, Chile, China, Georgia, India, Iran, Japan, Jordan, Kazakhstan, Moldova, Montenegro, Norway, Pakistan, Russia, Switzerland, Serbia, Ukraine, Egypt, Syria, Tunisia, Turkey, USA
Apple latent spherical virus	ALSV	No distribution data in EPPO PQR	Japan
Asian prunus virus	APV	No distribution data in EPPO PQR	China, Japan, USA
Cherry mottle leaf virus	CMLV	**Europe**: – **Africa**: South Africa **America**: Canada, USA **Asia**: – **Oceania**: –	USA, Canada, South Africa, the former Yugoslavia
Cherry twisted leaf virus	CTLV	No distribution data in EPPO PQR	Canada, USA
Apple fruit crinkle viroid	AFCVd	No distribution data in EPPO PQR	Japan
Apple scar skin viroid	ASSVd	No distribution data in EPPO PQR	India, USA, Canada, China, Japan, Korea

(a): Information refers to the little cherry disease.

3.5.3. Regulatory status

Because of the absence of commercial trade in pollen for the purposes of assisted pollination in the genus *Fragaria*, *Rubus*, *Ribes*, *Vitis* and *Cydonia*, viruses of these genera were excluded from the assessment and are not presented in the following section.

3.5.3.1. Regulatory status in the risk assessment area

Legislation directly related to the viruses and virus-like organisms

The viruses and virus-like organisms as subjects of this opinion are regulated harmful organisms in the EU and listed in Council Directive 2000/29/EC in the following sections:

Annex I, Part A—Harmful organisms whose introduction into, and spread within, all Member States shall be banned

Section I—Harmful organisms not known to occur in any part of the Community and relevant for the entire Community

(d) Viruses and virus-like organisms:

Species
3. *Tobacco ringspot virus*
4. *Tomato ringspot virus*
5. Viruses and virus-like organisms of *Cydonia* Mill., *Fragaria* L., *Malus* Mill., *Prunus* L., *Pyrus* L., *Ribes* L., *Rubus* L. and *Vitis* L., such as:
(b) Cherry rasp leaf virus (American)
(c) Peach mosaic virus (American)
(e) Peach rosette mosaic virus
(i) Plum line pattern virus (American)
(n) Non-European viruses and virus-like organisms of *Cydonia* Mill., *Malus* Mill., *Prunus* L., and *Pyrus* L

Annex II, Part A—Harmful organisms whose introduction into, and spread within, all Member States shall be banned if they are present on certain plants or plant products

Section I—Harmful organisms not known to occur in the Community and relevant for the entire Community

(d) Virus and virus-like organisms

Species	Subject of contamination
9. Little cherry pathogen (non-European isolates)	Plants of *Prunus cerasus* L., *Prunus avium* L., *Prunus incisa* Thunb., *Prunus sargentii* Rehd., *Prunus serrula* Franch., *Prunus serrulata* Lindl., *Prunus speciosa* (Koidz.) Ingram, *Prunus subhirtella* Miq., *Prunus yedoensis* Matsum., and hybrids and cultivars thereof, intended for planting, other than seeds

Section II—Harmful organisms known to occur in the Community and relevant for the entire Community

(d) Viruses and virus-like organisms

Species	Subject of contamination
7. Plum pox virus	Plants of *Prunus* L., intended for planting, other than seeds

Annex IV, Part A—Special requirements which must be laid down by all Member States for the introduction and movement of plants, plant products and other objects into and within all Member States

Section I—Plants, plant products and other objects originating outside the Community

Plant products and other objects	Special requirements
19.2. Plants of *Cydonia* Mill., […] *Malus* Mill., *Prunus* L., *Pyrus* L. […] intended for planting, other than seeds, originating in countries where the relevant harmful organisms are known to occur on the genera concerned The relevant harmful organisms are —on all species: —non-European viruses and virus-like organisms	Without prejudice to the provisions applicable to the plants where appropriate listed in Annex IIIA (9) and (18), and Annex IVAI (15) and (17), official statement that no symptoms of diseases caused by the relevant harmful organisms have been observed on the plants at the place of production since the beginning of the last complete cycle of vegetation
22.1. Plants of *Malus* Mill. intended for planting, other than seeds, originating in countries where the relevant harmful organisms are known to occur on *Malus* Mill. The relevant harmful organisms are: —Cherry rasp leaf virus (American) —Tomato ringspot virus,	Without prejudice to the provisions applicable to the plants, listed in Annex IIIA (9) and (18), Annex IIIB (1) and Annex IVAI (15), (17) and (19.2), official statement that: (a) the plants have been: —either officially certified under a certification scheme requiring them to be derived in direct line from material which has been maintained under appropriate conditions and subjected to official testing for at least the relevant harmful organisms using appropriate indicators or equivalent methods and has been found free, in these tests, from those harmful organisms or —derived in direct line from material which is maintained under appropriate conditions and subjected, within the last three complete cycles of vegetation, at least once, to official testing for at least the relevant harmful organisms using appropriate indicators or equivalent methods and has been found free, in these tests, from those harmful organisms (b) no symptoms of diseases caused by the relevant harmful organisms have been observed on plants at the place of production, or on susceptible plants in its immediate vicinity, since the beginning of the last complete cycle of vegetation
23.1. Plants of following species of *Prunus* L., intended for planting, other than seeds, originating in countries where Plum pox virus is known to occur: —*Prunus amygdalus* Batsch —*Prunus armeniaca* L. —*Prunus blireiana* Andre —*Prunus brigantina* Vill. —*Prunus cerasifera* Ehrh. —*Prunus cistena* Hansen —*Prunus curdica* Fenzl and Fritsch —*Prunus domestica* ssp. *Domestica* L. —*Prunus domestica* ssp. *insititia* (L.) C.K. Schneid —*Prunus domestica* ssp. *Italica* (Borkh.) Hegi. —*Prunus glandulosa* Thunb. —*Prunus holosericea* Batal. —*Prunus hortulana* Bailey	Without prejudice to the provisions applicable to the plants, listed in Annex IIIA (9) and (18), and Annex IVAI (15) and (19.2), official statement that: (a) the plants, other than those raised from seed, have been: —either officially certified under a certification scheme requiring them to be derived in direct line from material which has been maintained under appropriate conditions and subjected to official testing for, at least, *Plum pox virus* using appropriate indicators or equivalent methods and has been found free, in these tests, from that harmful organism or —derived in direct line from material which

Plant products and other objects	Special requirements
—*Prunus japonica* Thunb. —*Prunus mandshurica* (Maxim.) Koehne —*Prunus maritima* Marsh —*Prunus mume* Sieb and Zucc. —*Prunus nigra* Ait. —*Prunus persica* (L.) Batsch —*Prunus salicina* L. —*Prunus sibirica* L. —*Prunus simonii* Carr. —*Prunus spinosa* L. —*Prunus tomentosa* Thunb. —*Prunus triloba* Lindl. —other species of *Prunus* L. susceptible to Plum pox virus	is maintained under appropriate conditions and has been subjected, within the last three complete cycles of vegetation, at least once, to official testing for at least *Plum pox virus* using appropriate indicators or equivalent methods and has been found free, in these tests, from that harmful organism (b) no symptoms of disease caused by *Plum pox virus* have been observed on plants at the place of production or on susceptible plants in its immediate vicinity, since the beginning of the last three complete cycles of vegetation (c) plants at the place of production which have shown symptoms of disease caused by other viruses or virus-like pathogens, have been rogued out
Plants of *Prunus* L., intended for planting (a) originating in countries where the relevant harmful organisms are known to occur on *Prunus* L. (b) other than seeds, originating in countries where the relevant harmful organisms are known to occur (c) other than seeds, originating in non-European countries where the relevant harmful organisms are known to occur The relevant harmful organisms are: —for the case under (a): —Tomato ringspot virus —or the case under (b): —Cherry rasp leaf virus (American) —Peach mosaic virus (American) —Peach phony rickettsia —Peach rosette mycoplasm —Peach yellows mycoplasm —Plum line pattern virus (American) —Peach X-disease mycoplasm —or the case under (c): —Little cherry pathogen	Without prejudice to the provisions applicable to the plants, where appropriate listed in Annex IIIA (9) and (18) or Annex IVAI (15), (19.2) and (23.1), official statement that (a) the plants have been: —either officially certified under a certification scheme requiring them to be derived in direct line from material which has been maintained under appropriate conditions and subjected to official testing for at least the relevant harmful organisms using appropriate indicators or equivalent methods and has been found free, in these tests, from those harmful organisms, or —derived in direct line from material which is maintained under appropriate conditions and has been subjected, within the last three complete cycles of vegetation, at least once, to official testing for at least the relevant harmful organisms using appropriate indicators or equivalent methods and has been found free, in these tests, from those harmful organisms (b) no symptoms of diseases caused by the relevant harmful organisms have been observed on plants at the place of production or on susceptible plants in its immediate vicinity, since the beginning of the last three complete cycles of vegetation

Legislation related to the hosts of the viruses and virus-like organisms
Annex III, Part A—Plants, plant products and other objects the introduction of which shall be prohibited in all Member States

Description	Country of origin
9. Plants of […] *Cydonia* Mill., […] *Malus* Mill., *Prunus* L., *Pyrus* L., […] intended for planting, other than dormant plants free from leaves, flowers and fruit	Non-European countries
18. Plants of *Cydonia* Mill., *Malus* Mill., *Prunus* L. and *Pyrus* L. and their hybrids […] and *Fragaria*, intended for planting, other than seeds	Without prejudice to the prohibitions applicable to the plants listed in Annex IIIA (9), where appropriate, non-European countries, other than Mediterranean countries, Australia, New Zealand, Canada, the continental states of

Annex V—Plants, plant products and other objects which must be subject to a plant health inspection (at the place of production if originating in the Community, before being moved within the Community—in the country of origin or the consigner country, if originating outside the community) before being permitted to enter the Community

Part B—Plants, plant products and other objects originating in territories, other than those territories referred to in Part A

Section I—Plants, plant products and other objects which are potential carriers of harmful organisms of relevance for the entire Community and which must be accompanied by a plant passport

1. Plants, intended for planting

1.1. Plants intended for planting, other than seeds of the genera *Cydonia* Mill., *Malus* Mill., *Prunus* L., other than *Prunus laurocerasus* L. and *Prunus lusitanica* L. and *Pyrus* L.

2. Plants, plant products and other objects produced by producers whose production and sale is authorised to persons professionally engaged in plant production, other than those plants, plant products and other objects which are prepared and ready for sale to the final consumer, and for which it is ensured by the responsible official bodies of the Member States, that the production thereof is clearly separate from that of other products.

2.1. Plants intended for planting other than seeds of the genera *Fragaria* L., *Prunus laurocerasus* L., *Prunus lusitanica* L., *Rubus* L.

Section II—Plants, plant products and other objects which are potential carriers of harmful organisms of relevance for certain protected zones, and which must be accompanied by a plant passport valid for the appropriate zone when introduced into or moved within that zone

1. Plants, plant products and other objects.

1.3. Plants, other than fruit and seeds, of *Cydonia* Mill., *Malus* Mill., *Pyrus* L. and *Vitis* L.

1.4. Live pollen for pollination of *Cydonia* Mill., *Malus* Mill., *Pyrus* L.

Legislation related to other agents with a link to pollen

Council Directive 2000/29/EC lists *Erwinia amylovora* on plants for planting, other than seeds, of various species, including *Malus*, *Pyrus* and *Cydonia* in Annex IIAII. The same bacteria is also listed on 'Parts of plants, other than fruit, seeds and plants intended for planting, but including live pollen' of the same species in Annex IIB. As a consequence, import of live pollen for pollination of the species concerned is banned in Annex IIIB (1 and 2) in the defined protected zones, unless it originates from Switzerland, or from countries free of *E. amylovora* or from *E. amylovora* pest-free areas. Some of these requirements are further elaborated in Annex IVB (21) while Annex VAII states that pollen of the species concerned must be accompanied by a valid plant passport in order to be introduced or moved within the described protected zones.

3.5.3.2. Regulatory status outside the risk assessment area

The regulatory status of the viruses and viroids analysed in the present opinion was assessed using the EPPO PQR database (EPPO PQR, 2012). No further efforts were made to ensure the completeness of the information.

Table 10: Quarantine or alert pest status outside of the risk assessment area of the viruses and viroids listed in Council Directive 2000/29/EC and that have natural host plants in the *Prunus*, *Pyrus* and *Malus* genera

Virus	Acronym	Quarantine pathogen in	A1 listed in	A2 listed in
Tobacco ringspot virus	TRSV	Israel, Norway	East Africa, Argentina, Paraguay, Turkey, Ukraine	APPPC, EPPO, Canada, China
Tomato ringspot virus	ToRSV	Canada, Israel, Norway	APPPC, IAPSC, Argentina, Paraguay, Uruguay, China, Ukraine	EPPO, Turkey
Cherry rasp leaf virus (American)	CRLV	Israel, Norway	EPPO, IAPSC, Kazakhstan, Russia, Turkey, Ukraine	Canada
Peach mosaic virus (American)	PcMV	Norway	EPPO, Canada, Uzbekistan, Turkey	
Peach rosette mosaic virus	PRMV	Israel, Jordan	EPPO, Kazakhstan, Russia, Turkey, Ukraine	Canada
Plum line pattern virus (American)	APLPV	Israel, Jordan, Norway	EPPO, Uzbekistan, Moldova, Turkey	Canada
Little cherry virus 1	LChV1	Israel, Jordan	Paraguay, Turkey, Ukraine	Canada
Little cherry virus 2	LChV2	Israel, Jordan	Turkey, Ukraine	
Plum pox virus	PPV	USA, Israel, Norway, New Zealand	IAPSC, East Africa, South Africa, Argentina, Brazil, Canada, Paraguay, Uruguay, Kazakhstan, Uzbekistan	COSAVE, EPPO, Chile, Russia, Turkey, Ukraine
Cherry mottle leaf virus	CMLV		Canada	
Apple scar skin viroid	ASSVd	Israel	Canada	

APPPC, Asia and Pacific Plant Protection Commission; EPPO, European and Mediterranean Plant Protection Organization; COSAVE, Comité de Sanidad Vegetal; IAPSC, Inter-African Phytosanitary Council.

3.5.4. Probability of entry in the risk assessment area

3.5.4.1. Interceptions

There is no information on the volume of trade in pollen for pollination entering the EU[7], nor of the fraction of the shipments that are tested by national phytosanitary authorities. Nevertheless, the EUROPHYT database provides information about three intercepted shipments in which plant viruses were detected. Among the viruses thus identified, PNRSV and *Prune dwarf virus* (PDV) are very frequent quality agents in *Prunus* species (Caglayan et al., 2011; Hammond, 2011). CLRV is also a

[7] However, personal communications (Scott Beddard, FirmYield Pollen, August 2013; and Neil McClure, Firman Pollen Company, August 2013) indicate that in 2013, in total, these companies will trade 55 kg of pollen of the species concernend, enough to pollinate about 550 hectares of orchards. These communications further indicate that in 2013 pollen trade by these two companies involved Germany, France, Italy, Spain, Austria, the Netherlands, Belgium and Slovakia. In one company, pollen trade has been going on for 15 years.

frequent quality pathogen in cherry (Büttner et al., 2011). It should be noted that both PNRSV and CLRV are listed in Annex IIA of Council Directive 2000/29/EC when present on plants of *Rubus* L., intended for planting. The last virus identified, *Cherry rasp leaf virus*, an agent able to infect a range of hosts including *Prunus* species and *Malus* (James, 2011a; Martelli and Uyemoto, 2011), is listed in Annex IA of Council Directive 2000/29/EC.

Table 11: Viruses intercepted on pollen according to the EUROPHYT database

Virus	Host	Country of origin	Country of destination	Date of interception
Cherry leafroll nepovirus	*Prunus domestica*	USA	Slovakia	24 April 2013
Cherry leafroll nepovirus *Prunus necrotic ringspot virus* *Prune dwarf virus*	*Prunus* sp.	USA	Austria	20 March 2013
Cherry leafroll nepovirus *Cherry rasp leaf nepovirus* (listed) *Prunus necrotic ringspot virus*	*Prunus* sp.	USA	Austria	7 March 2013

Thus, despite the efforts that pollen-selling companies make to ensure freedom from viruses[8], at least some shipments of pollen for pollination appear to be contaminated by either quality or listed agents, an observation that is not very surprising given the wide prevalence of some of the agents considered.

3.5.4.2. Evaluation of the probability of entry in the risk assessment area

Four parameters are generally considered when analysing the potential for entry of a pathogen through a given pathway: (i) association with the pathway at origin; (ii) survival during transport and storage along the pathway; (iii) survival of existing pest management procedures; and (iv) transfer to a suitable host. In the particular case of viruses and the pollen for pollination pathway, pollen will, for obvious reasons, be kept alive, ensuring the survival of the viruses or viroids present, while transfer to a suitable host is guaranteed by the intended use of the pollen.

Therefore, the sole parameters that may have a significant influence on the potential for entry are the association of the viruses and/or viroids with the pollen collected by commercial companies and the impact of existing pest management procedures. In this respect, it should be noted that the current legislation in Annex IIIA, paragraphs 9 and 18, of Council Directive 2000/29/EC bans the import of plants for planting (the definition of which includes live pollen) from countries other than Mediterranean countries, Australia, New Zealand, Canada and continental USA, while Annex IV establishes certification or testing requirements for pollen imported from countries under an import ban derogatory status.

Of the agents considered in the present opinion, ALSV and AFCVd have so far been reported only from Japan. AFCVd seems to be widely distributed there (Koganezawa and Ito, 2011b), whereas ALSV was recorded only once, from a single tree (Koganezawa and Ito, 2011a). However, since ALSV does not cause symptoms in apple, no specific efforts have been made to improve understanding of its prevalence and geographic distribution. For these two agents, given the restrictions on import of pollen from Japan, the Panel rated the probability of entry through the pollen for pollination pathway as very unlikely. This evaluation is, however, associated with high uncertainty, given the lack of precise surveys for these two agents outside Japan.

[8] See websites at http://www.firmanpollen.com/index.html, http://www.firmyieldpollen.com/index.html, http://www.pollencollectionandsales.com/, http://www.californiaagsupply.com/pollen.htm

All other agents analysed in the present opinion are reported to be present, to variable extents, in the USA and Canada (and some of them also in Mediterranean countries, Australia or New Zealand). Their probability of association with traded pollen would therefore be linked with their prevalence in those countries (which is difficult to assess precisely), with the ability of companies commercialising pollen for pollination to screen out infected plants as pollen donors and with the enforcement of the provisions of Council Directive 2000/29/EC. However, recent interceptions of pollen shipments contaminated by multiple viruses, included a listed one (see above), indicate that this protection is likely to be incomplete. It should be noted that, in reaching its conclusion on the probability of entry, the Panel did not consider the possibility that a non-pollen-transmitted virus may never contaminate a pollen shipment, because many non-pollen-transmitted viruses have been found to contaminate another tissues or pollen samples of host plants (Hamilton et al., 1977; Mink, 1993; Card et al., 2007). This simplification has, however, no consequences for the overall assessment of the probability of introduction as pollen transmissibility is explicitly integrated in the analysis of the establishment phase (see below). As a consequence of the above factors, the Panel concludes that the probability of entry of the viruses and viroids considered, other than ALSV and AFCVd, through the pollen for pollination pathway is unlikely to moderately likely, with a high degree of uncertainty mostly associated with the lack of data on the prevalence of the agents concerned in the countries from which pollen may be collected for sale and on the lack of information on how the requirements of Council Directive 2000/29/EC are understood and addressed.

3.5.5. Probability of establishment and spread in the risk assessment area

3.5.5.1. Evaluation of the probability of establishment through infection of pollinated plants (horizontal transmission)

As indicated in Table 7 and in section 3.5.1.4, through its review of the limited literature available, the Panel determined that the viruses and viroids under consideration belong to horizontal transmission group 1 or 2.

Group 1 is composed of agents with the lowest probability of being transmitted in this fashion since it consists of agents (i) for which there is no evidence of horizontal transmission and (ii) that belong to viral genera in which no agent is known to be horizontally transmitted. The probability of establishment of group 1 agents through infection of mother plants is therefore rated as very unlikely. This evaluation is, however, associated with high uncertainty given the very limited data available on this question in the literature.

Group 2 is composed of agents for which there is no evidence of horizontal transmission but which belong to genera in which one or more agents are known to be transmitted in this rare manner. In the absence of conclusive evidence, the Panel considered the probability of establishment of group 2 agents through infection of mother plants as very unlikely to unlikely. This evaluation is associated with high uncertainty given the very limited data available.

Overall, considering the evidence and ratings for horizontal transmission group 1 and 2 agents, the Panel concludes that the probability of establishment through horizontal transmission of any of the viruses or viroids considered is very unlikely to unlikely, with high uncertainty.

3.5.5.2. Evaluation of the probability of establishment through infection of seeds (vertical transmission)

For a particular agent to become established through infection of seeds (vertical transmission), two events have to occur sequentially. The first is the production of at least one contaminated seed through vertical pollen transmission. The second is the germination of this seed and its survival to become a growing infected plant. This second step is not expected to be trivial under the conditions in a production orchard, the most likely, if not the sole, situation in which pollen for pollination is expected to be used. Indeed, most fruits are expected to be collected, sold and consumed and their seeds ultimately discarded in ways that will prevent their germination. Likewise, in the case of fruits remaining in an orchard and falling to the ground, only a small fraction of the seeds are expected to germinate and the vast majority of the ensuing seedlings are expected to be destroyed by orchard management practices such as weed control and mowing. However, scenarios can certainly be envisaged in which a contaminated seed might be deposited in an environment (ditch, orchard border or hedge) conducive to its development. Most of these scenarios are, however, regarded by the Panel as occurring with a low probability, reducing the overall probability of establishment through vertical transmission for the viruses considered.

As indicated in Table 6 and in section 3.5.1.3, through its review of the limited literature available, the Panel determined that the viruses and viroids under consideration belong to vertical transmission group 1, 2, 3 or 4.

Similarly to the analysis of horizontal transmission described above, vertical transmission groups 1 and 2 are composed of agents for which there is no evidence of vertical transmission, besides the existence of agents transmitted in this fashion in the same genera for group 2 viruses. The probability of establishment of groups 1 and 2 agents through production of infected seeds is therefore rated as very unlikely to unlikely. This evaluation is, however, associated with high uncertainty given (i) the very limited or complete absence of data available on pollen transmission of these agents in the literature and (ii) the lack of precise information on the actual probability that seeds from the fruits of a production orchard may ultimately develop into infected plants.

Group 3 is composed of two viruses, TRSV and ToRSV, for which vertical transmission has been demonstrated in at least one herbaceous host but for which there is no evidence that they might be vertically transmitted in *Prunus*, *Malus* or *Pyrus*. In the absence of conclusive evidence, the Panel considered that the probability of establishment of TRSV and ToRSV through infection of seeds of these three genera following pollination with contaminated pollen should be rated as unlikely. This evaluation is associated with high uncertainty given (i) the very limited data available on this question in the literature and (2) the absence of information on the actual probability that seeds developing from the fruits of a production orchard may ultimately develop into infected plants.

Vertical transmission group 4 is composed of a single agent, *Apple latent spherical virus* (ALSV), for which conclusive evidence about its vertical transmission in apple has been obtained (Nakamura et al., 2011). There is, therefore, little doubt that pollination with ALSV-contaminated pollen would result in the production of at least some infected seeds. For successful establishment, those contaminated apple seeds would then need to meet conditions suitable for their germination and ensuing development into infected plants, a scenario which, as discussed above, has a limited probability. In view of these elements, the Panel concludes that the probability of establishment through vertical transmission of ALSV following pollination with contaminated pollen should be rated as moderately likely to likely. This evaluation is associated with medium uncertainty because of the lack of precise information on the probability that seeds may develop from the fruits of an apple production orchard into contaminated plants.

Table 12: Synthesis of the ratings for the probability of establishment and for the associated uncertainty for the viruses belonging to the different vertical transmission groups

Virus	Acronym	Probability of establishment	Uncertainty
Vertical transmission group 1—No evidence for vertical pollen transmission of the agent and no known pollen transmitted agent in the same genus			
Asian prunus virus	APV	Very unlikely to unlikely	High
Little cherry virus 1	LChV1		
Little cherry virus 2	LChV2		
Cherry mottle leaf virus	CMLV		
Cherry twisted leaf virus	CTLV		
Peach mosaic virus (American)	PcMV		

Virus	Acronym	Probability of establishment	Uncertainty
Apple fruit crinkle viroid	AFCVd		
Apple scar skin viroid	ASSVd		
Vertical transmission group 2—No evidence for vertical pollen transmission of the agent but known pollen transmitted agent(s) in the same genus			
Cherry rasp leaf virus (American)	CRLV	Very unlikely to unlikely	High
Peach rosette mosaic virus	PRMV		
Plum line pattern virus (American)	APLPV		
Plum pox virus	PPV		
Vertical transmission group 3—Agent known to be vertically transmitted by pollen in hosts other than *Prunus*, *Malus* or *Pyrus* and known pollen-transmitted agent(s) in the same genus			
Tobacco ringspot virus	TRSV	Unlikely	High
Tomato ringspot virus	ToRSV		
Vertical transmission group 4—Agent known to be vertically transmitted by pollen in *Prunus*, *Malus* or *Pyrus* and known pollen-transmitted agent(s) in the same genus			
Apple latent spherical virus	ALSV	Moderately likely to likely	Medium

In conclusion, the Panel determined that the probability of establishment through vertical transmission of the viruses analysed in the present opinion should be rated as very unlikely to unlikely with the exception of ALSV, for which the probability is evaluated as moderately likely to likely. The uncertainty associated with these ratings is high for all viruses and viroids considered, with the exception of ALSV, for which it is rated as moderate.

3.5.5.3. Evaluation of the potential for spread

The viruses addressed in the present opinion have by definition *Prunus*, *Malus* or *Pyrus* as their natural hosts. Even without considering the possibility that they may have a more extended natural host range, it is thus clear that susceptible hosts are widely available for these viruses in a range of EU countries. In addition, as for viral agents in general, these viruses are not expected to face significant ecoclimatic limitations in areas where their host(s) can be grown. Likewise, currently used cultural practices for the plant species considered are not expected to significantly impact the agents considered.

Assuming that one of these agents becomes established through the use of contaminated pollen, one can conclude that the agent in question can be transmitted by pollen, either vertically or horizontally. Horizontal pollen-mediated transmission is an efficient and, for obvious reasons, difficult to control spread mechanism. However, as analysed above, it is unlikely that any of the viruses or viroids considered in the present opinion is transmitted in this manner (see section 3.5.4.2). Spread by vertical transmission, through the production of contaminated seeds, is considered a less efficient spreading mechanism, because in a variety of situations, as in commercial orchards, the probability that a seed will germinate and develop into an infected flowering tree despite orchard management is likely to be low. Such a scenario can, however, be considered in other situations, as in orchard margins and hedges or in wild plants interfertile with orchard crops in neighbouring areas (wild *Malus*, *Pyrus*, wild cherries, etc.). It should be noted that in some of these situations, as in wild relatives of cultivated crops, even efforts to eradicate them by the relevant phytosanitary authorities would probably prove difficult.

Although the majority of the viruses and viroids considered either do not have any known vector or have only vector species largely restricted to North and Central America (see Table 4; Martelli and Taylor, 1989; James, 2011b; Larsen and James, 2011; Martelli and Uyemoto, 2011), at least two of them, PPV and LChV2, have vectors that are widely present in the EU and may also contribute to the spread of these agents (Barba et al., 2011; Jelkmann and Eastwell, 2011).

Given the wide availability of suitable hosts, the absence of limiting ecoclimatic conditions in most of the EU, the existence of the pollen-mediated transmission mechanism and, at least for PPV and

LChV2, the existence of vector species in the EU, the Panel concludes that the probability of spread following establishment is moderately likely to likely. This rating is, however, associated with high uncertainty, in particular to acknowledge the lack of any information on the probability that an infected seed might, under a range of situations, produce an infected tree capable of further pollen production and disease propagation.

3.5.6. Potential for consequences in the risk assessment area

As indicated in the section describing the scope of the present opinion, the Panel decided not to address in detail the consequences of the establishment and spread of the considered agents in the risk assessment area, in terms of potential damages caused.

All the viruses and viroids considered are listed in Annex I or II of Council Directive 2000/29/EC, and 13 of them have been described as being responsible for diseases and damage in the three genera considered (*Prunus*, *Malus*, *Pyrus*). The two exceptions are APV, for which there are currently no data on the symptoms it may cause in its *Prunus* hosts (Candresse et al., 2011), and ALSV, which is reported to give asymptomatic infections in apple and to be asymptomatic or poorly symptomatic in its experimental hosts (Koganezawa and Ito, 2011a).

The direct consequence of the introduction and spread of one of these agents would be a breach of the current EU quarantine legislation and require that the affected Member State(s) take action to limit the spread and impact of the agent concerned.

3.5.7. Conclusion of the assessment of the viruses and viroids listed in Council Directive 2000/29/EC for *Prunus*, *Malus* and *Pyrus*

The 13 viruses and two viroids analysed in the present opinion are well known or recently described agents for which efficient diagnostic techniques are available. Most of them have no known vector(s) or have vectors that are absent or of restricted distribution in the risk assessment area. However, two of them, LChV2 and PPV, have vectors with EU-28-wide distribution. All are recognised plant pathogens causing damage in at least some of their hosts, with the possible exception of APV (no information on symptoms available) and ALSV (reported to cause asymptomatic infections in apple).

Given that all the agents analysed, with the exception of AFCVd and ALSV, are present to variable extents in countries from which pollen can be imported, and given the limited impact of other parameters (survival during transport, transfer to suitable hosts), the Panel concludes that the probability of entry of the viruses and viroids considered is unlikely to moderately likely, with a high degree of uncertainty. For ALSV and AFCVd, which have a distribution limited to Japan, a country from which pollen import is banned, the corresponding probability is estimated to be very unlikely with high uncertainty.

For all these agents, except ALSV, the probability of establishment through vertical or horizontal transmission following pollination with contaminated pollen was evaluated as very unlikely to unlikely, with high uncertainty. Owing to the existence of reliable data demonstrating its vertical transmission in apple, the corresponding probability for ALSV was evaluated as moderately likely to likely, with moderate uncertainty.

Given the wide availability of suitable hosts, the absence of limiting ecoclimatic conditions in most of the EU-28 and the fact that the establishment of these agents under the conditions studied would demonstrate their pollen transmissibility, the Panel determined that the probability of spread once one of these agents becomes established would be moderately likely to likely, with high uncertainty.

Lastly, as all the viruses and viroids considered are listed in Annex I or II of Council Directive 2000/29/EC the direct consequence of their establishment and spread would be a requirement for the affected Member State(s) to take action (i.e. to eradicate the pathogen), in accordance with the current EU legislation.

3.6. Analysis of the risks posed by the import of pollen from the genera *Prunus* L., *Cydonia* Mill., *Fragaria* L., *Malus* Mill., *Pyrus* L., *Ribes* L., *Rubus* L. and *Vitis* L. for the introduction of viruses and virus-like organisms into the EU

As stated in section 3.2, the Panel was unable to identify any evidence indicating that pollen of *Fragaria*, *Rubus*, *Ribes*, *Vitis* and *Cydonia* is sold commercially for assisted pollination purposes, probably reflecting the self-fertile status of cultivated crops in these genera. For these five genera, the risk of introduction of viruses listed in Council Directive 2000/29/EC through this pathway was therefore assessed by the Panel to be negligible, with low uncertainty. The development of such a pathway seems improbable given that the species concerned are self-fertile, which strongly limits the interest of assisted pollination

Concerning the genera *Prunus*, *Malus* and *Pyrus*, for which a pollen trade was identified, the risks posed by pollen import can be analysed by first identifying the listed agents that might be associated with pollen of each genus and then considering the probability of entry and establishment of each virus through the pollen for pollination pathway, as evaluated in the assessment section above (see section 3.5.). Tables 12, 13 and 14 present the results of such an analysis separately for *Prunus*, *Malus* and *Pyrus* pollen.

It should be noted that, in order to evaluate which viruses might be associated with pollen, only natural host range data have been considered. The fact that a given plant species might be an experimental host of the virus (as is the case, for example, with *Prunus* for ALSV) was merely considered as increasing the uncertainty of the evaluation. It should also be noted that when they have been evaluated experimentally, viruses that can infect apple have nearly always been found to be able to infect *Pyrus* species (Hadidi et al., 2011, and chapters therein).

Table 13: Viruses having possible association with *Prunus* pollen, their probability of entry and of establishment through the pollen for pollination pathway and the corresponding uncertainty ratings

Virus with *Prunus* as natural hosts	Acronym	Probability of entry	Uncertainty associated with probability of entry	Probability of establishment	Uncertainty associated with probability of establishment
Tobacco ringspot virus	TRSV	Unlikely to moderately likely	High	Very unlikely to unlikely	High
Tomato ringspot virus	ToRSV	Unlikely to moderately likely	High	Very unlikely to unlikely	High
Cherry rasp leaf virus	CRLV	Unlikely to moderately likely	High	Very unlikely to unlikely	High
Peach mosaic virus	PcMV	Unlikely to moderately likely	High	Very unlikely to unlikely	High
Peach rosette mosaic virus	PRMV	Unlikely to moderately likely	High	Very unlikely to unlikely	High
American plum line pattern virus	APLPV	Unlikely to moderately likely	High	Very unlikely to unlikely	High
Little cherry virus 1	LChV1	Unlikely to moderately likely	High	Very unlikely to unlikely	High
Little cherry virus 2	LChV2	Unlikely to moderately likely	High	Very unlikely to unlikely	High
Plum pox virus	PPV	Unlikely to moderately likely	High	Very unlikely to unlikely	High
Asian prunus virus	APV	Unlikely to moderately likely	High	Very unlikely to unlikely	High
Cherry mottle leaf virus	CMLV	Unlikely to moderately likely	High	Very unlikely to unlikely	High

Virus with *Prunus* as natural hosts	Acronym	Probability of entry	Uncertainty associated with probability of entry	Probability of establishment	Uncertainty associated with probability of establishment
Cherry twisted leaf virus	CTLV	Unlikely to moderately likely	High	Very unlikely to unlikely	High
Apple scar skin viroid	ASSVd	Unlikely to moderately likely	High	Very unlikely to unlikely	High

Table 14: Viruses having possible association with *Malus* pollen, their probability of entry and of establishment through the pollen for pollination pathway and the corresponding uncertainty ratings

Virus with *Malus* as natural host	Acronym	Probability of entry	Uncertainty associated with probability of entry	Probability of establishment	Uncertainty associated with probability of establishment
Tobacco ringspot virus	TRSV	Unlikely to moderately likely	High	Very unlikely to unlikely	High
Tomato ringspot virus	ToRSV	Unlikely to moderately likely	High	Very unlikely to unlikely	High
Cherry rasp leaf virus (American)	CRLV	Unlikely to moderately likely	High	Very unlikely to unlikely	High
Apple latent spherical virus	ALSV	Very unlikely	High	Moderately likely to likely	Medium
Apple fruit crinkle viroid	AFCVd	Very unlikely	High	Very unlikely to unlikely	High
Apple scar skin viroid	ASSVd	Unlikely to moderately likely	High	Very unlikely to unlikely	High

Table 15: Viruses having possible association with *Pyrus* pollen, their probability of entry and of establishment through the pollen for pollination pathway and the corresponding uncertainty ratings

Virus with *Malus* as natural host	Acronym	Probability of entry	Uncertainty associated with probability of entry	Probability of establishment	Uncertainty associated with probability of establishment
Apple scar skin viroid	ASSVd	Unlikely to moderately likely	High	Very unlikely to unlikely	High

3.7. Conclusions on the risks posed by the import of pollen from the genera *Prunus* L., *Cydonia* Mill., *Fragaria* L., *Malus* Mill., *Pyrus* L., *Ribes* L., *Rubus* L. and *Vitis* L. for the introduction of viruses and virus-like organisms into the EU

Because of the absence of an identified commercial trade in pollen for pollination for the genera *Fragaria*, *Rubus*, *Ribes*, *Vitis* and *Cydonia* (probably a consequence of the reproductive biology of these species), the risk of introduction of viruses listed in Council Directive 2000/29/EC through this pathway was assessed by the Panel to be negligible, with low uncertainty, mostly associated with the possibility that pollen of these five genera could be traded for pollination purposes despite the fact that the Panel was unable to identify such trade. However, such a possibility seems unlikely given that the species concerned are self-fertile, which strongly limits the interest of assisted pollination.

It should be stressed, however, that if, for unforeseen reasons, commercial trade in pollen of *Fragaria*, *Rubus*, *Ribes*, *Vitis* and *Cydonia* for pollination purposes were to develop, the above assessment would need to be re-analysed, as some viruses of these species listed in Council Directive 2000/29/EC may have the potential to be pollen-borne.

Concerning pollen of the genera *Prunus*, *Malus* and *Pyrus*, trade was identified and the risk of this pathway was, therefore, evaluated in detail. For 12 viruses and one viroid, the probability of entry was rated as unlikely to moderately likely and the probability of establishment as very unlikely to unlikely. In the case of the two remaining agents, ALSV and AFCVd, the probability of entry was rated as very unlikely and was associated with a moderately likely to likely (ALSV) or very unlikely to unlikely (AFCVd) probability of establishment. This indicates that, for all 15 agents considered, very significant limitations to their introduction were identified. Almost all of these ratings were, however, associated with high uncertainty because of the absence of relevant information on many important aspects.

As a consequence of the above analysis, the Panel considers that the risk of introduction of listed viruses and viroids by pollen for pollination of the genera *Prunus*, *Malus* and *Pyrus* is negligible to low, with high uncertainty.

4. Risk reduction options

4.1. Introduction

As per the Commission request, the evaluation of risk reduction options is limited to the assessment of options to (i) prevent entry of viruses into the EU and to (ii) avert their establishment. Pollen imports for commercial purposes, to enhance pollination of fruit trees of the genera under consideration, are of a manageable amount. Pollen for the purpose of assisting pollination is offered by only a few companies and so far seems to be produced in and to arrive from only the USA.

In what follows, the risk reduction options to reduce the probability of entry of pollen-transmitted viruses with pollen for pollination of the genera *Prunus*, *Malus* and *Pyrus* were systematically identified and only those applicable are discussed and evaluated. Since the risks associated with pollen of the genera *Fragaria*, *Rubus*, *Ribes*, *Vitis* and *Cydonia* were analysed to be negligible owing to the absence of trade, no specific efforts were made to identify or analyse risk reduction options for pollen of these five genera.

4.2. Analysis of the current legislation

In the Council Directive 2000/29/EC, importation of plants for planting of *Malus*, *Prunus* and *Pyrus* is covered by Annex IIIA (9) prohibiting imports and (18) specifying exemptions for dormant plants originating from non-European Mediterranean countries, Australia, New Zealand, Canada and the continental USA. Pollen, albeit not specifically stated in the regulation, is considered as dormant plants for planting and hence import of pollen from the above countries is permitted while other regulations, e.g. Annexes I, II and IV, apply. Indirectly, restrictions applying to pollen of *Malus* and *Pyrus* (but not *Prunus*) in relation to *E. amylovora* may further reduce the countries or areas from which pollen for pollination of these two genera can be imported.

It should be stressed that the current legislation is complex and difficult to understand and that its interpretation when it comes to the specific case of pollen for pollination purposes is far from obvious. A good example of this complexity is the situation of *Malus* and *Pyrus* pollen in relation to *E. amylovora*. This bacterium is listed in Annex IIAII of Council Directive 2000/29/EC on plants for planting of these species, which would include pollen for pollination by the current definition. A ban on entry, applying to the entire EU-28, of pollen with a potential to be contaminated with this agent would logically be expected in Annex III to support the Annex IIAII requirements. However, this ban is found only in Annex IIIB and is limited to the protected zones defined by the listing of *E.*

amylovora in Annex IIB, as if pollen for pollination purposes was in fact excluded from the definition of plants for planting.

The complexity of the current legislation may lead to misinterpretation, with the potential consequence of undermining its effectiveness, as exemplified in an exchange of the Panel with a representative of the Firman Pollen Company (Scott McClure, personal communication, August 2013; see Appendix B). This situation could be greatly improved by making explicit whether live pollen for pollination purposes is included in the definition of plants for planting and/or by making the Annex III of the legislation more explicit for pollen.

Specific requirements for quarantine viruses listed in Annexes I and II and for plants of *Prunus*, *Malus* and *Pyrus* regulate their introduction into the EU and movement within Member States. As far as plant imports and their movement within Member States are concerned, Annex IV applies and plants (including, although not explicitly stated, live pollen) have to be either officially certified under a certification scheme or derived in direct line from material that has been maintained under appropriate conditions and subjected to official testing at least once during the last three complete cycles of vegetation.

Pollen used for assisted pollination is produced by collecting flowers and extracting the pollen from those flowers. This allows the pollen to be obtained from well-defined varieties and from specifically identified trees. The origin of the pollen is therefore traceable and the mother plant(s) and/or an entire plantation can be subject to surveillance, monitoring and inspection/testing. Testing pollen for the presence of viruses is also feasible. Both strategies can be applied to efficiently support the phytosanitary documents that should accompany shipments.

As discussed in section 4.2.2, the availability of detection tests for the viruses and viroids addressed in the present opinion permits the efficient testing in mother plants and in pollen consignments. However, it is important to consider that visual inspection of symptoms is not sufficient or reliable to guarantee pest freedom of mother plants or of areas of production as several of the viruses concerned cause only mild symptoms or their symptom expression is transient or some virus infections may remain latent, as, for example, in the case of ALSV.

The current legislation, if properly understood and applied by all parties, has, therefore, the potential to provide a substantial level of risk reduction. However, as indicated above, the current legislation does not explicitly address pollen, and its requirements are difficult to interpret when considering pollen. As a consequence, competent authorities in exporting countries may misinterpret or altogether fail to comply with its requirements. Including specific statements for pollen in Annex IV, and stating that the observation of donor trees alone is not sufficient to fulfil Annex IV requirements, would facilitate the understanding of these rules and improve the production, quality and trustworthiness of the required certificates.

4.3. Options to reduce the probability of entry of pollen-transmitted viruses and virus-like organisms with pollen of *Prunus*, *Malus* and *Pyrus* into the EU

4.3.1. Options for consignments—prohibition

Prohibition is a phytosanitary regulation forbidding the importation or movement of specified pests or commodities (IPPC, 2012). As explained above, pollen imports from non-European Mediterranean countries, Australia, New Zealand, Canada and continental USA are permitted by the current legislation and, thus, an effective protection exists against entry of ALSV and AFCVd, which are reported from Japan only. Extending this partial prohibition to cover all non-EU countries, by removing for pollen the exemptions in Annex IIIA(18), would increase the level of protection against the entry of all agents considered in the present opinion.

Effectiveness: Very high.

Technical feasibility: Very high.

Uncertainty: Low.

4.3.2. Options for consignments—pest freedom: testing

Detection methods are available for all viral or viroid pathogens analysed in the present opinion, hence the absence of the viruses and viroids can be reliably assessed in pollen shipments if appropriate detection methods and prescribed protocols are used. This is confirmed by the reports of virus interceptions of PNRSV, CLRV, PDV and CRLV in pollen consignments of *Prunus*. Likewise, it can be assumed that it is also possible to test for the presence of other viruses addressed in the present opinion. In addition to the import requirements established in Annex IV, a general request could be made for the individual testing of pollen shipments. Such a measure would further reduce the risk that a contaminated pollen shipment is imported into the EU-28.

Effectiveness: High to very high depending on the agent considered, because in some cases there is little information on the ability of existing diagnostic tests to detect all strains/isolates of the agent considered.

Technical feasibility: High. Only a limited number of pathogens are to be tested for and only a small number of small samples are necessary.

Uncertainty: Low to medium. The limited knowledge of the diversity of some of the pathogens concerned might contribute to ambiguous testing results because of the failure of molecular tests to detect a variant isolate/strain.

4.4. Options to reduce probability of establishment

4.4.1. Eradication

Application of pollen to assist pollination is either achieved either using bees or by dusting pollen over a specific area or field. Dusting applications allow more control over the pollination process; however, in both cases pollen can also reach other plant species, including those wild relatives that can also be pollinated. Pollen transmission is a means of virus spread that is hard to control, in particular when it comes to horizontal transmission. Consequently, there are no options available to prevent virus spread after establishment, and therefore eradication to prevent virus establishment is the only means of control.

Pollen transmission of the viruses considered here is very unlikely to result in infection of the pollinated plants. It will be the seeds of pollinated fruit trees germinating and developing into plants that will be virus infected and from which virus-infected pollen will be produced to further spread the viruses. Considering pollen application in commercial orchards, the small chance that seedlings develop comes from windfall only. These seedlings are subject to crop management measures that eventually remove seedlings prior to flowering. Only if compatible fruit tree varieties or wild species, scattered in unmanaged areas, were to be pollinated would virus establishment in seedlings potentially occur.

For effective eradication, actions have to be taken on the crop plants and on all susceptible plants in the area where eradication is undertaken. Considering the case of virus transmission by pollen to plantation crops such as fruit trees, the main focus of eradication has to lie on identification of ruderal plants affected. The success of eradication would depend upon the early detection of virus establishment, which would be a challenging task when no obvious symptoms are expressed in those plants.

Effectiveness: Very high within an orchard but only moderate when considering the surrounding environment.

Technical feasibility: Moderate.

Uncertainty: Medium.

4.5. Conclusions on the identification of risk reduction options and evaluation of their effectiveness in reducing the risk to plant health of entry and establishment of pollen-transmitted viruses and virus-like organisms with pollen of *Prunus*, as well as pollen of *Malus* and *Pyrus* into the EU

As requested by the Commission, the Panel concentrated its analysis on risk reduction options having an impact on the introduction of virus and virus-like agents through the pollen for pollination pathway. Analysis of the current legislation demonstrated that it already provides a substantial level of risk reduction, by banning pollen importation from a range of countries and by requesting that imported pollen is either officially certified under a certification scheme or derived in a direct line from material that has been maintained under appropriate conditions and subjected to official testing at least once during the last three complete cycles of vegetation. However, risks could be further reduced by addressing pollen specifically in Annexes III and IV of Council Directive 2000/29/EC, in order to facilitate the understanding and the proper implementation of its requirements, and by making clear that visual observation of plants from which pollen is collected is not an appropriate and efficient testing technique.

The Panel identified two risk reduction options with high or very high effectiveness and feasibility to reduce the risk of entry: the extension of the existing prohibition on *Prunus*, *Malus* and *Pyrus* pollen importations to all non-EU countries and a request for an individual testing of all imported pollen shipments. Uncertainties on the effectiveness and feasibility of these measures were assessed as low and low to medium, respectively.

Eradication, the only risk reduction option with impact on establishment, was evaluated as having moderate effectiveness when considering the orchards and their environment, with medium technical feasibility and medium uncertainty.

CONCLUSIONS

According to Council Directive 2000/29/EC pollen is included in the definition of plants intended for planting. Because of the absence of an identified commercial trade in pollen for pollination from the genera *Fragaria*, *Rubus*, *Ribes*, *Vitis* and *Cydonia*, the risk of introduction of the viruses listed in Council Directive 2000/29/EC through this pathway was assessed by the Panel to be negligible, with low uncertainty, mostly associated with the possibility that pollen from these five genera could be traded for pollination purposes despite the fact that the Panel was unable to identify such trade. However, such a possibility seems unlikely given that the species concerned are self-fertile, which strongly limits the interest of assisted pollination.

It should be stressed, however, that if, for unforeseen reasons, commercial trade in pollen of *Fragaria*, *Rubus*, *Ribes*, *Vitis* and *Cydonia* for pollination purposes were to develop, the above assessment would need to be re-analysed, since some viruses of the species listed in Council Directive 2000/29/EC may have the potential to be pollen-borne.

Concerning pollen of the genera *Prunus*, *Malus* and *Pyrus*, trade was identified and the risk of this pathway was, therefore, evaluated in detail. For 12 viruses and one viroid, the probability of entry was rated as unlikely to moderately likely and the probability of establishment as very unlikely to unlikely. In the case of the two remaining agents, ALSV and AFCVd, the probability of entry is very unlikely and is associated with a moderately likely to likely (ALSV) or very unlikely to unlikely probability of establishment (AFCVd). This indicates that for all 15 agents considered, very significant limitations to their introduction were identified. Almost all of these ratings were, however, associated with high uncertainty because of the absence of relevant information on many important aspects.

As a consequence of the above analysis, the Panel considers that the risk of introduction of listed viruses and viroids by pollen for pollination of the genera *Prunus*, *Malus* and *Pyrus* is negligible to low, with high uncertainty.

As requested by the Commission, the Panel concentrated its analysis on risk reduction options having an impact on the introduction of virus and virus-like agents through the pollen for pollination pathway. Analysis of the current legislation demonstrated that it already provides a substantial level of risk reduction, by banning the importation of pollen (as included in plants for planting) from a range of countries and by requesting that imported pollen is either officially certified under a certification scheme, or derived in a direct line from material that has been maintained under appropriate conditions and subjected to official testing at least once during the last three complete cycles of vegetation. However, risks could be further reduced by addressing pollen specifically in Annexes III and IV of Council Directive 2000/29/EC, in order to facilitate the understanding and the proper implementation of its requirements, and by making clear that visual observation of plants from which pollen is collected is not an appropriate and efficient testing technique.

The Panel identified two risk reduction options with high or very high effectiveness and feasibility to reduce the risk of entry: the extension to all non-EU countries of the existing prohibition on *Prunus*, *Malus* and *Pyrus* pollen imports and a request for an individual testing of all imported pollen shipments. The uncertainties of the effectiveness and feasibility of these measures were assessed as low and low to medium, respectively.

Eradication, the only risk reduction option with impact on establishment, was evaluated as having moderate effectiveness when considering the orchards and their environment, with medium technical feasibility and medium uncertainty.

DOCUMENTATION PROVIDED TO EFSA

1. Request to provide a scientific opinion on the risks posed by *Prunus* pollen, as well as pollen from seven additional genera, for the introduction of viruses and virus-like organisms into the EU. SANCO.E2 GC/ap (2013) 1341784, 30 May 2013. Submitted by European Commission, DG SANCO (Directorate-General for Health and Consumers).

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APPENDICES

Appendix A. Ratings and descriptors

In order to follow the principle of transparency as described under Paragraph 3.1 of the Guidance document on the harmonised framework for risk assessment (EFSA PLH Panel, 2010) – '… Transparency requires that the scoring system to be used is described in advance. This includes the number of ratings, the description of each rating … the Panel recognizes the need for further development …' – the Plant Health Panel has developed specifically for this opinion rating descriptors to provide clear justification when a rating is given.

1. Ratings used in the conclusion of the pest risk assessment

In this opinion of EFSA's Plant Health Panel for the risk assessment of pollen of the genera *Fragaria*, *Rubus*, *Ribes*, *Vitis*, *Cydonia*, *Prunus*, *Malus* and *Pyrus*, a rating system of five levels with their corresponding descriptors has been used to formulate separately the conclusions on entry, establishment and spread as described in the following tables.

1.1. Rating of probability of entry

Rating for entry	Descriptors
Very unlikely	The likelihood of entry would be very low because the pest: 1. is not or is only very rarely associated with the pathway at the origin 2. cannot survive during transport or storage 3. cannot survive the current pest management procedures existing in the risk assessment area 4. cannot transfer to a suitable host in the risk assessment area
Unlikely	The likelihood of entry would be low because the pest: 1. is rarely associated with the pathway at the origin 2. can survive at only a very low level during transport or storage 3. is strongly limited by the current pest management procedures existing in the risk assessment area 4. has effective limitations for transfer to a suitable host in the risk assessment area
Moderately likely	The likelihood of entry would be moderate because the pest: 1. is occasionally associated with the pathway at the origin 2. can survive at only a low level during transport or storage 3. is limited by the current pest management procedures existing in the risk assessment area 4. has some limitations for transfer to a suitable host in the risk assessment area
Likely	The likelihood of entry would be high because the pest: 1. is frequently associated with the pathway at the origin 2. can survive during transport or storage 3. is unlikely to be limited by the current pest management procedures existing in the risk assessment area 4. has very few limitations for transfer to a suitable host in the risk assessment area
Very likely	The likelihood of entry would be very high because the pest: 1. is always or almost always associated with the pathway at the origin 2. always survives during transport or storage 3. is not limited by the current pest management procedures existing in the risk assessment area 4. has no limitations for transfer to a suitable host in the risk assessment area

1.2. Rating of probability of establishment

Rating for establishment	Descriptors
Very unlikely	The likelihood of establishment would be very low because of: absence or very limited availability of host plants; unsuitable environmental conditions; occurrence of other considerable obstacles preventing establishment
Unlikely	The likelihood of establishment would be low because of: limited availability of host plants; unsuitable environmental conditions over the majority of the risk assessment area; occurrence of other obstacles preventing establishment
Moderately likely	The likelihood of establishment would be moderate because: hosts plants are abundant in few areas of the risk assessment area; environmental conditions are suitable in few areas of the risk assessment area; no obstacles to establishment occur
Likely	The likelihood of establishment would be high because: hosts plants are widely distributed in some areas of the risk assessment area; environmental conditions are suitable in some areas of the risk assessment area; no obstacles to establishment occur. Alternatively, the pest has already established in some areas of the risk assessment area
Very likely	The likelihood of establishment would be very high because: hosts plants are widely distributed; environmental conditions are suitable over the majority of the risk assessment area; no obstacles to establishment occur. Alternatively, the pest has already established in the risk assessment area

1.3. Rating of probability of spread

Rating for spread	Descriptors
Very unlikely	The likelihood of spread would be very low because: 1. the pest has only one, specific, way of spreading (e.g. a specific vector, specific assisting virus, etc.) which is not present in the risk assessment area 2. highly effective barriers to spread exist 3. the hosts are not or very rarely present in the area of possible spread
Unlikely	The likelihood of spread would be low because: 1. the pest has one to few, specific, ways of spreading (e.g. specific vectors, specific assisting virus) and their occurrence in the risk assessment area is rare 2. effective barriers to spread exist 3. the hosts are occasionally present
Moderately likely	The likelihood of spread would be moderate because: 1. the pest has few, specific, ways of spreading (e.g. specific vectors, specific assisting virus) and their occurrence in the risk assessment area is limited 2. partially effective barriers to spread exist 3. the hosts are abundant in few parts of the risk assessment area
Likely	The likelihood of spread would be high because: 1. the pest has some, non-specific, ways of spreading (e.g. mechanical transmission), which occur in the risk assessment area 2. no effective barriers to spread exist 3. the hosts are widely present in some parts of the risk assessment area
Very likely	The likelihood of spread would be very high because: 1. the pest has multiple, non-specific, ways of spreading (e.g. mechanical transmission), all of which occur in the risk assessment area 2. no effective barriers to spread exist 3. the hosts are widely present in the whole risk assessment area

2. Ratings used for the evaluation of the risk posed by the import of pollen for pollination purposes for the introduction of listed viruses and virus-like organisms into the EU

Rating	Descriptors
Negligible	The import of pollen for pollination purposes does not carry a risk or carries a minimal risk of introduction of listed viruses and virus-like organisms into the EU
Low	The import of pollen for pollination purposes carries a small risk of introduction of listed viruses and virus-like organisms into the EU
Moderate	The import of pollen for pollination purposes carries a substantial risk of introduction of listed viruses and virus-like organisms into the EU
High	The import of pollen for pollination purposes carries a very substantial risk of introduction of listed viruses and virus-like organisms into the EU
Very high	The import of pollen for pollination purposes essentially makes certain or near certain the risk of introduction of listed viruses and virus-like organisms into the EU

3. Ratings used for the evaluation of the risk reduction options

The Panel developed the following ratings with their corresponding descriptors for evaluating the effectiveness of the risk reduction options to reduce the level of risk.

3.1. Rating of the effectiveness of risk reduction options

Rating	Descriptors
Negligible	The risk reduction option has no practical effect in reducing the probability of entry, establishment or spread, or of the potential consequences
Low	The risk reduction option reduces, to a limited extent, the probability of entry, establishment or spread, or of the potential consequences
Moderate	The risk reduction option reduces, to a substantial extent, the probability of entry, establishment or spread, or of the potential consequences
High	The risk reduction option reduces, to a major extent, the probability of entry, establishment or spread, or of the potential consequences
Very high	The risk reduction option essentially eliminates the probability of entry, establishment or spread, or of any potential consequences

3.2. Rating of the technical feasibility of risk reduction options

Rating	Descriptors
Negligible	The risk reduction option is not in use in the risk assessment area, and the many technical difficulties involved (e.g. changing or abandoning the current practices, implementing new practices and or measures) make their implementation in practice impossible
Low	The risk reduction option is not in use in the risk assessment area, but the many technical difficulties involved (e.g. changing or abandoning the current practices, implementing new practices and or measures) make its implementation in practice very difficult

Rating	Descriptors
Moderate	The risk reduction option is not in use in the risk assessment area, but it can be implemented (e.g. changing or abandoning the current practices, implementing new practices and or measures) with some technical difficulties
High	The risk reduction option is not in use in the risk assessment area, but it can be implemented in practice (e.g. changing or abandoning the current practices, implementing new practices and or measures) with limited technical difficulties
Very high	The risk reduction option is already in use in the risk assessment area or can be easily implemented with no technical difficulties

4. Ratings used for describing the level of uncertainty

For the risk assessment chapter—entry, establishment, spread and impact—as well as for the evaluation of the effectiveness of the management options, the level of uncertainty has been rated separately in coherence with the descriptors that have been defined specifically by the Panel in this opinion.

Rating	Descriptors
Low	No or little information or no or few data are missing, incomplete, inconsistent or conflicting. No subjective judgement is introduced. No unpublished data are used
Medium	Some information is missing or some data are missing, incomplete, inconsistent or conflicting. Subjective judgement is introduced with supporting evidence. Unpublished data are sometimes used
High	Most information is missing or most data are missing, incomplete, inconsistent or conflicting. Subjective judgement may be introduced without supporting evidence. Unpublished data are frequently used

REFERENCES

EFSA PLH Panel (EFSA Panel on Plant Health), 2010. Guidance on a harmonised framework for pest risk assessment and the identification and evaluation of pest risk management options by EFSA. EFSA Journal 2010;8(2):1495, 66 pp. doi:10.2093/j.efsa.2010.1495

Appendix B. Personal communications

Having identified that pollen of *Prunus* L., *Malus* Mill. and *Pyrus* L. is sold commercially by four US companies,[9] the Panel on Plant Health contacted these companies to investigate whether pollen of the other five genera addressed in the Commission request (*Cydonia* Mill., *Fragaria* L., *Ribes* L., *Rubus* L. and *Vitis* L.) are commercially traded. The information provided by three companies is extracted below:

1. Neil McClure, CEO, Firman Pollen Company Inc., August 2013

i. **Is pollen of the genera *Fragaria*, *Ribes*, *Rubus* and *Vitis* sold commercially for pollination purposes?** We do not handle any of those products and I am not familiar with any US companies that do. To my knowledge the US commercial pollination industry supplies pollen for apples, pears, cherries, plums, prunes, apricots, kiwi, peaches, and almonds.

ii. **Do you export pollen to the European Union?** We export a limited amount of pollen to the EU and have done so for over 15 years.

iii. **How often and in which periods of the year?** Pollen is shipped during the springtime, from March through April.

iv. **To which European countries do you export pollen?** We currently export to France, Italy, Spain, Austria, the Netherlands, Belgium, and also to Germany.

v. **Can you give an estimate of the amount of pollen per year exported into the European Union?** During the spring of 2013 we exported approximately 25 000 grams. Enough for about 210 hectares.

The thoughts below are only mine and through me the beliefs of the Firman Pollen Company. But in developing them I have worked closely with Certified Testing Laboratories, the USDA [United States Department of Agriculture] and the Washington State Department of Agriculture.

First let me say that the Firman Pollen Company does not believe that pollen should have free movement across borders if the importing country has any concerns about the spread of harmful organisms that could be found in pollen. It is our belief that an effective testing protocol should be in place to allay those concerns. The protocol that we are working on is to develop an effective testing regime that would be used on all shipments to the EU.

Now I will give you some additional information that may be helpful in your discussions concerning the cross border movement of fruit pollens.

Until the late spring of 2012 the importation of pollen into Europe was relatively unregulated. It only required a phytosanitary certificate that stated what the shipment contained. There were several protected regions of the EU that did not allow the importation of pollen but in general the free movement of pollen was allowed. In late April of 2012 that open movement changed. At that time the EU began to enforce very different requirements for pollen. New EU regulations lumped pollen into the same category as 'living plants.' We had already made all of our shipments for that year so our 2012 shipments were not affected. As we prepared to meet the new regulations for our pollen shipments for year 2013 the classification of 'living plants' created large problems. During the summer and fall [autumn] of 2012 we worked extensively with certified laboratories in the USA on testing protocols for pollen so that pollen shipments could meet the virus and bacteria concerns of the EU. However, there were inconsistencies within the EU requirements that we could not overcome. Most of the regulations allowed for testing to establish that the pollen would be free of the viruses and

[9] See websites at http://www.firmanpollen.com/index.html, http://www.firmyieldpollen.com/index.html, http://www.pollencollectionandsales.com/, http://www.californiaagsupply.com/pollen.htm

bacteria but in a few of the requirements the regulations were 'tree specific'. And even though tests were readily available to establish that the pollen was free of the offending bacteria or virus the 'tree specific' regulations did not allow for a test to satisfy the regulation. These inconsistencies in the regulations seemed to make pollen importations into Europe impossible.

As we broke this news to our customers they all insisted that the regulations had not changed. In response the US government said that if the individual countries would supply a letter stating that, then the US government would follow the same regulations that had been used during the previous decade, issuing a phytosanitary certificate stating only that the shipment contained pollen without any additional declarations. That was the policy that covered our shipments for 2013.

Unknown to us and to our government this policy was not correct, and two shipments, one to Austria and one to Italy, were tested in the EU and found to have viruses that are common in the USA and also in the EU. We had not tested the shipments for the viruses because our customers and their respective governments had given us information that it was not necessary to make any changes from past shipping policies. We are now aware that that information was not correct.

We are currently working with the US government and through them in discussions with the EU to try to eliminate the inconsistencies in the regulations. It is our hope that the accurate testing of pollen could be established throughout the regulations so that pollen importations could continue.

Using the idea that accurate, certified testing is allowed, our internal discussions have been on how to make the shipment of pollen safe and effective. The protocols that we believe would make the shipment of pollen into the EU safe and effective are as follows: All shipments should be tested after packaging yet prior to export to the EU. This would eliminate the possibility that untested pollen could be added to a shipment. The fact that it was tested and for what organisms would be listed on the phytosanitary certificate, as required by law. The US government would not issue a phytosanitary certificate unless the tests were conducted at a certified lab, no self testing would be allowed, also required by the US government. The discussions we are currently having with the virologist at Washington State University is whether an Elisa [enzyme-linked immunosorbent assay] test or a PCR [polymerase chain reaction] test should be required. The virologist seems to believe that an Elisa test would be adequate. A PCR test is an accurate test but false positives are much more common due to the machinery, reactive agents, and techniques that are required. The general feeling is that the Elisa test is fast, accurate and would find all the harmful organisms at levels that were biologically significant.

The following references are from Council Directive 2000/29/EC Annex IV, Part A 'Special Requirements', Section 1 'From outside the EU.' Section 2 is for 'Plants from within the EU' but the requirements seem to be the same as for plants outside the EU. The first area within Section 1 is # 15. It refers to *Monilinia fructicola* and the only solutions are 'tree specific.' There is an accurate test to determine the presence of *M. fructicola* but that option is not allowed under # 15. This is what I have called an inconsistency. As I will list later in this email, testing to determine that the product is free of the relevant organisms is normal throughout this section. Even # 16 in this section recognizes that a testing protocol is acceptable to determine that fruit is free of *M. fructicola*. Adding a testing requirement to # 15 that is similar to the testing protocols referenced in the following areas within this section would make # 15 uniform with those other protocols. # 17 within Section 1 refers to *Erwinia amylovora*. But I believe *E. amylovora* in live pollen is dealt with in a separate area of the Council Directive, Annex III, Part B. My reading of Annex III, Part B seems to allow the movement of live pollen without regard to *E. amylovora* as long as it is not into the listed protected areas. An accurate test does exist for *E. amylovora* and if my understanding of Annex III, Part B is incorrect then adding a testing requirement to # 17 would make # 17 consistent with other requirements within this section. # 20 refers to pear decline mycoplasm; this does not occur in pollen but all of its requirements are 'tree specific,' no testing allowed. This is inconsistent with # 22.2, apple proliferation mycoplasm, which also does not occur in pollen, but testing is clearly allowed in (b) (aa). The following I am going to lump together. # 22.1 concerns cherry leaf roll virus and tomato ringspot virus, # 23.1 concerns plum pox virus, # 23.2 covers a long list of viruses. Each of these numbered sections allows for a testing requirement or 'tree specific' requirements.

In summary, if I could make a recommendation to clarify the regulations on pollen importation it would be one of two possibilities. The most appropriate would be to recognize that pollen is sufficiently different from live plants to warrant a separate 'Part' within the existing Annexes. Within that 'Part' clearly defined testing protocols could be established. Once established any modifications needed could be handled more easily within one area of the regulations rather then the current problem of being scattered throughout the regulations. Understanding that that solution may not be quickly implemented or politically feasible, my second recommendation would be to build a consistency throughout the existing regulations that allowed for 'testing and found free of' within all sections.

I hope that this information is of some help in understanding the issues that currently affect the movement of pollen. Our company absolutely wishes to conform to all required regulations. And we have done much in establishing a working protocol that would allow for the safe movement of pollen from country to country.

2. Scott Beddard, General Manager, FirmYield Pollen, August 2013

i. **Is pollen of the genera *Fragaria*, *Ribes*, *Rubus* and *Vitis* sold commercially for pollination purposes?** We only collect and sell pollen from the *Prunus* and *Malus* genera.

ii. **Do you export pollen to the European Union?** Yes.

iii. **How often and in which periods of the year?** March and April.

iv. **To which European countries do you export pollen?** The Netherlands and Slovakia.

v. **Can you give an estimate of the amount of pollen per year exported into the European Union?** Approx. 30 000 grams.

3. Rebb Firman, Pollen Collection and Sales, August 2013

i. **Is pollen of the genera *Fragaria*, *Ribes*, *Rubus* and *Vitis* sold commercially for pollination purposes?** The only pollens we sell are tree fruit and tree nut pollens. We do not sell any type of the pollens you listed.

ii. **Do you export pollen to the European Union?** Yes, we export male kiwi fruit (*Actinidia*) pollen and fennel (*Foeniculum vulgare*) pollen to the EU.

iii. **How often and in which periods of the year?** Kiwi pollen is exported in May only. Fennel pollen year round as it is used for cooking.

iv. **To which European countries do you export pollen?**

v. **Can you give an estimate of the amount of pollen per year exported into the European Union?**