Pest Risk Analysis for *Pepino mosaic virus*

Project no. 044189

PEPEIRA
Pepino mosaic virus*: epidemiology, economic impact and pest risk analysis.*

SPECIFIC TARGETED RESEARCH OR INNOVATION PROJECT
Thematic Priority Sustainable management of Europe's natural resources

PRA Format used:
European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes
Version 09- 15190
PM 5/3 (4)

Project Co-ordinator:

Dr René van der Vlugt, Plant Research International – Business Unit Biointeractions & Plant Health, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

Pest Risk Analysis (Workpackage 4.3) co-ordinator:

Arjen Werkman MSc, National Reference Laboratory, Plant Protection Service,
P.O. Box 9102, 6700 HC Wageningen, The Netherlands (partner 19)

2nd author
Dr Claire Sansford, Plant Health Consultancy Team, The Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, United Kingdom (partner 2)

Reviewers: PEPEIRA partners (See overview below)

Citation:

Werkman A.W. & Sansford C.E. (2010). Pest Risk Analysis for *Pepino mosaic virus* for the EU. Deliverable Report 4.3. EU Sixth Framework Project Project PEPEIRA. http://www.pepeira.com

Date:
11th June 2010

List of participants and contributors:

Partner 1 (coordinator)
Dr R van der Vlugt
Plant Research International
P.O. Box 16, NL-6700 AA Wageningen, The Netherlands
Phone: +31 (0) 317 480675
E-mail: Rene.vandervlugt@wur.nl

Partner 2
Dr R Mumford
Food and Environment Research Agency (FERA)
Sand Hutton, York, YO41 1LZ, United Kingdom
Phone: +44 (0) 1904 462140
E-mail: rick.mumford@fera.gsi.gov.uk
Dr. J Peters jeff.peters@fera.gsi.gov.uk

Partner 3
Prof. Dr. F García-Arenal Rodríguez
Centro de Biotecnologiá y Genómica de Plantas UPM-INIA
Autopista MV, km38, 28223, Madrid, Spain
Phone: +34 91336 4550/4539
E-mail: fernando.garciaarenal@upm.es

Partner 4
Ana Alfaro-Fernández
Instituto Agrofestal Del Mediterraneao
Camino de Vera s/n, 46022, Valencia, Spain
Phone: +34 963879259
E-mail: analfer1@doctor.upv.es

Partner 5
Dr. B. Gábor
Csongrád Megyei Mezőgazdasági Szakigazgatási Hivatal
Növény- és Talajvédelmi Igazgatóság
Rárósi út 110, PO Box 99, 6801, Hódmezovásárhely, Hungary
Phone: + 36 62 535773
E-mail: Bese.Gabor@csongrad.ontsz.hu

Partner 6
Dr. S.L. Nielsen
University of Aarhus, Faculty of Agricultural Sciences
Department of Integrated Pest Management
Forsøgsvej 1, DK-4200, Slagelse, Denmark
Phone: +45 89 99 35 00
E-mail: steenl.nielsen@agrsci.dk

Partner 7
Dr.ir. M. Schenk
Wageningen University and Research Centre ,
Greenhouse Horticulture
PO box 20, 2665 MV, Bleiswijk, The Netherlands
Phone: +31 317 485752
E-Mail: martijn.schenk@wur.nl

Partner 8
Dr. L.Tomasolli
Centro di Ricerca per la Patologia Vegetale
Via Carlo Giuseppe Bertero, 22, 00156, Roma, Italy
Phone : +39 0682070292
E-mail: laura.tomassoli@entecra.it

Partner 11
Prof. Dr. M. Ravnikar
National Institute of Biology
Vecna pot 111, SI-1001 Ljubljana, Slovenia
Phone: + 386 1 4233388
E-mail: Maja.Ravnikar@nib.si
Dr. I. Gutierrez Ion.Gutierrez@nib.si
Dr. N. Mehle Natasa.Mehle@nib.si

Partner 12
Dr. K. Petrusis
Agricultural Research Centre (ARC)
Estonia
Phone: + 37 2 6729161
E-mail: karme.petrutis@pmk.agri.ee

Partner 13
Dr. L. Papayiannis
Agricultural Research Institute (ARI)
Athalassa, PO Box 22016, 1516, Nicosia, Cyprus
Phone: + 35 722403203
E-mail: l.papayiannis@arinet.ari.gov.cy

Partner 14
Prof. Dr. H. Pospieszny
Institute for Plant Protection (IOR)
W. Wegorka 20, 60-318, Poznan, Poland
Phone: + 48 618649094
E-mail: H.Pospieszny@ior.poznan.pl

Partner 15
Dr. C. Varveri
Benaki Phytopathological Institute
8 St. Delta street, 14561, Kifissia, Greece
Phone: + 30 210 2128036
E-mail: c.varveri@bpi.gr

Partner 16
Dr. Dag-Ragnar Blystad
Bioforsk, Norwegian Inst. For Agricultural and Environmental Research
Høgskoleveien 7, N-1432, Aas, Norway
Phone: + 47 908 72 588
E-mail: dag-ragnar.blystad@bioforsk.no

Partner 17
Prof. Dr. D. Hristova
Plant Protection Institute (PPI)
2230 Kostinbrod, Bulgaria
Phone: + 35 9 721 6 60 61
E-mail: di_hristova@yahoo.com

Partner 18
Prof. Dr. A.M. Nazaré Pereira
Universidade de Trás-os-Montes e Alto Douro (UTAD)
Departamento de Agronomia
5000-801 Vila Real Portugal
Phone: + 351 259350506
E-mail: anazare@utad.pt

Partner 9
M. X. Tassus
Laboratoire National de la Protection des Végétaux
Rue Dixméras 7, 49044, Cedex 1, Angers, France
Phone: +33 (0) 2 41 72 32 40
E-mail: xavier.tassus@agriculture.gouv.fr

Partner 10
Dr. I. Hanssen
Scientia Terrae Research Institute
Fortsesteenweg 30a, 2860 Sint-Katelijne-Waver, Belgium
Phone: + 32 15 30 55 90
E-mail: iha@scientiaterrae.org

Partner 19
Dr. J.W. Roenhorst
Plant Protection Service
PO Box 9102, 6700 HC, Wageningen, The Netherlands
Phone: + 31 317 496850
E-mail: j.w.roenhorst@minlnv.nl
Dr. D.J. van der Gaag d.j.van.der.gaag@minlnv.nl

Partner 20
Dr.H.J. Vetten
Julius Kühn Institute, Federal Research Centre for Cultivated Plants
Messeweg 11-12
38104 Braunschweig, Germany
Phone: +49 513 2993720
E-mail: heinrich-josef.vetten@jki.bund.de
Dr. E Pfeilstetter ernst.pfeilstetter@jki.bund.de

Table of Contents

SUMMARY: PEST RISK ANALYSIS FOR *PEPINO MOSAIC VIRUS*

This summary presents the main features of a Pest Risk Analysis (PRA) which has been conducted on *Pepino mosaic virus* as a key deliverable from the EU-funded PEPEIRA Project. The PRA was prepared according to the EPPO Standard '*Guidelines on Pest Risk Analysis: Decision-support scheme for quarantine pests*' version 09-15190 (PM 5/3 (4). This summary is based upon the template for the EPPO '*Report of a Pest Risk Analysis*', version 06-12731, now superceded by 08-13988. Elements of both versions are included.

Pest: *Pepino mosaic virus*

PRA area: European Union (27 Member States).

Assessors: Arjen Werkman[1] and Claire Sansford[2]. [1]Plant Protection Service, P.O. Box 9102 , 6700 HC Wageningen, The Netherlands and [2]The Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, United Kingdom.

Reviewers: PEPEIRA Partners.

Citation: Werkman A.W. & Sansford C (2010). Pest Risk Analysis for *Pepino mosaic virus* for the EU. Deliverable Report 4.3. EU Sixth Framework Project Project PEPEIRA. http://www.pepeira.com

Date: 11th June 2010.

STAGE 1: INITIATION

Reason for doing PRA: This PRA is being produced to account for the findings of the EU Sixth Framework RTD project 'Pepeira' www.pri.wur.nl/UK/research/projects/pepeira/ whose aim is to produce an EU-wide PRA for *Pepino mosaic virus* (PepMV) accounting for the results of the project as well as a review of the literature. The PRA is intended to be used to review the current European Commission (EC) emergency legislation for PepMV in 2010 (Commission Decision 2004/200/EC).

Taxonomic position of pest: Kingdom: Virus / Family: Flexiviridae / Genus: Potexvirus / Species: *Pepino mosaic virus* / Acronym: PepMV

Note that there are currently four genotypes *(also referred to in the Summary and in the PRA as strains)* that are recognised:

> The original Peruvian (LP), the European tomato (EU), the American genotype (US1) and the Chilean (CH2). For more information on genotypes see the answer to question 8 in the PRA.

STAGE 2: PEST RISK ASSESSMENT

Probability of introduction

Entry

Geographical distribution:

North America: There are several reports of PepMV in Canada and the United States.
Central and South America: Findings of PepMV have been reported in Chile, Ecuador, Guatemala and Peru.

Africa: PepMV has been reported in Morocco (which is also part of the EPPO region – see below).

Asia: It has been reported in China, the current status is unknown.

EU and EPPO region: Since 1999, PepMV has been reported as causing outbreaks of disease/being detected in 19 out of the current 27 Member States (MS) of the EU; the first affected being the UK and the Netherlands, with sporadic reports from various countries since. The most recent information on the status of PepMV comes from the official EU surveys. Currently, the requirements for the surveys are for MS to survey 4 categories: tomato seed, plants for planting, fruit production sites and fruit being marketed.

In reviewing the surveys for this PRA it is important to note that not all MS appear to have reported to the EC or there are no data available, and, of those that have undertaken surveys, some have not reported on all 4 categories. The intensity of surveillance has also varied between years and MS. The summary below only reflects the overview reports from the EC either presented at the Standing Committee for Plant Health or in an overview table. More detailed information should be available from each EU MS but in general it is not presented here because it was not available to the authors.

In the period 2007-2009 PepMV was found during official surveys in 17 MS: Austria, Belgium, Cyprus, Czech Republic (declared eradicated), Denmark, France, Germany, Hungary, Ireland, Italy, the Netherlands, Poland, Romania, Slovak Republic, Spain, Sweden and the United Kingdom. The incidence of detection has varied between countries and years with some reports showing more prevalence of PepMV than others. In several EU countries where PepMV has been found, attempts have been made to eradicate the virus.

In the EPPO region PepMV has also been reported in the Canary Islands, Norway (2001 – eradicated), Morocco, Switzerland and the Ukraine (current status unknown).

Major host plants or habitats:	The main host of PepMV is tomato. Also pepino, potato and several weed species have been reported to be a natural host of the virus. Recently basil has been reported to be a natural host in Italy, however attempts to obtain infectious isolates or confirm infection failed. Therefore the status of basil as a host of PepMV is not clear. Eggplant is readily infected by PepMV and an infection has been found in a Belgium greenhouse where plants of eggplant were grown next to infected tomato plants. However, more research is needed on eggplant to determine whether it is likely to become an important host of PepMV. Pepper and a number of other species can be infected mechanically with PepMV, but no natural findings are known.
Which pathway(s) is the pest likely to be introduced on:	Four main '*commodity types*' are identified and assessed in this PRA: 1. Tomato fruit 2. Seed of tomato 3. Plants for planting of tomato 4. Insect vectors (bumble bees) The pathways plants for planting of pepino, fruit of pepino, plants for planting of basil and human assistance have been identified but based on different considerations not further assessed. Because PepMV has been recorded already in the PRA area (in 19 out of the 27 EU MS) the pathways of spread within the PRA area are also considered here with risk management options being determined in the same way as for pathways of entry into the EU. Therefore probabilities of entry for each commodity type are assessed for two groups: A) non-EU countries where PepMV has been reported, albeit the current status in some of these countries is unknown: Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China and B) EU countries where the virus has been reported during the official surveys in the period 2007-2009 (17 out of 27 MS) unless the NPPO has confirmed that the virus has been eradicated: Austria, Belgium, Cyprus, Czech Republic (considered eradicated), Denmark, France, Germany, Hungary, Ireland, Italy, the Netherlands, Poland, Romania, Slovak Republic, Spain, Sweden and the United Kingdom. Countries from where there are no official reports of PepMV but from where the virus has been intercepted in the EU (e.g. on material from Israel) are not included in this analysis. ***Tomato fruit*** The overall probability of entry/movement spread by trade of tomato fruit is estimated as ranging from **unlikely** to **very likely** depending on the origin. The main uncertainty

is exact information on distribution of the virus in the different countries of origin.

- Entry into the PRA area: Most of the imported tomato fruit originates from Morocco. Although the current status of PepMV in Morocco is unknown, there are several interception reports of PepMV on Moroccan tomato fruit suggesting that PepMV is present in tomato production areas.
- Movement within the PRA area: The majority of the massive amount of tomato fruit traded within the EU originates from countries where PepMV has been reported.

PepMV will remain infectious during shipment and is likely not to be detected during existing management procedures. Tomato fruit is shipped very widely throughout the PRA area and during suitable times of the year for transfer of the virus to a crop. The risk of transfer depends on the nature of the processing of the tomato fruit, especially sorting and packing. If there are not sufficient (hygiene) measures the virus is moderately likely to transfer.

Tomato seeds

The overall probability of entry/movement by tomato seeds is estimated as ranging from **unlikely** to **likely**, depending on origin. Although the risk of the virus being associated with seed and being capable of infecting plants is estimated as being low, the amount of seed traded is massive and the risks associated with one infected seed is potentially high. The main uncertainty is exact information on distribution of the virus in the different countries of origin.

- Entry into the PRA area: Most tomato seed sown in the EU is produced in third countries. In many of these countries PepMV has been reported to occur. Moreover, there are many interception reports of PepMV on seed.
- Movement within the PRA area: Inside the PRA area tomato seed is produced and traded, mainly in countries where PepMV is known to occur.

The rate of seed transmission of PepMV has been shown to be very low. Growing out of untreated seeds harvested from infected fruit gives a very low transmission rate. Moreover existing phytosanitary measures (acid-extraction) has been shown to effectively reduce the transmission rate. However, one seed giving rise to an infected seedling in a batch of young plants is very likely to spread PepMV to other plants and finally infect the whole crop. This pathway might be especially important for the risk of introduction of new strains (genotypes) into the PRA area and for further spread.

Tomato plants for planting

The overall probability of entry by tomato seedlings is estimated as ranging from **very unlikely** to **moderately**

likely. The main uncertainty is exact information on distribution of the virus in the different countries of origin.

- Entry into the PRA area: Only Norway, Switzerland, the Canary Islands and Morocco are considered and hardly any tomato plants are imported from the first two countries; the situation with respect to the Canary Islands and Morocco is not known but <u>assumed</u> unlikely.
- Movement within the PRA area: Most seedlings used in a country are grown in this country, although in some country plants are introduced from other EU countries, mainly originating from the Netherlands. There are occasional findings of PepMV on plants for planting.

Since tomato plants are directly introduced at tomato production sites, it is very likely that infected plants will aid transfer of PepMV to suitable hosts.

Bumble bees

This pathway is not considered to pose a risk of entry into the PRA area from third countries (due to existing bee health legislation).

Although a large quantity of commercially produced bumble bees is moved within the PRA area, the probability of movement is estimated as **very unlikely** because the probability of association is very unlikely. This is because bumble bees are produced on a diet of bee-collected pollen and sugar water. The main risk from bumble bees is associated with spread within an infected area (See 1.30 in the PRA).

Establishment

<u>Plants or habitats at risk in the PRA area:</u> Tomato is the main host of PepMV and the main crop at risk in the PRA area. Pepino is only grown experimentally and on a small-scale in the PRA area, principally in Mediterranean countries such as Spain. For potato, basil and eggplant the importance of these crops as a host of PepMV is uncertain. Some weed species are known to harbour the virus but there is no identified risk associated with these species, other than their potential to act as sources of infection for tomato crops if infected weeds occur in close proximity to them.

<u>Climatic similarity of present distribution with PRA area (or parts thereof):</u> Climatic conditions affecting pest establishment of PepMV in the PRA area are considered completely similar.

Tomato is grown throughout the PRA area. In the northern part of the PRA area, tomato is only grown commercially under protected cultivation. In domestic gardens, tomato can be grown outdoors in the summer months. In the southern part of the PRA area tomato is commercially grown outdoors as well as under protection. PepMV already occurs in both northern and southern parts of the PRA area. Most reports on PepMV in tomato in northern Europe, Southern Europe and North America are on crops grown

	under protected cultivation. The only report of an infection with PepMV in outdoor tomato cultivation originates from Cyprus, although there is not yet any information available on the possible survival of PepMV in the field. Infections with PepMV in outdoor tomato cultivation probably occur more often although no further data are available. Additionally there have been reports of findings of PepMV in weeds in infected tomato fields in Spain.
Aspects of the pest's biology that would favour establishment:	The managed environment in tomato crops is highly favourable for establishment of PepMV. It is very easily mechanically transmitted. Since tomato is a crop where crop-handling procedures are very intensive there is a high risk of mechanical spread. Since the first findings of PepMV in tomato the main focus has been on hygiene measures. Nowadays strict hygiene protocols apply in protected cultivation. PepMV has already been reported in 19 of the 27 EU MS. In several countries attempts to eradicate the virus have been made but it still persists at many sites. However, the chance of the virus surviving eradication programmes is mainly dependent on the intensity of tomato production in a certain area. Infections in isolated greenhouses are more easily eradicated then infections in greenhouses in dense production areas. Infections that persist between crops in plant debris (poor hygiene) are likely to carry-over into the next crop. There have been reports of bumble bees being capable of spreading the virus in greenhouses although the exact mechanism of transmission has not been determined. Especially in dense tomato production areas bumble bees might act as a vector for PepMV facilitating spread between greenhouses.
Characteristics (other than climatic) of the PRA area that would favour establishment:	Tomato fruit is very widely distributed and traded in the PRA area. Most tomato seed sown in the EU is produced in third countries (e.g. China, Thailand, India, Chile) and shipped to the EU in bulk, although there is also some production in the EU. Most plants for planting are grown in the MS from imported seed. Only the Netherlands is exporting substantial numbers of young plants, mainly to neighbouring countries.
Which part of the PRA area is the endangered area:	The whole PRA area where tomato production takes place.

Potential Geographical Distribution of *Pepino mosaic virus* in the EU

PepMV has already been reported in 19 EU Member States, in including southern, northern, western and eastern parts of the EU. Therefore the potential geographical distribution of PepMV is the whole of the EU.

POTENTIAL ECONOMIC CONSEQUENCES

How much economic impact does the pest have in its present distribution:

The impact of PepMV depends on several factors.

The main factors are virus isolate, cultivation conditions including climate, and the tomato cultivar. As mentioned in this PRA (Question 8) no correlation between virus strain (genotype) and symptoms has been shown yet. However, it is known that some isolates of the virus consistently induce more severe symptoms than other isolates when tested, even within a genotype (strain). The type of virus isolate present in a crop will influence the effect of PepMV. For example in the PEPEIRA field trials the 'mild' isolate had a minimal effect on both yield and quality while the 'aggressive' isolate had a minor effect on yield but a significant effect on quality. However, the effect of individual isolates is unpredictable until they are subject to testing. If isolates of different strains of PepMV occur simultaneously in a crop the impact might be more severe. Combined infections of PepMV with other viruses, bacteria or fungi can lead to a more severe impact.

The climate and possibly the cultivation conditions of the crop (e.g. crop management, nutrient balance) may influence the effect of PepMV. In the past, it has been observed that light and temperature influence symptomatology of PepMV (Jones & Lammers, 2005). In several papers it has been stated that a low light intensity will induce more severe symptoms, although in other trials the opposite effect was observed. In the Pepeira trials most effect was observed in the first part of the growing season.

Circumstantial evidence suggests that the tomato cultivars used may influence the effect of PepMV.

Overall it is difficult to quantify the effect of climate, cultivar, cultivation and isolate on the effect of PepMV.

Economic effects of PepMV are usually the result of reductions in quality. In most, but not all trials, yield losses are limited. However, it should be noted that even a small yield loss can result in economic losses for individual growers. Since it has been shown that PepMV affects fruit quality and therefore may result in downgrading of Class 1 fruit, the level of economic loss will depend upon differences in market price and marketing.

The overall conclusion of the effect of PepMV on yield and quality is that PepMV will have a minor effect on yield and a moderate effect on fruit quality. The effects will mainly depend on the isolate present and this is difficult to predict. Under optimal climatic conditions for the crop, in combination with a mild isolate, the effects will be minimal, while under negative climatic conditions in combination with an aggressive isolate the effect can be very serious.

CONCLUSIONS OF PEST RISK ASSESSMENT

Summarize the major factors that influence the acceptability of the risk from this pest:

- *Pepino mosaic virus* is moving in trade throughout the year from both non-EU countries where the virus occurs and within the EU
- Both tomato fruit and tomato seed are distributed widely throughout the PRA area
- Tomato seedlings are mainly propagated within each EU Member State but some are traded within the EU from a few countries including the Netherlands
- It is very likely that the virus will survive or could remain undetected during existing emergency phytosanitary measures which are only specified for seed and currently are only for seed treatment rather than for testing
- PepMV is already present in several geographical parts of the EU, including the main tomato fruit production areas.
- PepMV is very readily mechanically transmitted and spread by human assistance is very likely.
- Up to now, four different strains (genotypes) of PepMV have been identified. Although there is no distinct correlation between strains and severity of symptoms, the potential for introduction of new strains poses a risk as well as for further spread of the existing known strains.
- Although in some cases yield loss in tomato has been reported, the main economic impact is based on reduction of quality of fruit.

Estimate the probability of entry:

The overall probability of entry (and spread within the EU) of PepMV is estimated as **high**

Estimate the probability of establishment:

The probability of establishment in the PRA area is **high**.

PepMV is already present in many parts of the PRA area and was first reported in 1999 from the UK and the Netherlands. It is known to have occurred in 19 of the current 27 EU MS, including the main tomato production areas, both in protected cultivation and in outdoor grown tomato crops. It has also been found in weeds surrounding tomato production facilities in Spain. Although strict hygiene measures can prevent establishment of PepMV the fact that the virus is very readily transmitted mechanically poses great risks. Thus, the probability of establishment is very high, with a low uncertainty.

Estimate the potential economic impact:

The potential economic impact is **medium**.

The main economic impact is associated with downgrading of good quality tomato fruit due to symptoms caused by PepMV. The size of the impact depends on whether the crop is infected with a mild or aggressive isolate of PepMV. There may be other influences including cultivation practices, temperature and light, and possibly the type of cultivar, but the effect is not predictable. The classification system of fruit for marketing in a country and the current market price will influence the size of the impact resulting from an outbreak of PepMV. From both the results of experimental trials and from observations in commercial crops, very low to very high economic damage is known to occur. Therefore the overall economic importance is estimated as medium.

Degree of uncertainty

The main uncertainties are:

<u>Distribution</u>

- One current uncertainty is the distribution of PepMV in third countries. The PRA is based on official reports on the presence of PepMV in countries, although interception data suggest that the distribution might be wider.
- There are also uncertainties on the exact distribution in the PRA area. Although the virus is considered widely distributed in some of the main European tomato production areas as well as in some that produce much less fruit, there are countries that claim the virus is absent or where no official surveys have been reported to the European Commission.

<u>Pathways</u>

- The probability of crops other than tomato being natural hosts of PepMV is uncertain. This applies especially for basil, eggplant and pepper.
- The importance of bumble bees for spread of PepMV is uncertain.

<u>Economic impact</u>

- The main uncertainty in assessing the economic impact is linked to the occurrence of the different isolates. Since symptom development and the consequent downgrading of fruit are strongly correlated with the aggressiveness of an isolate, the economic impact is dependent on the type of isolate that is present. The aggressiveness of individual isolates can vary within a genotype (strain) and effect of the isolate is not predictable without testing. Moreover, new variants of the virus might be introduced.

OVERALL CONCLUSIONS

PepMV is already present in the PRA area but based solely on the incidence of the virus reported in official surveys, by phytosanitary definition, the virus is considered to be '*not widely distributed*' (see question 13 of the PRA). However, there is uncertainty regarding the exact distribution of PepMV both within the PRA area and in third countries.

The first reports of the virus in the EU were in 1999 in 2 MS; by 2009 it had been reported from 19 of the current 27 MS.

There is a high risk of further entry, establishment and spread of PepMV in the PRA area.

The economic impact of PepMV is influenced by the isolate that is infecting the tomato crop (this is not predictable), as well as possibly by some cultivation practices, and, by the marketing system and the current market price for tomato fruit. Under certain circumstances the impact of the virus can be high.

Therefore, management options may be needed to prevent further entry and spread of PepMV, including existing and new genotypes. Pathway xiv – bumble bees from within the EU is not considered for risk management.

STAGE 3: PEST RISK MANAGEMENT

IDENTIFICATION OF THE PATHWAYS

Pathways studied in the pest risk management section *(numbers in brackets are pathway numbers assigned in the PRA)*

1. Tomato fruit (i – non-EU, viii – EU)
2. Seed of tomato (ii – non-EU, ix – EU)
3. Plants for planting of tomato (iii – non-EU, x – EU)

Other pathways identified but not studied	None

IDENTIFICATION OF POSSIBLE MEASURES

* Possible measure - realistic
** Possible measure - not likely to be practical
*** Pre-existing phytosanitary measures that have an impact on PepMV (including current emergency measures as well as those that are not specific to the pest).

A) i tomato fruit non-EU, viii tomato fruit EU MS:
- specified testing**
- specified growing conditions**
- pest-freedom of crop, pest-free place of production or pest-free area**
- internal surveillance and/or eradication campaign**

B) ii tomato seed non-EU, ix tomato seed EU MS:
- pre-existing pest-specific phytosanitary measures (Commission Decision 2004/200/EC; Anon., 2004) and non-pest-specific phytosanitary measures (EC Plant Health Directive; point 48, Annex IVAI and point 27 of Annex IVAII; Anon., 2000)***
- specified testing* (could be used to support †)
- import under special licence/permit and post-entry quarantine**. This is effectively the same as specified seed testing but it is only appropriate for small quantities of seed for research or trialling and not for commercial quantities of tomato seed.
- specified treatment* (could be used to support †)
- specified growing conditions** (although difficult to implement this would be a necessary measure in support of other options)
- certification scheme**
- pest-freedom of crop, pest-free place of production or pest-free area*† . This is already a requirement for seed in the emergency measures (Anon., 2004) but it may need to be further refined which could be difficult, but can be considered if choices are offered in the way this is determined as described below.
- internal surveillance and/or eradication campaign**

C) iii tomato plants non-EU, x tomato plants EU MS:
- pre-existing (non-pest-specific) phytosanitary measures (EC Plant Heath Directive; point 48 of Annex IVAI and point 27 of Annex IVAII; Anon., 2000)***
- specified testing**
- specified growing conditions**
- certification scheme**
- pest-freedom of crop, pest-free place of production or pest-free area**

- internal surveillance and/or eradication campaign**

EVALUATION OF THE MEASURES IDENTIFIED IN RELATION TO THE RISKS PRESENTED BY THE PATHWAYS

The risks presented by the pathways have been ranked from **high** to **very low** depending upon the type of commodity as well as the origin.

Degree of uncertainty

CONCLUSION:

Recommendation for possible measures:

Based upon the findings of this PRA, it may be necessary for decision-makers to consider changing from the emergency measures for PepMV (Commission Decision 2004/200/EC; Anon., 2004) to permanent listing of PepMV in the EC Plant Health Directive 2000/29/EC (Anon., 2000). The rationale behind this and the possible options are described below:

Pest listing
Since the virus already occurs in parts of the EU, including the main tomato production areas, if the emergency legislation is dropped, for measures to continue to be taken, PepMV would require listing in Annex IIAII (harmful organisms known to occur in the community and relevant for the entire community).

Tomato fruit
For tomato fruit, no realistic or reliable phytosanitary measures have been identified. To reliably guarantee pest- freedom, very high numbers of tomato fruit would have to be tested. This, in combination with economic losses resulting from destruction of the fruit, makes the imposition of phytosanitary measures for tomato fruit unrealistic. However, to prevent the introduction of PepMV to fruit production sites where these co-exist with packing houses, strict hygiene practices would be required. In areas of the EU with a high density of fruit production this will be especially difficult, however, there are published hygiene protocols which can be followed and are already implemented in some EU Member States on a voluntary basis.

Tomato plants
For tomato plants for planting, if these are grown from seeds free of PepMV and strict hygiene measures are taken, a pest-free place of production for young plants could be established. However, reliable testing to confirm absence of PepMV in young plants is difficult. Moreover, destruction of plants and a possible shortage of supply of plants to growers might lead to economic losses. Therefore, the imposition of phytosanitary measures for tomato plants for planting is probably unrealistic.

Tomato seed
As measures for two of the main pathways i.e. tomato fruit and plants for planting are considered most likely to be unrealistic, if decision-makers conclude that PepMV should be permanently listed in the EC Plant Health Directive, the subject of contamination in Annex IIAII would be seeds of tomato (listed as *Lycopersicon lycopersicum*; noting that the correct scientific name for tomato is now *Solanum lycopersicum*). This pathway is believed to be an important route for introduction of PepMV into a new area or for further introduction into an existing area. It also poses a risk of introduction of new variants of PepMV.

The risk management option that is recommended to decision-makers for consideration for phytosanitary measures is principally for seed treatment and virus-testing (seed/mother plant). This could be used as the basis for a requirement for seed to originate in a pest-free crop, place of production or area. This is already the basis of the pre-existing emergency phytosanitary measures which requires acid extraction of tomato seed AND: a pest-free area, OR a pest-free place of production, OR official seed testing (which is equivalent to a pest-free crop).

With respect to seed treatment, if seeds were only to be acid–extracted (or an equivalent method) this is already facilitated within the requirements for seeds in Annex IVAI, article 48 for seeds entering the EU and Annex IVAII, article 27 for seeds originating within the EU.

If seeds were to be acid-extracted and subject to an additional treatment, this would require an additional article in Annex IVAI and IVAII, specific to PepMV. More research on the efficacy of an additional seed treatment would be necessary. Different treatments are already in use by some seed houses.

If seed is required to come from a pest-free area, pest-free place of production or pest-free crop this could be catered for within the pre-existing articles for tomato seed in Annex IVAI and IVAII of the EU directive 2000/29/EC with the addition of PepMV. The options for this include those described already including seed treatment; as well as symptom-free and virus-free (by testing) mother plants, and/or seed testing. If mother plants and/or seed is/are to be tested, a harmonised testing protocol would be helpful. An outcome of the Pepeira project will be an EPPO protocol for the detection of PepMV.

Since there is no consistent difference between genotypes in terms of their biology and aggressiveness, and because isolates of the same genotype can behave differently, it is not appropriate to regulate by genotype.

It is recommended that these measures are considered by decision-makers in light of the findings of the PRA to determine future policy for PepMV in the EU.

PEST RISK ANALYSIS FOR *PEPINO MOSAIC VIRUS*

Stage 1: Initiation

The aim of the initiation stage is to identify the pest(s) and pathways which are of phytosanitary concern and should be considered for risk analysis in relation to the identified PRA area.

1. Give the reason for performing the PRA

This PRA is being produced to account for the findings of the EU Sixth Framework RTD project 'Pepeira' (www.pepeira.wur.nl/UK). The aim of the project is to produce an EU-wide PRA for *Pepino mosaic virus* (PepMV) accounting for the results of the project as well as a review of the literature. The PRA is intended to be used to review the current European Commission (EC) emergency legislation for PepMV in 2010 (Commission Decision 2004/200/EC) (Anon., 2004).

Go to 2

2. Specify the pest or pests of concern and follow the scheme for each individual pest in turn. For intentionally introduced plants specify the intended habitats.

Kingdom: Virus
Family: *Flexiviridae*
Genus: *Potexvirus*
Species: *Pepino mosaic virus*. Named as such as it was first isolated from *Solanum muricatum* (pepino). (Jones *et al*., 1980)
Acronym: PepMV

Pepino mosaic virus (PepMV) was first described in 1980 after it was found to infect pepino (*Solanum muricatum*) in Peru (Jones *et al*., 1980). Many years after the first description, in 1999 the virus was reported to infect tomato (*Solanum lycopersicum*) in the Netherlands and the United Kingdom (UK) (Wright & Mumford, 1999; Van der Vlugt *et al*., 2000). Subsequently, outbreaks were reported in many other countries including other EU Member States and EPPO countries.

Different genotypes of PepMV are distinguished: the original Peruvian (LP), the European tomato (EU), the American genotype (US1) and the Chilean (CH2). For more information on genotypes see the answer to question 8 in the present PRA.

Go to 3

3. Clearly define the PRA area.

The PRA area is the European Union (27 Member States)

Go to 4

<u>Earlier analysis</u>

4. Does a relevant earlier PRA exist?

Yes. In 2005 a joint Pest Risk Analysis was produced by the United Kingdom and the Netherlands (Jones & Lammers., 2005) as well as an EPPO-style datasheet upon which the PRA was based (Jones *et al*., 2005). The PRA was based on earlier PRAs written by the United Kingdom and the Netherlands. Parts of the 2005 PRA and datasheet as well as the earlier PRAs have been used in the present PRA.

if yes **Go to 5**
if no Go to 6

5. Is the earlier PRA still entirely valid, or only partly valid (out of date, applied in different circumstances, for a similar but distinct pest, for another area with similar conditions)?

The earlier PRAs are only partly valid. New information on the biology, epidemiology and impact of the virus has been published since and data have been specifically-generated in the Pepeira project to help address uncertainties in the earlier PRAs.

if entirely valid End
if partly valid proceed with the PRA, but compare as much as possible with the earlier PRA **Go to 6**
if not valid Go to 6

6. Specify all host plant species (for pests directly affecting plants) or suitable habitats (for non parasitic plants). Indicate the ones which are present in the PRA area.

<ins>Natural Hosts:</ins>

Tomato (*Solanum lycopersicum*) is the most important natural host of PepMV (Wright & Mumford, 1999; Van der Vlugt *et al*., 2000). PepMV is mainly found in this crop, which is widely-grown in the PRA area.

Pepino (*Solanum muricatum*) is a host in Peru and China (Jones *et al*., 1980; Soler *et al*., 2002; Zhang *et al*., 2003). Efforts have been made to grow pepino as a fruiting crop at a commercial scale under greenhouse conditions in the Mediterranean area of the EU but this has not yet been successful (Prohens *et al*., 2000; Prohens *et al*., 2005). Pepino is also reported to be grown on a small scale in Spain (EPPO, 2000). However, the most recent information is that there is no commercial production of pepino in Spain. There are some experimental greenhouses that are trialling the cultivation of pepino. Occasionally fruits harvested from the trials are sold at local markets (J. Prohens, UPV, Spain, *personal communication* to A. Alfaro-Fernandez, UPV, Spain, February 2010).

In Spain, symptomless infections of PepMV were found in weed species (*Amaranthus* sp*., Malva parviflora, Nicotiana glauca, Solanum nigrum* and *Sonchus oleraceus*) near to greenhouses with PepMV infected tomato plants (Jordá *et al*., 2001a). In a later publication, the weed species *Bassia scoparia, Claystegia sepium, Chenopodium murale, Convolvulus althaeoides, Convulvulus arvensis, Conyza albida, Coronopus* sp*., Diplotaxis erucoides, Echium creticum, Echium humile, Heliotropium europaeum, Moricandia arvensis, Onopordum* sp*., Piptatherum multiflorum, Plantago afra, Rumex* sp*., Sisymbrium irio, Sonchus tenerrimus* and *Taraxicum vulgare*, which were growing in or around tomato fields in Murcia and Almeria provinces of Spain, tested positive for PepMV (Córdoba *et al*., 2004). No artificial inoculation studies have been performed to determine the nature of these infections and therefore the exact role of these weed species in the epidemiology of PepMV is not known.

In surveys in Peru, PepMV has been found to be naturally present in wild *Solanum* species (S*. chilense, S. chmielewskii, S. parviflorum* and *S. peruvianum*). These species do not occur naturally in the PRA area (Peralta & Spooner, 2000; Tutin *et al*., 2002). Only one out of five plants of S*. peruvianum* infected with PepMV had symptoms (Soler *et al*., 2002). PepMV was also detected in tomato and pepino in the same surveys.

PepMV has also been detected in potato (*Solanum tuberosum* cv. 'Yungay') in the field in the Andes in Peru. In addition, 14% of tested accessions in the potato germplasm collection at the Centro Internacional de la Papa (CIP) in Peru have been found with PepMV (L. Salazar, CIP, Peru, *personal communication*, to D. Jones, ex-Central Science Laboratory, UK). Under experimental conditions potato was found to be infected by different strains of PepMV by mechanical inoculation but with a very low success rate, and rarely local or systemic symptoms are observed. Only on one occasion could the virus be detected in plants grown from tubers harvested from an inoculated potato plant (Pepeira final report WP2, 2010). Potato is commonly grown in the PRA area.

Recently, basil (*Ocimum basilicum*), a herb which is widely-grown in the PRA area, was reported to be a natural host of PepMV in greenhouse-grown plants in Sicily, Italy (Davino *et al*., 2009). Infected, symptomatic plants were detected in July 2008 in greenhouses in an area where tomato plants were found to be infected by PepMV 3 years earlier. Subsequent to this report investigations were undertaken to determine whether this would be a significant new host. However, the original isolate that was obtained was not infectious and attempts to confirm infection were therefore unsuccessful (L. Tomassoli, ISPV, Italy & R. van der Vlugt, PRI, The Netherlands, *personal communication* to A.W. Werkman, PPS, the Netherlands, January 2010). Moreover, attempts to inoculate other isolates of PepMV onto basil did not result in infected basil (H. Pospieszny, IOR, Poland & L. Tomassoli, ISPV, Italy *personal communication* to A.W. Werkman, PPS, the Netherlands, January 2010). Therefore the status of basil as a natural host of PepMV is doubtful.

<u>Experimental hosts</u>
Several species have been found to be experimentally-susceptible to infection by PepMV following artificial inoculation. These are known as experimental hosts. Most of these species belong to the *Solanaceae* family. An overview of experimental hosts that have been tested can be found in the Pepeira final report WP3 (2010).

Two plant species that are grown as economically-important crops are considered to be experimental hosts:

Eggplant: Eggplant (*Solanum melongena*) was found to be very readily infected by different strains (EU, US1, CH2) of PepMV by mechanical inoculation. The virus could be detected in inoculated plants in high virus titres and sometimes severe local and systemic symptoms were observed (Pepeira final report WP2, 2010). In Belgium, eggplant has been tested and found to be infected in greenhouses where plants of eggplant were grown next to a PepMV-infected tomato crop (I. Hanssen, Scientia Terrae, Belgium, *personal communication* to A.W. Werkman, PPS, the Netherlands, 2010). Since eggplant is easily infected by PepMV this crop might play a role in the epidemiology of PepMV. However, there are no further data on the natural occurrence of PepMV in eggplant and no surveys have been done on the occurrence in EU MS. Therefore more research is needed on the exact role of eggplant in the epidemiology of PepMV and the possible distribution of the virus in eggplant. Eggplant is not considered further in this PRA. This appraisal may change if further information become available, e.g. during future survey activities.

Pepper: In pepper (*Capsicum annuum)* no natural infections are known. Inoculated leaves can be infected by different strains of PepMV by mechanical inoculation but with a low success rate (Pepeira final report WP3, 2010). No systemic infection of pepper was observed in this study.

Go to 7

7. Specify the pest distribution

PepMV is present on at least four continents: The Americas (North, Central and South), Europe, Asia and Africa. Over the period 1999 to 2010, PepMV has been reported from 19 out of the 27 EU Member States. In Table 1 and Annex 1, the available information from published reports, and official EU MS survey data is summarised. It should be noted that not all outbreaks have been reported and that EU MS survey data are sometimes incomplete. Not all EU MS appear to have reported their data to the EC and in some cases no data are available. In addition, the intensity of the surveillance has varied between years and MS. Therefore, this overview represents the current knowledge of the distribution of PepMV, but it should not be used as a reference to support a statement of absence of PepMV in a certain area or country. Although the authors of this PRA have tried to give as objective an assessment of the data which are available, it is acknowledged that individual MS may have a different perspective for their country based upon their experience. This is not easily accounted for in a PRA for the whole of the EU.

Moreover there are also non-EU countries where there are no official reports of PepMV or that claim that the virus is absent, although there are notifications of non-compliance.

Table 1. Distribution of *Pepino mosaic virus* with references to occurrence and status

North America:	**Canada:** First reported in 2001 (French *et al*., 2001). Since then several reports (Verhoeven *et al*., 2003; French et al., 2005; EPPO, 2009). A survey of greenhouse tomatoes in 2006 reports findings in the provinces British Columbia and Ontario (Ling *et al.,* 2008). **USA:** First reported in 2001 (French *et al*., 2001). Since then several reports (Maroon-Lango *et al*., 2003; Verhoeven *et al*., 2003; Ling *et al.,* 2008; EPPO, 2009). A survey of greenhouse tomatoes in 2006 reports findings in the states of Alabama, Arizona, California, Colorado and Texas (Ling *et al*., 2008). Tomato seed imported from the USA was tested and found to be positive for PepMV in the 2008 official UK survey (UK NPPO, 2008).
Central America:	**Guatemala:** (EPPO, 2009) (current status unknown).
South America:	**Peru:** PepMV was first discovered in 1974 in two field crops of pepino at Imperial in the Canete Valley of coastal Peru (Jones *et al*., 1980) and again in 2000 when it was also found in tomato and four wild *Lycopersicon* spp. (Soler *et al*., 2002); on the EPPO Alert List with no details (EPPO, 2009). **Chile:** On tomato (Muñoz *et al*., 2002 <u>in</u> Soler *et al*., 2005a) (EPPO Alert List, 2009 – no details). **Ecuador:** First report in Ecuador in surveys of *Lycopersicon* spp. detected PepMV in wild *Lycoperiscon pimpinellifolium* (currant tomato) but not in tomato (Soler *et al*., 2005a); (EPPO, 2009 – no details). There are no details of the current status of PepMV in these countries.
Caribbean:	No record
Europe (EU Member States):	**Summary:** Since 1999, PepMV has been reported as causing outbreaks of disease/being detected in 19 out of the current 27 Member States (MS) of the EU; the first affected being the UK and the Netherlands, with sporadic reports from various countries since. (See below). The most recent information on the status of PepMV comes from the official EU surveys. Currently, the requirements for the surveys are for MS to survey 4 categories: tomato seed, plants for planting, fruit production sites and fruit being marketed.

	In reviewing the surveys for this PRA it is important to note that not all MS appear to have reported to the EC or there are no data available, and, of those that have undertaken surveys, some have not reported on all 4 categories. The intensity of surveillance has also varied between years and MS. The summary below only reflects the overview reports from the EC either presented at the Standing Committee for Plant Health or in an overview table. More detailed information should be available from each EU MS but in general is not presented here because it was not available to the authors. According to survey reports from the EC Standing Committee for Plant Health (SCPH) presented on the 26th of May 2009, PepMV was reported as being detected in 9 out of 27 EU MS in 2007, while it was detected in 11 MS in 2008 (Anon., 2009b). These data are not detailed and do not allow an informed assessment of the status of the virus in each MS to be easily made. In survey results for 2009 the virus was reported as being detected in 15 MS – the survey results are more detailed than those of the previous two years (Anon., 2010a). In the period 2007-2009 PepMV was found during official surveys in 17 MS: Austria, Belgium, Cyprus, Czech Republic (declared eradicated), Denmark, France, Germany, Hungary, Ireland, Italy, the Netherlands, Poland, Romania, Slovak Republic, Spain, Sweden and the United Kingdom. The incidence of detection has varied between countries and years with some reports showing more prevalence of PepMV than others. In several EU countries where PepMV has been found, attempts have been made to eradicate the virus. Details are difficult to summarise as not all the information that is needed is freely available. All records listed below are presumed to be in tomato. For an overview of survey activities and survey results for the EU see **Annex 1**. **Austria.** Reports of findings in tomato in 1999 (Verhoeven *et al*., 2003). Also found in fruit production sites and marketed fruit in the official surveys of 2007 (Anon., 2009b). In the 2008 official survey there were no findings (Anon., 2009b). In the 2009 official survey 3 outbreaks in tomato fruit production were reported and 3 lots of infected marketed fruit (origin the Netherlands) (Anon., 2010a) **Belgium.** PepMV was reported to occur at tomato production sites in 2001/2002 (Verhoeven *et al*., 2003). Publications indicate that in the period 2004-2006 a gradual increase of the number of PepMV infected tomato greenhouses was seen (Hanssen *et al*., 2008; Hanssen *et al*., 2009a). In official surveys in 2007 and 2008 no findings were reported (Anon., 2009b). In the 2009 official survey found in 2 lots of plants for planting (seed originated from another EU MS), three imported seed lots and tomato fruit on the market originating from a domestic tomato producer (plants from another EU MS) (Anon., 2010a) **Bulgaria.** Found in 1 location in 2004 (subject to eradication) (EPPO, 2009). In official surveys conducted in 2007, 2008 and 2009 no findings were reported (Anon., 2009b; Anon., 2010a). **Cyprus.** In 2007 there were no findings in official surveys whilst for 2008 no data are available (Anon., 2009b). In the 2009 official surveys 20 outbreaks at fruit production sites were reported and 4 lots of marketed fruit (some from the Netherlands, some from Cyprus) (Anon., 2009b; Anon., 2010a). **Czech Republic.** In 2007 there were no findings in official surveys (Anon., 2009b). Found in 2008 at 1 site (tomato), under eradication (EPPO, 2009); however, considered eradicated in the EC survey return (Anon., 2009b). In 2009 no findings in the official survey

	(Anon. 2010a) **Denmark.** PepMV was found at one tomato nursery in 2001 and 2002 (Anon., 2002; Anon., 2003). In 2007 there were no findings in the official survey but in 2008 a finding was reported at a fruit production site (Anon., 2009b). In the official survey of 2009 found at one plant producer (seeds originated in the Netherlands) and one tomato fruit production site (Anon., 2010a) **Estonia.** In 2007 there were no findings in the official survey, for 2008 no data are available (Anon., 2009b). In 2009 no findings in the official survey (Anon. 2010a) According to the Estonian Agricultural Board there were also no findings in the surveys of 2007-2008 (Anon. 2010b). **Finland.** Found in tomato in 2001 and 2003 (subject to eradication) (EPPO, 2009); in the surveys of 2007, 2008 and 2009 no findings were reported (Anon., 2009b; Anon., 2010a). **France.** Since the first report in 2002 few outbreaks in tomato, (Cotillon *et al*., 2002; Martin & Mousserion, 2002; Verhoeven *et al*., 2003; EPPO, 2009). In the official surveys of 2007 found in plants for planting and fruit production, and in 2008 in fruit production (Anon., 2009b). In the official survey of 2009 found in three domestic seed lots, 100 imported seed lots (of various origins) and three outbreaks at fruit production sites reported (Anon. 2010a). **Germany.** Several cases have been reported in tomato, eradication was achieved in most cases and the virus was considered to be transient (Lesemann *et al*., 2000; Verhoeven *et al*., 2003; EPPO, 2009). In the official surveys of 2007 found in fruit production and marketed fruit of German origin and in 2008 in fruit production (Anon., 2009b). In the official survey of 2009 four outbreaks at fruit production sites reported (plants of German origin at one site and originating from the Netherlands at the other three sites) (Anon. 2010a). **Greece.** For 2007 no survey data are available, in 2008 and 2009 no findings in the official surveys (Anon., 2009b; Anon. 2010a). **Hungary.** First found in 2004 in tomato (EPPO, 2009). In the official surveys of 2007 and 2008 findings were reported in fruit production (Anon., 2009b). In the official survey of 2009 two outbreaks at fruit production sites and found in five lots of marketed fruit originating in Hungary (two), Italy (two) and Spain (one) (Anon. 2010a). **Ireland.** Found in 2002 at one production site and eradicated (Anon., 2003). In the official survey of 2007 no findings except in marketed fruit from another country, in 2008 found in fruit production (Anon., 2009b). In the official survey of 2009 three outbreaks at fruit production sites, origin of the plants was the Netherlands (Anon. 2010a). **Italy**. Findings reported in tomato (Roggero *et al*., 2001; Salomone & Roggero, 2002; EPPO, 2009) and recently found in basil (Davino *et al*., 2009). In the official survey of 2007 no findings except in marketed fruit from another country, in 2008 found in fruit production (Anon., 2009b). In the official survey of 2009 6 outbreaks at fruit production sites (growing material originated in Sicily (5 sites) and Sardinia (1 site)) (Anon. 2010a). **Latvia.** In the official survey in 2007 no findings reported, for 2008 no data. (Anon., 2009b). In 2009 no findings in the official survey except in two lots of marketed fruit originating in the Netherlands and Poland (Anon., 2010a) **Lithuania.** In the past an interception only (Anon., 2003). In the official surveys of 2007, 2008 and 2009 no findings reported (Anon., 2009b; Anon. 2010a). **Luxembourg.** For the period 2007-2009 no data available. In 2007 Luxembourg indicated to the EU that surveys carried out from 2004

	to 2006 gave no indication of PepMV presence in Luxembourg (Steven Jones, FVO, EU, *personal communication* to A. Werkman, PPS, The Netherlands, January 2010) **Malta.** In 2007 there were no findings in the official survey, for 2008 no data are available (Anon., 2009b). In 2009 no findings in the official survey (Anon. 2010a) **Netherlands.** Present at fruit production sites (van der Vlugt *et al*., 2000; van der Vlugt *et al*., 2002; Verhoeven *et al*., 2003; EPPO, 2009). In official surveys in 2007 found in fruit production, domestic and imported seed and in plants for planting, in 2008 found in fruit production (Anon., 2009b). In the UK official surveys, in 2008 and 2009 tomato fruit from the Netherlands was found to be infected with PepMV (UK NPPO; 2008 and 2009). In the Netherlands official survey of 2009 one finding at a plant producer and 27 outbreaks at tomato production sites reported – some were related to contamination from fruit production and others due to inoculation of plants by growers for '*cross-protection'* (Anon., 2010a) **Poland.** Found in 2001 at a research station, and eradicated. (Pospieszny *et al*., 2002); few outbreaks since (Pospieszny & Borodynko, 2006; Pospieszny *et al*., 2008; EPPO Alert List, 2009). In official surveys of 2007 found in fruit production and marketed fruit of domestic origin, in 2008 found in fruit production (Anon., 2009b). In the UK official survey of 2008 tomato fruit from Poland was found to be infected with PepMV (UK NPPO, 2008). In the Polish official survey of 2009, 20 outbreaks at tomato production sites and 60 lots of marketed fruit of various origins (Anon., 2010a) **Portugal.** In official surveys conducted in 2007 and 2008 no findings were reported (Anon., 2009b). In 2009 no findings in the official survey (Anon. 2010a) **Romania.** Reported in fruit production in the official survey of 2007, no findings in the survey of 2008 (Anon., 2009b). In 2009 no findings in the official survey (Anon. 2010a) **Slovak Republic.** Found in 1 location in 2004, further surveys confirmed eradication (EPPO, 2009). For 2007 no survey data are available, in 2008 no findings in the official surveys (Anon., 2009b). In the 2009 official survey found in fruit from commercial chain originating from Slovak Republic (Anon., 2010a) **Slovenia.** In official surveys conducted in 2007, 2008 and 2009 no findings were reported (Anon., 2009b; Anon., 2010a). **Spain.** Present at fruit production sites (Jordá *et al*., 2000; Soler *et al*., 2000; Martínez-Culebras *et al*., 2002; Mansilla *et al*., 2003; Verhoeven *et al*., 2003; Cordoba *et al*., 2004; EPPO, 2009). In official surveys of 2007 found in plants for planting and fruit production, and in 2008 in fruit production (Anon., 2009b). In the UK official surveys, in 2008 and 2009 tomato fruit from Spain was found to be infected with PepMV (UK NPPO, 2008 and 2009). Publications from 2005 and 2006 suggests that in 1998 PepMV was already present in tomato in Spain (Soler *et al.*, 2005; Pagan *et al*., 2006). In the official survey of 2009, 61 outbreaks at tomato production sites reported (Anon., 2010a) **Sweden.** Found once in 2001 in 1 tomato glasshouse (EPPO, 2009). In official surveys conducted in 2007 and 2008 no findings were reported except in imported seed (2007) (Anon., 2009b). In the official survey of 2009 seven outbreaks at tomato production sites reported, plants originated in Denmark (Anon., 2010a) **UK.** Since 1999 several outbreaks were reported in various regions of the country, but most of them were eradicated at the end of each growing season (Mumford and Metcalfe, 2001; Wright and Mumford, 1999; Verhoeven *et al*., 2003; EPPO, 2009). Findings were reported in the official surveys of 2007 in seed, fruit production and marketed

	fruit and in 2008 in fruit production (Anon. 2009b). In the 2009 official survey there were six outbreaks of PepMV at tomato fruit production sites of which five could be traced to a UK origin and one to the Netherlands, also found in marketed fruit with a range of origins (Anon., 2010a).

EPPO region:	As in Africa (Morocco) below and Europe (EU Member States) above plus: **Canary Islands.** Several reports (Jordá *et al*., 2001b; Martínez-Culebras *et al*., 2002; Verhoeven *et al*., 2003). In the period 2003-2008 several findings on Gran Canaria and Tenerife (Alfaro-Fernandez *et al.,* 2009b. The UK has intercepted tomato fruit from the Canary Islands infected with PepMV, most recently in the 2008 and 2009 official surveys (UK NPPO, 2008 and 2009). Sweden also detected infected fruit from Gran Canaria in their survey of marketed fruit in 2009 (Anon., 2010a). **Israel**. There are no published reports or official records of PepMV in Israel. However, the UK intercepted PepMV on 2 lots of seed out of 15 tested from Israel in the official survey of 2008 (UK NPPO, 2008). Also in 2009 found in seed during official surveys by Belgium (one lot) and France (five lots) (Anon., 2010a). **Norway.** Absent found once in 2001 and eradicated (EPPO, 2009). **Switzerland.** Found in 2004 in '*the French-speaking part*' but then eradicated, other outbreaks have been found in Ticino and Zurich cantons (EPPO, 2009). **Ukraine.** (Verhoeven *et al*., 2003). Current status unknown.
Africa:	**Morocco.** Introduced into tomato crops in 2002 (Hanafia & Schnitzler, 2002). Current status unknown. However, the UK has intercepted tomato fruit from Morocco infected with PepMV most recently in the 2008 and 2009 official surveys (UK NPPO, 2008 and 2009). Poland has also detected fruit from Morocco infected with PepMV in the 2009 official survey (Anon., 2010a).
Middle East:	No record.
Asia:	**China.** (Zhang *et al*., 2003). Current status unknown.
Oceania:	No record.

Go to Stage 2

Stage 2: Pest Risk Assessment

Section A: Pest categorization

> *At the outset, it may not be clear which pest(s) identified in Stage 1 require(s) a PRA. The categorization process examines for each pest whether the criteria in the definition for a quarantine pest are satisfied. In the evaluation of a pathway associated with a commodity, a number of individual PRAs may be necessary for the various pests potentially associated with the pathway. The opportunity to eliminate an organism or organisms from consideration before in-depth examination is undertaken is a valuable characteristic of the categorization process.*
> *An advantage of pest categorization is that it can be done with relatively little information; however information should be sufficient to adequately carry out the categorization.*

<u>Identify the pest (or potential pest)</u>

8. Is the organism clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

Yes. PepMV can be distinguished from other Potexviruses. The closest related Potexvirus species *Narcissus mosaic virus (*NMV) shares only 49% nucleotide sequence identity with PepMV, so that differentiation based on nucleotide sequence determination or specifically developed RNA based molecular tools is possible. In addition, PepMV specific poly- and monoclonal antibodies from different suppliers are commercially available.

Currently, four PepMV genotypes can be distinguished (Stijger and van der Vlugt, 2008; Hanssen *et al*. 2009b; Hanssen *et al*., 2010a):

- the Peruvian PepMV genotype (**LP**) which was first isolated from *Lycopersicon peruvianum* (Peruvian tomato) and is similar to the original pepino (*S. muricatum*) isolate (Lopez *et al*., 2005; Pagán *et al*., 2006);
- the European tomato genotype (**EU**), which was first reported in greenhouse tomato production in Europe (Mumford & Metcalfe, 2001; Aguilar *et al*., 2002; Cotillon *et al*., 2002; Verhoeven *et al*., 2003; Pagán *et al*., 2006);
- the **CH2** genotype, which was first isolated from tomato seeds from Chile (Ling, 2007);
- the **US1** genotype, which was first described in the United States (Maroon-Lango *et al*., 2005).

The EU and LP genotypes share a nucleotide sequence homology of 96% and cluster phylogenetically.

The CH2 genotype is rather different as it displays only 78 to 80% sequence homology with the EU and LP genotype groups.

The US1 genotype shares 78% sequence homology with CH2 and 82% with EU/LP genotypes.

As differences between genotypes at the nucleotide level are considerable, several molecular assays, including an RT-PCR-RFLP method, a TaqMan RT-qPCR method and a multiplex RT-PCR method combined with RFLP have been developed to discriminate these four PepMV genotypes (Hanssen *et al*., 2008; Gutiérrez-Aguirre *et al.*, 2009; Alfaro-Fernández *et al.,* 2009c).

Importantly however, these different genotypes cannot be distinguished based on biological characteristics, as biological differences between isolates from the same genotype can be considerable (Córdoba-Sellés *et al*., 2007; Hanssen *et al*., 2009b; Hasiów-Jaroszewska *et al*., 2009). Mild, moderate and aggressive isolates sharing over 99% sequence identity have been reported for both the EU and CH2 genotypes, indicating that minor differences at the viral genome level can account for considerable differences in symptomatology (Hanssen *et al*., 2009b; Schenk *et al*., 2010; Hasiów-Jaroszewska *et al*., 2009).

if yes indicate the correct scientific name and taxonomic position **Go to 10**
if no Go to 9

9. Even if the causal agent of particular symptoms has not yet been fully identified, has it been shown to produce consistent symptoms and to be transmissible?
if yes Go to 10
if no Go to 19

<u>Determining whether the organism is a pest</u>

10. Is the organism in its area of current distribution a known pest (or vector of a pest) of plants or plant products?
Yes. In the areas where it is known to occur it is a primary pathogen of tomato and pepino. It has also been detected on weed species in Spain, some of which were symptomatic. It has been found once affecting cultivated basil in Sicily but no other reports have been made and investigations have not confirmed the susceptibility of basil to PepMV (see 6.). Other plants that have been found infected are eggplant (Belgium, once) and potato (Peru) but these reports are uncommon (see 6.).

if yes, the organism is considered to be a pest **Go to 12**
if no Go to 11

11. Does the organism have intrinsic attributes that indicate that it could cause significant harm to plants?
Note: Some organisms may not be known to be harmful in their area of current distribution, but may nevertheless have the potential to become pests in the PRA area. This possibility may have to be considered in certain circumstances.

if yes or uncertain, the organism may become a pest of plants in the PRA area Go to 12
if no Go to 19

Presence or absence in the PRA area and regulatory status (pest status)

12. Does the pest occur in the PRA area?

Note: occurrence: the presence in an area of a pest officially recognized to be indigenous or introduced and/or not officially reported to have been eradicated [FAO, 1990; revised FAO, 1995; formerly occur]. This includes organisms which have been introduced intentionally and which are not subject to containment (notably cultivated plants). Organisms present for scientific purposes under adequate confinement (e.g. in botanic gardens) are not included.

Yes.

A full record of published findings of PepMV in the EU is given in Stage 1, initiation, question 7.

The first published reports of PepMV in the EU came from the Netherlands and the UK early in 1999 (Van der Vlugt *et al*., 2000; Wright and Mumford 1999). Publications from 2005 and 2006 suggests that in 1998 PepMV was already present in tomato in Spain (Soler *et al.*, 2005; Pagan *et al*., 2006). Up unto 2009, outbreaks had been reported in 19 out of the current 27 EU MS. Based on EC official surveys for PepMV, in the period 2007-2009 the virus has been found at tomato production sites in 17 out of the 27 MS (Anon., 2009b; 2010a).

In the Pepeira project a questionnaire among the partners has been held on the knowledge of the occurrence of the different strains (genotypes) in the different countries. An overview is given in Table 2. The EU and CH2 strains are the most predominant, followed by the LP strain, while the US1 has only been reported from Spain. It should be noted that this is just an indication (Pepeira final report WP1, 2010).

Table 2 Overview of strains (genotypes) of PepMV that were detected in the countries of the Pepeira partners (Y: Yes; N: No; ?: No information)

	Belgium	Bulgaria	Cyprus	Denmark	Estonia	France	Germany	Greece	Hungary	Italy	Netherlands	Norway	Poland	Portugal	Slovenia	Spain	United Kingdom	Total
Peruvian	Y	N	N	N	?	N	Y	N	N	N	Y	N	N	N	N	Y	N	4
EU-tomato	Y	N	N	N	?	Y	Y	N	Y	N	Y	N	Y	N	N	Y	Y	8
Chile-2	Y	N	Y	Y	?	Y	Y	N	N	Y	Y	N	Y	N	N	Y	Y	10
US1	N	N	N	N	?	N	N	N	N	N	N	N	N	N	N	Y	N	1

In Spain, weed species around tomato production sites have been found infected with PepMV (Jordá *et al.*, 2001a; Córdoba *et al*., 2004).

if yes **Go to 13**
if no Go to 14

13. Is the pest widely distributed in the PRA area?

Note: a quarantine pest may be 'present but not widely distributed'. This means that the pest has not reached the limits of its potential area of distribution either in the field or in protected conditions; it is not limited to its present distribution by climatic conditions or host-plant distribution. There should be evidence that, without phytosanitary measures, the pest would be capable of additional spread. If the pest is present but not widely distributed in the PRA area, it may already be under official control, with the aim of eradication or containment. If it is not already under official control and if the conclusion of this PRA is that it should be regulated as a quarantine pest, then the pest should also be placed under official control.

No. Although since 1999 findings of the virus have been reported in 19 of the current 27 EU MS (excluding in fruit and on interceptions), the number of reported outbreaks is relatively low. However, the virus is considered widely distributed in some of the main European tomato production areas (e.g. the Netherlands, Spain) as well as some that produce much less fruit (e.g. Cyprus where it was detected in 20 places of production out of 36 premises inspected in 2009 – Anon., 2010a). In other countries the virus has a very restricted distribution and usually is present only in some companies. It is not considered to have reached the limits of its potential area of distribution and without phytosanitary measures it has the potential for further spread. 'New' genotypes (e.g. US1) appear not to be widespread in the PRA area yet (See Table 2).

Some EU MS where PepMV was detected now claim the virus is absent, although survey data confirming this absence are not in all cases consistently available (see Table 1).

In several EU MS attempts have been made to eradicate PepMV by destroying all affected plants and controlling the distribution of potentially infected fruit from affected production sites.

if not widely distributed	**Go to 14**
if widely distributed	Go to 19

<u>Potential for establishment and spread in the PRA area</u>

For a pest to establish, it should find host plants or suitable habitat in the PRA area. Natural hosts should be of primary concern but, if such information is lacking, plants which are recorded as hosts only under experimental conditions or accidental/very occasional hosts may also be considered. The pest should also find environmental conditions suitable for its survival, multiplication and spread, either in natural or in protected conditions.

14. Does at least one host-plant species (for pests directly affecting plants) or one suitable habitat (for non parasitic plants) occur in the PRA area (outdoors, in protected cultivation or both)?

Note: if the PRA is conducted on a pest which indirectly affects plants through effects on other organisms, these organisms should also be present in the PRA area. Some pests require more than one host plant species to complete their life cycle and this should be taken into account when answering this question.

Yes.

<u>Tomato</u>

Tomato crops would seem to be most at risk in the PRA area. Tomato is grown in all EU countries and is an extremely valuable crop.

In 2008, 293,300 ha of tomato was grown in the 27 Member States of the EU resulting in a production of 16,187,454 tonnes of tomato fruit (FAO, 2009a).

Table 3. Tomato production statistics for EU Member States in 2008 (FAO, 2009a).

Member State	**Area harvested (ha)**	**Production (tonnes)**		**Member State**	**Area harvested (ha)**	**Production (tonnes)**
Austria	185	42109		Latvia	13	41
Belgium	500	200000		Lithuania	200	1300
Bulgaria	3474	134131		Luxembourg	1	85
Cyprus	330	33178		Malta	400	16600
Czech Republic	1202	27899		Netherlands	1500	720000
Denmark	50	20000		Poland	14640	702546
Estonia	200	7500		Portugal	13000	1100000
Finland	116	40467		Romania	51460	814376
France	4122	714635		Slovakia	2939	56585
Germany	308	65096		Slovenia	187	4704
Greece	25000	1338600		Spain	55300	3847800
Hungary	2400	206000		Sweden	50	16200
Ireland	30	12000		United Kingdom	216	88690
Italy	115477	5976912		**Total**	293300	16187454

The biggest producer based on these data was Italy. These data include both indoor and outdoor production. For example, indoor production in Italy in 2009 was 7,280 ha with a production of 516,260 tonnes (L. Tomassoli, ISPV, Italy *personal communication* to A.W. Werkman, PPS, the Netherlands, April 2010)

<u>Pepino</u>
Pepino (*Solanum muricatum*) is a host in Peru and China (Jones *et al*., 1980; Soler *et al*., 2002; Zhang *et al*., 2003). The most recent information is that there is no commercial production of pepino in Spain. There are some experimental greenhouses that are trialling the cultivation of pepino. Occasionally fruits harvested from the trials are sold at local markets. (J. Prohens, UPV, Spain, *personal communication* to A. Alfaro-Fernandez, UPV, Spain, February 2010).

<u>Potato</u>
In Peru, PepMV has been found in field-grown potato. However there is no information on whether the plants were symptomatic and whether there was any effect on yield or quality of the tubers. Thus, the importance of PepMV in potato production is not clear. Potato is an economically very important crop in the EU. In 2008, total production of the 27 Member States was 61,582,974 tonnes (FAO, 2009b).

<u>Basil</u>
Recently, basil (*Ocimum basilicum*) was reported to be a symptomatic natural host of PepMV in Italy (Davino *et al*., 2009) although the importance is not yet clear and subsequent investigations question its status as a natural host (see 6.). Basil is an important culinary herb and production takes place outdoors or under protection in the EU. In Italy, a total production of 504 tonnes was obtained from 174 ha, Sicily is less important with 40 tonnes being produced on 0.04 ha (L. Tomassoli, ISPV, Italy *personal communication* to A.W. Werkman, PPS, the Netherlands, April 2010)

<u>Weed species</u>
PepMV has been detected in weed species in Spain, some of which are symptomatic. However, the environment is not considered as being at risk as a result of PepMV.

if yes **Go to 15**
if no Go to 19

15. If a vector is the only means by which the pest can spread, is a vector present in the PRA area?
(if a vector is not needed or is not the only means by which the pest can spread ***go to 16****) Note: if a vector is the only natural means by which the pest can spread and when it is absent from the PRA area, a separate PRA to determine the risk of introduction of the vector may be needed.*

PepMV is highly contagious and can be spread by contact because it is mechanically-transmitted in sap. Therefore it does not require a vector for spread to occur.

However, in experiments bumblebees (*Bombus* spp.) have been shown to spread the virus in plastic houses in Spain and in greenhouses in Canada although the mechanism of transmission has not been determined (Lacasa *et al*., 2003; Shipp *et al.*, 2008). Bumblebees are widely used for pollination in tomato production. However, their role in transmission will be mainly limited to production places already infected by PepMV. In dense tomato production areas bumble bees might act as a vector for PepMV between greenhouses. Otterstatter and Thomson (2008) indicate that bumble bees disperse from greenhouses. The estimated foraging range of a bumble bee has been shown in one study to be a maximum of 758 m (Knight *et al.*, 2005).

Alfaro-Fernandéz (2009a) demonstrated the possibility of the fungal vector *Olpidium virulentus* to transmit PepMV. However, this might only play a role in spread inside an infected production facility.

Since isolated populations of wild *Lycopersicon* species in Peru have been found naturally infected, it is thought that spread may be due to some unknown vector (Soler *et al.*, 2002). In Peru, no evidence was found for transmission by the aphid *Myzus persicae* (Jones *et al.*, 1980).

Since the ease of mechanical transmission of PepMV has been demonstrated, it has been suggested that other animals (i.e. insects, birds, rodents) might play a role in spread of the virus, however, there are no published reports to support this theory.

if yes **Go to 16**
if no Go to 19

16. Does the known area of current distribution of the pest include ecoclimatic conditions comparable with those of the PRA area or sufficiently similar for the pest to survive and thrive (consider also protected conditions)?

Yes. PepMV is already in the PRA area (the EU) and the virus is known to survive and thrive here under protected as well as field conditions, so the ecoclimatic conditions are suitable for the virus in the PRA area.

if yes or uncertain **Go to 17**
if no Go to 19

Potential for economic consequences in PRA area.

17. With specific reference to the plant(s) or habitats which occur(s) in the PRA area, and the damage or loss caused by the pest in its area of current distribution, could the pest by itself, or acting as a vector, cause significant damage or loss to plants or other negative economic impacts (on the environment, on society, on export markets) through the effect on plant health in the PRA area?

Note: "through the effect on plant health" means that the organism should have a direct or indirect effect on plants. ISPM n° 11 states that "*Environmental effects and consequences considered should result from effects on plants. Such effects, however, on plants may be less significant than the effects and/or consequences on other organisms or systems. For example, a minor weed may be significantly allergenic for humans or a minor plant pathogen may produce toxins that seriously affect livestock. However, the regulation of plants solely on the basis of their effects on other organisms or systems (e.g. on human or animal health) is outside the scope of this standard. If the PRA process reveals evidence of a potential hazard to other organisms or systems, this should be communicated to the appropriate authorities which have the legal responsibility to deal with the issue."*

Yes. PepMV is a recognised plant pest which has caused losses in tomato crops.

The 2005 joint Dutch/UK PRA and Datasheet presented a range of experimental data, observations and personal communications on the impact of PepMV on the yield and quality of tomato fruit and no consensus on the impact was reached at that time. To estimate the effect of PepMV on yield and quality several trials have been performed. In the PEPEIRA project it was shown in field trials that both yield reductions and downgrading in quality because of fruit symptoms can occur (Pepeira final report WP2, 2010).

if yes or uncertain	**Go to 18**
if no	Go to 19

Conclusion of pest categorization

18. This pest could present a phytosanitary risk to the PRA area (Summarize the main elements leading to this conclusion)

PepMV has been known to occur in the PRA area since 1999 when it was found affecting tomato crops in the UK and the Netherlands. It is known to cause quality losses in tomato crops and has been reported to cause yield losses in some instances. It has been subject to EC emergency phytosanitary legislation since 2000 (Anon., 2000) and is currently subject to legislation that was revised in 2004 (Anon., 2004). Excluding interceptions it has been recorded in 19 Member States in total with some Member States having taken eradication action. In the period 2007 to 2009 it was reported in 17 EU MS. (See Table 1). However, not all MS appear to have reported the results of their surveys to the EC and there is some variation in the breadth of reporting between MS and years. The EC emergency legislation covers the risk of spread by the movement of seeds of tomato. This legislation is due to be reviewed in 2010 in part based upon the findings of the Pepeira project including this PRA.

PepMV is also listed on the EPPO Alert List (http://www.eppo.org/QUARANTINE/Alert_List/alert_list.htm, 1st of February 2010).

More details are given in Section B of the Risk Assessment stage of this PRA.

Go to section B

19. The pest does not qualify as a quarantine pest for the PRA area and the assessment for this pest can stop (summarize the main reason for stopping the analysis).

There should be clear indications that the pest is likely to have an unacceptable economic impact in the PRA area. Unacceptable economic impact is described in ISPM No. 5 Glossary of phytosanitary terms, Supplement No. 2: Guidelines on the understanding of potential economic importance and related terms. Climatic and cultural conditions in the PRA area should be considered to decide whether important economic (including environmental or social) damage or loss to plants may occur in the PRA area. The effect of the presence of the pest on exports from the PRA area should also be allowed for. In some cases, the pest may only be potentially harmful, as suggested by its intrinsic attributes.

For a pathway analysis, go to 4 and proceed with the next pest. If no further pests have been identified the PRA may stop at this point.

Section B: Assessment of the probability of introduction and spread and of potential economic consequences

Note
During pest categorization (Section A), the assessor may have identified factors which have a major influence on the overall evaluation (e.g. the climatic conditions for establishment appear to be critical). In such situations it is recommended that the assessor first considers the questions in section B that are relevant to these factors. Based on the evaluation of such questions, and if the conclusion is that the risk is very low or low, it may not be necessary to answer other parts of the scheme.
This part of the risk assessment process firstly estimates the probability of the pest being introduced into the PRA area (its entry and establishment) and secondly makes an assessment of the likely economic impact if that should happen. From these assessments, it should be possible to estimate the level of risk associated with the pest, which can then be used in the pest risk management phase to determine whether it is necessary to take phytosanitary measures to prevent the introduction of the pest, and if the measures chosen are appropriate for the level of risk.
The evaluation is based on the replies to a series of questions, mostly expressed in the first instance as the choice of an appropriate phrase out of a set of five alternatives (e.g. very unlikely, unlikely, moderately likely, likely, very likely). It is important to identify especially high or especially low risks. The user of the scheme should add to all replies any details which appear relevant indicating the source of information used. In addition the level of uncertainty attached to each answer should be given. Note that for the time being, no specific methods for combining scores neither for quantifying uncertainty are recommended; such methods are being developed in the framework of a European project (PRATIQUE).
Answer as many of the following questions as possible. If any question does not appear to be relevant for the pest concerned, it should be noted as "irrelevant". If any question appears difficult to answer no judgement should be given but the user should note whether this is because of lack of information or uncertainty.

1. Probability of introduction and spread

Note: Introduction, as defined by the FAO Glossary of Phytosanitary Terms, is the entry of a pest resulting in its establishment.

Probability of entry of a pest

<u>Identification of pathways</u>

Note: Pathway is defined in the Glossary as "any means that allows the entry or spread of a pest" [FAO, 1990; revised FAO, 1995].
Pathways can be identified principally in relation to the geographical distribution and host range of the pest. Consignments of plants and plant products moving in international trade are the principal pathways of concern and existing patterns of such trade will, to a substantial extent, determine which pathways are relevant. Other pathways such as other types of commodities, packing materials, persons, baggage, mail, conveyances and the exchange of scientific material should be considered where appropriate. Entry by natural means should also be assessed, as natural spread is likely to reduce the effectiveness of phytosanitary measures.
Closed pathways may also be considered, as the pests identified may support existing phytosanitary measures. Furthermore, some pathways may be closed by phytosanitary measures which might be withdrawn at a future date. In such cases, the risk assessment may need to be continued. Data on detections in imported consignments may indicate the ability of a pest to be associated with a pathway. For a PRA initiated by the identification of a pathway, this is the main pathway to be considered.
If the PRA is being conducted on a pest that is intentionally imported, e.g. a plant for planting or a biological control agent, and this is the only pathway of entry, an

assessment of its entry potential is not required. However, it is still important to record the volume, frequency and distribution of imports (the assessor should answer the following questions of the scheme: 1.5, 1.6, 1.10 and 1.13). If other pathways of entry also exist, these should be assessed following standard procedures. Spread from the intended habitat to the unintended habitat which is an important judgement for intentionally imported plants is covered by questions 1.30 to 1.32.

1.1. Consider all relevant pathways and list them.

Note: Relevant pathways are those with which the pest has a possibility of being associated (in a suitable life stage), on which it has the possibility of survival, and from which it has the possibility of transfer to a suitable host. Make a note of any obvious pathways that are impossible and record the reasons.

Pepino mosaic virus is most likely to <u>enter</u> the PRA area (EU 27 Member States) from the pathways listed below. More detail on the significance of each pathway is given in question 1.2.

For PRA purposes, this question normally tackles the assessment of the risk of entry on pathways originating <u>outside</u> of the PRA area. However, the pathways are relevant to both entry of PepMV <u>into</u> the PRA area as well as to <u>spread within</u>. Because PepMV has been recorded in the PRA area (in 19 out of the 27 EU MS) we need to determine the most appropriate risk management options to try to control movement of the virus both within and between MS. For this reason EPPO have agreed that the pathways of spread within the PRA area should also be listed here with risk management options being determined in the same way as for pathways of entry (F. Petter, EPPO, France, *personal communication* to C. Sansford, Fera, UK, November 2009).

The main commodities on which PepMV could be moved are fruit, seed and plants of tomato; also, plants and fruit of pepino. True seed of potato and pepino are not considered as it is not known whether the virus is transmitted via seed of these hosts and true seed of potato is prohibited entry into the EU. Tubers and plants of potato are also not considered for the same reasons. Although basil recently has been reported as a natural host, it is not further considered as a pathway since further investigations have indicated the status of basil as a natural host is doubtful. Since more information is needed on the possible role of eggplant in the epidemiology of PepMV this pathway is also not considered further.

Countries of origin for pathways of entry are those non-EU countries where PepMV has been reported (albeit the current status in some of these countries is unknown). These are listed in Table 1 (Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China). Countries where there are no official reports but from which PepMV has been intercepted (e.g. India, Israel, Thailand etc.) are not included in this analysis. The most likely countries of origin for pathways of spread within the PRA area are those EU countries where the virus has been reported during the official surveys in the period 2007-2009 (17 out of 27 MS) unless the NPPO has confirmed that the virus has been eradicated. The 17 countries are: Austria, Belgium, Cyprus, Czech Republic (considered eradicated), Denmark, France, Germany, Hungary, Ireland, Italy, the Netherlands, Poland, Romania, Slovak Republic, Spain, Sweden and the United Kingdom. All EU countries have been assessed for the probability of association with the pathway at origin (1.3) and as appropriate in response to the remaining questions in this section of the PRA.

The main potential pathways of entry are as follows:

i. Tomato fruit originating from non-EU countries where PepMV has been reported: Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China.

ii. Seed of tomato originating from non-EU countries where PepMV has been reported: Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China. Currently, seed of tomato is allowed to enter and move within the EU provided it has been subjected to acid-extraction or an equivalent measure (point 48 Annex IVAI and point 27 Annex IVAII of the EC Plant Health Directive; Anon., 2000).

iii. Plants for planting of tomato originating from non-EU countries where PepMV has been reported. Only non-EU European and Mediterranean countries are considered since import of plants of the Solanaceae from other third countries is prohibited (Point 13 of Annex IIIA of the EC Plant Health Directive; Anon., 2000). Thus Canary Islands, Norway, Switzerland and Morocco are the only countries considered here.

iv. Plants for planting of pepino originating from non-EU countries where PepMV has been reported – as for tomato this will only be from non-EU European and Mediterranean countries, i.e. Canary Islands, Norway, Switzerland and Morocco, due to the EC phytosanitary requirements for Solanaceae.

v. Fruits of pepino originating from non-EU countries where PepMV has been reported: Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China.

vi. Human assistance e.g. contaminated packaging materials related to import and spread by contact from non-EU countries where PepMV has been reported: Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China.

vii. Insect vectors (bumble bees) imported from non-EU countries where PepMV has been reported: Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China.

The same pathways are considered for the risk of movement between EU MS and within MS:

viii. Tomato fruit
ix. Seed of tomato
x. Plants for planting of tomato
xi. Plants for planting of pepino
xii. Fruit of pepino
xiii. Human assistance e.g. contaminated packaging materials related to import and spread by contact
xiv. Insect vectors (bumble bees)

Go to 1.2

1.2. Select from the relevant pathways, using expert judgement, those which appear most important. If these pathways involve different origins and end uses, it is sufficient to consider only the realistic worst-case pathways. The following group of questions on pathways is then considered for each relevant pathway in turn, as appropriate, starting with the most important.

Direct principal pathways of entry:

i. Tomato fruit originating from non-EU countries where PepMV has been reported: Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China. PepMV is known to occur in high concentrations in fruit of tomato. Tomato fruit is regularly imported from some third countries where PepMV is known to be present. Also large quantities of fruit are traded within the EU (pathway viii. below). Tomatoes are traded as pre-packed tomatoes or bulk/unpacked tomatoes. Since sorting of tomato sometimes takes place at tomato production sites there is a possibility of spread of PepMV from imported and traded fruit to tomato crops by contact transmission.

ii. Seed of tomato originating from non-EU countries where PepMV has been reported: Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China. Currently, seed of tomato is allowed to enter and move within the EU provided it has been subjected to acid-extraction or an equivalent measure (point 48 Annex IVAI and point 27 Annex IVAII of the EC Plant Health Directive; Anon., 2000). Although seed transmission has only been found to occur in experiments under conditions that might not be directly comparable to the common practice of seed companies and without normal phytosanitary treatments, it is considered to be a potential pathway of entry. If seeds are harvested from PepMV-infected plants the seed may be contaminated. If a seedling raised at a propagation nursery from contaminated seed becomes infected, there is a risk that once moved to a place of production the crop and fruit may become infected. Most tomato seed sown in the EU is produced in third countries (e.g. China, Thailand, India, Chile) and shipped to the EU in bulk. Although acid-extraction is considered to eliminate virus present on the seed there have been several interceptions of contaminated seed lots from third countries. This pathway has a potential for further introduction of PepMV including the introduction of new variants.

iii. Plants for planting of tomato originating from non-EU countries where PepMV has been reported. Only the European and Mediterranean countries are considered (Canary Islands, Norway, Switzerland and Morocco) since import from third countries is prohibited (Point 13 of Annex IIIA of the EC Plant Health Directive; Anon., 2000).

Less significant direct or indirect pathways of entry:

iv. Plants for planting of pepino from non-EU countries where PepMV occurs. As for tomato this will only be from non-EU European and Mediterranean countries i.e. Canary Islands, Norway, Switzerland and Morocco, due to the EC phytosanitary requirements for Solanaceae. This pathway is considered to be less important because PepMV has only been reported from pepino in Peru (Jones *et al*., 1980; Soler *et* al., 2002; Zhang *et al*, 2003). Records in Norway, Switzerland and Morocco were on tomato. Pepino is not commonly-grown in Europe; trials have been undertaken in Mediterranean countries including Spain; Spain is also reported to have cultivated it on a small-scale (EPPO, 2000) but it is not commercially-grown. It is not known to be grown in Norway or Switzerland. It was introduced to Morocco in 1952 and planted in the south of the country. Harvested fruits were shipped to France and England (Prohens *et al*., 1996). The current status of pepino as a crop in Morocco is not known. According to Prohens *et al.* (1996), Spain shipped seeds of pepino to Tenerife (Canary Islands) in 1788; the plant adapted well to the environment there where it is still grown, however, its status as a crop is unknown. This pathway is not considered further.

v. Fruit of pepino from non-EU countries where PepMV occurs: Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China. This pathway of entry into the EU is currently considered to be less important because information is lacking on possible infestation of fruit or on possible transmission from the frui*ts.*
This pathway is therefore not considered further.

vi. Human assistance e.g. contaminated packaging materials related to imports of fruit from countries where PepMV occurs: Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China. For entry of the virus into the PRA area from third countries this pathway is minor and is only related to contamination from the import of affected tomato fruit.
Because fruit itself is considered to be a pathway and is *consi*dered further in this PRA, including for risk management options, this pathway is not considere*d furt*her.

vii. Insect vectors (bumble bees) imported from countries where PepMV occurs: Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China. Bumblebees have been shown by experiment to spread the virus in plastic houses in Spain and in greenhouses in Canada although the mechanism of transmission has not been determined (Lacasa *et al*., 2003; Shipp *et al*., 2008). Bumblebees are widely used for pollination in tomato production. There are imports of bumblebees into the EU but there are controls in place related to the health of the bees (honey bees and bumble bees). Bees are only allowed into the EU from certain countries (Council Decision 79/542/EEC). Interpretation of a summary of this legislation by Fera (2009) suggests that all countries where PepMV has been reported are eligible to export bumblebees to the EU except Peru, Ecuador, Canary Islands and Norway. However, according to Fera (2009) only Argentina, Australia, New Zealand and the US State of Hawaii can meet the Decision requirements with respect to bee health. So there appears to be no pathway of entry from the countries where PepMV has been reported.
This pathway is not considered further.

Direct principal pathways of movement between EU MS and spread within:

viii. Tomato fruit from EU MS where PepMV has been reported: PepMV is known to occur in high concentrations in fruit of tomato. Large quantities of tomato fruit are traded within the EU. Tomatoes are traded as pre-packed tomatoes or bulk/unpacked tomatoes. Since sorting of tomato sometimes takes place at or close to tomato production sites there is a possibility of spread of PepMV from fruit produced and traded within the EU to tomato crops by contact transmission.

ix. Seed of tomato from EU MS where PepMV has been reported: Currently, seed of tomato is allowed to move within the EU provided it has been subjected to acid-extraction or an equivalent measure (Point 48 Annex IVAI and Point 27 Annex IVAII of the EC Plant Health Directive; Anon., 2000). Although seed transmission has only been found to occur in experiments under conditions that are not directly comparable to the common practice of seed companies and without normal phytosanitary treatments, it is considered to be a potential pathway of entry. If seeds are harvested from PepMV-infected plants the seed will be contaminated. If a seedling raised at a propagation nursery from contaminated seed becomes infected, there is a risk that once moved to a place of production the crop and fruit may become infected.

x. Plants for planting of tomato from EU MS where PepMV has been reported: Most plants are raised within a Member State, however plants are traded between MS.

Less significant direct or indirect pathways of movement between EU MS and spread within:

xi. Plants for planting of pepino - pepino is cultivated experimentally on a small-scale in Spain but there are no major areas of cultivation in the EU and there is no known trade in plants or cuttings between EU countries.
This pathway is therefore not considered further.

xii. Fruit of pepino – this pathway is currently considered to be less important because information is lacking on possible infestation of fruit or on possible transmission from the fruits.
This pathway is therefore not considered further.

xiii. Human assistance e.g. contaminated packaging materials related to import of fruit and spread by contact. Because fruit itself is considered to be a pathway and is considered further in this PRA including for risk management options this pathway is not considered further.

xiv. Insect vectors - bumble bees are commercially-produced on a diet of sugar-water and pollen (C. Jilesen, PPS, The Netherlands, *personal communication* to A.W. Werkman, PPS, The Netherlands, 2010) which makes the pathway less significant. This is a potential pathway of spread but only where bumble bees that have been used to pollinate tomato crops in one EU MS are moved intentionally or spread naturally to tomato crops in the same MS or to other EU MS.
This pathway is considered further.

Go to 1.3

Probability of the pest being associated with the individual pathway at origin.

1.3. How likely is the pest to be associated with the pathway at origin taking into account factors such as the occurrence of suitable life stages of the pest, the period of the year?

very unlikely, unlikely, moderately likely, likely, very likely.

Level of uncertainty:	**Low**	**Medium**	**High**

For the purposes of this PRA the exact distribution of PepMV and incidence in both EU and non-EU countries where PepMV has been reported is not known (see question 7). With few exceptions this makes judgements on the likelihood of pest association with the pathway at origin highly speculative. Recent official surveys from some of the EU MS have been used to try to respond to this question. In order to give as objective an assessment as possible using the available data, the main countries of origin for pathways of spread within the PRA area are those EU countries where the virus has been found during the official surveys in the three year period 2007-2009 (17 out of 27 MS) unless the NPPO has confirmed that the virus has been eradicated. In the Czech Republic PepMV was reported in 2008 at a plant producer, but was stated to be eradicated. The 16 other countries are: Austria, Belgium, Cyprus, Denmark, France, Germany, Hungary, Ireland, Italy, the Netherlands, Poland, Romania, Slovak Republic, Spain, Sweden and the United Kingdom.

For the remaining ten EU Member States the known situation with respect to PepMV depends upon the survey reports that are available. Official surveys have been required since PepMV was first subject to EC legislation in 2000. Several of these countries have acceded to the EU fairly recently and so may not have undertaken surveillance prior to accession. This is summarised in the table below:

Table 4. Summary of official survey reports for 2007 and 2008 (source Anon., 2009b) and 2009 (source 2010a) and previous reports (Table 1) for EU MS where PepMV has <u>not</u> been reported between 2007-2009

	Categories surveyed*			**Previous status****
Country	**2007**	**2008**	**2009**	
Bulgaria	All	All	All	One finding, 2004, eradication undertaken. Joined EU 2007.
Estonia	FP	FP, PfP ***	FP	No previous reports Joined EU 2004.
Finland	PfP	All	PfP, FP	Found in 2001 and 2003, eradication undertaken.
Greece	None	All	PfP, FP, FM	No previous reports.
Latvia	PfP, FP	None	PfP, FP, FM	No previous reports. Joined EU 2004.
Lithuania	All	All	All	Interception only (2003). Joined EU 2004.
Luxembourg	None	None	None	No previous reports
Malta	FP, FM	None	All	No previous reports. Joined EU 2004.
Portugal	S, PfP, FP	All	S, PfP, FM	No previous reports
Slovenia	S, PfP, FP	All	All	No previous reports. Joined EU 2004.

*Seed - S, plants for planting - PfP, fruit production sites - FP, marketed fruits – FM
** From Table 1.
*** Anon., 2010b

Luxembourg is the only EU MS for which there are no survey reports for the past 3 years and so there is a high level of uncertainty associated with the status of PepMV in this country. Luxembourg is not one of the major producing countries for tomato in the EU but it may export some of its crop. Production data for 2008 (see Table 3) shows that Luxembourg only produced 85 tonnes of tomato fruit from 1ha of crop. Production in 2007 amounted to 100 tonnes (FAO, 2009a), but strangely, exports of tomato fruit in 2007 (2008 not available) exceeds this amount at 349 tonnes (FAO, 2009b). Which of these figures is correct is uncertain.

Bulgaria and Lithuania have reported on all 4 categories during the period 2007-2009 (Table 4. This, along with the situation with respect to earlier findings can be considered to lower the risk (with low uncertainty) with respect to PepMV being associated with pathways of entry. Presumed absent with low uncertainty.

The remaining EU MS have reported on some surveillance in the past 3 years reducing the uncertainty associated with the current status of PepMV.

The likelihood of association of PepMV with each of the pathways is summarised in Tables 5 from third countries) and 6 from EU MS). Although the risk analysts have tried to give as objective an assessment of the data available, it is acknowledged that individual MS may have a different perspective based upon their experience. This is not easily accounted for in a PRA for the whole of the EU.

<u>Pathway i: Tomato fruit from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China</u>
<u>Pathway viii: Tomato fruit from EU MS</u>
Most reports on PepMV are on tomato plants and fruit harvested from these plants. If the virus is present at a site it is most likely infecting tomato plants and therefore likely to be associated with tomato fruit. The likelihood of association of PepMV with tomato fruit will vary according to geographical origin.

In Canada and the USA there are several published reports and a survey in 2006 indicates the presence of PepMV in two provinces in Canada (British Colombia and Ontario) and five states in the USA (Alabama, Arizona, California, Colorado and Texas) (Ling *et al*., 2008).

For Canada and the USA the probability of association with tomato fruit is likely (from the affected areas) with high uncertainty.

For the Canary Islands there are several findings reported in the period 2003-2008 at fruit production sites on Gran Canaria and Tenerife (Alfaro-Fernandez *et al.*, 2009b). The UK detected PepMV in fruit imported from the Canary Islands in 2008 and 2009 (UK NPPO, 2008 and 2009). Sweden detected PepMV in marketed fruit in the 2009 survey including in fruit from Gran Canaria (Anon., 2010a). The probability of association with tomato fruit from the Canary Islands is very likely with low uncertainty.

For Switzerland there are few reports in different areas (EPPO, 2009) and the probability is estimated moderately likely with high uncertainty.

For Guatemala, Peru, Chile, Ecuador, Ukraine, Morocco and China there are only initial published records on pest status and the current status is unclear. However, the UK detected PepMV in fruit imported from Morocco in 2008 and 2009 (UK NPPO, 2008 and 2009); also Poland detected fruit with PepMV from Morocco in 2009 (Anon., 2010a). Therefore, with the exception of Morocco the probability of association with tomato fruit is estimated as moderately likely with high uncertainty. For Morocco the probability of association is estimated as likely with low uncertainty.

In Norway the current status is absent, found once (in 2001) and eradicated (EPPO, 2009). The probability of association with tomato fruit is therefore estimated as very unlikely with low uncertainty.

In the PRA area the range of distribution of PepMV at fruit production sites varies considerably and some of the countries which have recently joined the EU have obviously only just started reporting on official surveys. The probability of association is estimated as unlikely to very likely with a range of uncertainties according to the information that is available to complete the PRA.

Probability of association
(Non-EU countries): Very unlikely – Very likely / Uncertainty: Variable
(EU MS): Unlikely – very likely / Uncertainty: Variable

<u>Pathway ii: Seed of tomato from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China</u>
<u>Pathway ix: Seed of tomato from EU MS</u>
There has been an abundance of experimental work and discussion on the likelihood of true seed transmission of PepMV in tomato seed and this has to be considered when determining the likelihood of association of PepMV with seed as well as under question 1.12 (*How likely is the pest to be able to transfer from the pathway to a suitable host or habitat*?).

The association with seed and the mode of transmission is not consistently understood. Ling (2008) performed a study on virus location in tomato seeds. He detected the presence of PepMV in the seed coat fraction, but not in the embryo. During growing-out assays with contaminated seed (10,000 seedlings) no PepMV infection was observed, but inoculation of seed extract onto plants of *Nicotiana benthamiana* resulted in infection. This indicates a possible risk of spread of PepMV by seed, via mechanical transmission. Also, Córdoba-Selles *et al.* (2007) suggest that during germination, tomato seedlings can be inoculated by virus present on the seed coat.

Some reports have shown that seeds are contaminated but when the seed is subjected to growing-out assays PepMV was not transmitted to the seedlings (Salomone & Roggero, 2002; Ling & Carpenter, 2005; Ling, 2008). In other studies seed transmission has been demonstrated (Krinkels, 2001; Córdoba-Selles *et al*., 2007; Hanssen *et al,* 2010a). In these studies, rates of transmission were up to 2%, depending upon the seed cleaning and disinfection methods applied.

In the EU PEPEIRA project a large trial was performed with more than 87,000 tomato seedlings grown from seeds harvested from infected fruit. The seeds were pectinase-extracted, but acid extraction was not performed in order to try to determine the likelihood of seed transmission in the absence of phytosanitary measures. In this experiment an overall low level of transmission (0.026%) was obtained. However, the observed seed transmission rates varied from 0.005% to 0.057%, depending on the seed batch used. Results showed that PepMV can be transmitted from seeds contaminated with virus to seedlings albeit at a low rate (Hanssen *et al*., 2010a).

Official EU surveys have detected PepMV in seed (see Annex 1). However, because of the relatively low transmission rates and the likelihood that PepMV is a contaminant of the seed coat (i.e. not present in the embryo), the probability of association of PepMV with tomato seed is (with the exception of Norway) considered unlikely to moderately likely with the association dependent upon the geographical origin of the seed and with high uncertainty. Our knowledge on the status of PepMV in seed by origin is considered to be similar to that for fruit (pathways i and viii).

Probability of association
(Non-EU countries): Very unlikely to moderately likely / Uncertainty: Low to high
(EU MS): Unlikely to moderately likely / Uncertainty: High

<u>Pathway iii: Plants for planting of tomato from Canary Islands, Norway, Switzerland and Morocco</u>
<u>Pathway x: Plants for planting of tomato from EU MS</u>
Since tomato plants can become infected at an early stage, PepMV can be associated with plants for planting but this will depend upon either the seed that is used to produce the seedlings being contaminated, or poor hygiene at the propagation nursery leading to carry-over where outbreaks of PepMV have already occurred.

From outside the EU only the Canary Islands, Norway, Switzerland, and Morocco are considered. In Norway the status of PepMV is considered absent while in Switzerland there are only few reports (see question 7). Therefore the probability of association of PepMV with plants for planting from these countries is scored as very unlikely with low uncertainty for Norway, and unlikely with medium uncertainty for Switzerland. In Morocco, the status of PepMV has not been reported since 2002 when it was first reported to have been introduced. Tomato fruit from Morocco was found infected in EU MS surveys in 2008 (UK) and 2009 (UK and Poland). It is assumed that plants for planting of tomato from Morocco are likely to be infected with high uncertainty. Similarly because of reports of interceptions of PepMV on tomato fruit from the Canary Islands, it is assumed that plants for planting from there are also likely to be infected, but with high uncertainty.

In the PRA area the status of PepMV at propagation nurseries is required to be recorded as part of the official surveys.

The official surveys for the UK in 2008 and 2009 did not detect PepMV in seedlings (63 and 67 premises inspected, some more than once with 10 and 21 samples taken respectively and no PepMV found), UK NPPO (2008 and 2009). The 2007 overview of official surveys for the EU MS has limited details; but France, the Netherlands and Spain detected PepMV in plants for planting produced in these countries; in 2008 only details from 12 MS were presented (this does not include France, the Netherlands or Spain) and of these none reported PepMV in plants for planting (Anon., 2009b). In the 2009 MS surveys a total of 9548 samples originating from 1760 premises have been tested. Four outbreaks were reported. Two from Belgium, one from Denmark and one from the Netherlands (Anon., 2010a).

The probability of association with plants for planting (tomato) from the EU is estimated as unlikely to moderately likely with medium uncertainty according to the information that is available to complete the PRA.

Probability of association
(Non-EU countries): Very unlikely - Likely / Uncertainty: low to high
(EU MS): Unlikely to moderately likely / Uncertainty: medium

Pathway xiv: Insect vectors (bumble bees) from EU MS
Bumblebees that are commercially-produced for pollination of crops of tomato are reared on a diet of pollen and sugar water. (C. Jilesen, Netherlands, *personal communication*, 2010). For this reason they are very unlikely to be associated with PepMV.

Probability of association
(EU MS): Very unlikely / Uncertainty: low

Go to 1.4

Table 5. (a) Estimated likelihood of PepMV being associated with tomato fruit, seed and plants for planting at the origin of the pathway outside of the EU in the absence of phytosanitary measures, ranked according to the following scheme:
VU, Very unlikely; **U**, Unlikely; **ML**, Moderately likely; **L**, Likely; **VL**, Very Likely;
(b) associated levels of uncertainty : **H** = High, **M** = Medium, **L** = Low.

(a) Estimated likelihood of PepMV being associated with commodity pathways originating outside of the EU												
Commodity pathway	***Canada ****	***USA*****	***Guatemala***	***Peru***	***Chile***	***Ecuador***	***Canary Islands***	***Norway***	***Switzerland***	***Ukraine***	***Morocco***	***China***
(i) Tomato fruit	L	L	ML	ML	ML	ML	VL	VU	ML	ML	L	ML
(ii) Seed of tomato	U-ML	U-ML	U-ML	U-ML	U-ML	U-ML	U-ML	VU	U-ML	U-ML	U-ML	U-ML
(iii)Plants for planting of tomato***	-	-	-	-	-	-	L	VU	UL	-	L	-

(b) Estimated levels of uncertainty for the estimated likelihood of PepMV being associated with commodity pathways originating outside of the EU												
Commodity pathway	***Canada ****	***USA*****	***Guatemala***	***Peru***	***Chile***	***Ecuador***	***Canary Islands***	***Norway***	***Switzerland***	***Ukraine***	***Morocco***	***China***
(i) Tomato fruit	H	H	H	H	H	H	L	L	H	H	L	H
(ii) Seed of tomato	H	H	H	H	H	H	H	L	H	H	H	H
(iii)Plants for planting of tomato***	-	-	-	-	-	-	H	L	M	-	H	-

* British Columbia, Ontario
** Alabama, Arizona, California, Colorado, Texas
*** Only permitted from the Canary Islands, Norway, Switzerland and Morocco

Table 6. (a) Estimated likelihood of PepMV being associated with tomato fruit, seed and plants for planting, and bumblebee packages at the origin of the pathway within the EU ranked according to the following scheme: **VU**, Very unlikely; **U**, Unlikely; **ML**, Moderately likely; **L**, Likely; **VL**, Very Likely; (b) **H** = High, **M** = Medium, **L** = Low. In order to give as objective an assessment of available data as possible, the estimation for tomato is based on the survey reports of EU MS in the period 2007-2009. If during the period the virus has not been found the estimation is U. If the virus has been found during these three years the estimation is VL or L for fruit, and U to ML for seed and plants. This all with a Medium to High uncertainty.

(a) Estimated likelihood of PepMV being associated with commodity pathways originating within the EU

Commodity pathway	***AT***	***BE***	***BG***	***CY***	***CZ***	***DK***	***EE***	***FI***	***FR***	***DE***	***EL***	***HU***	***IT***	***IE***	***LV***	***LT***	***LU***	***MT***	***NL***	***PL***	***PT***	***RO***	***SK***	***SI***	***ES***	***SE***	***UK***
(viii) Tomato fruit	L	L	U	L	ML	L	U	U	L	L	U	L	L	L	U	U	U	U	VL	L	U	L	L	U	VL	L	L
(ix) Seed of tomato	U-ML	U-ML	U	U-ML	U-ML	U-ML	U	U	U-ML	U-ML	U	U-ML	U-ML	U-ML	U	U	U	U	U-ML	U-ML	U	U-ML	U-ML	U	U-ML	U-ML	U-ML
(x)Plants for planting of tomato	U-ML	U-ML	U	U-ML	U-ML	U-ML	U	U	U-ML	U-ML	U	U-ML	U-ML	U-ML	U	U	U	U	U-ML	U-ML	U	U-ML	U-ML	U	U-ML	U-ML	U-ML
(xiv) Bumblebee packages	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU	VU

(b) Estimated levels of uncertainty for the estimated likelihood of PepMV being associated with commodity pathways originating within the EU

Commodity pathway	*AT*	*BE*	*BG*	*CY*	*CZ*	*DK*	*EE*	*FI*	*FR*	*DE*	*EL*	*HU*	*IT*	*IE*	*LV*	*LT*	*LU*	*MT*	*NL*	*PL*	*PT*	*RO*	*SK*	*SI*	*ES*	*SE*	*UK*
(viii) Tomato fruit	M	M	M	M	M	M	M	M	M	M	H	M	M	M	H	M	H	H	L	M	M	M	H	M	M	M	M
(ix) Seed of tomato	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
(x)Plants for planting of tomato	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
(xiv) Bumblebee packages	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L

1.4. How likely is the concentration of the pest on the pathway at origin to be high, taking into account factors like cultivation practices, treatment of consignments?

Note: these are practices mainly in the country of origin, such as plant protection product application (including herbicides for plants), use of specific cultivars, removal of substandard produce, kiln-drying of wood, cultural methods, sorting and cleaning of commodities. Note that cultivation practices may change over time. Phytosanitary measures are not considered in this question (see 1.9).

Impossible**/very unlikely, unlikely, moderately likely, likely, very likely.**

Level of uncertainty:	**Low**	**Medium**	High

In this PRA a '*high concentration*' is interpreted as the portion of the commodity that is infected, for example the percentage infected fruit, plants or seeds.

For this and the questions that follow in this section it is assumed that the commodities are coming from areas of countries where PepMV is present.

<u>Pathway i: Tomato fruit from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China</u>
<u>Pathway viii: Tomato fruit from EU MS</u>
Because PepMV is mechanically transmitted, planting, pruning and fruit-picking are activities that can easily spread the virus through the crop at tomato fruit production sites, resulting in more infected fruit. To prevent introduction and spread of PepMV and other harmful organisms it is normal good practice for hygiene protocols to be used. If these are not deployed, without changing clothes, PepMV may spread between glasshouses by personnel moving between crops within a few days of working with an infected crop. The concentration of PepMV strongly depends on the crop handling practices and hygiene at the production site.
Likelihood of the concentration of the pest being high
(Non-EU countries): Likely to very likely / Uncertainty: medium
(EU MS): Likely to very likely / Uncertainty: medium

<u>Pathway ii: Seed of tomato from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China</u>
<u>Pathway ix: Seed of tomato from EU MS</u>
In the absence of phytosanitary measures, various treatments that are used to harvest and clean tomato seed have been shown to efficiently reduce the seed transmission rate of PepMV. Córdoba-Selles *et al*. (2007) showed that the virus was '*largely eradicated*' by immersing affected seeds in 10% trisodium phosphate for 3 hours. Heat treatments did not eradicate the virus in seeds (24h at 80ºC and 48h at 74ºC). Ling (2010) performed experiments on effectiveness of seed treatment through bioassay. Sodium hypochlorite and trisodium phosphate effectively reduced infection rate, depending on use. Other treatments, such as hydrochloric acid, hot water and dry heat had only a partial effect.

A study within the Pepeira project (Hanssen *et al.*, 2010a) showed that in a '*worst-case scenario*', seeds harvested from an infected tomato crop up to 15 weeks after infection with PepMV and subject to minimal cleaning using pectinase (rather than any normal phytosanitary treatment such as acid-extraction) and grown-on still only gave a very low transmission rate of 0.026%. See:
http://documents.plant.wur.nl/pri/pepeira/explanitarynotes.pdf

With respect to infectious virus particles (rather than remnants of virus particle associated with seed which will not be capable of infection), the following judgements have been made. If an efficient treatment has been applied the concentration is unlikely to be high. If poor cleaning has been applied the concentration might be moderately likely.

Likelihood of the concentration of the pest being high
(Non-EU countries): unlikely to moderately likely / Uncertainty: medium
(EU MS): unlikely to moderately likely / Uncertainty: medium

<u>Pathway iii: Plants for planting of tomato from Canary Islands, Norway, Switzerland and Morocco</u>
<u>Pathway x: Plants for planting of tomato from EU MS</u>

The production practises for plants for planting are much less intensive than at fruit production sites, and very strict hygiene measures are usually taken. Spread in a production nursery will therefore be limited.

The percentage of plants in a lot being infected is considered most likely to be low.

The official surveys for the UK in 2008 and 2009 did not detect PepMV in seedlings (63 and 67 premises inspected, some more than once with 10 and 21 samples taken respectively and no PepMV found), UK NPPO (2008 and 2009). In 2007 the overview of official surveys for the EU MS has limited details; but France, the Netherlands and Spain detected PepMV in plants for planting produced in these countries; in 2008 only details from 12 MS were presented (this does not include France, the Netherlands or Spain) and of these none detected PepMV in plants for planting (Anon., 2009b). In the 2009 MS surveys a total of 9548 samples originating from 1760 premises have been tested. Four outbreaks were reported. Two from Belgium (seeds originated from another MS), one from Denmark and one from the Netherlands, in both cases plants had been grown from seed originating from the Netherlands (Anon., 2010a).

Likelihood of the concentration of the pest being high
(Non-EU countries): unlikely / Uncertainty: medium
(EU MS): unlikely / Uncertainty: medium

<u>Pathway xiv: Insect vectors (bumble bees) from EU MS</u>
Bumblebees have been shown by experiment to spread PepMV in plastic houses in Spain and in greenhouses in Canada although the mechanism of transmission has not been determined (Lacasa *et al*., 2003; Shipp *et al*., 2008).

Bumblebees are reared indoors using pollen bought from bee keepers and sugar syrup as a food source (Velthuis & Van Doorn, 2006). So it is very unlikely that the concentration of PepMV associated with bumblebees at the origin of the pathway will be high.

Likelihood of the concentration of the pest being high
(EU MS): Very unlikely / Uncertainty: Low

Go to 1.5

1.5. How large is the volume of the movement along the pathway?
Note: This should be estimated on the basis of quantities of the traded commodity, packing materials, persons, baggage, mail and conveyances, on a yearly basis. For natural spread, movement of the pest should be estimated as far as possible (usually little information is available).

minimal, minor, **moderate, major, massive.**

Level of uncertainty:	**Low**	**Medium**	High

Pathway i: Tomato fruit from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China
Pathway viii: Tomato fruit from EU MS
Tomato fruit is regularly imported from non-EU countries where PepMV has been reported to occur, e.g. Morocco, Canary Islands and countries in South America. Data sourced from EUROSTAT (2010) and presented in Table 7 shows that Morocco is by far the greatest source of tomato fruit from outside of the EU (but note that the Canary Islands data are included in data for Spain). The most recent data on trade between countries in the FAOSTAT Tradestat database is for 2005 (FAO, 2009b). It differs to the Eurostat database, for example, it shows that in 2005 Canada and China both exported relatively small quantities of tomatoes to the EU (1 tonne from Canada to France, 12 tonnes from China to Germany) – Eurostat shows zero exports from these countries; FAOSTAT (FAO, 2009b) showed that Norway exported 9 tonnes to Germany and 18 tonnes to the Netherlands; Eurostat shows 0.7 tonnes in total for Norway to the whole of the EU.

Imports from Morocco have increased over the last couple of years. France is the main importer of tomatoes from Morocco, receiving about 85% of the import. Part of this imported fruit is further shipped throughout the EU. This is confirmed by the rising export figures for France in the period October-May. (Salm, 2008).

Within the EU large quantities of tomato fruit are traded (EUROSTAT, 2010 / Salm, 2008, Annex Trade). In 2008 a total 2.5 billion tonnes of tomato was traded within the EU. Spain (includes data for the Canary Islands) and the Netherlands are the main traders of fresh tomato fruit, with over 925,747 and 861,682 tonnes of intra-EU trade (exports to other EU MS) of tomatoes respectively in 2008 (Table 8). The 17 countries that have reported findings of PepMV during the last three years (see 1.3) are responsible for almost 95% of the trade of tomatoes within the EU. Luxembourg (which has not reported on official surveys for the past 3 years) appears to have some trade in tomatoes (see comments on production and trade data in 1.3) amounting to 349 tonnes in 2007 (FAO, 2009a). In 2005 it exported tomato fruits to Belgium (62 tonnes), France (149 tonnes) and the Netherlands (15 tonnes) (FAO, 2009b). It should be noted that again Eurostat and FAO data are not in all cases consistent. The trade data in Table 8 for Luxembourg and the Netherlands is higher than the production data in Table 3. A possible explanation might be re-export of imported fruit.

Table 7. Import of tomato fruit into the EU from third countries where PepMV is reported to occur (100kg; Source: Eurostat, 2010 (extracted 22-02-2010))

	Year			
Origin	**2005**	**2006**	**2007**	**2008**
Canada	0	0	0	0
Chile	231	291	0	423
China	0	0	0	0
Ecuador	20	0	0	0
Guatemala	0	0	0	0
Morocco	2150266	2199234	3017850	3056113
Norway	7	286	0	0
Peru	0	1	126	2
Switzerland	638	372	230	697
Ukraine	4759	0	354	0
United States	256	25	66	0

Table 8. Trade ('export' to other MS) of tomato fruit within the EU (Quantity in 100kg; Source: Eurostat, 2010 (extracted 22-02-2010)

	Year			
Origin	**2005**	**2006**	**2007**	**2008**
Austria	121762	84865	114957	125624
Belgium	1.844.944	1.794.798	1.758.664	1.600.481
Bulgaria	178	4.140	8.503	55.935
Cyprus	478	332	105	6
Czech Republic	60.885	94.418	113.099	132.424
Denmark	8.677	3.954	4.305	5.163
Estonia	1.956	671	5	355
Finland	571	432	2.379	4.497
France	1.109.085	1.099.353	1.582.700	1.657.561
Germany	349.678	401.449	383.122	391.503
Greece	11.838	8.102	16.346	22.506
Hungary	18.796	10.256	31.345	16.192
Ireland	19.975	25.209	29.518	26.523
Italy	860.120	959.287	1.044.365	1.016.641
Latvia	11.807	5.292	3.562	10.840
Lithuania	21.671	32.891	43.632	41.471
Luxembourg	*2.487*	*3.771*	*3.487*	*2.848*
Malta	0	0	0	35
Netherlands	*7.455.536*	*7.929.540*	*7.928.700*	*8.616.815*
Poland	546.572	523.233	674.054	810.396
Portugal	517.400	424.090	1.017.932	1.169.886
Romania	605	548	1.680	5.494
Slovak Republic	34.792	71.318	77.391	101.194
Slovenia	4.978	21.149	37.843	46.498
Spain	9.135.018	9.623.452	8.571.287	9.257.470
Sweden	8.946	10.532	4.639	4.958
United Kingdom	41.306	41.239	42.810	45.472
Total	*22.190.061*	*23.174.321*	*23.496.430*	*25.168.788*

(NB: See comments under 1.3 on potential discrepancies in production (FAO) and trade (EUROSTAT data).

Since the first findings of PepMV in tomato in the EU in 1999 there have been many interception reports of PepMV infected fruit. Even though there is no regulation of PepMV on fruit, the official surveys carried out by EU MS, include marketed fruit and test for PepMV.

In the period 2000 – May 2010 there were 261 notifications of non-compliance on tomato fruit for PepMV (Europhyt, see Annex 2). The origins of the fruit were both EU countries (Belgium, France, Germany, Italy, Poland, Spain and the Netherlands) as well as non-EU countries (Canary Islands, Morocco). In the 2009 official survey 80 out of 461 tested lots of tomato fruit on the market was reported to be found infected by PepMV. Origin of the positive lots was Belgium, Canary Isles, Cyprus, France, Hungary, Italy, Morocco, Netherlands, Poland, Spain and Slovak Republic (Anon, 2010a).

Volume of movement
(Non-EU countries): Massive / Uncertainty: low
(EU MS): massive / Uncertainty: low

Pathway ii: Seed of tomato from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China
Pathway ix: Seed of tomato from EU MS
Most tomato seed sown in the EU is produced in third countries (e.g. China, Thailand, India, Chile) and shipped to the EU in bulk.

It is difficult to obtain data on imports of tomato seed. Recent investigation of imports of tomato seed to the UK for the PRA for *Columnea latent viroid* (CLVd) (Sansford & Morris, 2009) proved difficult and it was mainly through personal communications with Plant Health and Seed Inspectors for England and Wales that some information was obtained. Use of imported tomato seed by UK plant propagators is common practice because the UK does not have a tomato seed production industry. Seed apparently originating in other EU MS may have been imported from third countries, repackaged and relabelled and then shipped to the UK. Some seed originated from Canada, the USA as well as from Taiwan. Tomato seed is also introduced to the UK from within Europe including from the Netherlands, Germany, Belgium and France (Sansford & Morris, 2009). The overview of surveys performed by the MS also shows the wide range of countries of origin (Anon., 2010a).

There have been many interception reports of PepMV on seeds. In the period 2000 – May 2010 there were 64 notifications of non-compliance on seed (Europhyt, Annex 2). Countries of origin were both EU (France and the Netherlands) as well as non-EU countries (Chile, China, Thailand, Guatemala, India, Israel, Madagascar, Senegal, Taiwan and the United States).

There are no published reports or official records of PepMV in Israel. However, the UK intercepted PepMV on 2 lots of seed out of 15 from Israel in the official survey of 2008. The UK also intercepted PepMV on 2 lots of seed of 14 from the USA (UK NPPO, 2008). In the 2009 survey Belgium has reported interceptions on seed from China, Israel and Guatemala (1 lot each) and France reported interceptions on seed from China (43 lots), Thailand (34), Chile (9), Israel (5), India (3), Morocco (2), Senegal (2) and Vietnam (2). France also reported findings on 3 seed lots produced in France (Anon., 2010a).

Volume of movement
(Non-EU countries): Massive / Uncertainty: low
(EU MS): Moderate / Uncertainty: low

Pathway iii: Plants for planting of tomato from Canary Islands, Norway, Switzerland and Morocco
Pathway x: Plants for planting of tomato from EU MS
There is hardly any import of tomato plants from Norway and Switzerland. Inside the EU there is some trade, although most plants are grown in the EU MS from imported seed. The Netherlands exports about 50% of their young plants to other EU MS, especially neighbouring countries. Tomato plants are permitted to be imported from the Canary Islands and Morocco but whether any trade exists into the EU is not known.

Most tomato seedlings used in the UK are grown in the UK; however some are introduced from the Netherlands. Import figures for tomato seedlings/plants from other EU countries to the UK are not collected. (Sansford and Morris, 2009).

In the period 2000 – May 2010 there were 4 notifications of non-compliance for PepMV on plants for planting, all on plants originating from the Netherlands (Europhyt, Annex 2).

The official EU survey requires that EU MS undertake surveys of plants for planting (seedlings). The UK did not find PepMV on seedlings in the 2008 and 2009 surveys (UK NPPO, 2008 and 2009). In the 2009 EU survey reports 4 outbreaks have been reported; two in Belgium (seed originated from another EU MS), one in Denmark and one in the Netherlands – both derived from seed from the Netherlands (Anon., 2010a).

Volume of movement
(Non-EU countries): Minimal / Uncertainty: low
(EU MS): Major / Uncertainty: low

<u>Pathway xiv: Insect vectors (bumble bees) from EU MS</u>

The Netherlands, Belgium and Slovakia produce a lot of bumblebee packages which are traded within the EU (and to third countries) (C. Jilesen, PPS, The Netherlands, *personal communication* to A.W. Werkman, PPS, The Netherlands, 2010). Details of the trade are not known. In 2006 it was estimated that in excess of 10,000 bumblebee pollination units were imported into the UK every year; major EU companies producing these units were located in Belgium and the Netherlands. Other companies that are known to produce such units but for which the status of exports was unknown were located in Austria, Italy, Spain, the Netherlands and, the non-EU country of Norway (Wilkins, 2006, *unpublished report*).

Volume of movement
(EU MS): Major/ Uncertainty: medium

Go to 1.6

1.6. How frequent is the movement along the pathway?

Note: This should be estimated on the basis of quantities of the traded commodity, packing materials, persons, baggage, mail and conveyances, on a yearly basis. For natural spread, movement of the pest should be estimated as far as possible (usually little information is available).

very rarely, rarely, **occasionally, often, very often.**

Level of uncertainty:	**Low**	Medium	High

See under 1.5.

<u>Pathway i: Tomato fruit from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China</u>
<u>Pathway viii: Tomato fruit from EU MS</u>
Tomato fruit is imported and traded during the whole year.

From the Canary Islands and Morocco the main period of import to the EU is November-April.

In southern European countries (mainly Spain) the main export period is December-May while in northern European countries (e.g. Belgium, the Netherlands and Poland) the main export period is May-October (Salm, 2008). Outside these periods the trade is less frequent, but still occurs.

Details for individual EU MS are not readily available. However, the UK has some information on the availability of fresh tomato fruits on the UK market by country and month but with no details of quantities (Anon., 2008) thus:

Tomato fruit from third countries where PepMV had been recorded is available all year round from: the USA and Peru.

Fruit from other third countries where PepMV has been recorded is available over shorter periods: the Canary Islands – October to May; Morocco – October to June.

Fruit from EU countries where PepMV has been recorded is available all year round from: Belgium, Cyprus, France, the Netherlands, and Spain

Fruit from other EU countries where PepMV has been recorded is available over shorter periods: from Ireland - March to November; from Hungary - April to November; from Poland - April to December; from Italy - February to September; from Portugal - July to November.

Frequency of movement
(Non-EU countries): often / Uncertainty: low
(EU MS): very often / Uncertainty: low

<u>Pathway ii: Seed of tomato from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China</u>
<u>Pathway ix: Seed of tomato from EU MS</u>
Tomato seed is imported and traded during the whole year.
Frequency of movement
(Non-EU countries): often / Uncertainty: low
(EU MS): often / Uncertainty: low

<u>Pathway iii: Plants for planting of tomato from Canary Islands, Norway, Switzerland and Morocco</u>
<u>Pathway x: Plants for planting of tomato from EU MS</u>
Within the EU there is some trade in seedlings, but most plants are grown in the MS from imported seed.

The main peak of trade of tomato plants for planting in the northern part of the PRA area is in the autumn/winter period when the new crop is planted. In southern parts it will take place throughout the year. In Greece, trading of plants occurs throughout the year (C. Varveri, BPI, Greece, *personal communication* to A. Werkman, PPS, The Netherlands, January 2010) and in Italy, the trade period is mainly December – January and July-August, depending on the region (L. Tomassoli, ISPV, Italy, *personal communication* to A. Werkman, PPS, The Netherlands, January 2010).

Frequency of movement
(non EU countries): Very rarely / Uncertainty: low
(EU MS): Often / Uncertainty: low

<u>Pathway xiv: Insect vectors (bumble bees) from EU MS</u>
Bumble bees are brought into tomato crops to pollinate them during the growing season which varies from the north to the south of the PRA area but occurs throughout the year. A bumble bee hive normally lasts 8-12 weeks (Velthuis & Van Doorn, 2006). This means that there will be several introductions in a greenhouse throughout the year.

Frequency of movement
(EU MS): Often / Uncertainty: low

Go to 1.7

<u>Probability of survival during transport or storage</u>

1.7. How likely is the pest to survive during transport /storage?

Note: consideration should be given to:
- *speed and conditions of transport;*
- *vulnerability of the life-stages likely to be transported (for plants: viability of seeds or other propagules);*
- *whether the life cycle is of sufficient duration to extend beyond time in transit;*
- *commercial procedures (e.g. refrigeration) applied to consignments in transport or at destination*

Data on detections in imported consignments may be used to indicate the ability of a pest to survive in transit.

very unlikely, unlikely, moderately likely, **likely, very likely.**

Level of uncertainty:	**Low**	Medium	High

<u>Pathway i: Tomato fruit from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China</u>
<u>Pathway viii: Tomato fruit from EU MS</u>
Like most viruses PepMV will survive and be infectious as long as it is present in living plant material. If tomato fruit is infected with PepMV they will therefore remain infected during transport. Official survey data and interception data (see 1.5) shows that PepMV can be detected in marketed tomato fruit.
Survival during transport:
(Non EU countries): Very likely / Uncertainty: low
(EU MS): Very likely / Uncertainty: low

<u>Pathway ii: Seed of tomato from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China</u>
<u>Pathway ix: Seed of tomato from EU MS</u>
PepMV present on the seed might lose some of its infectivity due to transport conditions. However, the effect will be minimal as long as the seed is being shipped under optimal conditions for seed health (germinability). Córdoba-Sellés *et al.* (2007) showed that heat treatment of seed at 70°C for 96 hours still resulted in infected seedlings indicating that virus which is infective before transport might remain infective during transport.
Official survey data and interception data (see 1.5) shows that PepMV can be detected on seed.

Survival during transport:
(Non EU countries): Likely / Uncertainty: low
(EU MS): Likely / Uncertainty: low

<u>Pathway iii: Plants for planting of tomato from Canary Islands, Norway, Switzerland and Morocco</u>
<u>Pathway x: Plants for planting of tomato from EU MS</u>
Like most viruses PepMV will be infectious as long as it is present in living plant material. If tomato plants are infected with PepMV they will remain infected during transport.

Official survey data and interception data (see 1.5) shows that PepMV can be detected on plants for planting.

Survival during transport:
(Non EU countries): Very likely / Uncertainty: low
(EU MS): Very likely / Uncertainty: low

<u>Pathway xiv: Insect vectors (bumble bees) from EU MS</u>
Bumblebees have been shown by experiment to spread the virus in plastic houses in Spain and in greenhouses in Canada although the mechanism of transmission has not been determined (Lacasa *et al*., 2003; Shipp *et al*., 2008). For this reason it is not known how likely PepMV is to survive on/in the bees and no judgment can be made.

Go to 1.8

1.8. How likely is the pest to multiply/increase in prevalence during transport /storage?

Note: Some pests do not multiply/increase in prevalence during transport/storage; in this case it should be rated impossible.

Impossible/**very unlikely, unlikely,** moderately likely, **likely,** very likely**.**

Level of uncertainty:	**Low**	Medium	High

<u>Pathway i: Tomato fruit from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China</u>
<u>Pathway viii: Tomato fruit from EU MS</u>
Tomato fruit is systemically infected by PepMV. It is unlikely that the virus will multiply in transport or in storage of the fruit.

Multiplication in transport or storage:
(Non EU countries): Unlikely / Uncertainty: low
(EU MS): Unlikely / Uncertainty: low

<u>Pathway ii: Seed of tomato from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China</u>
<u>Pathway ix: Seed of tomato from EU MS</u>
The most likely location for the virus on the seed is on the outside in dried residual material of the fruit pulp. The virus is very unlikely to multiply on seed in transport or in storage.

Multiplication in transport or storage:
(Non EU countries): Very unlikely / Uncertainty: low
(EU MS): Very unlikely / Uncertainty: low

<u>Pathway iii: Plants for planting of tomato from Norway, Switzerland and Morocco</u>
<u>Pathway x: Plants for planting of tomato from EU MS</u>
Tomato plants are systemically-infected and multiplication in infected plants is possible.

Multiplication in transport or storage:
(Non EU countries): Moderately likely / Uncertainty: low
(EU MS): Moderately likely / Uncertainty: low

<u>Pathway xiv: Insect vectors (bumble bees) from EU MS</u>
Bumblebees have been shown by experiment to spread the virus in plastic houses in Spain and in greenhouses in Canada although the mechanism of transmission has not been determined (Lacasa *et al*., 2003; Shipp *et al*., 2008). For this reason it is not known how likely PepMV is to multiply on/in the bees during transport and no judgment can be made.

Go to 1.9

<u>Probability of the pest surviving existing pest management procedures</u>

1.9. How likely is the pest to survive or remain undetected during existing management procedures (including phytosanitary measures)?

Note: existing phytosanitary measures (e.g. inspection, testing or treatments) are most probably required as a protection against other (quarantine) pests and applied in the exporting country or the importing country. The assessor should bear in mind that such measures could be removed in the future if the other pests are re-evaluated.

The likelihood of detecting the pest during inspection or testing will depend on a number of factors including:

- *ease of detection of the life stages which are likely to be present. Some stages are more readily detected than others, for example insect adults may be more obvious than eggs or seeds and bulbs for plants; Availability of identification tests (for certain micro organisms).*
- *location of the pest on the commodity - surface feeders may be more readily detected than internal feeders;*
- *symptom expression - many diseases may be latent for long periods, at certain times of the year, or may be without symptoms in some hosts or cultivars and virulent in others;*
- *distinctiveness of symptoms - the symptoms might resemble those of other pests or sources of damage such as mechanical or cold injury;*
- *the intensity of the sampling and inspection regimes;*
- *distinguishing the pest from similar organisms.*

very unlikely, unlikely, **moderately likely, likely,** very likely.

Level of uncertainty:	Low	**Medium**	High

<u>Pathway i: Tomato fruit from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China</u>
<u>Pathway viii: Tomato fruit from EU MS</u>
There are no phytosanitary measures in place for imports or intracommunity trade in tomato fruit in the EU. However, as part of the official surveys, EU MS are visiting premises where fruit is marketed (e.g. shops, supermarkets, or public markets). As part of the official surveys and Europhyt notifications, marketed fruit from several third countries as well as from EU MS has been detected with infection by PepMV (see 1.5).

PepMV can induce symptoms on the fruit, e.g. marbling and uneven ripening. However, the virus is also known to be symptomless (Spence *et al., 2006;* Hanssen *et al*, 2009a; Pepeira final report WP2, 2010; Schenk *et al*, 2010). Environmental conditions seem to play an important role in symptomatology (Jordá *et al*., 2001a; Spence *et al.*, 2006). Although no correlation between symptoms and genotypes has been found, symptoms can differ between PepMV isolates belonging to the same genotype (Hanssen *et al*., 2008; Hanssen *et al*.; 2009b; Hasiów-Jaroszweska *et al.,* 2009). In the PEPEIRA field trials it was observed that fruit symptoms mainly occurred in the first trusses after inoculation (Pepeira final report WP2, 2010). Later in the season hardly any symptoms were observed. Therefore, if mature fruit is inspected there is a reasonable chance the virus will not be detected. Since there is no current legislation for fruit there is no compulsory testing and therefore the virus is likely to remain undetected. Also where symptoms are detected, the fruits may not be rejected since the pest is not regulated for fruit in Decision 2004/200/EC (Anon., 2004b). There is a market for Class 2 fruit in most EU MS.

Probability of survival or remaining undetected during existing pest management procedures including phytosanitary procedures:
(Non-EU countries): Likely / Uncertainty: medium
(EU MS): likely / Uncertainty: medium

<u>Pathway ii: Seed of tomato from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China</u>
<u>Pathway ix: Seed of tomato from EU MS</u>
Seed of tomato is only allowed to enter and move within the EU provided it has been subjected to acid-extraction or an equivalent measure (Point 48 of Annex IVAI and Point 27 of Annex IVAII of the EC Plant Health Directive; Anon., 2000). It has been shown that this treatment can effectively reduce the transmission rate (Córdoba-Selles *et al*., 2007; Ling, 2010).

Moreover, intensive testing of tomato seeds for the presence of PepMV takes place. Seed-producing companies test both plants used for seed production as well as harvested seed. Also NPPO's test for presence of PepMV in seed lots. According to the EU official survey report in 2009, 265 samples from seeds of EU origin and 927 samples from third countries have been tested (Anon., 2010a). Three samples with EU origin and 103 samples with non-EU origin were found infected. It is unknown whether these findings concerned infectious virus. However, since contaminated seed lots are still intercepted at regular intervals there remains some uncertainty on the efficacy of existing phytosanitary measures.

Probability of survival or remaining undetected during existing pest management procedures including phytosanitary procedures:
(Non-EU countries): Moderately likely / Uncertainty: medium
(EU MS): Moderately likely / Uncertainty: medium

<u>Pathway iii: Plants for planting of tomato from Canary Islands, Norway, Switzerland and Morocco</u>
<u>Pathway x: Plants for planting of tomato from EU MS</u>
EC Decision 2004/200/EC (Anon., 2004) dropped requirements for tomato seedlings to be either from PepMV free areas/nurseries or to be tested for PepMV. It is understood that this was because tomato seedlings infected by PepMV were said not to exhibit symptoms and so could not be visually identified at nurseries. Extensive sample testing in the Netherlands showed that tomato plants on propagation nurseries rarely tested positive (Jones *et al*., 2005). Nevertheless, one of the requirements of the official surveys is for EU MS to survey plants for planting for PepMV. In 2009, 9548 samples were reported to be tested during official surveys with only 4 positive lots (Anon., 2010a). Whether this is representative of the prevalence of PepMV in seedlings being traded is not certain.

Plants for planting of tomato are traded (rarely, but mainly from the Netherlands) at a very young stage. At this stage PepMV is either asymptomatic or hardly shows any symptoms. Therefore, if seedlings are only tested when symptomatic, the virus will not be detected. Moreover, the amount of virus in young plants might be too low to be detected by the methods that are currently available.

Probability of survival or remaining undetected during existing pest management procedures including phytosanitary procedures:
(Non-EU countries): Likely to very likely / Uncertainty: medium
(EU MS): Likely to very likely / Uncertainty: medium

<u>Pathway xiv: Insect vectors (bumble bees) from EU MS</u>

There are no management practices or phytosanitary measures in place for PepMV associated with bumblebees.

Probability of survival or remaining undetected during existing pest management procedures including phytosanitary procedures:
(EU MS): Very likely / Uncertainty: medium

Go to 1.10

Probability of transfer to a suitable host or habitat

1.10. In the case of a commodity pathway, how widely is the commodity to be distributed throughout the PRA area?
Note: the more scattered the destinations, the more likely it is that the pest might find suitable habitats.

very limited, limited, moderately widely, widely, **very widely**

Level of uncertainty:	**Low**	**Medium**	High

Pathway i: Tomato fruit from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China
Pathway viii: Tomato fruit from EU MS
Tomato fruit originating from the Canary Islands is distributed throughout the PRA area. The bulk of tomato fruit originating from Morocco is imported (most recently) into France. However, after import this fruit is moved throughout the PRA area (Salm, 2008). Tomato fruit produced in the EU is also distributed throughout the PRA area.

Breadth of distribution of the commodity throughout the PRA area:
(Non-EU countries): Very widely / Uncertainty: low
(EU MS): Very widely / Uncertainty: low

Pathway ii: Seed of tomato from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China
Pathway ix: Seed of tomato from EU MS
Tomato seed is distributed throughout the PRA area, wherever tomato production takes place.
Breadth of distribution of the commodity throughout the PRA area:
(Non-EU countries): Very widely / Uncertainty: low
(EU MS): Very widely / Uncertainty: low

Pathway iii: Plants for planting of tomato from Canary Islands, Norway, Switzerland and Morocco
Pathway x: Plants for planting of tomato from EU MS
Tomato plants for planting that are distributed are most likely to be raised from seed within the EU MS where tomato production takes place (throughout the PRA area). The exception is plants for planting produced in the Netherlands which can be sent to other EU MS.

Plants for planting from the Canary Islands, Norway, Switzerland and Morocco are thought unlikely to be imported into the EU in considerable quantities.
Breadth of distribution of the commodity throughout the PRA area:
(Non-EU countries): Very limited / Uncertainty: low
(EU MS): Moderately widely / Uncertainty: medium

Pathway xiv: Insect vectors (bumble bees) from EU MS
Bumblebees are used to pollinate tomato crops throughout the PRA area. In 2004, worldwide a total of 40,000 ha of tomato production was pollinated using bumble bees (Velthuis & Van Doorn, 2006). This includes the main indoor tomato production areas in the EU.

Breadth of distribution of the commodity throughout the PRA area:
(EU MS): Very widely / Uncertainty: Low

Go to 1.11

1.11. In the case of a commodity pathway, do consignments arrive at a suitable time of year for pest establishment?

Note: introduction at many different times of the year will increase the probability that entry of the pest will occur at a life stage of the pest or the host which is suitable for establishment or when habitat or environmental conditions are favourable.

Pathway i: Tomato fruit from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China
Pathway viii: Tomato fruit from EU MS
Pathway ii: Seed of tomato from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China
Pathway ix: Seed of tomato from EU MS
Pathway iii: Plants for planting of tomato from Canary Islands, Norway, Switzerland and Morocco
Pathway x: Plants for planting of tomato from EU MS

Yes. Tomato fruit from third countries is mainly imported in the autumn and winter when the supply of EU tomatoes is low. At this time the new season of tomato cultivation commences and there is a risk that PepMV present on all of these pathways can spread to the new crop and lead to an infection. Intra-EU trade takes place throughout the year (see question 1.6).

Tomato plants and tomato seed are imported for crop production and so will be immediately associated with tomato production facilities.

Pathway xiv: Insect vectors (bumble bees) from EU MS

Bumblebees are brought into greenhouses several times during a growing season and so they will arrive at a suitable time of year to aid establishment (Velthuis & Van Doorn, 2006).

if yes **Go to 1.12**
if no Go to 1.14

1.12. How likely is the pest to be able to transfer from the pathway to a suitable host or habitat?

Note: consider innate dispersal mechanisms or the need for vectors, and how close the pathway on arrival is to suitable hosts or habitats.

very unlikely, unlikely, **moderately likely,** likely, **very likely.**

Level of	**Low**	**Medium**	High

uncertainty:			

Pepino mosaic virus is very easily mechanically transmitted (Jones *et al.*, 1980; Mumford and Metcalfe 2001).

<u>Pathway i: Tomato fruit from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China</u>
<u>Pathway viii: Tomato fruit from EU MS</u>
Imported tomato fruit is traded as pre-packed tomatoes or bulk/unpacked tomatoes. In the Netherlands sorting of these tomatoes usually takes place at central sites amid greenhouses for production of tomatoes. In the UK some packing facilities and production greenhouses are in very close proximity on the same site. In these cases there is a possibility of spread from imported fruit to the production by contact transmission.

Experiments carried-out in January 2010 showed that hands that have been in contact with infected fruit are able to infect tomato plants (M. Schenk, WUR-Glastuinbouw, The Netherlands unpublished data, February 2010). Moreover, it was also shown that transmission of PepMV is very efficient when healthy tomato plants come in contact with rigid plastic containers and clothing that have been contaminated with infected fruit sap. This occurred after a 1-hour and a 48-hour interval had elapsed between contamination with infected fruit sap and contact with healthy plants. This work shows that there is a risk of transfer of PepMV occurring when workers packing infected imported tomatoes at production facilities come into contact with the growing crop.

However, most tomato production facilities in at least the Netherlands and the United Kingdom have strict hygiene practices to prevent cross-transfer of mechanically-transmitted pathogens including PepMV and *Potato spindle tuber viroid* (PSTVd) details of which are outlined in guidelines on best practice for tomato production. These are aimed at reducing the risk of introduction and spread of PepMV (Fletcher, 2000; O'Neill *et al*., 2003), PSTVd (O'Neill & Mumford, 2006) and the combination of PepMV, *Verticillium* spp. and *Clavibacter michiganensis* sups. *michiganensis* (Anon., 2007).The hygiene practices might be less strict with small producers or those who are cultivating tomato together with a variety of other crops.

Tomato fruits are finally sold to the end-consumer. Consumers will eat the tomato fruits or dispose them in the domestic waste if they are not suitable for consumption. The only potential pathway of spread is by consumers handling infected fruit and domestic tomato crops.

It is unlikely that the pest will spread by movement of infested fruit by consumers, with the exceptions of consumers who work in nurseries or tomato production facilities.

Transfer to suitable host
(Non-EU countries) moderately likely / Uncertainty: medium
(EU MS): moderately likely / Uncertainty: medium

<u>Pathway ii: Seed of tomato from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China</u>
<u>Pathway ix: Seed of tomato from EU MS</u>
In the case of plants grown from seeds that are contaminated with infectious virus, the virus is very likely to spread to other plants or lots grown at the same site.

Transfer to suitable host
(non-EU countries): Very likely / Uncertainty: low
(EU MS): Very likely / Uncertainty: low

Pathway iii: Plants for planting of tomato from Canary Islands, Norway, Switzerland and Morocco
Pathway x: Plants for planting of tomato from EU MS
In the case of tomato plants for planting that are infected with PepMV the virus is very likely to spread to other plants or lots grown at the same site.

Transfer to suitable host
(non-EU countries): Very likely / Uncertainty: low
(EU MS): Very likely / Uncertainty: low

Pathway xiv: Insect vectors (bumble bees) from EU MS
Bumblebees have been shown by experiment to spread the virus in plastic houses in Spain and in greenhouses in Canada although the mechanism of transmission has not been determined (Lacasa *et al*., 2003; Shipp *et al*., 2008). Once the virus is introduced in a crop, it is possible that bumble bees that have pollinated the infected tomato crop and which escape from a greenhouse could transfer PepMV to other tomato plants or to susceptible weeds which could act as a reservoir for the virus.

(EU MS): Moderately likely / Uncertainty: low

Go to 1.13

1.13. In the case of a commodity pathway, how likely is the intended use of the commodity (e.g. processing, consumption, planting, disposal of waste, by-products) to aid transfer to a suitable host or habitat?
Note: Some uses are associated with much higher probability of introduction (e.g. planting) than others (e.g. processing). Consider whether the intended use of the commodity would destroy the pest or whether the processing, planting or disposal might be done in the vicinity of suitable hosts or habitats.

N/A, very unlikely, unlikely, **moderately likely,** likely, **very likely.**

Level of uncertainty:	**Low**	**Medium**	High

See 1.12

Pathway i: Tomato fruit from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China
Pathway viii: Tomato fruit from EU MS
(Non-EU countries): moderately likely / Uncertainty: medium
(EU MS): moderately likely / Uncertainty: medium

Pathway ii: Seed of tomato from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China
Pathway ix: Seed of tomato from EU MS
(Non-EU countries): very likely / Uncertainty: low
(EU MS): very likely / Uncertainty: low

Pathway iii: Plants for planting of tomato from Canary Islands, Norway, Switzerland and Morocco
Pathway x: Plants for planting of tomato from EU MS
(Non-EU countries): very likely / Uncertainty: low
(EU MS): very likely / Uncertainty: low

Pathway xiv: Insect vectors (bumble bees) from EU MS
(EU MS): Moderately likely / Uncertainty: low

Go to 1.14

Consideration of further pathways
In principle, all the relevant pathways selected at point 1.2 may in turn be considered. However, the replies given for the pathway(s) so far considered indicate that it is not necessary to consider any more.

1.14. Do other pathways need to be considered?

if yes	Go back to 1.2 for the next pathway
if no	**Go to conclusion on the probability of entry and then to 1.15**

Conclusion on the probability of entry

The overall probability of entry should be described and risks presented by different pathways should be identified.

Below, we assess the probability of entry into and spread within the PRA area of *Pepino mosaic virus* based on the answers of questions 1.3 – 1.14. A summary is given in table 9. For a more detailed summary of risk of association per country see also tables 5 and 6 under Question 1.3

Table 9. Overview of answers for the different pathways related to probability of introduction
Prob. (Probability)**:** **VU**, Very unlikely; **U**, Unlikely; **ML**, Moderately likely; **L**, Likely; **VL**, Very Likely; **Mas**, Massive; **Maj**, Major; **Mod**, Moderate; **Min**, Minimal; **VO**, Very Often; **O**, Often; **Occ**, Occasionally; **Vra**, Very Rarely; **Vwi**, Very Widely; **Vli**, Very Limited; **Li**, Limited
Unc (levels of uncertainty) : **H** = High, **M** = Medium, **L** = Low. NJ = No judgement

Questions		**tomato fruit**				**tomato seed**				**plants for planting**				**bumble bee**	
		i non-EU		viii EU		ii non-EU		ix EU		iii non-EU		x EU		xiv EU	
		Prob.	Unc.	Prob.	Unc.	Prob.	Unc.	Prob.	Unc.	Prob.	Unc.	Prob.	Unc.	Prob.	Unc.
1.3	association at origin	VU-VL	Var.	U-VL	Var.	VU-ML	L-H	U-ML	H	VU-L	L-H	U-ML	M	VU	M
1.4	concentration being high	L-VL	M	L-VL	M	U-ML	M	U-ML	M	U	M	U	M	VU	M
1.5	volume of movement	Mas	L	Mas	L	Mas	L	Mod	L	Min	L	Maj	L	Maj	M
1.6	frequency of movement	Oft	L	VO	L	Oft	L	Oft	L	Vra	L	Oft	L	Oft	L
1.7	survival during transport/storage	VL	L	VL	L	L	L	L	L	VL	L	VL	L	NJ	NJ
1.8	multiplcation during transport/storage	U	L	U	L	VU	L	VU	L	ML	L	ML	L	NJ	NJ
1.9	Survival/detection during existing procedu	L	M	L	M	ML	M	ML	M	L-VL	M	L-VL	M	VL	M
1.10	distribution throughout PRA area	Vwi	L	Vwi	L	Vwi	L	Vwi	L	Vli	L	Mod	M	Vwi	L
1.11	Arrival at suitable time of year	Yes		Yes		Yes		Yes		Yes		Yes		Yes	
1.12	transfer from pathway to host/habitat	ML	M	ML	M	VL	L	VL	L	VL	L	VL	L	ML	L
1.13	aid of intended use for transfer	ML	M	ML	M	VL	L	VL	L	VL	L	VL	L	ML	L
Overall probability of entry		**Unlikely to Very likely**				**Unlikely to Likely**				**Very Unlikely to Moderately likely**				**Very Unlikely**	

Tomato fruit

The overall probability of entry/movement by tomato fruit is estimated as ranging from **unlikely** to **very likely** depending on the origin. The main uncertainty is exact information on distribution of the virus in the different countries of origin.

- Entry into the PRA area: Most of the imported tomato fruit originates from Morocco. Although the current status of PepMV in Morocco is unknown, there are several interception reports of PepMV on Moroccan tomato fruit suggesting that PepMV is present in tomato production areas.
- Movement within the PRA area: The majority of the massive amount of tomato fruit traded within the EU originates from countries where PepMV has been reported.

PepMV will remain infectious during shipment and is likely not to be detected during existing management procedures. Tomato fruit is shipped very widely throughout the PRA area during suitable times of the year. The risk of transfer of the virus to a crop depends on the nature of the processing of the tomato fruit, especially sorting and packing. If there are not sufficient (hygiene) measures the virus is moderately likely to transfer.

Tomato seeds

The overall probability of entry/movement by tomato seeds is estimated as ranging from **unlikely** to **likely**, depending on origin. Although the risk of the virus being associated with seed and being capable of infecting plants is estimated as being low, the amount of seed traded is massive and the risks associated with one infected seed is potentially high. The main uncertainty is exact information on distribution of the virus in the different countries of origin.

- Entry into the PRA area: Most tomato seed sown in the EU is produced in third countries. In many of these countries PepMV has been reported to occur. Moreover, there are many interception reports of PepMV on seed.
- Movement within the PRA area: Inside the PRA area tomato seed is produced and traded, mainly in countries where PepMV is known to occur.

The rate of seed transmission of PepMV has been shown to be very low. Growing out of untreated seeds harvested from infected fruit gives a very low transmission rate. Moreover existing phytosanitary measures (acid-extraction) has been shown to effectively reduce the transmission rate. However, one seed giving rise to an infected seedling in a batch of young plants is very likely to spread to other plants and finally infect the whole crop. This pathway might be especially important for the risk of introduction of new strains (genotypes) into the PRA area and for further spread.

Tomato plants for planting

The overall probability of entry by tomato seedlings is estimated as ranging from **very unlikely** to **moderately likely**. The main uncertainty is exact information on distribution of the virus in the different countries of origin.

- Entry into the PRA area: Only Canary Islands, Norway, Switzerland and Morocco are considered and hardly any tomato plants are imported from the first two countries; the situation with respect to Canary Islands and Morocco is not known but <u>assumed</u> unlikely.
- Movement within the PRA area: Most seedlings used in a country are grown in this country, although in some country plants are introduced from other EU countries, mainly originating from the Netherlands. There are occasional findings of PepMV on plants for planting.

Since tomato plants are directly introduced at tomato production sites, it is very likely that infected plants will aid transfer of PepMV to suitable hosts.

Bumble bees

This pathway is not considered to pose a risk of entry into the PRA area from third countries (due to existing bee health legislation).

Although a large quantity of commercially produced bumble bees is moved within the PRA area, the probability of movement is estimated as **very unlikely** because the probability of association is very unlikely. This is because bumble bees are produced on a diet of bee-collected pollen and sugar water. The main risk from bumble bees is associated with spread within an infected area (See 1.30 in the PRA).

Probability of Establishment

For plants which are intentionally imported, the assessment of the probability of establishment concerns the unintended habitat.

Availability of suitable hosts or suitable habitats, alternate hosts and vectors in the PRA area

1.15. Estimate the number of host plant species or suitable habitats in the PRA area (see question 6).

Very few, **Few**, Moderate number**,** Many, Very many**.**

Level of uncertainty:	**Low**	Medium	High

Tomato is the main host of PepMV. Other known natural hosts that are grown in the EU are potato and pepino. It is not certain whether basil is a natural host, and the role of eggplant as a natural host should be further investigated (see 6.). In Spain some weed species were found to be a host for PepMV (Cordoba *et al*., 2004).
The number of host plant species is therefore estimated as few.

1.16. How widespread are the host plants or suitable habitats in the PRA area? (specify)

very limited, limited, moderately widely, widely**, very widely**

Level of uncertainty:	**Low**	Medium	High

See question 6 for more detail on the host species.

Tomato

Tomato crops are most at risk in the PRA area. Tomato is grown in all EU countries and is an extremely valuable crop.

In 2008, 293,300 ha of tomato was grown in the 27 Member States of the EU resulting in a production of 16,187,454 tonnes of tomatoes (see Table 3 for details).

Pepino

Efforts are being made to grow pepino under greenhouse conditions for fruit production in the Mediterranean region. Although there are reports of commercial growing of pepino seemingly being unsuccessful (Prohens *et al*, 2000; Prohens *et al*, 2005) there was a report that '*pepino dulce*' (*Solanum muricatum*) is cultivated on a small-scale in Spain (EPPO, 2000). Recent information suggests that there is no real production of pepino in Spain. There are some experimental greenhouses that are trialling the cultivation of pepino. Occasionally fruits are sold at local markets. (J. Prohens, UPV, Spain, *personal communication* to A. Alfaro-Fernandez, UPV, Spain, February 2010)

Potato

In Peru, PepMV has been found in field-grown potato. However there is no information on whether the plants were symptomatic and whether there was any effect on yield or quality of the tubers. Results from the Pepeira project show that it is a poor host (Pepeira final report WP3, 2010). Thus, the importance of PepMV in potato production is not clear. Potato is a very important crop in the EU. In 2008, total production of the 27 Member States was 61,582,974 tonnes (FAO, 2009c).

<u>Weeds</u>
In Spain, symptomless infections of PepMV were found in weed species near to glasshouses with PepMV infected tomato plants (Jordá *et al*., 2001a; Córdoba *et al*., 2004). Some of these weeds species occur throughout the PRA area. The wild *Lycopersicon* species found to be infected in Peru (Soler *et al.*, 2002), are not known to occur in the PRA area (Peralta & Spooner, 2000; Tutin *et al*., 2002).

1.17. If an alternate host or another species is needed to complete the life cycle or for a critical stage of the life cycle such as transmission (e.g. vectors), growth (e.g. root symbionts), reproduction (e.g. pollinators) or spread (e.g. seed dispersers), how likely is the pest to come in contact with such species?
Note: Is the species present, widespread and abundant could it be introduced or could another species be found?

N/A, very unlikely, unlikely, moderately likely, likely, very likely

Level of uncertainty:	Low	Medium	High

Not applicable, there is no need for alternate hosts.

<u>Suitability of the environment</u>

Specify the area where host plants (for pests directly affecting plants) or suitable habitats (for non parasitic plants) are present (cf. QQ 1.15-1.17). This is the area for which the environment is to be assessed in this section. If this area is much smaller than the PRA area, this fact will be used in defining the endangered area.

1.18. How similar are the climatic conditions that would affect pest establishment, in the PRA area and in the current area of distribution?
Note: the climatic conditions in the PRA area to be considered may include those in protected cultivation. When comparing climates in a pest's current distribution with those in the PRA area, it is important to ensure that, as far as possible, the variables selected are relevant to the pest's ability to exploit conditions when these are favourable for growth and reproduction and to survive unfavourable periods, such as those of extreme cold, heat, wetness or drought.

not similar, slightly similar, moderately similar, largely similar, **completely similar**

Level of uncertainty:	**Low**	Medium	High

Tomato is grown throughout the PRA area. In the northern part of the PRA area tomato is only grown commercially under protected cultivation. However, tomato plants are grown outdoors in domestic gardens in the summer. In the southern part of the PRA area tomato is commercially grown outdoors as well as under protection. PepMV already occurs in both northern and southern parts of the PRA area (question 7). Most reports on PepMV in tomato in northern Europe, southern Europe and North America are on crops grown under protected cultivation (Van der Vlugt *et al.*, 2000; Jorda *et al*., 2001a; Ling & Carpenter, 2005). In Cyprus an infection with PepMV in outdoor tomato cultivation was found in 2009 (L. Papayiannis, ARI, Cyprus, *personal communication* to A. Werkman, PPS, The Netherlands, January 2010). There is not yet any information available on the possible survival of PepMV from 2009 to 2010 in the field in Cyprus. However, it is not uncommon that tomato debris and old plants are left in the fields. Therefore survival outdoors might occur (L. Papayiannis, ARI, Cyprus, *personal communication* to A. Werkman, PPS, The Netherlands, April 2010). Infections with PepMV in outdoor tomato cultivation probably occur more often although no further data are available. Additionally there have been reports of findings of PepMV in weeds in infected tomato fields in Spain (Córdoba *et al.*, 2004; Pagán *et al*., 2006). In Peru the virus has been found to infect wild *Lycopersicon* species and pepino in the field (Soler et al., 2002). Climatic conditions affecting pest establishment of PepMV in the PRA area are considered completely similar.

1.19. How similar are other abiotic factors that would affect pest establishment, in the PRA area and in the current area of distribution?

Note: the major abiotic factors to be considered are the physical and chemical characteristics of the soil; others are, for example, environmental pollution, topography/orography. For organisms having an aquatic stage pH, salinity, current and temperature are important factors to consider.

No judgement, not similar, slightly similar, moderately similar, largely similar, completely similar

Level of uncertainty:	Low	Medium	High

Not applicable to viruses as far as establishment is concerned.

1.20. If protected cultivation is important in the PRA area, how often has the pest been recorded on crops in protected cultivation elsewhere?

N/A, never, very rarely, rarely, occasionally, **often,** very often

Level of uncertainty:	**Low**	Medium	High

PepMV is mainly described on tomato in protected cultivation and has been reported in many countries both in and outside of the PRA area. See question 1.18 and Table 1.

1.21. How likely is it that establishment will occur despite competition from existing species in the PRA area, and/or despite natural enemies already present in the PRA area?

Note: For pest plants, how likely is the pest plant to build up monospecific stands? Is the species a freshwater macrophyte? Is the species allelopathic? Is the species able to fix nitrogen? Natural enemies include antagonists (herbivores, predators and parasites). The assessor should also consider if the species is unpalatable to grazing animals or toxic.

very unlikely, unlikely, moderately likely, likely, **very likely.**

Level of uncertainty:	**Low**	Medium	High

Competition with other viruses is not likely to have a limiting effect on PepMV. In fact PepMV has been found in mixed infections with *Tomato chlorosis virus* and *Tomato infectious chlorosis virus* in Italy (Davino *et al.,* 2008; L. Tomassoli, ISPV, Italy, personal communication to A.W. Werkman, PPS, the Netherlands, April 2010), and mixed infections with *Tomato yellow leaf curl virus* and Tomato torrado virus are reported from Spain (Soler *et al*., 2005a; Alfaro-Fernandez *et al.*, 2009b). Natural enemies are not applicable for viruses.

Cultural practices and control measures

1.22. To what extent is the managed environment in the PRA area favourable for establishment?

Note: factors that should be considered include cultivation practices such as the time of year that the crop is grown, soil preparation, method of planting, irrigation, whether grown under protected conditions, surrounding crops, time of harvest, method of harvest, soil water balance, fire regimes, disturbance, etc. Factors to consider for pest plants are for instance the regular mowing of road sides, cleaning of water courses, etc.

Not at all favourable, slightly favourable, moderately favourable, **highly favourable,** very highly favourable

Level of uncertainty:	**Low**	Medium	High

The managed environment in tomato crops is highly favourable for establishment of PepMV. The virus has been reported in 19 out of 27 countries in the PRA area, and in the period 2007-2009 it has been reported in 17 EU countries (question 7). It is very easily mechanically transmitted (Jones *et al.*, 1980; Mumford & Metcalfe, 2001). Since tomato is a crop where crop-handling procedures are very intensive there is a high risk of establishment. Since the first findings of PepMV in tomato the main focus has been on hygiene measures. Nowadays strict hygiene protocols apply in protected cultivation. For example in the Netherlands, tomato producing companies often forbid their employees to consume tomatoes brought from home to prevent introduction of PepMV. In the UK and the Netherlands there are published guidelines on best practice for hygiene specific to pests such as PepMV (Fletcher, 2000; O'Neill *et al*., 2003; Anon., 2007) and a later publication on *Potato spindle tuber viroid* (PSTVd) gives more up-to-date guidance on pest control in tomatoes for organisms that are mechanically-transmitted (O'Neill and Mumford, 2006).

1.23. How likely is it that existing pest management practice will fail to prevent establishment of the pest?

Note: for pest plants is the species poorly controlled by herbicides? Is the species intolerant or suffer from mutilation, cultivation or fire?

very unlikely, unlikely, **moderately likely**, likely, very likely.

Level of uncertainty:	**Low**	Medium	High

Good crop management practices including good hygiene measures can prevent establishment of PepMV. However, since the virus is readily transmitted mechanically where it is present it can be easily spread within and between crops and can therefore establish if its presence is not detected early in the growing season. Individual infested plants present in a lot of young plants used for planting in a fruit production company will lead to complete infestation of the whole crop in a rather short time.

1.24. Based on its biological characteristics, how likely is it that the pest could survive eradication programmes in the PRA area?

Note: Some pests can be eradicated at any time (survival is very unlikely), others at an early stage (moderately likely) and others never (very likely). Similarly, incursions of some pests may be difficult to find and/or delimit (very likely). Note that intentionally imported plants may need to be eradicated from the intended habitat as well as from the unintended habitat. Some plants should be eradicated before fructification.

very unlikely, unlikely, **moderately likely**, likely, very likely**.**

Level of uncertainty:	Low	**Medium**	High

PepMV has already been reported in 19 of the 27 EU MS, and in the period 2007-2009 it has been reported in 17 countries in the PRA area (question 7). In several countries attempts to eradicate have been made but the virus still persists at many sites. However, the chance of the virus surviving eradication programmes is mainly dependent on the intensity of tomato production in a certain area. Infections in isolated greenhouses are more easily eradicated then infections in greenhouses in dense production areas. Moreover, infections that persist between crops in plant debris (poor hygiene) are likely to carry-over into the next crop.

<u>Other characteristics of the pest affecting the probability of establishment</u>

1.25. How likely is the reproductive strategy of the pest and the duration of its life cycle to aid establishment?

Note: consider characteristics which would enable the pest to reproduce effectively in a new environment, such as parthenogenesis/self-crossing, short life cycle, number of generations per year, resting stage, high intrinsic rate of increase, self fertility, vegetative propagation, production of viable seeds, prolific seed production, formation of a persistent seed bank or offspring bank. For a pest transmitted by a vector the reproductive strategy of the vector should also be taken into account.

very unlikely, unlikely, moderately likely, likely, **very likely**

Level of uncertainty:	**Low**	Medium	High

The virus reproduces itself readily within its host plants. It does not require a vector. Due to its extremely high infectivity mechanical transmission e.g. during cultivation practises is a very efficient way of establishment and spread.

1.26. How likely are relatively small populations to become established?

Note: if very small populations are known to survive for long periods in their area of current distribution, such evidence may be used to answer this question. For plants, is the species able to hybridise freely? Is the species polymorphic, with, for example, subspecies? Is the species self-compatible? Does the species reproduce by vegetative fragmentation?

No judgment, very unlikely, unlikely, moderately likely, **likely,** very likely

Level of uncertainty:	Low	**Medium**	High

Small populations are likely to become established. Since PepMV is very easily transmitted, one infected plant may lead to establishment. If at the end of the growing season no strict hygiene measures are taken between the successive crops the virus is likely to remain present at the site. Again, this is also dependent on intensity of tomato production in an area (See 1.24.)

1.27. How adaptable is the pest?

Note: is the species polymorphic, with, for example, subspecies or pathotypes? Is it known to have a high mutation rate? Does it occur in a wide range of climate and habitats? Such evidence of variability may indicate that the pest has an ability to withstand environmental fluctuations, to adapt to a wider range of habitats or *hosts, to develop resistance to plant protection products and to overcome host resistance.*

Adaptability is:
very low, low**, moderate,** high, very high

Level of uncertainty:	Low	**Medium**	High

PepMV is a moderately adaptable virus. Its host-range is relatively limited and it is mainly confined to protected cropping conditions but it has been recorded in the field.

PepMV is currently known to have four distinct genotypes: the original Peruvian genotype, the European tomato genotype EU, the American genotype US1 and the Chilean genotype CH2 (Hanssen *et al*., 2010a). Initially, all reported outbreaks of PepMV in European tomato production were caused by isolates from the EU genotype. In recent years however, the CH2 genotype has largely replaced the EU genotype in commercial tomato production in several European countries and has become the dominant genotype in surveys, whereas in the United States and Canada the EU genotype is predominant (French *et al*., 2005; Gómez *et al*., 2008; Hanssen *et al.*, 2008; Hanssen and Thomma, 2010; Ling, 2008; Gomez *et al.*, 2009). Interestingly, a study in Spain revealed that the Peruvian genotype was present on the Canary Islands in 2000 and that CH2-like sequences were already present in Spain in 2004 (Pagán *et al*., 2006). Recently the US1 genotype has been reported from the Canary Islands; this is the first finding of this genotype in a different location than originally reported (North-America; Alfaro-Fernández *et al*., 2008).

It can thus be assumed that all four currently known PepMV genotypes are present in European tomato production. In addition, naturally occurring mixed infections of different genotypes have been reported, including the presence of recombinants (Pagán *et al*., 2006; Hanssen *et al*., 2008). Sequence analyses suggest that the formerly reported genotype US2 (Maroon-Lango *et al*., 2005) is a recombinant of US1 and CH2. Altogether these reports show that recombinant events occur in mixed infection, but the biological relevance of the resulting recombinants is currently not known.

Factors contributing to PepMV population dynamics, like the recent population shift in European tomato production with the EU genotype gradually being overtaken by the CH2 genotype, are currently not known (Hanssen and Thomma, 2010). It was suggested that the CH2 genotype has a biological advantage over the EU genotype, as it seemed to spread faster within a crop (Hanssen *et al*., 2008). This was confirmed by a recent study on evolutionary dynamics of the PepMV population in Spain, in which RT-qPCR analyses in inoculated tomato plants showed that a CH2 isolate (PS5) accumulated faster and to higher viral loads than a EU isolate (Sp13) (Gómez *et al*., 2009). However, in this respect the low incidence of the CH2 genotype in North America is remarkable and might reflect different PepMV dissemination pathways linked to a different, less intensive structure of tomato growth facilities in North America as compared to Europe, where PepMV is mainly prevalent in dense greenhouse tomato cultivation areas. In the American situation mechanical transmission through workers or bumblebees may be subordinate to the long-distance transmission through young plants and seeds (Hanssen and Thomma, 2010). Recently, the existence of a population bottleneck during seed transmission was reported, with an apparent advantage of the EU genotype in transmission through seeds harvested from a mother crop co-infected by the EU and CH2 genotypes (Hanssen *et al*., 2010a). As seed transmission was suggested as a major dissemination route of PepMV in 1999 and 2000, before strict phytosanitary regulations were in place (Córdoba-Sellés *et al*., 2007), this putative population bottleneck might be related to the original dominance of the EU genotype in European countries.

As PepMV is a RNA virus, a high mutation rate can be expected. However, mutation analyses performed on several Spanish isolates revealed very few non-synonymous substitutions, reflecting strong purifying selection (Gómez *et al*., 2009). Also in a Belgian study it was shown that the number of mutations in the RNA sequence of PepMV isolates throughout a greenhouse trial period was rather limited and that most of the mutations that took place had no clear biological relevance (Hanssen *et al.,* 2009b, Chapter 3).

1.28. How often has the pest been introduced into new areas outside its original area of distribution? (specify the instances, if possible)

Note: if this has happened even once before, it is important proof that the pest has the ability to pass through most of the steps in this section (i.e. association with the pathway at origin, survival in transit, transfer to the host or habitat at arrival and successful establishment). If it has occurred often, it suggests an aptitude for transfer and establishment.

never, very rarely, rarely, occasionally, **often,** very often

Level of uncertainty:	**Low**	Medium	High

PepMV is believed to have originated in southern America, as it was first reported in Peru on pepino and has since been found in Peru on wild species of *Lycopersicon* (Jones *et al*., 1980; Soler *et al*., 2002). Since the first report of PepMV infecting tomato (found in the UK and the Netherlands in 1999) there have been several reports of the virus throughout the world with the exception of Oceania. In the EU it was reported in the Netherlands and the United Kingdom, and has now been reported in 19 of the 27 EU MS (question 7; Wright & Mumford, 1999; Van der Vlugt *et al*., 2000). The rapid spread of new genotypes like CH2 and also US1 suggests multiple introductions into the PRA area.

1.29. If establishment of the pest is very unlikely, how likely are transient populations to occur in the PRA area through natural migration or entry through man's activities (including intentional release into the environment)?

Note: Non-applicable applies when establishment has already been observed in the PRA area. Transience is defined as the presence of a pest that is not expected to lead to establishment

N/A, very unlikely, unlikely, moderately likely, likely, very likely

Level of uncertainty:	Low	Medium	High

Non applicable. PepMV has been reported in 19 of the 27 EU MS with the first reports dating back to 1999 in the UK and the Netherlands.

<u>Conclusion on the probability of establishment</u>

The overall probability of establishment should be described.

PepMV is already present in many parts of the PRA area and was first reported in 1999 from the UK and the Netherlands. It is known to have occurred in 19 of the current 27 EU MS, including the main tomato production areas, both in protected cultivation and in outdoor grown tomato crops. It has also been found in weeds surrounding tomato production facilities in Spain. Although strict hygiene measures can prevent establishment of PepMV the fact that the virus is very readily transmitted mechanically poses great risks. Thus, the probability of establishment is very high with a low uncertainty.

Probability of spread

Spread potential is an important element in determining how quickly impact is expressed and how readily a pest can be contained. In the case of intentionally imported plants, the assessment of spread concerns spread from the intended habitat or the intended use to an unintended habitat, where the pest may establish. Further spread may then occur to other unintended habitats. The nature and extent of the intended habitat and the nature and amount of the intended use in that habitat will also influence the probability of spread. Some pests may not have injurious effects on plants immediately after they establish, and in particular may only spread after a certain time. In assessing the probability of spread, this should be considered, based on evidence of such behaviour.

1.30. How likely is the pest to spread rapidly in the PRA area by natural means?

Note: consider the suitability of the natural and/or managed environment, potential vectors of the pest in the PRA area, and the presence of natural barriers. Spread depends on the capacity of a pest to be dispersed (e.g. wind dispersal) as well as on the quantity of pest that can be dispersed (e.g. volume of seeds).
Natural spread can result from movement of the pest by flight (of an insect), wind or water dispersal, transport by vectors such as insects, birds or other animals (internally through the gut or externally on the fur), natural migration, rhizomial growth. Spread is defined as the expansion of the geographical distribution of a pest within an area [FAO, 2007]

very unlikely, **unlikely**, moderately likely, likely, very likely

Level of uncertainty:	Low	**Medium**	High

PepMV is spread mainly with the aid of humans (see 1.31).

There have been reports of bumble bees being capable of spreading the virus in greenhouses although the exact mechanism of transmission has not been determined (Lacasa *et al.*, 2003; Shipp *et al*., 2008). In dense tomato production areas bumble bees might act as a vector for PepMV between greenhouses. Otterstatter and Thomson (2008) indicate that bumble bees disperse from greenhouses. The estimated maximum foraging range that a bumble bee will have is 758 m according to one study (Knight *et al.*, 2005)

Birds have been purported to spread PepMV by contact transmission between greenhouses although this is only a hypothesis based on experiences and discussions with tomato growers (Hanssen *et al.*, 2009a; pers. comm. A. Werkman). It also has been suggested that there might be other insects, as well as birds and rodents that might transmit the virus mechanically, although there are no published reports of this occurring.

1.31. How likely is the pest to spread rapidly in the PRA area by human assistance?

Note: consider the potential for movement with commodities or conveyances, the fact that the species is intentionally dispersed by people, the ability of the pest to be unintentionally dispersed along major transport routes. As for 1.30, consider the capacity to be spread as well as the quantity that can be spread. For intentionally introduced plants consider spread to the unintended habitat.

very unlikely, unlikely, moderately likely, likely**, very likely**

Level of uncertainty:	**Low**	Medium	High

The main pathways of spread for PepMV are plant material (mainly plants for planting, infected fruit and contaminated seed albeit with a low transmission rate). There is also potential for spread on contaminated packing material.

Since there is a lot of movement of this material and the virus is very easily transmitted the pest is very likely to spread rapidly with human assistance.

In dense tomato production areas, PepMV is known to spread rapidly between greenhouses where hygiene measures have not been observed by workers. Experiments carried-out in January 2010 have shown that hands, clothing and rigid plastic containers that have been in contact with infected fruit are able to lead to infection of tomato plants (see 1.12).

In the Netherlands and Belgium there are tomato growers that have deliberately inoculated their crops with a mild PepMV isolate in the belief that it offers protection against subsequent infection by an aggressive isolate (Spence *et al.,* 2006*;* Hanssen *et al.*, 2009a). This action will readily facilitate spread by human assistance.

1.32. Based on biological characteristics, how likely is it that the pest will not be contained within the PRA area?

Note: consider the biological characteristics of the pest that might allow it to be contained in part of the PRA area. For intentionally introduced plants consider spread to the unintended habitat.

very unlikely, unlikely moderately likely, likely, **very likely.**

Level of uncertainty:	Low	**Medium**	High

Since the virus is very easily spread by contact (mechanical transmission) it is very likely that the pest will not be contained within the PRA area.

Go to conclusion on the probability of spread

<u>Conclusion on the probability of spread</u>

The overall probability of spread should be described.

The probability of spread is considered as very likely. Given the ease of mechanical transmission the virus is very likely to spread by human assistance, especially in dense production areas. In such dense production areas there is also a moderate risk of spread by natural means, e.g. bumble bees and birds that might transmit the virus mechanically.

Go to Conclusion on the probability of introduction and spread

<u>Conclusion on the probability of introduction and spread</u>

The overall probability of introduction and spread should be described. The probability of introduction and spread may be expressed by comparison with PRAs on other pests.

The probability of introduction, establishment and spread of PepMV is very likely. This is supported by the occurrence of the virus in many parts of the PRA area.

Go to 1.33

Conclusion regarding endangered areas

1.33. Based on the answers to questions 1.15 to 1.32 identify the part of the PRA area where presence of host plants or suitable habitats and ecological factors favour the establishment and spread of the pest to define the endangered area.

Note: The PRA area may be the whole EPPO region or part of it. The endangered area may be the whole of the PRA area, or part or parts of the area (i.e. the whole EPPO region or whole or part of several countries of the EPPO region). It can be defined ecoclimatically, geographically, by crop or by production system (e.g. protected cultivation such as glasshouses) or by types of ecosystems.

The endangered area is the whole of the EU where tomato production takes place.

Go to 2 Assessment of potential economic consequences

2. Assessment of potential economic consequences

The main purpose of this section is to determine whether the introduction of the pest will have unacceptable economic consequences. It may be possible to do this very simply, if sufficient evidence is already available or the risk presented by the pest is widely agreed. Start by answering Questions 2.1 - 2.10. If the responses to question 2.2 is "major" or "massive" and the answer to 2.3 is "with much difficulty" or "impossible" or any of the responses to questions 2.4, 2.5, 2.7, 2.9 and 2.10 is "major" or "massive" or "very likely" or "certain", the evaluation of the other questions in this section may not be necessary and you can go to 2.16 unless a detailed study is required or the answers given to these questions have a high level of uncertainty. In cases where the organism has already entered and is established in part of the PRA area, responses to questions 2.1, 2.6 and 2.8, which refer to impacts in its area of current distribution, should be based on an assessment of current impacts in the PRA area in addition to impacts elsewhere. *Expert judgement is used to provide an evaluation of the likely scale of impact. If precise economic evaluations are available for certain pest/host plant combinations, it will be useful to provide details.* *The replies should take account of both short-term and long-term effects of all aspects of agricultural, environmental and social impact.* *In any case, providing replies for all hosts (or all habitats) and all situations may be laborious, and it is desirable to focus the assessment as much as possible. The study of a single worst-case may be sufficient. Alternatively, it may be appropriate to consider all hosts/habitats together in answering the questions once. If a selection is made, it should be justified. Only in certain circumstances will it be necessary to answer the questions separately for specific hosts/habitats.*

Consider potential hosts/habitats identified in question 6 when answering the following questions:

Pest effects

2.1. How great a negative effect does the pest have on crop yield and/or quality to cultivated plants or on control costs within its current area of distribution?

Note: factors to consider are types, amount and frequency of damage and crop losses in yield and quality, together with costs of treatment.

minimal, **minor, moderate, major,** massive

Level of uncertainty:	Low	**Medium**	High

Opinions vary as to the economic damage caused by PepMV on tomato, other than the general view that there is likely to be some damage to fruit resulting from infection of tomato plants with this virus. Hence the range of scores assigned to the response for this question could vary from minor to major but with medium uncertainty.

Several experiments have been performed to try to determine the impact of the virus on tomato yield and quality. The emergence of new genotypes (strains) of PepMV has complicated the assessment of the impact. As stated in response to question 8, the different genotypes cannot be distinguished based on biological characteristics including their effect on yield and quality. This is because isolates of PepMV of the same genotype can differ in their effect.

An overview of trials and experiences is given below:

Pepeira trials 2007-2009 (Pepeira final report WP2, 2010)**:**

<u>Trial design</u>

In the PEPEIRA project replicate greenhouse trials over two full seasons have been performed in four countries. The countries were Hungary, the Netherlands, Spain and the United Kingdom. In these greenhouse trials the effects of two isolates of PepMV on tomato cultivar 'Cedrico' was determined. In the first year an isolate known to be mild, belonging to the European tomato strain, was tested (isolate 1066). In the second year an aggressive CH2 isolate was used (isolate PCH-06/104). In both years a healthy control treatment was included. Each treatment consisted of four replicate plots of 20 tomato plants each. Crop management was in line with local commercial practise, including very strict hygiene measures. Plants were inoculated when the fifth truss was flowering, and shortly before the first harvest, and success of inoculation was tested by ELISA. Several characteristics were scored during the growing season: PepMV concentration, flowering and fruit set, leaf symptoms, fruit symptoms, yield and shelf-life. Since the isolates were assessed in different growing seasons, although they were tested under controlled cropping conditions, the infected crops were likely to have experienced different temperature and watering regimes. This might have influenced the results.

<u>Results</u>
For an overview of the results see Table 10. Because the two isolates were not tested in the same cropping season this may have influenced the results. Therefore the effects of each isolate can only be compared to the healthy controls and not with the other isolate.

No clear effects on flowering, fruit set or shelf life were observed. Leaf symptoms were observed for both isolates. The plots inoculated with the aggressive (PCH-06/104) isolate showed more pronounced symptoms than the plots inoculated with the mild isolate (1066).

When comparing yield across all four sites, the 1066 isolate had no measurable effect on total yield of Class I, Class II and waste combined. The PCH-06/104 isolate reduced the total yield of Class I, Class II and waste combined by 4%. However, this reduction was not found statistically significant at the 5% significance level. When considering the results of all countries separately, the 1066 isolate again had no significant effect on total yield. The PCH-06/104 isolate only had a significant effect on total yield in Spain, where yield was reduced by 10%. For the Hungarian and the UK trials there was a tendency towards a lower yield in the PCH-06/104-infected strains compared to healthy plants, although this effect was not significant. In the Netherlands, yield was unaffected.

When looking at fruit quality the mild PepMV isolate (1066) had only very little effect (Table 11). The occurrence of discoloration in the infected plots did not differ statistically with discoloration occurring in uninfected plots. In the trial with the more aggressive PepMV isolate (PCH-06/104) an effect on fruit quality was observed (Table 12). Class I yield in plants that were infected with PCH-06/104 was overall reduced by around 15% over a 15-week period. This was a significant effect. The effects were most pronounced during the early part of the harvesting period. Also at each trial location this significant effect was observed, except for Spain where mean yields of Class I fruit were very low.

Efford trials, United Kingdom (Spence *et al*, 2006)**:**
In the United Kingdom two fully replicated trials with PepMV on tomato were undertaken in 2001-2002 and 2003. Conditions were similar to commercial tomato production. In the first trial one tomato cultivar was tested, in the second trial two were tested. In both experiments, bulk yield was not found to be reduced significantly. However, it was reported that PepMV affected quality and size of tomatoes. In both trials, after infection with PepMV, size of fruit was affected resulting in a reduction of fruit numbers of the preferred size category. Although in the first trial no distinct virus symptoms were seen on fruits, blotchy ripening and gold marbling disorders were observed more often in the PepMV inoculated plants then in the

healthy controls. This combination resulted in losses of class 1 fruit across the season that amounted to 6.5%. In the 2003 trial clear virus symptoms were observed. In this second trial, overall 55% of tomatoes of PepMV affected plants were graded as class 1, compared with 88.7% from non-inoculated plants. Moreover in the trials it was observed that PepMV had a greater effect on quality if plants were inoculated later in the season. Although in other reports low light conditions are thought to result in more damage (Jordá *et al.,* 2001a), in the Efford trials a greater effect in quality of fruit was observed in the sunny 2003 year compared to the 2001-2002 trial which was conducted under lower light conditions.

Trials at PPO Glastuinbouw, the Netherlands (De Buck and Stijger, 2002):
In 2001, a glasshouse experiment with PepMV was undertaken in Naaldwijk. The effects on the yields and quality of fruit of six cultivars of a PepMV infection alone and a combined infection with *Verticillium* were determined. Although the results could not be statistically analysed, no clear yield effects were observed when the plants were infected with PepMV alone. Interaction with a *Verticillium* infection resulted in yield loss for five out of six cultivars. The results indicate that there may be a difference in cultivar response to a combined infection of PepMV and *Verticillium*. In the same experiment, there was no clear effect on quality of PepMV or mixed PepMV and *Verticillium* infections as compared to controls.

European ringtest, 2001-2002 (Anon., 2003; Werkman *et al.*, Netherlands, 2003, unpublished results)**:**
In the winter of 2001/2002, a ring test was performed in the Netherlands, Spain, Germany and the UK to study the effect of tomato cultivar, virus isolates and climatic conditions on symptomatology. Because it was not possible to obtain standardised conditions and the plot sizes were small, it was not possible to make a well-sustained statistical analysis. However, there were no clear indications of an effect on yield and hardly any symptoms were observed on the fruit (Werkman *et al.*, Netherlands, 2003, unpublished results). Because no statistically valid conclusions could be reached from the data obtained, the UK decided to undertake comprehensive research that could be statistically analysed. Hence the trials at Efford as reported above were initiated.

Surveys on symptom expression and damage of PepMV in Belgium (Hanssen et al., 2009a):
In the years 2005-2006 an extensive survey among tomato growers was conducted among Belgium tomato growers. In the study the major tomato growing areas in Flanders were covered. In the 2005 survey most of the growers considered fruit quality as the main problem associated with PepMV infection. Around 70% of the growers estimated the percentage of lower quality tomatoes as a result of PepMV infection as less than 5%. The remaining group gave a higher estimation, up to more than 20%. In the 2006 survey a significant loss in production was observed and attributed to PepMV infection. In about 50% of the PepMV infected production sites the production loss was estimated at more than 5%, and in one fifth of the cases even more than 10%. However, reported quality losses were less than in 2005.

Trials on cross-protection in Belgium (Hanssen *et al*., 2010b)
In Belgium a trial in plastic tunnels was performed to assess the potential of a mild PepMV isolate (belonging to the LP strain) to protect tomato plants against a more aggressive isolate (belonging to the CH2 strain; same isolate as used in PEPEIRA field trials). There were four treatments: 1) non-infected control (mock inoculated), 2) LP mild isolate alone, 3) CH2 aggressive isolate alone and 4) LP mild isolate followed three weeks later by inoculation of the CH2 aggressive isolate. Two types of fruit symptoms were scored, fruit marbling and fruit flaming. Overall percentage of marbling/flaming was 0.1%/3.8% for the control plants, 1.3%/5.0% for the LP mild

reference plants, 4.2%/16.5% for the CH2 aggressive reference plants and 17.9%/12.6% for the LP mild pre-inoculated plants. Overall yield loss compared to the non-infected control was highest in the LP mild reference plants (13%). In the CH2 aggressive reference plants yield was reduced by 6%. In comparison, the overall yield reduction by this isolate in the Pepeira trials was 4%. In the LP mild pre-inoculated plants yield was reduced by 3%. Although statistical analysis has been performed in these trials, there are no data on the significance of these specific results.

Trials on cross-protection in The Netherlands (Schenk *et al*., 2010)
In the Netherlands, trials were performed in greenhouses to examine whether tomato plants can be protected against PepMV by a preceding infection with an attenuated isolate. In the trials three tomato cultivars were tested, all cultivars were grafted and non-grafted. Two attenuated isolates were used for inoculation: EU-Att1 belonging to the EU strain, PE-Att2 (PE strain). As challenging isolates, two EU isolates were used, EU-Nec1 and EU-Chl1. There were nine treatments: 1) virus-free control, 2-5) single infections of all four isolates and 6-9) cross-protection treatments.
Average yield was higher in grafted plants then in non-grafted plants, but there was no significant interaction between treatment and grafting. Compared to the uninfected treatment, there were significant overall yield losses by the EU-Nec1 isolate (24%), and the EU-Chl1 isolate (8%). For the attenuated isolates there was no significant effect on yield. In the cross-protection treatment yield losses of EU-Nec1 and EU-Chl1 were reduced to 0-3%. The cross-protection by EU-Att1 resulted in a significant improvement of the yield. In all treatments no fruit symptoms were observed that could be related to PepMV.

Report on the Pepeira stakeholder meeting (Pepeira final report WP1, 2010)**:**
During the Pepeira stakeholder meeting in Wageningen in February 2010, 46 stakeholders and the PEPEIRA partners discussed several issues on PepMV. Although it concerned only discussions and there is no direct scientific basis under the report, the views during these discussions are used in this PRA to give an indication of experiences in the field.
One question was on the damage caused by PepMV. There was consensus that PepMV can cause considerable damage and that the virus can have a very large economic impact. The figures observed in the PEPEIRA field trials were confirmed by several stakeholders. Quality loss is generally considered as the biggest problem. It was discussed that the direct economic effect of this loss is very difficult to calculate as it depends on the market prices of Class I versus Class II fruit. Prices are directly influenced by the relative amounts of each class that are available on that market. A distinct quality loss will directly influence prices and will have a negative influence on the price of good quality tomatoes. This damages the sales as well as the sector. Another distinct side effect of PepMV is that it has led to mistrust among key players in the industry. Since the sector is most cost-effective when there is trust among stakeholders, PepMV has an indirect negative effect.

Conclusion:

The impact of PepMV depends on several factors.

The main factors are virus isolate, cultivation conditions including climate, and the tomato cultivar. As mentioned earlier in this PRA (Question 8) no correlation between virus strain (genotype) and symptoms has been shown yet. However, it is known that some isolates of the virus consistently induce more severe symptoms than other isolates when tested, even within a strain. The type of virus isolate present in a crop will influence the effect of PepMV. For example in the PEPEIRA field trials the 'mild' isolate had a minimal effect on both yield and quality while the 'aggressive' isolate had a minor effect on yield but a major effect on quality. However, the effect of individual isolates is unpredictable until they are subject to testing. If isolates of different strains of PepMV occur simultaneously in a crop the impact might be more severe. If combined infections of PepMV with other viruses, bacteria or fungus occur, this synergism can lead to a more severe impact.

The climate and possibly the cultivation conditions of the crop (e.g. crop management, nutrient balance) may influence the effect of PepMV. In the past it has been observed that light and temperature influence symptomatology of PepMV (Jones & Lammers, 2005). In several papers it has been stated that a low light intensity will induce more severe symptoms, although in the Efford trials the opposite effect was observed. In the Pepeira trials most effect was observed in the first part of the growing season.

Finally, circumstantial evidence suggests that the tomato cultivars used may influence the effect of PepMV.

Overall it is difficult to quantify the effect of climate, cultivar, cultivation and isolate on the effect of PepMV.

Economic effects of PepMV are usually the result of reductions in quality. In most, but not all trials, yield losses are limited. However, it should be noted that even a small yield loss can result in economic losses for individual growers. Since it has been shown that PepMV affects fruit quality and therefore may result in downgrading of Class 1 fruit, the level of economic loss will depend upon differences in market price and marketing.

The overall conclusion of the effect of PepMV on yield and quality is that PepMV will have a minor effect on yield and a moderate effect on fruit quality. The effects will mainly depend on the isolate present and this is difficult to predict. Under optimal climatic conditions for the crop, in combination with a mild isolate, the effects will be minimal, while under negative climatic conditions in combination with an aggressive isolate the effect can be very serious.

Table 10. Summary of the effects of the PepMV isolates *1066* and *PCH-06/104* on tomato plants grown under conditions similar to the commercial practice in the UK, Hungary, Spain and the Netherlands. Each isolate was tested in a different year. (No effect = no significant effect at p =0.05; percent figures are significant reductions)

Isolate	***1066* (mild isolate)**				***PCH-06/104* (agressive isolate) (% values are reductions – significant at p = 0.05)**			
Country **Effect on:**	**UK**	**Hungary**	**Spain**	**Netherlands**	**UK**	**Hungary**	**Spain**	**Netherlands**
Flowering	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*
Fruit set	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*
Symptoms in plant heads and foliage	Mild symptoms: Yellow spots	Mild symptoms: Yellow spots	Mild symptoms: One plant with yellow spots, necrosis on petals	Mild symptoms: Yellow spots and discoloration	leaf bubbling, mild mosaic	leaf bubbling, scorch, yellow spots	Mild symptoms: yellow spots, necrosis on petals	Clear symptoms shortly after inoculation, mild symptoms later on
Total yield - weight	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	10%	*No effect*
Total yield - No. of fruits	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*
Fruit size distribution	*No effect*	*No effect*	Slightly larger fruits	No effect	*Smaller fruits early season*	smaller fruits	No general effect on size	*No effect*
Fruit quality (% downgrading from Class I to Class II)	*No effect*	*No effect*	*No effect*	*No effect*	10%	29%	23%	13%
Fruit symptoms	*No effect*	Uneven ripening observed, but not PepMV related	*No effect*	Uneven ripening observed, but not PepMV related	Marbling and uneven ripening	Marbling and uneven ripening observed	*No effect*	Increased occurrence of uneven ripening
Shelf-life	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	*No effect*	Small negative effect early in the season

Table 11. Effect of PepMV mild isolate 1066 on yield of tomato (g/plant/week) over a 14 week harvest period. Values in brackets are changes in yield within each class category as a result of PepMV infection (% yield compared with healthy control plots). A minus value represents a yield reduction due to virus infection. * and ** represent significant yield effects at *P*<0.05 and *P*<0.01 respectively. Note, analysis was done using REML for repeated measures because of the unbalanced data set.

	Class I		Class II		Waste	
Country	Healthy	Inoculated	Healthy	Inoculated	Healthy	Inoculated
Hungary	165.2	144.7 (-12.4%)	59.4	60.4 (1.6%)	47.2	69.2 (46.4%)**
Netherlands	661.4	642.9 (-2.8%)	57.2	61.9 (8.2%)	2.1	3.1 (47.1%)
Spain	17.1	25.9 (50.8%)	134.1	144.2 (7.5%)	215.3	204.4 (-5.1%)
UK	580.6	567.7 (-2.2%)	55.2	90.7 (64.6%)*	4.3	3.8 (-10.7%)
Country Mean	**266.6**	**256.1 (-4.0%)**	83.2	96.0 **(15.4%)**	88.5	91.3 **(3.2%)**

Table 12. Effect of PepMV aggressive isolate PCH-06/104 on yield of tomato (g/plant/week) over a 14-week harvest period[1]. Values in brackets are changes in yield within each class category as a result of PepMV infection (% yield compared with healthy control plots). A minus value represents a yield reduction due to virus infection. *, ** and *** represent significant yield effects at P<0.05, P<0.01, and P<0.001 respectively. Analysis was done using ANOVA for repeated measures.

	Class I		Class II		Waste	
Country	Healthy	Inoculated	Healthy	Inoculated	Healthy	Inoculated
Hungary	218.0	153.7 (-29.5%)**	84.7	118.3 (39.7%)**	48.6	62 (27.6%)
Netherlands	642.7	558.3 (-13.1%)***	87.6	167.1 (90.7%)***	21.4	34.4 (60.7%)
Spain	63.5	48.7 (-23.3%)	161.6	123.8 (-23.4%)**	280.0	277.2 (-1.0%)
UK	593.6	530.3 (-10.4%)**	60.1	93.9 (56.2%)**	37.5	37.8 (0.8%)
Country Mean	**376.2**	**320.4 (-14.8%)*** **	**98.3**	**123.9 (26.0%)*** **	**96.9**	**102.8 (6.1%)**

[1] The means presented in the analysis of variance are calculated after estimating the values for the weeks, treatments, countries and replicate blocks when data were missing. As a result of this, the predicted means will therefore not match the observed means exactly for the treatments for each country where missing values were present (and in the case of the Netherlands, data were collected beyond 14 weeks).

2.2. How great a negative effect is the pest likely to have on crop yield and/or quality in the PRA area without any control measures?

Note: the ecological conditions in the PRA area may be adequate for pest survival but may not be suitable for pest populations to build up to levels at which significant damage is caused to the host plant(s). Rates of pest growth, reproduction, longevity and mortality may all need to be taken into account to determine whether these levels are exceeded. Consider also effects on non-commercial crops, e.g. private gardens, amenity plantings.

PepMV is already in the PRA area and is subject to varying degrees of control (including phytosanitary controls). Therefore it is difficult to assess the negative effect in the absence of any control measure. The answer for 2.1 is based upon field trials and surveys. The survey results most likely include responses based upon crops where phytosanitary and or cultural controls have been applied. The effect on yield and quality in the absence of controls will also depend upon the dominant isolate, as described under 2.1. If no controls at all are applied there are chances that more aggressive isolates will become predominant and there will be a major negative effect on tomato production.

Since PepMV is mainly a problem for fruit quality and to a lesser extent (variable) for yield, the effect on non-commercial tomato growing will be minimal.

minimal, minor, moderate, **major**, massive

Level of uncertainty:	Low	Medium	**High**

2.3. How easily can the pest be controlled in the PRA area without phytosanitary measures?

Note: *Consider the existing control measures and their efficacy against the pest. Difficulty of control can result from such factors as lack of effective plant protection products against this pest, resistance to plant protection products, difficulty to change cultural practices, occurrence of the pest in natural habitats, private gardens or amenity land, simultaneous presence of more than one stage in the life cycle, absence of resistant cultivars.*

The probability of introduction, establishment and spread of PepMV is very likely (See Questions 1.23, 1.24 and 1.32). This is supported by the occurrence of the virus in many parts of the PRA area despite attempts to eradicate PepMV in some countries. However, there are options to control the virus without phytosanitary measures:

<u>Hygiene measures</u>

The main control strategy should be to ensure PepMV is not introduced to a production facility. Moreover, spread through virus-contaminated hands, tools and clothes of workers should be prevented. Diseased plants within a production facility should be destroyed as soon as they are detected to avoid transfer by plant-to-plant contact and by crop management. Also, thorough cleaning of materials/tools etc. should take place to eliminate the virus. However, when the virus has entered a compartment it will be very difficult to contain.

In seed production and in plant production facilities these measures are feasible if measures are strictly applied. In the Netherlands, nurseries have developed protocols to ensure freedom of seeds and plants from harmful organisms (PEPEIRA stakeholder meeting report). For fruit production sites hygiene protocols are also available (Fletcher, 2000; O'Neill *et al.*, 2003; O'Neill & Mumford, 2006; Anon., 2007). However, especially in dense production areas it might be difficult to control the virus in production facilities due to the higher chance of contact spread by humans and to a lesser extent by insects or birds.

Resistance:
Some resistance breeding activities work has been undertaken to determine if some cultivars of pepino and wild solanaceous species are resistant, but no resistant varieties of tomato are expected to become available in the near future (Ling & Scott, 2007).

Since the main control option is hygiene it should be possible to control the virus. The main uncertainty is to what extent these measures are cost-effective. Prevention of entry is likely to be cost-effective, eradication of the virus from an infected crop during the growing season is not.

very easily, easily, **with some difficulty,** with much difficulty, impossible

Level of uncertainty:	**Low**	Medium	High

2.4. How great an increase in production costs (including control costs) is likely to be caused by the pest in the PRA area?

Note: both normal farm practice costs and costs of control should be included, in particular:
- ease of detection of the pest: species that are difficult to detect will require a greater surveillance and monitoring effort which will indirectly result in higher production costs.
- treatment: treatment options may vary (plant protection products, physical removal,…). Treatment costs may be divided into operating (e.g. chemical, fuel, equipment) and labour (i. e. hours per ha).

PepMV is already in the PRA area and some of the costs associated with production are related to phytosanitary measures and some are those taken by different sectors of the tomato industry to control PepMV as well as other pests. Estimates of the increase in production costs (including control costs) caused by the pest in the PRA area are difficult to separate out. The costs for each sector are listed without quantitative estimates:

Seed industry:
When producing tomato seeds strict hygiene measures are common to prevent introduction of a wide range of diseases. Since PepMV is very easily contact transmitted and already present in parts of the PRA area, in most cases there is even more emphasis on hygiene. There are also rigorous monitoring programmes including testing of mother plants and seed lots for the presence of PepMV.

Plant producers:
When producing tomato plants for planting, strict hygiene measures are common to prevent introduction of a wide range of diseases. Since PepMV is very easily contact transmitted and already present in parts of the PRA area, in most cases there is even more emphasis on hygiene. Also regular testing takes place.

Growers - Tomato production:
Main emphasis in tomato production is prevention of introduction of PepMV and spread within a production place. In general there are hygiene measures, but experiences with PepMV has shown that in some countries a lot of investments

have been made to 'lock' the premises to prevent infection of PepMV. This ranges from changing of clothes to automated gates that can only be passed if one is disinfected.

Since some of the control measures are already common practice in the different sectors the additional increase of production costs is estimated as moderate.

minimal, minor, **moderate,** major, massive

Level of uncertainty:	Low	**Medium**	High

2.5. How great a reduction in consumer demand is the pest likely to cause in the PRA area?

In general, plant diseases have a minimal effect on consumer demand of edible products, particularly of staple foods such as tomatoes, but they can influence supply. PepMV will not have large or sudden effects on total yield but it can influence the quality of individual grower's crops. PepMV can result in downgrading of Class 1 fruit to Class 2 or even to waste. Consumers will still 'demand' tomatoes; the demand for a particular type of tomato may still be the same but the supply of Class 1 fruit may decrease and that of Class 2 may increase.

The effect of this is that the price of Class 1 fruit may increase and there may be a marginal fall in demand as a result, but there is limited information to make a judgement for the PRA area with great certainty. Similarly, if the supply of Class 2 fruit increases the price may fall and the demand may increase. The net effect depends on the relative prices of Class 1 and 2 fruit and the elasticities but there are uncertainties in information on elasticity (and therefore price changes due to the disease) and relatively poor information on the price of Class 2 fruit. The overall effect on demand is likely to be minor but with medium uncertainty. (G. Jones, ADAS, UK, personal communication to C. Sansford, Fera, 2010).

minimal**, minor,** moderate, major, massive

Level of uncertainty:	Low	**Medium**	High

2.6. How important is environmental damage caused by the pest within its current area of distribution?

Note: effects of introduced pests may include: reduction of keystone species; reduction of species that are major components of ecosystems, and of endangered species; significant reduction, displacement or elimination of other species; indirect effects on plant communities (species richness, biodiversity); significant effects on designated environmentally sensitive areas; significant change in ecological processes and the structure, stability of an ecosystem (including further effects on plant species).
Pests which principally have effects on crop yield or quality may also have environmental side-effects. If the main effects are already large and unacceptable, detailed consideration of such side-effects may not be necessary. On the other hand, other pests principally have environmental effects and the replies to this and the following question are then the most important of this part of the analysis.

PepMV already occurs in the PRA area and there are no reports of environmental damage either in the area itself or elsewhere. Although PepMV has been reported to occur on some weed species in both Peru and Spain no direct negative effects of the virus have been observed or are expected.

minimal, minor, moderate, major, massive

Level of uncertainty:	**Low**	Medium	High

2.7. How important is the environmental damage likely to be in the PRA area (see note for question 2.6)?

See the response to 2.6.

minimal, minor, moderate, major, massive

Level of uncertainty:	**Low**	Medium	High

2.8. How important is social damage caused by the pest within its current area of distribution?

Note: Social effects may arise as a result of impacts to commercial or recreational values, life support/human health, biodiversity, aesthetics or beneficial uses. Social effects could be, for example, changing the habits of a proportion of the population (e.g. limiting the supply of a socially important food) damaging the livelihood of a proportion of the human population, affecting human use (e.g. water quality, recreational uses, tourism, animal grazing, hunting, fishing). Effects on human or animal health, the water table and tourism could also be considered, as appropriate, by other agencies/authorities.

PepMV already occurs in the PRA area. There are no reports found of social damage here or elsewhere. Locally, large yield reduction and/or quality losses might cause social damage due to income change.

minimal, minor, moderate, major, massive

Level of uncertainty:	**Low**	Medium	High

2.9. How important is the social damage likely to be in the PRA area?

See the response to 2.8

minimal, minor, moderate, major, massive

Level of uncertainty:	**Low**	Medium	High

2.10. How likely is the presence of the pest in the PRA area to cause losses in export markets?

Note: *consider the extent of any phytosanitary measures likely to be imposed by trading partners.*

PepMV already occurs in some important tomato producing parts of the PRA area.
Currently, PepMV is only regulated as a quarantine pest on tomato seeds in the EU, India and Israel. Although there have been several interception reports in seed, these reports are mainly made by EU Member States. If there are interceptions the lots might be destroyed or more strict requirements might be added, however regarding the current trade is not likely that there will be a loss

of export markets. Since there is currently no legislation for tomato fruit and plants for planting, loss of export markets is unlikely for these products.

Impossible/very unlikely, **unlikely**, moderately likely, likely, very likely/certain

Level of uncertainty:	**Low**	Medium	High

As noted in the introduction to section 2, the evaluation of the following questions may not be necessary if the response to question 2.2 is "major" or "massive" and the answer to 2.3 is "with much difficulty" or "impossible" or any of the responses to questions 2.4, 2.5, 2.7, 2.9 and 2.10 is "major" or "massive" or "very likely" or "certain". You may go directly to point 2.16 unless a detailed study of impacts is required or the answers given to these questions have a high level of uncertainty.

2.11. How likely is it that natural enemies, already present in the PRA area, will not reduce populations of the pest below the economic threshold?

Note: For pest plants, natural enemies include herbivores and pathogens.

The question is not applicable to viruses since they have no known natural enemies.

very unlikely, unlikely, moderately likely, likely, **very likely**

Level of uncertainty:	**Low**	Medium	High

2.12. How likely are control measures to disrupt existing biological or integrated systems for control of other pests or to have negative effects on the environment?

Hygiene measures to prevent introduction or spread of PepMV might interfere with biological or integrated systems for control of other pests. However since measures are mainly aimed at minimizing cultural practises and producing clean seed and plants for planting, the effects will be minimal. Depending on how they are used, disinfectants used for hygiene, such as sodium hypochlorite, might have a local impact on the environment.

impossible, very unlikely**, unlikely,** moderately likely, likely, very likely, certain

Level of uncertainty:	**Low**	Medium	High

2.13. How important would other costs resulting from introduction be?

Note: costs to the government, such as project management and administration, enforcement, research, extension/education, advice, publicity, certification schemes; costs to the crop protection industry.

Since the first outbreaks of PepMV in 1999 a lot of research has been performed and financed by government, institutes and industry. These activities include: monitoring activities by governments, research and development work, and development and large scale use of reliable testing methods and the development of hygiene protocols. The costs also depend on the phytosanitary status that the virus has.

minimal, minor, **moderate**, major**,** massive

Level of uncertainty:	**Low**	Medium	High

2.14. How likely is it that genetic traits can be carried to other species, modifying their genetic nature and making them more serious plant pests?

Recombination between PepMV isolates are known to occur (Question 1.27). However, there are no reports of recombination of PepMV with other virus species.

impossible**, very unlikely,** unlikely, moderately likely, likely, very likely, certain

Level of uncertainty:	**Low**	Medium	High

2.15. How likely is the pest to cause a significant increase in the economic impact of other pests by acting as a vector or host for these pests?

PepMV is not known and very unlikely to act as vector for other pests.

Impossible/very unlikely, unlikely, moderately likely, likely, very likely/certain

Level of uncertainty:	**Low**	Medium	High

Conclusion of the assessment of economic consequences

2.16. Referring back to the conclusion on endangered area (1.33), identify the parts of the PRA area where the pest can establish and which are economically most at risk.

The whole EU area where tomato production takes place. In some of the main production areas PepMV is already present in tomato production and growers are dealing with the virus.

Go to degree of uncertainty

Degree of uncertainty

Estimation of the probability of introduction of a pest and of its economic consequences involves many uncertainties. In particular, this estimation is an extrapolation from the situation where the pest occurs to the hypothetical situation in the PRA area. It is important to document the areas of uncertainty (including identifying and prioritizing of additional data to be collected and research to be conducted) and the degree of uncertainty in the assessment, and to indicate where expert judgement has been used. This is necessary for transparency and may also be useful for identifying and prioritizing research needs.

It should be noted that the assessment of the probability and consequences of environmental hazards of pests of uncultivated plants often involves greater uncertainty than for pests of cultivated plants. This is due to the lack of information, additional complexity associated with ecosystems, and variability associated with pests, hosts or habitats.

The main uncertainties are:

Distribution

-One current uncertainty is the distribution of PepMV in third countries. The PRA is based on official reports on the presence of PepMV in countries, although interception data suggest that the distribution might be wider.

-There are also uncertainties on the exact distribution in the PRA area. Although the virus is considered widely distributed in some of the main European tomato production areas as well as in some that produce much less fruit, there are countries that claim the virus is absent or where no official surveys have been reported to the European Commission.

Pathways

-The probability of crops other than tomato being natural hosts of PepMV is uncertain. This applies especially for basil, eggplant and pepper.

-The importance of bumble bees for spread of PepMV is uncertain.

Economic impact

-The main uncertainty in assessing the economic impact is linked to the occurrence of the different isolates. Since symptom development and the consequent downgrading of fruit are strongly correlated with the aggressiveness of an isolate, the economic impact is dependent on the type of isolate that is present. The aggressiveness of individual isolates can vary within a genotype (strain) and effect of the isolate is not predictable without testing. Moreover, new variants of the virus might be introduced, or new genotypes (strains) may result from recombinations of existing variants

For Pest-Initiated Risk Assessments:	**Go to conclusion of the risk assessment**
For Pathway-Initiated Risk Assessments:	**Go to back to 1.3 to evaluate the next pest, if all pests have been evaluated go to conclusion of the risk assessment**

Conclusion of the pest risk assessment

Entry: *Evaluate the probability of entry and indicate the elements which make entry most likely or those that make it least likely. Identify the pathways in order of risk and compare their importance in practice.*

Both entry into the PRA area as well as spread within are considered. The overall probability of introduction and spread in the absence of phytosanitary measures is estimated as **high.**

The relative importance of the pathways is given below (based upon a five word ranking system where **very low** and **very high** are extremes). (See also Table 9 – probability of introduction).

<u>Pathway (i)</u>
Tomato fruit from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China: **High risk**

<u>Pathway (ii)</u>
Tomato seed from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China: **Low to medium risk,** depending on origin

<u>Pathway (iii)</u>
Tomato plants for planting from Canary Islands, Morocco, Norway and Switzerland: **Low risk**

<u>Pathway (viii)</u>
Tomato fruit from EU MS: **High risk**

<u>Pathway (ix)</u>
Tomato seed from EU MS: **Low to medium risk,** depending on origin

<u>Pathway (x)</u>
Tomato plants for planting from EU MS: **Low risk**

<u>Pathway (xiv)</u>
Bumble bees from EU MS: **Very low risk**

Establishment
Evaluate the probability of establishment, and indicate the elements which make establishment most likely or those that make it least likely. Specify which part of the PRA area presents the greatest risk of establishment.

The virus has been present in the PRA area since at least 1999 when it was first reported in the UK and the Netherlands and it has increased its distribution. It has now been recorded in 19 EU MS. The risk of (further) establishment is therefore estimated as very high.

Because of the ease of spread of PepMV, entry can easily lead to establishment. The extent as to what the virus can be contained depends on hygiene measures and on measures to prevent introductions into new areas and into companies not yet infected

Economic importance
List the most important potential economic impacts, and estimate how likely they are to arise in the PRA area. Specify which part of the PRA area is economically most at risk.

The main economic impact is associated with downgrading of good quality tomato fruit due to symptoms caused by PepMV. In most cases yield losses are limited although because of the importance of tomato even a small yield loss can result in an overall high economic loss. The size of the impact depends on whether the crop is infected with a mild or aggressive isolate of PepMV. There may be other influences including cultivation practice, temperature and light and type of cultivar, but the effect is not predictable. The classification system of fruit for marketing in a country and the current market price will influence the size of the impact resulting from an outbreak of PepMV. From both the results of experimental trials and from observations of the growing crop, very low to very high economic damage is known to occur. Therefore the overall economic importance is estimated as medium.

Overall conclusion of the pest risk assessment

The risk assessor should give an overall conclusion on the pest risk assessment and an opinion as to whether the pest or pathway assessed is an appropriate candidate for stage 3 of the PRA: the selection of risk management options, and an estimation of the associated pest risk.

PepMV is already present in the PRA area but based solely on the incidence of the virus reported in official surveys, by phytosanitary definition, the virus is considered to be '*not widely distributed*' (see question 13 of the PRA). However, there is uncertainty regarding the exact distribution of PepMV both within the PRA area and in third countries. Up to 2009, the virus had been reported from 19 out of 27 MS. There is a high risk of further entry, establishment and spread of PepMV in the PRA area. This is supported by the increase in the number of EU MS where it has been reported since it was first detected in only 2 EU MS in 1999. The economic impact of PepMV is particularly influenced by the isolate that is infecting the tomato crop (this is not predictable), as well as possibly some cultivation practices, and, by the marketing system and the current market price for tomato fruit. Under certain circumstances the impact of the virus can be high. Therefore, management options may be needed to prevent further entry and spread of PepMV, including existing and new genotypes. Pathway xiv – bumble bees from within the EU is not considered for risk management.

Stage 3: Pest risk management

The pest risk management stage is the third stage in pest risk analysis. It provides a structured analysis of the measures that can be recommended to minimize the risks posed by a pest or pathway. The pest risk management part may be used to consider measures to prevent entry, establishment or spread of a pest . It explores options that can be implemented (i) at origin or in the exporting country, (ii) at the point of entry or (iii) within the importing country or invaded area.

Before considering the available risk management options, a judgement on the acceptability of the risk posed by the pest or pathway is required. In this scheme, the methods whereby risk management options are selected differ according to whether the introduction is intentional or unintentional, whether the organism is absent or already present in the PRA area and the type of entry pathway. The options are structured so that, as far as possible, the least stringent options are considered before the most expensive/disruptive ones. Options to prevent unintentional entry on commodities are distinguished from options to prevent natural spread/movement or entry with other pathways such as passenger luggage. It should be noted that measures recommended for intentional introductions are often restricted to prohibiting imports and to actions that can be taken in the importing country.

The scheme requires a judgement on the reliability of each potential measure identified. A reliable measure is understood to mean one that it is efficient, feasible and reproducible. Limitations of application in practice should be noted. Once all potential measures have been identified, the extent to which they are cost-effective and can be combined with other measures is evaluated. A pest may enter by many different pathways and a pathway may transport many pests. It is therefore important to repeat the process for all relevant pests and pathways of concern.

In considering your responses to the following questions, please note that helpful information may be obtained from the pest risk assessment stage, particularly from the section concerning the entry of a pest (1.1-1.14). References to the relevant sections of the risk assessment stage have been added.

Risk associated with major pathways

Acceptability of the risk

A decision has to be made to determine whether the risk from any pest/pathway combination is an acceptable risk. This decision will be based on the relationship between the level of risk identified in the pest risk assessment stage (i.e. the combination of the probability of introduction and the potential economic impact) and the importance/desirability of the trade that carries the risk of introduction of the pest.

3.1. **Is the risk identified in the Pest Risk Assessment stage for all pest/pathway combinations an acceptable risk?**

If yes: STOP

If no: Proceed through the risk management scheme following the instructions below

No.

Types of pathways

In most cases, the pathways to be studied will be particular commodities of plants and plant products, of stated species, moving in international trade and coming from countries where the pest is known to occur, and the questions are intended primarily for these situations. However, the pathways identified in the pest risk assessment may also include other types of pathways, e.g. natural pathway (pest spread), transport by human travellers, conveyances packing material and traded commodities other than plants and plant products, and these also need to be assessed for suitable measures. Therefore, this section explains how to analyze the other types of pathways. For *plants, it is particularly important to prioritize the pathways and to identify their relative importance, as some important pathways may not currently be regulated (grain, wool, hides, sand, gravel...).*

Instructions for working through the Risk Management stage

Pest-Initiated Analysis

In the case of an analysis concerning an unintentional introduction of a pest, go to question 3.2 and proceed through steps 3.2-3.10, which relate to different pathways on which the pest being analyzed may be carried. Thereafter continue with the questions concerned with the measures that might be applied to each pathway. Repeat the process for every major pathway.

For the intentional import of pest plants, the focus should be on measures preventing the establishment and spread of the organism in unintended habitats within the PRA area. The main pathway for these plants is usually the trade with ornamental plants intended for planting. For such cases go directly to question 3.29 (measures that can be taken in the importing country). This still allows the option of prohibiting import (3.37) to be considered. However, if the organism is also entering the area unintentionally, then measures may be required to prevent introduction through unintentional pathways and steps 3.2-3.28 should also be followed. Options for managing the unintentional introduction of pest plants are covered by following the procedures for pathway-initiated analysis.

Pathway-Initiated Analysis for a commodity of plants and plant products

In the case of a pathway-initiated analysis for a commodity of plants and plant products, since the precise pathway is already known, begin with question 3.11 to consider possible measures for this pathway and repeat the process as far as question 3.41 for each of the pests identified in the pest risk assessment as presenting a risk to the PRA area. When all the pests have been considered, go to 3.42 to integrate the measures for the commodity. (Note that the probabilities for entry of a particular pest with other pathways, including existing pathways, may also need to be investigated).

In considering your responses to the following questions, please note that helpful information may be obtained from the pest risk assessment stage, particularly from the section concerning entry (1.1-1.14). References to the relevant sections of the risk assessment stage have been added.

3.2. **Is the pathway that is being considered a commodity of plants and plant products?**

If yes **go to 3.11**
If no go to 3.3

A) i tomato fruit non-EU, viii tomato fruit EU MS:
Yes.

B) ii tomato seed non-EU, ix tomato seed EU MS:
Yes.

C) iii tomato plants non-EU, x tomato plants EU MS:
Yes.

3.3. Is the pathway that is being considered the natural spread of the pest? (see answer to question 1.30)

Note: Natural spread includes movement of the pest by flight (of an insect), wind or water dispersal, transport by vectors such as insects or birds, natural migration, rhizomial growth.

If yes go to 3.4
If no go to 3.9

3.4. Is the pest already entering the PRA area by natural spread or likely to enter in the immediate future? (see answer to question 1.30)

If yes *go to 3.5*
If no *go to 3.38*

3.5. Is natural spread the major pathway?

If yes *go to 3.29*
If no *go to 3.6*

3.6. Could entry by natural spread be reduced or eliminated by control measures applied in the area of origin?

If yes *possible measures: control measures in the area of origin*
go to 3.7

3.7. Could the pest be effectively contained or eradicated after entry? (see answer to question 1.24, 1.32)

If yes *possible measures: internal containment and/or eradication campaign*
Go to 3.8

3.8. Was the answer "yes" to either question 3.6 or question 3.7?

If yes *Go to 3.29*
If no *Go to 3.38*

3.9. Is the pathway that is being considered the entry with human travellers?

If yes *possible measures: inspection of human travellers, their luggage, publicity to enhance public awareness on pest risks, fines or incentives. Treatments may also be possible*
Go to 3.29
If no *Go to 3.10*

3.10. Is the pathway being considered contaminated machinery or means of transport?

If yes *possible measures: cleaning or disinfection of machinery/vehicles*
Go to 3.29

For other types of pathways (e.g. commodities other than plants or plant products, exchange of scientific material, packing material, grain, wool, hides, sand, gravel ...), not all of the following questions may be relevant; adapt the questions to the type of pathway.

Go to 3.12

Existing phytosanitary measures

Phytosanitary measures (e.g. inspection, testing or treatments) may already be required as a protection against other (quarantine) pests (see stage 2: question 1.9). The assessor should list these measures and identify their efficacy against the pest of concern. The assessor should nevertheless bear in mind that such measures could be removed in the future if the other pests are re-evaluated.

3.11. **If the pest is a plant, is it the commodity itself?**

If yes — Go to 3.29

If no (the pest is not a plant or the pest is a plant but is not the commodity itself) — go to 3.12

A) i tomato fruit non-EU, viii tomato fruit EU MS:
No.

B) ii tomato seed non-EU, ix tomato seed EU MS:
No.

C) iii tomato plants non-EU, x tomato plants EU MS:
No.

3.12. **Are there any existing phytosanitary measures applied on the pathway that could prevent the introduction of the pest?**

if appropriate, list the measures and identify their efficacy against the pest of concern.

Go to 3.13

A) i tomato fruit non-EU, viii tomato fruit EU MS:
No.

B) ii tomato seed non-EU, ix tomato seed EU MS:
Yes. Seed of tomato is only allowed to enter and move within the EU provided it has been subjected to acid-extraction or an equivalent measure (point 48 of Annex IVAI and point 27 of Annex IVAII of the EC Plant Health Directive; Anon., 2000). This measure is not specifically aimed at PepMV, but will reduce the risks.

There is also the current European Commission emergency legislation for PepMV (Commission Decision 2004/200/EC) (Anon., 2004) that has specific measures for the seed pathway. In this legislation, seed of tomato is also only allowed to enter and move within the EU provided it has been subjected to an appropriate acid-extraction method, but in addition: the seed must originate in an area where PepMV is known not to occur (pest-free area), OR there must have been no symptoms of PepMV on the plants producing the seed at the place of production throughout their complete cycle of vegetation (pest-free place of production), OR the seed must be officially tested for PepMV and found free from it (pest-free crop).

C) iii tomato plants non-EU, x tomato plants EU MS:
Yes. Import of plants of the Solanaceae from third countries other than European and Mediterranean countries is prohibited (point 13 of Annex IIIA of the EC Plant Health

Directive; Anon., 2000). This measure is not specifically aimed at PepMV, but it will reduce the risks of entry. However, this does not prevent entry from non-EU European and Mediterranean countries where PepMV has been reported (Norway - eradicated; Switzerland, Canary Islands and Morocco).

For tomato plants from EU MS there are also phytosanitary measures in relation to Potato stolbur phytoplasm (point 18.6 of Annex IVAII), *Ralstonia solanacearum* (point 18.7 of Annex IVAII), *Liriomyza huidobrensis* and *L. trifolii* (point 23 of Annex IVAII), *Clavibacter michiganensis* ssp. *sepedonicus*, *Globodera pallida*, *G. rostochiensis*, *Synchytrium endobioticum* (point 24 of Annex IVAII) and *Tomato yellow leaf curl virus* (point 26.1 of Annex IVAII). These provisions will not prevent the introduction and spread of PepMV.

Identification of appropriate risk management options

This section (questions 3.13 to 3.31) examines the characteristics of the pest to determine if it can be reliably detected in consignments by inspection or testing, if it can be removed from consignments by treatment or other methods, if limitation of use of the commodity would prevent introduction, or if the pest can be prevented from infecting/infesting consignments by treatment, production methods, inspection or isolation. "Reliably" should be understood to mean that a measure is efficient, feasible and reproducible. Measures can be reliable without being sufficient to reduce the risk to an acceptable level. In such cases their combination with other measures to reach the desired level of protection against the pest should be envisaged (see question 3.32). When a measure is considered reliable but not sufficient, the assessor should indicate this. The efficiency, feasibility and reproducibility of the measures should be evaluated by the assessor for each potential management option identified. Limitations of application of measures in practice should be noted. ***Cost effectiveness and impact on trade are considered in the section "evaluation of risk management options" (questions 3.34 to 3.36).***

Options for consignments

<u>Detection of the pest in consignments by inspection or testing</u>

3.13. **Can the pest be reliably detected by a visual inspection of a consignment at the time of export, during transport/storage or at import?**

<u>A) i tomato fruit non-EU, viii tomato fruit EU MS:</u>
No. Fruit can show symptoms, but symptoms are not always present.

<u>B) ii tomato seed non-EU, ix tomato seed EU MS:</u>
No. PepMV is not visible on the seed.

<u>C) iii tomato plants non-EU, x tomato plants EU MS:</u>
No. Symptoms are often absent in young plants.

If yes **possible measure: visual inspection.**

Go to 3.14

3.14. **Can the pest be reliably detected by testing (e.g. for pest plant, seeds in a consignment)?**

<u>A) i tomato fruit non-EU, viii tomato fruit EU MS:</u>

Yes. PepMV can be detected in fruit by e.g. ELISA, (real-time) RT-PCR and inoculating indicator plants. However, in the case of symptomless infections the reliability of testing will depend on the sampling size.

B) ii tomato seed non-EU, ix tomato seed EU MS:
Yes if the sample size and testing methology is well-defined. PepMV can be detected on seeds by e.g. ELISA and (real-time) RT-PCR.

In the Pepeira project a ringtest was performed to test the different methods. It was shown that testing a sample of 5 infected seeds in 245 healthy seeds gave a positive result in both ELISA and PCR. When a test gives a positive result this does not automatically mean that this is infectious virus, or that it will result in infected plants. However, it is an indication that remnants of the virus are present on the seed and that PepMV was present during harvest.

A harmonised protocol for seed testing is necessary. An outcome of the Pepeira project will be an EPPO protocol for the detection of PepMV (Pepeira final report WP4, 2010). The sampling and testing rate will need to be defined for reliable detection in a seed lot.

C) iii tomato plants non-EU, x tomato plants EU MS:
Not reliably. PepMV can be detected in young plants by e.g. ELISA and (Real-time) RT-PCR. However, it depends on the time of infection whether the virus level will be high enough to be detected. Moreover, the number of plants that will need to be tested to detect an infected plant in an infected consignment depends upon the rate of infection.

If yes **possible measure: specified testing.**

Go to 3.15

3.15. **Can the pest be reliably detected during post-entry quarantine?**
Note: ISPM no. 5 "Glossary of Phytosanitary Terms" defines quarantine as "official confinement for observation and research or for further inspection, testing and/or treatment of a consignment after entry".

If yes **possible measure: import under special licence/permit and post-entry quarantine.**
Go to 3.16

A) i tomato fruit non-EU, viii tomato fruit EU MS:
Not applicable for fruit

B) ii tomato seed non-EU, ix tomato seed EU MS:
Yes – in theory this is the same measure as 3.14; i.e. seed testing. However, import under special licence/permit and post-entry quarantine is rarely used in the EU and in this case would only be appropriate for small quantities of seed for research or trialling and not for commercial quantities of seed. Specified testing of seed (3.14) is the equivalent practical alternative for commercial tomato seed originating outside as well as within the EU.

C) iii tomato plants non-EU, x tomato plants EU MS:
As with 3.14, not reliably. PepMV can be detected in young plants by e.g. ELISA and (Real-time) RT-PCR. However, it depends on the time of infection whether the virus level will be high enough to be detected. Moreover, the number of plants that will

need to be tested to detect an infected plant in an infected consignment depends upon the rate of infection. As with seed, import or movement under special licence/permit and post-entry quarantine is rarely used in the EU and would not be appropriate for commercial numbers of plants.

Removal of the pest from the consignment by treatment or other phytosanitary procedures

3.16. **Can the pest be effectively destroyed in the consignment by treatment (chemical, thermal, irradiation, physical)?**

If yes **possible measure: specified treatment.**
Go to 3.17

A) i tomato fruit non-EU, viii tomato fruit EU MS:
No. The pest is a virus and treatment would destroy the fruit.

B) ii tomato seed non-EU, ix tomato seed EU MS:
Yes. Seed treatment after harvest is possible. For example, acid-extraction (which is currently prescribed, or an equivalent measure, in the phytosanitary requirements for tomato seed imported into and moved within the EU), and, possibly, the addition of another treatment such as e.g. sodium hypochlorite or trisodium phosphate. However, the efficacy of additional treatments such as these are dependent upon how they are applied. Moreover, methods/chemicals used for additional treatment might have negative side effects on germination. Some seed houses already apply sodium hypochlorite or trisodium phosphate in addition to acid extraction, however, controlled experiments would be needed to determine true efficacy.

C) iii tomato plants non-EU, x tomato plants EU MS:
No. The pest is a virus and any treatment would most likely mean destroying the plants.

3.17. **Does the pest occur only on certain parts of the plant or plant products (e.g. bark, flowers), which can be removed without reducing the value of the consignment? (This question is not relevant for pest plants).**

If yes **possible measure: removal of parts of plants from the consignment**
Go to 3.18

A) i tomato fruit non-EU, viii tomato fruit EU MS:
No.

B) ii tomato seed non-EU, ix tomato seed EU MS:
No.

C) iii tomato plants non-EU, x tomato plants EU MS:
No.

3.18. **Can infestation of the consignment be reliably prevented by handling and packing methods?**

If yes **possible measure: specific handling/packing methods**

Go to 3.19

A) i tomato fruit non-EU, viii tomato fruit EU MS:
No.

B) ii tomato seed non-EU, ix tomato seed EU MS:
No.

C) iii tomato plants non-EU, x tomato plants EU MS:
No.

Prevention of establishment by limiting the use of the consignment

3.19. **Could consignments that may be infested be accepted without risk for certain end uses, limited distribution in the PRA area, or limited periods of entry, and can such limitations be applied in practice?**

If yes **possible measure: import under special licence/permit and specified restrictions:**
Go to 3.20

A) i tomato fruit non-EU, viii tomato fruit EU MS:
Possibly. By preventing fruit coming in contact with tomato production. This is done in the UK when infected imported fruit is detected (based initially upon symptoms) by the Plant Health and Seeds Inspectors. Fruit is then sent to retailers direct i.e. it is not sent to packing houses which are often on sites of production. Not all fruit that is infected will show symptoms and so this does not guarantee absence. It is also necessary to take strict hygiene measures to prevent the virus entering a facility on clothes, tools and other items. Some fruit production nurseries prohibit workers from bringing tomatoes onto the premises (i.e. as part of their meals).

B) ii tomato seed non-EU, ix tomato seed EU MS:
No.

C) iii tomato plants non-EU, x tomato plants EU MS:
No.

Options for the prevention or reduction of infestation in the crop.

Prevention of infestation of the commodity

3.20. **Can infestation of the commodity be reliably prevented by treatment of the crop?**

If yes **possible measure: specified treatment and/or period of treatment**
Go to 3.21

A) i tomato fruit non-EU, viii tomato fruit EU MS:
No. Cross-protection has been used by growers in some countries in an attempt to protect their crops from severe damage. Research has shown that success with this method might only be achieved when using a mild isolate of a PepMV genotype (strain) to protect against infection with another potentially more aggressive isolate of the same genotype (Hanssen & Thomma, 2010; Schenk *et al.*, 2010). If the genotype of the isolate used to cross-protect the crop differs from the genotype that it has to give protection against, the severity of the symptoms may be increased (Hanssen &

Thomma, 2010). Since it is not always known which genotypes occur in an area, and new genotypes might be introduced this approach bears great risks. Moreover, official legislation requires that cross-protection strains (genotypes) are registered as crop protection agents; a process that can take many years. Experience from the UK in gaining official approval for a weak strain of *Zucchini yellow mosaic virus* (ZYMV) clearly demonstrated the difficulties and cost of the registration process (Pepeira final report, stakeholder meeting). Therefore, cross-protection is considered not to be a reliable or realistic treatment option. In addition, cross-protection does not prevent infestation of the commodity but rather leads to intentional infestation of tomato plants and fruits with PepMV.

B) ii tomato seed non-EU, ix tomato seed EU MS:
No.

C) iii tomato plants non-EU, x tomato plants EU MS:
No.

3.21. **Can infestation of the commodity be reliably prevented by growing resistant cultivars? (This question is not relevant for pest plants).**

If yes **possible measure: consignment should be composed of specified cultivars**
Go to 3.22

A) i tomato fruit non-EU, viii tomato fruit EU MS:
No. Resistance to PepMV in tomato is not currently available. Limited progress is being made.

B) ii tomato seed non-EU, ix tomato seed EU MS:
No. Resistance to PepMV in tomato is not currently available. Limited progress is being made.

C) iii tomato plants non-EU, x tomato plants EU MS:
No. Resistance to PepMV in tomato is not currently available. Limited progress is being made.

3.22. **Can infestation of the commodity be reliably prevented by growing the crop in specified conditions (e.g. protected conditions such as screened greenhouses, physical isolation, sterilized growing medium, exclusion of running water, etc.)?**

If yes, **possible measure: specified growing conditions**
Go to 3.23

A) i tomato fruit non-EU, viii tomato fruit EU MS:
Yes. If strict hygiene measures are taken infestation could be prevented. Practice has shown it can be difficult but best-practice guides are available for PepMV and PSTVd (UK, NL). Especially in dense tomato production areas it will be difficult. Healthy starting material should be the basis. This could be achieved by other measures.

B) ii tomato seed non-EU, ix tomato seed EU MS:
Yes. If strict hygiene measures are taken infestation could be prevented. Practice has shown it can be difficult but best-practice guides are available for PepMV and PSTVd

(UK, NL). Especially in dense tomato production areas it will be difficult. Healthy starting material should be the basis. This could be achieved by other measures.

C) iii tomato plants non-EU, x tomato plants EU MS:
Yes. If strict hygiene measures are taken infestation could be prevented. Practice has shown it can be difficult but best-practice guides are available for PepMV and PSTVd (UK, NL). Especially in dense tomato production areas it will be difficult. Healthy starting material should be the basis. This could be achieved by other measures

3.23. **Can infestation of the commodity be reliably prevented by harvesting only at certain times of the year, at specific crop ages or growth stages?**

If yes **possible measure: specified age of plant, growth stage or time of year of harvest**
Go to 3.24

A) i tomato fruit non-EU, viii tomato fruit EU MS:
No.

B) ii tomato seed non-EU, ix tomato seed EU MS:
No.

C) iii tomato plants non-EU, x tomato plants EU MS:
No.

3.24. **Can infestation of the commodity be reliably prevented by production in a certification scheme (i.e. official scheme for the production of healthy plants for planting)?**

If yes **possible measure: certification scheme**
Go to 3.25

A) i tomato fruit non-EU, viii tomato fruit EU MS:
Not applicable for fruit.

B) ii tomato seed non-EU, ix tomato seed EU MS:
In theory, by taking strict hygiene measures and implementing a testing scheme, healthy seed can be produced. However, detection of low levels of virus contaminating seed requires testing high numbers of seed. See 3.14. Moreover, currently such a certification scheme does not exist either nationally or at the EU level.

C) iii tomato plants non-EU, x tomato plants EU MS:
In theory, by taking strict hygiene measures and implementing a testing scheme for PepMV, healthy young plants can be produced. However, certification schemes for young plants propagated from seeds do not exist either nationally or at the EU level (they are normally for vegetatively propagated plant material). Also, those schemes that do exist only require visual freedom of plants from harmful organisms. For this reason, a certification scheme for young plants of tomato would not be effective. Symptoms of PepMV infection are usually not detectable in seedlings and plants would require laboratory testing. In addition, laboratory tests can be negative when the virus titre is very low (below detection limits) in young tomato plants. Therefore even an elaborate testing scheme would not guarantee that seedlings are free from virus.

Establishment and maintenance of pest freedom of a crop, place of production or area

Note that in this set of questions pest spread capacity is considered without prejudice to any other measure that can be recommended. For some pests, growing the plant in specific conditions can prevent natural spread (e.g. production in a glasshouse may provide protection against pest with high capacity for natural spread). These measures should have been identified in question 3.22.

In answering questions 3.25 to 3.27 refer to the answer to question 1.30 of the risk assessment section.

3.25. **Has the pest a very low capacity for natural spread?**

If yes **possible measures: pest freedom of the crop, or pest-free place of production or pest-free area**
Go to 3.28

If no Go to 3.26

Yes. PepMV is not spread rapidly by natural means (see answer to 1.30). Rapid spread is most likely to be of human assistance (see answer to 1.31).

3.26. Has the pest a low to medium capacity for natural spread?

If yes possible measures: pest-free place of production or pest free area.
Go to 3.28

If no Go to 3.27

3.27. The pest has a medium to high capacity for natural spread
Possible measure: pest-free area.
Go to 3.28

3.28. **Can pest freedom of the crop, place of production or an area be reliably guaranteed?**

Note : In order to guarantee freedom of a crop, place of production, place of production and buffer zone, or area, it should be possible to fulfil the requirements outlined in ISPM No. 4 and ISPM No. 10. Consider in particular the degree to which unintentional movement of the pest by human assistance could be prevented (see answer to question 1.31).

If no **Possible measure identified in questions 3.25-3.27 would not be suitable.**
Go to 3.29

A) i tomato fruit non-EU, viii tomato fruit EU MS:
This may be difficult especially in areas with a high density of tomato fruit production companies. Strict hygiene measures have to be taken at each place of production.

B) ii tomato seed non-EU, ix tomato seed EU MS:
Yes, if strict hygiene measures are taken in the seed crop, and the crop is grown from healthy starting material based upon intensive testing and seed treatment with the seed destined for marketing being tested and treated at harvest. See 3.14 and 3.16.

C) iii tomato plants non-EU, x tomato plants EU MS:

Yes in theory. If strict hygiene measures are taken, healthy starting material is used and intensive testing takes place, although reliable testing of young plants is not always possible. See 3.14.

Consideration of other possible measures

3.29. **Are there effective measures that could be taken in the importing country (surveillance, eradication) to prevent establishment and/or economic or other impacts?**

Note: For intentionally imported plants, see the EPPO Standard PM/3 67 on Guidelines for the management of invasive alien plants or potentially invasive alien plants which are intended for import or have been intentionally imported. When natural spread is the major pathway, international measures are not justified and risk should be accepted because it is not manageable.

If yes **Possible measures: internal surveillance and/or eradication campaign**

Go to 3.30

Yes, but this is highly dependent on several factors. If intensive surveys including testing take place and outbreaks are eradicated, establishment could be prevented. However, since the virus is readily mechanically transmitted this can be very difficult. In areas with a dense fruit production industry, eradication will be very difficult.

Evaluation of risk management options

This section evaluates the risk management options selected and considers in particular their cost effectiveness and potential impact on international trade.

3.30. **Have any measures been identified during the present analysis that will reduce the risk of introduction of the pest? List them.**

Yes. See Figure 1 for possible measures and their feasibility. This is also summarised below with an individual assessment of whether the measures are realistic or impractical.

Figure 1: Summary of Pest Risk Management options for PepMV

		Pathways					
No.	**Possible measure**	i tomato fruit non-EU	viii tomato fruit EU MS	ii tomato seed non-EU	ix tomato seed EU MS	iii tomato plants non-EU	x tomato plants EU MS
3.12	Phytosanitary measures						
3.13	visual inspection						
3.14	specified testing						
3.15	import under special licence/permit and post-entry quarantine						
3.16	specified treatment						
3.17	removal of parts of plants from the consignment						
3.18	specific handling/packing methods						
3.19	import under special licence/permit and specified restrictions						
3.20	specified treatment and/or period of treatment						
3.21	consignment should be composed of specified cultivars						
3.22	specified growing conditions						
3.23	specified age of plant, growth stage or time of year of harvest						
3.24	certification scheme						
3.25	pest freedom of crop or pest-free place of production or pest-free						
3.26	pest-free place of production or pest-free area						
3.27	pest-free area						
3.28	If no, measures identified in 3.25 to 3.27 would not be suitable						
3.29	internal surveillance and/or eradication campaign						

Pre-existing phytosanitary measures that have an impact on PepMV (including current emergency measures as well as those that are not specific to the pest).
Possible measure, realistic
Possible measure, not likely to be practical or reliable on its own
Measure ineffective
Considered under 3.25 beause the pest has a very low capacity for natural spread

* *Possible measure - realistic*

*** Possible measure not likely to be practical or reliable on its own*

**** Pre-existing phytosanitary measures that have an impact on PepMV (including current emergency measures as well as those that are not specific to the pest).*

<u>A) i tomato fruit non-EU, viii tomato fruit EU MS:</u>
- specified testing**
- specified growing conditions**
- pest-freedom of crop, pest-free place of production or pest-free area**
- internal surveillance and/or eradication campaign**

<u>B) ii tomato seed non-EU, ix tomato seed EU MS:</u>
- pre-existing pest-specific phytosanitary measures (Commission Decision 2004/200/EC; Anon., 2004) and non-pest-specific phytosanitary measures (EC Plant Health Directive; point 48, Annex IVAI and point 27 of Annex IVAII; Anon., 2000)***
- specified testing* (could be used to support †)

- import under special licence/permit and post-entry quarantine**. This is effectively the same as specified seed testing but it is only appropriate for small quantities of seed for research or trialling and not for commercial quantities of tomato seed.
- specified treatment* (could be used to support †)
- specified growing conditions** (although difficult to implement this would be a necessary measure in support of other options)
- certification scheme**
- pest-freedom of crop, pest-free place of production or pest-free area*† . This is already a requirement for seed in the emergency measures (Anon., 2004) but it may need to be further refined which could be difficult, but can be considered if choices are offered in the way this is determined as described below.
- internal surveillance and/or eradication campaign**

<u>C) iii tomato plants non-EU, x tomato plants EU MS:</u>
- pre-existing (non-pest-specific) phytosanitary measures (EC Plant Heath Directive; point 48 of Annex IVAI and point 27 of Annex IVAII; Anon., 2000)***
- specified testing**
- specified growing conditions**
- certification scheme**
- pest-freedom of crop, pest-free place of production or pest-free area**
- internal surveillance and/or eradication campaign**

If yes **Go to 3.31**
If no Go to 3.38

3.31. **Does each of the individual measures identified reduce the risk to an acceptable level?**

Not in isolation. The only measures that are likely to help reduce the risk from PepMV to an acceptable level <u>and</u> which are realistic to implement have been identified for tomato seeds. These are:

- Specified treatment (although the exact method of use and efficacy of treatments should be determined). See 3.16.
- Specified testing (albeit this is considered to be potentially difficult – see 3.14)
- Pest-free crop, pest-free area or pest-free place of production

Other measures were identified as either ineffective or not likely to be practical.

If yes Go to 3.34
If no **Go to 3.32**

3.32. **For those measures that do not reduce the risk to an acceptable level, can two or more measures be combined to reduce the risk to an acceptable level?**

Note: The integration of different phytosanitary measures at least two of which act independently and which cumulatively achieve the Appropriate Level of Protection against regulated pests are known as Systems Approaches (see ISPM 14: the use of integrated measures in a systems approach for Pest Risk Management). It should be noted that Pest free places of production identified as phytosanitary measures in questions 3.25 to 3.27 may correspond to a System Approach.

Specified treatment and specified testing in combination with strict hygiene measures could be used to help support a requirement for seed to originate in a pest-free crop, pest-free area or pest-free place of production

With respect to testing, freedom of symptoms in the mother plants and testing of these plants for PepMV may be a practical alternative to seed testing.

If yes **Go to 3.34**
If no Go to 3.33

3.33. **If the only measures available reduce the risk but not down to an acceptable level, such measures may still be applied, as they may at least delay the introduction or spread of the pest. In this case, a combination of phytosanitary measures at or before export and internal measures (see question 3.29) should be considered.**

Go to 3.34

3.34. **Estimate to what extent the measures (or combination of measures) being considered interfere with international trade.**

Note: *If this analysis concerns a pest already established in the PRA area but under official control, measures that are applied for international trade should not be more stringent than those applied domestically/internally.*

The measure of specified treatment (acid-extraction or equivalent) for tomato seed is no more restrictive than the measure that is already in place in the EC Plant Health Directive (Anon., 2000) and one of the measures in the emergency legislation (Anon., 2004). However, treatment in addition to acid-extraction has the potential to be more restrictive; nevertheless some seed houses already use these on a voluntary basis. In addition to acid extraction (or equivalent), the requirement for seed to originate either in a pest-free crop, pest-free place of production or a pest-free area are the three basic requirements in the emergency legislation for PepMV (Anon., 2004); the latter being based upon official testing. If specified testing becomes compulsory then this would be more restrictive than the current emergency measures.

Provided seed-producing countries are able to comply, this should not interfere with international trade.

Go to 3.35

3.35. **Estimate to what extent the measures (or combination of measures) being considered are cost-effective, or have undesirable social or environmental consequences.**

The measures for seed treatment are likely to be cost-effective as acid-extraction or an equivalent is already in place as a phytosanitary requirement for seed of tomato imported into and moved within the EU. The addition of another treatment such as sodium hypochlorite or trisodium phosphate would not be expensive and some seed houses already treat seed using this chemical.

However, there might be negative effects on germination of seed for some tomato cultivars, especially cultivars that are used for grafting.

Seed testing is likely to require testing high numbers of seed and this would be expensive, although it is already common practice in several seed companies.

Consideration could also be given to freedom of symptoms and testing in the mother plants for seed production as an alternative to, or in addition to seed testing.

Go to 3.36

3.36. **Have measures (or combination of measures) been identified that reduce the risk for this pathway, and do not unduly interfere with international trade, are cost-effective and have no undesirable social or environmental consequences?**

Yes.

If yes For pathway-initiated analysis, go to 3.39
For pest-initiated analysis, go to 3.38
If no Go to 3.37

3.37. **Envisage prohibiting the pathway.**
Note: Prohibition should be viewed as a measure of last resort. If prohibition of the pathway is the only measure identified for a commodity-initiated analysis, there may be no need to analyze any other pests that may be carried on the pathway. If later information shows that prohibition is not the only measure for this pest, analysis of the other pests associated with the pathway will become necessary.
For pathway-initiated analysis, go to 3.43 (or 3.39)
For pest-initiated analysis go to 3.38

3.38. **Have all major pathways been analyzed (for a pest-initiated analysis)?**

Yes.

If yes **Go to 3.41**
If no Go to 3.1 to analyze the next major pathway
Yes.

3.39. **Have all the pests been analyzed (for a pathway-initiated analysis)?**
If yes **Go to 3.40**
If no **Go to 3.1 (to analyze next pest)**

3.40. **For a pathway-initiated analysis, compare the measures appropriate for all the pests identified for the pathway that would qualify as quarantine pests, and select only those that provide phytosanitary security against all the pests.**
Note: the minimum effective measures against one particular pest may reduce the risk from other pests far more than necessary, but these measures would be the only ones appropriate for the pathway as a whole.

Go to 3.41

3.41. **Consider the relative importance of the pathways identified in the conclusion to the entry section of the pest risk assessment**
Note: the relative importance of the pathways is an important element to consider in formulating phytosanitary regulation. Regulation of pathways presenting similar risks should be consistent.

The relative importance of the pathways in terms of the risk of entry is given below (based upon a five word ranking system where **very low** and **very high** are extremes). (See also Table 8 – probability of introduction). Pathways where measures are likely to be effective and realistic are seed of tomato from non-EU and EU countries (Pathways ii and ix). Although the risk of entry for all four pathways is lower in comparison to fruit, the risk of establishment from these pathways is very high. Measures for fruit (high risk) are impractical due to the volume of trade. The risk this pathway poses to companies producing tomato fruit, tomato plants for planting or tomato seed can most effectively be mitigated by applying strict hygiene measures in the respective place of production.

<u>Pathway (i)</u>
Tomato fruit from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China: **High risk**

<u>Pathway (viii)</u>
Tomato fruit from EU MS: **High risk**

<u>Pathway (ii)</u>
Tomato seed from Canada, USA, Guatemala, Peru, Chile, Ecuador, Canary Islands, Norway, Switzerland, Ukraine, Morocco and China: **Low to medium risk** depending on the origin.

<u>Pathway (ix)</u>
Tomato seed from EU MS: **Low to medium risk** depending on the origin.

<u>Pathway (iii)</u>
Tomato plants for planting from Canary Islands, Morocco, Norway and Switzerland: **Low risk**

<u>Pathway (x)</u>
Tomato plants for planting from EU MS: **Low risk**

<u>Pathway (xiv)</u>
Bumble bees from EU MS: **Very low risk**

Go to 3.42

3.42. **All the measures or combination of measures identified as being appropriate for each pathway or for the commodity can be considered for inclusion in phytosanitary regulations in order to offer a choice of different measures to trading partners.**

Note: only the least stringent measure (or measures) capable of performing the task should be selected. Thus, if inspection is truly reliable, it should not be necessary to consider treatment or testing. Note also that some measures may counteract each other; for example the requirement for resistant cultivars may make detection more difficult. It may be that some or all of these measures are already being applied to protect against one or more other pests, in which case such measures need only be applied if the other pest(s) is/are later withdrawn from the legislation.

The minimum phytosanitary measure applied to any pest is the declaration in phytosanitary regulations that it is a quarantine pest. This declaration prohibits both the entry of the pest in an isolated state, and the import of consignments infested by the pest. If other phytosanitary measures are decided upon, they should accompany the declaration as a quarantine pest. Such declaration may occasionally be applied alone, especially: (1) when the pest concerned may be easily detected by phytosanitary inspection at import (see question 3.13), (2) where the risk of the pest's introduction is low because it occurs infrequently in international trade or its biological capacity for establishment is low, or (3) if it is not possible or desirable to regulate all trade on which *the pest is likely to be found. The measure has the effect of providing the legal basis for the NPPO to take action on detection of the pest (or also for eradication and other internal measures), informing trading partners that the pest is not acceptable, alerting phytosanitary inspectors to its possible presence in imported consignments, and sometimes also of requiring farmers, horticulturists, foresters and the general public to report any outbreaks.*

Based upon the findings of this PRA, it may be necessary for decision-makers to consider changing from the emergency measures for PepMV (Commission Decision 2004/200/EC; Anon., 2004) to permanent listing of PepMV in the EC Plant Health Directive 2000/29/EC (Anon., 2000). The rationale behind this and the possible options are described below:

Pest listing

Since the virus already occurs in parts of the EU, including the main tomato production areas, if the emergency legislation is dropped, for measures to continue to be taken, PepMV would require listing in Annex IIAII (harmful organisms known to occur in the community and relevant for the entire community).

Tomato fruit

For tomato fruit, no realistic or reliable phytosanitary measures have been identified. To reliably guarantee pest- freedom, very high numbers of tomato fruit would have to be tested. This, in combination with economic losses resulting from destruction of the fruit, makes the imposition of phytosanitary measures for tomato fruit unrealistic. However, to prevent the introduction of PepMV to fruit production sites where these co-exist with packing houses, strict hygiene practices would be required. In areas of the EU with a high density of fruit production this will be especially difficult, however, there are published hygiene protocols which can be followed and are already implemented in some EU Member States on a voluntary basis.

Tomato plants

For tomato plants for planting, if these are grown from seeds free of PepMV and strict hygiene measures are taken, a pest-free place of production for young plants could be established. However, reliable testing to confirm absence of PepMV in young plants is difficult. Moreover, destruction of plants and a possible shortage of supply of plants to growers might lead to economic losses. Therefore, the imposition of phytosanitary measures for tomato plants for planting is probably unrealistic.

Tomato seed

As measures for two of the main pathways i.e. tomato fruit and plants for planting are considered most likely to be unrealistic, if decision-makers conclude that PepMV should be permanently listed in the EC Plant Health Directive, the subject of contamination in Annex IIAII would be seeds of tomato (listed as *Lycopersicon lycopersicum*; noting that the correct scientific name for tomato is now *Solanum lycopersicum*). This pathway is believed to be an important route for introduction of PepMV into a new area or for further introduction into an existing area. It also poses a risk of introduction of new variants of PepMV.

The risk management option that is recommended to decision-makers for consideration for phytosanitary measures is principally for seed treatment and virus-testing (seed/mother plant). This could be used as the basis for a requirement for seed to originate in a pest-free crop, place of production or area. This is already the basis of the pre-existing emergency phytosanitary measures which requires acid extraction of tomato seed AND: a pest-free area, OR a pest-free place of production, OR official seed testing (which is equivalent to a pest-free crop).

With respect to seed treatment, if seeds were only to be acid–extracted (or an equivalent method) this is already facilitated within the requirements for seeds in Annex IVAI, article 48 for seeds entering the EU and Annex IVAII, article 27 for seeds originating within the EU.

If seeds were to be acid-extracted <u>and</u> subject to an additional treatment, this would require an additional article in Annex IVAI and IVAII, specific to PepMV. More research on the efficacy of an additional seed treatment would be necessary. Different treatments are already in use by some seed houses.

If seed is required to come from a pest-free area, pest-free place of production or pest-free crop this could be catered for within the pre-existing articles for tomato seed in Annex IVAI and IVAII of the EU directive 2000/29/EC with the addition of PepMV. The options for this include those described already including seed treatment; as well as symptom-free and virus-free (by testing) mother plants, and/or seed testing. If mother plants and/or seed is/are to be tested, a harmonised testing protocol would be helpful. An outcome of the Pepeira project will be an EPPO protocol for the detection of PepMV.

Since there is no consistent difference between genotypes in terms of their biology and aggressiveness, and because isolates of the same genotype can behave differently, it is not appropriate to regulate by genotype.

Go to 3.43

3.43. **In addition to the measure(s) selected to be applied by the exporting country, a phytosanitary certificate (PC) may be required for certain commodities. The PC is an attestation by the exporting country that the requirements of the importing country have been fulfilled. In certain circumstances, an additional declaration on the PC may be needed (see EPPO Standard PM 1/1(2): Use of phytosanitary certificates).**

A PC would be required for seed entering the EU and a Plant Passport for seed moving within the EU.

Go to 3.44

3.44. **If there are no measures that reduce the risk for a pathway, or if the only effective measures unduly interfere with international trade (e.g. prohibition), are not cost-effective or have undesirable social or environmental consequences, the conclusion of the pest risk management stage may be that introduction cannot be prevented. In the case of pest with a high natural spread capacity, regional communication and collaboration is important.**

Conclusion of Pest Risk Management.

Summarize the conclusions of the Pest Risk Management stage. List all potential management options and indicate their effectiveness. Uncertainties should be identified.

The only practical phytosanitary measure for reducing the risk of further entry into and spread of PepMV within the EU that has been identified is for seed of tomato.

The most inexpensive measure is that which already exists for tomato seed, i.e. a requirement for acid extraction or an equivalent measure. The addition of a requirement for treatment would potentially reduce the risk from PepMV further and would be inexpensive; indeed, some seed houses are already utilising this approach.

The addition of a requirement for a choice between seed originating from a pest-free crop, pest-free place of production or a pest-free area, (symptom-free mother plants and possibly testing; and/or seed testing), would enhance the efficacy of the risk management options being proposed. The EPPO protocol for detection of PepMV arising from this project could be usefully deployed to determine the best approach.

It is recommended that these measures are considered by decision-makers in light of the findings of the PRA to determine future policy for PepMV in the EU.

Monitoring and review

Performance of measure(s) should be monitored to ensure that the aim is being achieved. This is often carried out by inspection of the commodity on arrival, noting any detection in consignments or any entries of the pest to the PRA area.

Information supporting the pest risk analyses should be reviewed periodically by the pest risk analysts to ensure that any new information that becomes available does not invalidate the decision taken. The analysts should in particular be aware that new international trade may be initiated, host plants may newly be grown in the PRA area which were not grown at the time the PRA was conducted, climate may change, new policy decisions may influence the result of a previous analysis.

REFERENCES

Aguilar, J.M., Hernandez-Gallardo, M.D., Cenis, J.L. Lacasa, A. and Aranda, M.A. (2002). Complete sequence of the pepino mosaic virus RNA genome. Archives of Virology 147, 2009-2015.

Alfaro-Fernandez, A., Cebrian, M.C., Cordoba-Selles, C., Herrera-Vasquez, J.A. and Jorda, C. (2008). First report of the US1 strain of Pepino mosaic virus in tomato in the Canary Islands, Spain. Plant Disease 92, 1590.

Alfaro-Fernández, A., Córdoba-Selles, M.C., Herrera-Vásquez, J.A., Cebrián, M.C. and Jordá, C (2009a) Transmission of Pepino mosaic virus by the fungal vector Olpidium virulentus. Journal of Phytopathology

Alfaro-Fernández, A., Córdoba-Selles, M.C., Juárez, M., Herrera-Vásquez, J.A., Sánchez-Navarro, J.A., Cebrián, M.C., Font, M.I. and Jordá, C (2009b) Occurrence and geographical distribution of the 'Torrado' disease in Spain. Journal of Phytopathology p25

Alfaro-Fernández A, Sánchez-Navarro JA, Cebrián MC, Córdoba-Sellés MC, Pallás V, Jordá J., (2009c) Simultaneous detection and identification of Pepino mosaic virus (PepMV) isolates by multiplex one-step RT-PCR. European Journal of Plant Pathology 125, 143-158.

Anon, 2000 (*as amended*). 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. *Official Journal of the European Communities.* **43 no. L 169**, pp. 1 - 112.

Anon. (2003). Situation of Pepino Mosaic Potexvirus in EPPO countries. Reporting Service 2003, No 9, 132.

Anon., 2004. Commission Decision 2004/200/EC of 27 February 2004 on measures to prevent the introduction and spread within the Community of *Pepino mosaic virus*. *Official Journal of the European Union*. **L64, 43-44.**

Anon. (2007) Hygiëneprotocol tomaat 2007-09-27
http://www.tuinbouw.nl/files/HygprotocolTomaat28sept2007-10.pdf

Anon., 2008. re:fresh Directory 2008. An essential resource for the fresh produce industry. Publishers FPJ. 380pp.

Anon. (2009a) Notification of harmful organism – Pepino mosaic virus. Ministry of agriculture Republic of Cyprus. 2009-04-02

Anon. (2009b) Results of the surveys carried out by Member States in 2007 and 2008 for Pepino mosaic virus. Standing Committee of Plant Health 26-27 May 2009

Anon. (2010a) Results of the surveys carried out by Member States in 2009 for Pepino mosaic virus. Mail Steven Jones, 20/1/2010

Anon. (2010b) Estonian Agricultural Board, 2010. Results of Estonian official surveys of harmful pests and diseases, http://www.pma.agri.ee.

Córdoba, M.C., Martínez-Priego, L. and Jordá, C. (2004). New natural hosts of Pepino mosaic virus in Spain. Plant Disease 88, 906.

Cordoba-Selles, M.C., Garcia-Randez, A., Alfaro-Fernandez, A. and Jorda-Gutierrez, C. (2007). Seed transmission of pepino mosaic virus and efficacy of tomato seed disinfection methods. Plant Disease 91 (10) 1250-1254

Cotillon, A.C., Girard, M. and Ducouret, S. (2002). Complete nucleotide sequence of the genomic RNA of a French isolate of Pepino mosaic virus (PepMV). Archives of Virology 147, 2231-2238.

Davino, S, Davino, M., Bellardi, M.G. and Agosteo, G.E. (2008). Pepino mosaic virus and Tomato chlorosis virus causing mixed infection in protected tomato crops in Sicily. Phytopathologia Mediterranea 47 35-41

Davino, S., Accotto, G.P., Masenga, V., Torta, L. and Davino, M. (2009). Basil (Ocimum basilicum) a new host of Pepino mosaic virus. Plant Pathology 58 407

de Buck, A. and Stijger, I. (2002). Schade door pepinomazaïekvirus. Onderzoek en aanbevelingen. Praktijkonderzoek Plant & Omgeving B.V. Sector Glastuinbouw. Januari 2002. PPO 539.

EPPO, 2000. Pepino mosaic potexvirus found in Spain. EPPO Reporting Service, No. 9, 2000/132

EPPO, 2009. Pepino mosaic virus. EPPO Alert List entry January 2000, reviewed February 2009. http://www.eppo.org/QUARANTINE/Alert_List/viruses/PEPMV0.htm

EUROSTAT, 2010. http://epp.eurostat.ec.europa.eu/newxtweb/, extracted 22-02-2010

FAO (2009a). FAOSTAT Agricultural Production Statistical Database, Food and Agriculture Organization of the United Nations. Production: Crops. Database updated 16 December 2009. http://faostat.fao.org/site/567/default.aspx#ancor

FAO (2009b). FAOSTAT Agricultural Production Statistical Database, Food and Agriculture Organization of the United Nations. Trade: Tradestat. Detailed trade data. Database updated 2 August 2009. http://faostat.fao.org/site/535/default.aspx#ancor

FAO (2009c). FAOSTAT Agricultural Production Statistical Database, Food and Agriculture Organization of the United Nations. Trade: Tradestat. Detailed trade matrix. (Trade2005 between countries to 2005). http://faostat.fao.org/site/537/default.aspx#ancor

Fera (2009). The importation of bees into England. A guidance note for importers. 9pp. https://secure.fera.defra.gov.uk/beebase/pdfs/importingbees.pdf

Fletcher J, 2000, Pepino mosaic, a new disease of tomatoes. Horticultural Development council Factsheet 12/00. 8pp. http://www.hdc.org.uk/assets/pdf/33201200/4977.pdf

French, C.J., Bouthillier, M., Bernardy M., Ferguson, G., Sabourin, M., Johnson, R.C., Masters, C., Godkin, S. and Mumford, R. (2001). First report of Pepino mosaic virus in Canada and the United States. Plant Disease 85, 1121.

French, C., Bunckle, A., Ferguson, G. and Bernardy, M. (2005). Complete sequencing and phylogenetic analysis of tomato isolates of Pepino mosaic virus from Canada and other geographic regions. Phytopathology 95 (6) Supplement S31.

Gómez P, Sempere RN, Elena SF, Aranda MA, 2009. Mixed infections of Pepino mosaic virus strains modulate the evolutionary dynamics of this emergent virus. J. Virol. 83: 12378-12387.

Gutiérrez-Aguirre, I., Mehle, N., Delic, D., Gruden, K., Mumford, R. and Ravnikar, M. (2009) Real-time quantitative PCR based sensitive detection and genotype discrimination of Pepino mosaic virus. Journal of Virological methods 162: 46-55

Hanafia A, Schnitzler WH, 2002. Integrated production and protection in greenhouse tomato in Morocco. In: Proceedings of the VIIth International Symposium on Protected Cultivation in Mild Winter Climates. Acta Hort. 659, ISHS 2004. pp295-300.

Hanssen, I.M., Paeleman, A., Wittemans, L., Goen, K., Lievens, B., Bragard, C., Vanachter, A.C.R.C. and Thomma, B.P.H.J. (2008). Genetic characterization of pepino mosaic virus isolates from Belgian greenhouse tomatoes reveals genetic recombination. European Journal of Plant Pathology 121, 131-146.

Hanssen, I.M., Paeleman, A., Van Bergen, L., Vandewoestijne, E., Wittemans, L., Goen, K., Vanachter, A.C.R.C. and Thomma, B.P.H.J. (2009a) Survey of symptom expression and damage caused by Pepino mosaic virus (PepMV) in commercial tomato production in Belgium. Acta Hort (ISHS) 808, 185-92.

Hanssen, I.M., Paeleman, A., Vandewoestijne, E., Van Bergen, L., Bragard, C., Lievens, B., Vanachter, A.C.R.C. and Thomma, B.P.H.J. (2009b). Pepino mosaic virus isolates and differential symptomatology in tomato. Plant Pathology 58, 450-460.

Hanssen IM, Mumford R, Blystad D-R, Cortez I, Hasiów-Jareszewska B, Hristova D, Pagán I, Pepeira A-M, Peters J, Pospieszny H, Ravnikar M, Stijger I, Tomassoli L, Varveri C, van der Vlugt R, Nielsen SL, 2010a. Seed transmission of Pepino mosaic virus in tomato. Eur. J. Plant Pathol. 126: 145-152.

Hanssen, I.M., Gutiérrez-Aguirre, I., Paeleman, A., Goen, K., Wittemans, L., Lievens, B., Vanachter, A.C.R.C., Ravnikar, M. and Thomma, B.P.H.J. (2010b) Co-infection of greenhouse tomato with different Pepino mosaic virus isolates results either in cross protection or enhanced symptom display. Plant Pathology 59: 13-21

Hanssen IM, Thomma BPHJ, 2010. Pepino mosaic virus: a successful pathogen that rapidly evolved from emerging to endemic in tomato crops. Molecular Plant Pathology 11: 179-189.

Hasiow, B, Borodynko, N. and Pospieszny, H. (2008). Complete genomic RNA sequence of the Polish pepino mosaic virus isolate belonging to the US2 strain. Virus genes 36 209-214

Hasiów-Jaroszewska B, Pospieszny H, Borodynko N, 2009. New necrotic isolates of Pepino mosaic virus representing the CH2 genotype. J. Phytopathol. 157: 494-496.

Jones, R.A.C., Koenig, R. and Lesemann, D.E. (1980). Pepino mosaic virus, a new potexvirus from pepino (Solanum muricatum). Annals of Applied Biology 94, 61-68.

Jones, D.R. and Lammers, W. (2005) Pest Risk Analysis for Pepino Mosaic Virus. York, UK: Central Science Laboratory.
http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/documents/pepino.pdf

Jones, DR, Sansford CE, Lammers W (2005). Datasheet for Pest Risk Analysis of Pepino mosaic virus. 29 April 2005. 28pp.

Jordá, C., Lázaro, A., Font, I., Lacasa, A., Guerrero, M.M., Cano, A. (2000). Nueva enfermeded en el tomate. Phytoma-Espaňa 119, 23-28.

Jordá, C., Lázaro Pérez, A., Martinez, P.V. and Lacasa, A. (2001a). First report of Pepino mosaic virus on natural hosts. Plant Disease 85, 1292.

Jordá, C., Lázaro Pérez, A., Martinez, P.V., Lacasa, A. and Guerrero M.M. (2001b). First report of Pepino mosaic virus on Tomato in Spain. Plant Disease 85, 1292.

Knight, M.E., Martin, A.P., Bishop, S., Osborne, J.L., Hale, R.J., Sanderson, R.A. AND Goulson, D. (2005) An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. Molecular Ecology, 14, (6), 1811-1820.

Krinkels, M. (2001). Pepino Mosaic virus causes sticky problem. Prophyta: The Annual, May 2001, 30 – 33.

Lacasa, A., Guerrero, M.M., Hita, I., Martinez, M.A., Jordá, C., Bielza, P., Contreras, J., Alcazar, A. and Cano, A. (2003). Implication of bumble bees (Bombus spp.) on Pepino mosaic virus (PepMV) spread on tomato crops. Plagas 29, 393-403.

Lesemann, D.E., Dalchow, J., Winter, S., Pfeilstetter, E. (2000). Occurrence of Pepino mosaic virus in European tomato crops: identification, etiology and epidemiology. Mitteilungen aus der Biologisehen Bundesanstalt 376, 323.

Ling, K. and Carpenter, L. (2005). Pepino mosaic virus, an emerging disease in greenhouse tomato production worldwide: Is seed responsible?. Proceedings 1st IS on Tomato Diseases 43-50

Ling, K. (2007). The population genetics of pepino mosaic virus in North America greenhouse tomatoes. Phytopathology 97 (7) Supplement S65.

Ling, K. and Scott, J.W. (2007). Sources of resistance to pepino mosaic virus in tomato accessions. Plant Disease 91 (6) 749-753

Ling, K., Wintermantel, W.M. and Bledsoe, M (2008). Genetic composition of Pepino mosaic virus population in North American greenhouse tomatoes. Plant Disease 92 (12) 1683-1688

Ling, K. (2008). Pepino mosaic virus on tomato seed: Virus location and mechanical transmission. Plant Disease 92 (12) 1701-1705

Ling, K.S. (2010) Effectiveness of Chemo- and Thermotherapeutic Treatments on Pepino mosaic virus in Tomato Seed. Plant Disease 94 (3) 325-328

Lopez, C., Soler, S. and Nuez, F. (2005). Comparison of the complete sequences of three different isolates of Pepino mosaic virus: size variability of the TGBp3 protein between tomato and L. peruvianum isolates. Archives of Virology 150, 619-627.

Mansilla, C., Sanchez, F. and Ponz, F. (2003). The diagnosis of the tomato variant of pepino mosaic virus: an IC-RT-PCR approach. European Journal of Plant Pathology 109, 139-146.

Maroon-Lango, C., Guaragna, M.A., Jordan, R.L., Bandia, M., Marquardt, S. (2003). Detection and characterization of a US isolate of Pepino mosaic virus. Phytopathology 93 (6) Supplement S57.

Maroon-Lango, C.J., Guaragna, M.A., Jordan, R.L., Hammond, J., Bandla, M. and Marquardt, S.K. (2005). Two unique US isolates of pepino mosaic virus from a limited source of pooled tomato tissue are distinct from a third (European-like) US isolate. Archives of Virology 150, 1187-1201.

Martin, J. and Mousserion, C. (2002). Potato varieties which are sensitive to the tomato strain of Pepino mosaic virus (PepMV). Phytoma 552, 26-28.

Martínez-Culebras, P.V., Lázaro, A., Abad Campos, P., Jordá, C. (2002). A RT-PCR assay combined with RFLP analysis for detection and differentiation of isolates of Pepino mosaic virus (PepMV) from tomato. European Journal of Plant Pathology 108, 887-892.

Mumford R.A., and Metcalfe, E.J. (2001). The partial sequencing of the genomic RNA of a UK isolate of Pepino mosaic virus and the comparison of the coat protein sequence with other isolates from Europe and Peru. Archives of Virology 146, 2455-2460.

O'Neill T, Spence N, Mumford R, Skelton A, 2003. Pepino mosaic virus of tomato - new results on virus persistence and disinfection. Horticultural Development Council Factsheet 20/03. 4pp. http://www.hdc.org.uk/assets/pdf/33202003/4992.pdf

O'Neill T, Mumford R, 2006. Potato spindle tuber viroid in tomato and new viroid reports. Horticultural Development Council Factsheet 09/06. 8pp. http://www.hdc.org.uk/assets/pdf/33200906/5232.pdf

Otterstatter, M.C. and Thomson J.D. (2008). Does pathogen spillover from commercially reared bumble bees threaten wild pollinators? PloS ONE 3(7):e2771

Pagan, I., Cordoba-Selles, M, Martinez-Priego, L., Fraile, A., Malpica, J.M., Jorda, C. and Garcia-Arenal, F. (2006). Genetic structure of the population of Pepino mosaic virus infecting tomato crops in Spain. Phytopathology 96 (3) 274-279.

Pepeira final report (2010). www.pepeira.wur.nl/UK

Peralta, I.E. and Spooner, D.M. (2000). Classification of wild tomatoes: a review. Kurziana 28, 45-54

Pospieszny, H., Borodynko, N. and Palczewska, M. (2002). Occurrence of Pepino mosaic virus in Poland. Phytopathologia Polonica 26, 91-94.

Pospieszny, H. and Borodynko, N. (2006). New Polish isolate of Pepino mosaic virus highly distinct from European tomato, Peruvian, and US2 strains. Plant Disease 90, 1106.

Pospieszny, H, .Hasiow, B. and Borodynko, N. (2008). Characterization of two distinct Polish isolates of pepino mosaic virus. European Journal of Plant Pathology 122 443-445

Prohens J, Ruiz JJ, Nuez F. (1996). The pepino (Solanum muricatum, Solanaceae): a "new" crop with a history. Economic Botany 50, 355-368.

Prohens, J., Ruiz, J.J. and Nuez, F. (2000). Growing cycles for a new crop , the pepino, in the Spanish Mediterranean. Acta Hort. 523:53-60

Prohens, J., Rodriquez, A. and Nuez, F. (2005). Utilization of genetic resources for the introduction and adaptation of exotic vegetable crops: The case of pepino (Solanum muricatum). Euphytica 146: 133-142

Roggero, P., Masenga, V., Lenzi, R., Coghe, F., Ena, S. and Winter, S., (2001). First report of Pepino mosaic virus (Potexvirus) in tomato in Italy. Plant Pathology 50, 798.

Salm, P. van der. (2008). Productinfo Tomaat 2006/2007. Productschap Tuinbouw 2008/85

Salomone, A. and Roggero, P. (2002). Host range, seed transmission and detection by ELISA and lateral flow of an Italian isolate of Pepino mosaic virus. Journal of Plant Pathology 84, 65-68.

Sansford C, Morris J, 2009. Revised Summary Pest Risk Analysis for Columnea latent viroid. 35pp. 13th October 2009.
http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/documents/clvd.pdf

Schenk MF, Hamelink R, van der Vlugt RAA, Vermunt AMW, Kaarsenmaker RC, Stijger ICCMM, (2010). The use of attenuated isolates of Pepino mosaic virus for cross-protection. European Journal of Plant Pathology DOI 10.1007/s10658-010-9590-4.

Shipp, J.L., Buitenhuis, R., Stobbs, L., Wang, K., Kim, W.S. and Ferguson, G. (2008). Vectoring of pepino mosaic virus by bumble-bees in tomato greenhouses. Annals of Applied Biology 153 149-155

Soler, S., Cebolla-Cornejo, J., Prohens, J. and Nuez, F. (2000). El Pepino mosaic virus (PepMV), una nueva amenaza para el cultivo del tomate. II. Vida Rural 119, 48-52.

Soler, S., Lopez, C, Diez, M.J., Perez de Castro, A. and Nuez, F. (2005). Association of Pepino mosaic virus with tomato collapse. Journal of Phytopathology 153, 464-469.

Soler S, López C, Nuez F (2005a). Natural occurrence of viruses in Lycopersicon spp. in Ecuador. Plant Disease 89(11), p 1244

Soler, S., Prohens, J., Diez, M.J. and Nuez, F. (2002). Natural occurrence of Pepino mosaic virus in Lycopersicon species in Central and Southern Peru. Journal of Phytopathology 150 (2), 49-53.

Spence, N.J., Basham, J., Mumford, R.A., Hayman, G., Edmondson, R. and Jones, D.R. (2006). Effect of Pepino mosaic virus on the yield and quality of glasshouse-grown tomatoes in the UK. Plant Pathology 55, 595-606.

Stijger, C.C.M.M. and Vlugt, R.A.A. van der (2008). Pepino mosaic virus. In: Encyclopedia of Virology. Third Edition / Mahy, B., Regenmortel, M.H.V. van, 2008

Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M. and Webb, D.A. (2002) Flora Europaea: Volume 3 Diapensiaceae to Myoporaceae

UK NPPO, 2008. Monitoring for the presence of Pepino mosaic virus by the United Kingdom in 2008. UK survey report supplied by the National Plant Protection Organisation to the EC SCPH as required under Commission Decision 2004/200/EC. 3pp.

UK NPPO, 2009. Monitoring for the presence of Pepino mosaic virus by the United Kingdom in 2008. UK survey report supplied by the National Plant Protection Organisation to the EC SCPH as required under Commission Decision 2004/200/EC. 3pp.

Van der Vlugt, R.A.A., Cuperus, C., Vink, J., Stijger, C.M.M., Lesemann, D.E., Verhoeven, J.T.J. and Roenhorst, J.W. (2002). Identification and characterization of Pepino mosaic potexvirus in tomato. OEPP/EPPO Bulletin 32, 503-508

van der Vlugt, R.A.A., Stijger, C.C.M.M., Naaldwijk, A.A., Verhoeven, J. Th. J. and Lesemann, D.E. (2000). First report of Pepino mosaic virus on tomato. Plant Disease 84, 103.

Velthuis HHW, van Doorn A (2006). A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. Apidologie 37:421–451.

Verhoeven, J.T.J., van der Vlugt, R.A..A. and Roenhorst, J.W. (2003). High similarity between isolates of pepino mosaic virus suggests a common origin. European Journal of Plant Pathology 109, 419-425.

Wilkins S. Importation of non-native sub-species of bumblebees for glasshouse pollination. Central Science Laboratory internal report, UK. 5pp.

Wright, D. and Mumford, R. (1999). Pepino mosaic potexvirus (PepMV). First records in tomato in the United Kingdom. Plant Disease Notice No. 89. Central Science Laboratory, York, UK.

Zhang Y.L., Shen, Z.J., Zhong, J., Lu, X.L., Chjeng, G. and Li, R.D. (2003). Preliminary characterization of Pepino mosaic virus Shanghai isolate (PepMV-Sh) and its detection by ELISA. Acta Agriculturae Shanghai 19, 90-92.

ANNEX 1 Survey activities by EU Member States in the period 2007-2009

Overview survey results MS PepMV 2007

	1.1 Tomato Seeds		for planting	fruit	market		
Member State	domestic	Other country	domestic	domestic	domestic	other country	Considered 'present' in presentation SCPH 2007
Austria	no	no	no	yes	yes		yes
Belgium	no survey	no survey	no	no	no survey	no survey	
Bulgaria	no	no	no	no	no	no	
Cyprus	no	no	no	no survey	no	no	
Czech Republic	no	no	no	no	no	no	
Denmark	no	no	no	no survey	no survey	no survey	
Estonia	no survey	no survey	no survey	no	no survey	no survey	
Finland	no survey	no survey	no	no survey	no survey	no survey	
France	no	yes	tes	yes	no survey	no survey	yes
Germany	no	no	no	yes	yes	no	yes
Greece	no data	no data	no data	no data	no data	no data	
Hungary	no survey	no survey	no	yes	no	no	yes
Ireland	no survey	no survey	no survey	no	no	yes	
Italy	no	no	no	no	no	yes	
Latvia	no survey	no survey	no	no	no survey	no survey	
Lithuania	no	no	no	no	no	no	
Luxembourg	no data	no data	no data	no data	no data	no data	
Malta	no survey	no survey	no survey	no	no	no	
Netherlands	yes	yes	yes	no data	no data	no data	yes
Poland	no	no	no	yes	yes	no	yes
Portugal	no	no	no	no	no data	no data	
Romania	no	no	no	yes	no	no	yes
Slovak Republic	no data	no data	no data	no data	no data	no data	
Slovenia	no	no	no	no	no data	no data	
Spain	no	no	yes	yes	no	no	yes
Sweden	no	yes	no	no	no	no	
United Kingdom	yes	yes	no	yes	yes	no	yes
Total			0	0	0		

Overview survey results MS PepMV 2008

	1.1 Tomato Seeds		plants for	fruit	market		
Member State	domestic	Other country	domestic	domestic	domestic	other country	Considered 'present' in presentation SCPH 2008
Austria	no		no	no	no		
Belgium	no		no	no	no		
Bulgaria	no		no	no	no		
Cyprus	no		no	no	no		
Czech Republic				yes (eradicated)			
Denmark				yes			yes
Estonia							
Finland	no		no	no	no		
France				yes			yes
Germany				yes			yes
Greece	no		no	no	no		
Hungary				yes			yes
Ireland				yes			yes
Italy				yes			yes
Latvia	no data		no data	no data	no data		
Lithuania	no		no	no	no		
Luxembourg	no data		no data	no data	no data		
Malta	no data		no data	no data	no data		
Netherlands				yes			yes
Poland				yes			yes
Portugal	no		no	no	no		
Romania	no		no	no	no		
Slovak Republic	no		no	no	no		
Slovenia	no		no	no	no		
Spain				yes			yes
Sweden	no		no	no	no		
United Kingdom				yes			yes
Total							

Overview survey results MS PepMV 2009

	1.1 Tomato Seeds								1.2 Tomato plants for planting							1.3 Tomato fruit production						1.4 To
	Seeds of EU origin				Imported from third countries																	
Member State	Country of Origin	# lots	#samples	# positive lots	Country of Origin	# lots	#samples	# positive lots	# premises	# inspections	# samples	# positive	# positive lots	# outbreaks	Country of origin (+)	# premises	# inspections	# samples	# positive	# outbreaks	Comments	# premises
Austria	AT	1	1	0	Israel	2	2	0	0	9	3	0	0	0		0	298	27	3	3	Varieties: Dirk, Campari and Sarantos the origin of the seedlings were the Netherlands	0
Belgium					Thailand, China, Israel, Guatemala	5	5	3			24	2	2	2	The seeds originated from another MS							13
Bulgaria	NL	1	0	0	Japan, Turkey, Israel, Korea	7	10	0	21	33	38	0	0	0		83	145	106	0	0		21
Cyprus									17	19	19	0	0	0		36	36	36	20	20	3 areas in Limmasol District 1 area in Larnaca district	18
Czech Republic	CZ	4	2	0					121	123	12	0	0	0		30	30	1	0	0		27
Denmark	NL	1	1	0	China, Israel, Japan	8	8	0	15	30	33	2	1	1	NL seeds	1	1	1	1	1	Already present in 2008, plants of DK origin	
Estonia																19	19	24	0	0		
Finland									13	15	192	0	0	0		5	6	12	0	0		
France	FR, NL	38	75	3	CN, TH, CL, IN, MA, Sn, VN, KR, ZA, TR, BR, US, TW, PE, MX,	1059	686	100	102	119	26	0	0	0		260	306	50	4	3		
Germany					China, Korea, Israel, Chile, India, Taiwan, Ecuador	63	28	0	111	136	152	0	0	0		109	170	216	15	4	1) outbreak in small company: plants DE origin, 2) outbreak at a 6 ha company; plants NL origin, 3) outbreak in a company already contaminated lasxt year, plants NL, 4) outbreak in small company, Nl origin.	56
Greece									17	17	1034	0	0	0		250	259	2555	0	0		3
Hungary									23	27	29	0	0	0		34	45	50	5	2	In counties: Bács new outbreak of unknown origin at an experimental site Csongrád recurrent outbreak at 1 grower	4
Ireland																8	11	35	6	3	Plants supplied by NL	7
Italy					China, Japan, Chile, India, Vietnam	9	11	0	436	442	685	0	0	0		573	1065	599	63	6	1 Sardinia, 5 Sicily	59
Latvia									28	28	22	0				26	30	31	0	0		21
Lithuania	PL, NL	3	2	0					36	36	0	0	0	0		36	42	17	0	0		27
Luxembourg	no data available																					
Malta	Italy	1	1	0	Israel	3	3	0	8	8	22	0	0	0		24	24	61	0	0		5
Netherlands					Brasil, Chile, China, Guatemala, India, Indonesia, Israel, Japan, Kenya, Peru, Tanzania, Thailand, Turkey, USA	713	93	0	20	398	178	1	1	1	NL	78	167	30	27	27	1. Contact contamination from other fruit production sites 2. Introduced on purpose by growers early in the season for preventing introduction of PepMV later in the season	
Poland	PL, NL, DE, PT	36	36	0	China, Israel, Chile, India, Thailand	14	22	0	324	381	32	0	0	0		946	1134	378	3030	20		620
Portugal	ES, NL, FR	12	12	0	China, Israel, Thailand, India	50	50	0	55	69	330	0	0	0								0
Romania	NL, IT, HU, PL, FR, SI, DE	30	30	0					233	281	20	0	0	0		957	923	664	0	0		292
Slovak Republic									2	2	2	0	0	0		33	41	21	0	0		1
Slovenia	IT, AT, FR, GR	9	10	0	China	3	3	0	177	188	11	0	0	0		58	59	42	0	0		2
Spain	Andalucia, Cantabria, Murcia, La Rioja	0	94	0	USA/Chile Israel EEUU		6	0		548	6684	0	0	0			1919	2189	435	61		1
Sweden	DE	4	1	0	Costa Rica, Chile	2	0	0	1	1	0	0	0	0		24	29	55	18	7	DK	15

Overview findings and reports of PepMV in EU MS

Member State	d 'present' in presentation SCPH 2007	d 'present' in presentation SCPH 2008	in production initial reports 2009	Reported in surveys 2007-2009	EPPO Alert List 2010
Austria	yes		yes	yes	found in 2007, under eredication
Belgium			yes	yes	no data
Bulgaria					found in 1 location in 2004, under eradication
Cyprus			yes	yes	no data
Czech Republic		no, but found and stated eredicated		yes	found in 2008 in 1 site, under eradication
Denmark		yes	yes	yes	no data
Estonia					no data
Finland					first found in 2001 and again in 2003, under eradication
France	yes	yes	yes	yes	2 isolated findings in 2000/2001 were subsequently eradicated, few outbreaks were detected in Centre and Bretagne - under offical control
Germany	yes	yes	yes	yes	several cases have been reported in Hessen, Thüringen, Hamburg and Sachsen, eradication was achieved in most cases and the virus is considered as transient
Greece	no data				no data
Hungary	yes	yes	yes	yes	first found in 2004 in one glasshouse
Ireland		yes	yes	yes	no data
Italy		yes	yes	yes	first found in 2001 in Sardegna, in 2005 in Sicilia
Latvia		no data			no data
Lithuania					no data
Luxembourg	no data	no data	no data		no data
Malta		no data			no data
Netherlands	yes	yes	yes	yes	found in a few fruit-producing sites
Poland	yes	yes	yes	yes	found in 2001 on 2 tomato plants, eradicated
Portugal					no data
Romania	yes			yes	no data
Slovak Republic	no data		yes	yes	found in 1 location in 2004, further surveys confirmed eradication
Slovenia					no data
Spain	yes	yes	yes	yes	first found in 2000 - Almeria, Andalucia, Galicia, Comunidad Valencia, Murcia and Canary Islands, so far with little economic impact
Sweden			yes	yes	found
United Kingdom	yes	yes	yes	yes	since 1999 several outbreaks were reported in various regions of the country, but most of them were eradicated at the end of each growing season - in 2004, PepMV was only present in 5 prodcution sites
Total	9	10	15	17	

ANNEX 2 Overview of notifications of non-compliance of Pepino mosaic virus in the period 2000 – May2010

Source: Europhyt, extraction date 19 May 2010

Notifications of non-compliance of PepMV on seed of tomato

Year	Country of export	Country of Origin	Country of Destination	Reporting Country
2010	CN	CN	PL	PL
2009	US	US	FR	FR
2009	CN	CN	FR	FR
2009	CN	CN	FR	FR
2009	IN	IN	FR	FR
2009	SN	SN	FR	FR
2009	CN	CN	FR	FR
2009	TH	TH	FR	FR
2009	IL	IL	FR	FR
2009	TH	TH	FR	FR
2009	TH	TH	FR	FR
2009	US	CN	FR	FR
2009	TH	TH	FR	FR
2009	TH	TH	FR	FR
2009	TH	TH	FR	FR
2009	TH	TH	FR	FR
2009	IL	CN	NL	BE
2009	US	IN	FR	FR
2009	TH	TH	FR	FR
2009	GT	GT	NL	BE
2008	IN	IN	PL	PL
2008	IL	IL	GB	GB
2008	US	US	GB	GB
2008	IL	IL	GB	GB
2008	IL	IL	PL	PL
2008	NL	NL	PL	PL
2008	NL	NL	PL	PL
2008	US	CN	PL	PL
2008	NL	NL	PL	PL
2008	NL	NL	PL	PL
2008	NL	NL	PL	PL
2008	US	US	GB	GB
2008	CN	CN	PL	PL

Year	Country of export	Country of Origin	Country of Destination	Reporting Country
2007	US	CN	SE	SE
2007	FR	TW	GB	GB
2007	CL	CL	FR	FR
2007	CL	CL	FR	FR
2006	NL	NL	GB	GB
2006	NL	NL	GB	GB
2006	NL	CL	GB	GB
2006	NL	NL	GB	GB
2006	NL	NL	GB	GB
2006	CL	CL	FR	FR
2006	NL	NL	GB	GB
2006	NL	NL	GB	GB
2006	FR	FR	GB	GB
2006	NL	NL	GB	GB
2006	CL	CL	FR	FR
2005	CL	CL	FR	FR
2005	MG	MG	FR	FR
2004	CN	CN	FR	FR
2004	CL	CL	FR	FR
2004	IN	IN	FR	FR
2004	CL	CL	FR	FR
2004	CL	CL	FR	FR
2004	CL	CL	FR	FR
2004	CL	CL	FR	FR
2003	CN	CN	FR	FR
2003	NL	NL	GB	GB
2003	CL	CL	FR	FR
2003	CL	CL	CL	FR
2003	CL	CL	CL	FR
2003	CL	CL	CL	FR
2003	CL	CL	CL	FR

Notifications of non-compliance of PepMV on plants for planting of tomato

Year	Country of export	Country of Origin	Country of Destination	Reporting Country
2009	NL	NL	PL	PL
2008	NL	NL	GB	GB
2006	NL	NL	GB	GB
2006	NL	NL	GB	GB

Notifications of non-compliance of PepMV on fruit of tomato

	Country of Origin								
Year	**BE**	**DE**	**ES**	**FR**	**IT**	**MA**	**NL**	**PL**	**IC**	*Total*
2000	1		16				8		8	*33*
2001			6			1	4		6	*17*
2002			1				1		2	*4*
2003			10				1		9	*20*
2004			4				3		8	*15*
2005			1			1	6	1	6	*15*
2006			4			1	11	1	3	*20*
2007			6			2	9	1	8	*26*
2008	3	1	22	2	4		22		4	*58*
2009	1		13	2	1	5	20	1	3	*46*
2010			3			1	3			*7*
Total	*5*	*1*	*86*	*4*	*5*	*11*	*88*	*4*	*57*	*261*