Netherlands Food and
Consumer Product Safety Authority
Ministry of Economic Affairs, Agriculture & Innovation

Pest Risk Analysis for *Blueberry scorch virus*

Including an inventory of highbush blueberry pests and diseases present in North America and absent in the Netherlands

October 2012

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Summary

This document describes the relevant aspects to access the potential risk of *Blueberry scorch virus* (BlScV) for the Netherlands. In addition, the import of plants for planting and fruit of *Vaccinium corymbosum* is considered in a broader scope, by evaluation of other pests and diseases associated with this crop in North America where *V. corymbosum* originates from.

Pest Risk Analysis BlScV

<u>Biology</u>
Blueberry scorch virus (BlScV) causes damage (Blueberry scorch disease) to *Vaccinium corymbosum* (highbush blueberry). The virus can also infect some other *Vaccinium* species (*V. macrocarpon*, *V. membranaceum* and *V. ashei*) but these species do not appear to develop (strong) symptoms. Damage on *V. corymbosum* depends on cultivar and virus strain. Some cultivars may show complete necrosis others may not show symptoms at all. The virus can be transmitted in a non-persistent mode by several aphid species. The most relevant vector species is *Ericaphis* (*Fimbriaphis*) *fimbriata*. Thus far, mechanical transmission has not been reported.

<u>Geographical distribution</u>
BlScV probably originates in North America. It is present in Canada and USA. Outside North America, it has been locally found in Italy (year of first finding 2004), the Netherlands (one field, year of first finding 2008) and Poland (first finding in 2008).

<u>Reason for performing the PRA</u>
In 2001 and 2003 two *Vaccinium corymbosum* samples with BlScV - like symptoms were sent to the NPPO of the Netherlands. The presence of BlScV in the samples was confirmed in 2003, once reliable tests had been developed. However, the origin of the samples was unknown. The detection of BlScV in the samples was reason for performing a PRA in 2004, which was finalized in January 2005 (with a minor update in 2007) and conducting surveys in the Netherlands. The pest was not detected during surveys in 2004 and 2005. However, the virus was found during a survey in 2008 at a production site in the southern part of the Netherlands. This first finding of BlScV in the Netherlands and the availability of new scientific information on the biology and epidemiology of BlScV since the last PRA were the reasons to update the PRA from January 2005. Note that in the EU, BlScV was found for the first time in Piedmont in Italy in 2004 (Ciuffo et al., 2005), in the Netherlands and in Poland in 2008. Thus far, it has not been reported from other EU-countries.

In addition to the detailed pest risk analysis for BlScV, a brief inventory was made of highbush blueberry pests in North America which are not (yet) present in the Netherlands, but which could be introduced with imports from North America.

<u>Current regulatory status</u>
Reported as a harmful organism for which statutory measures are taken by the Netherlands to the European Commission in line with requirements of Art. 16.2 of Council Directive 2000/29/EC. Recommended by EPPO for regulation as a quarantine pest (EPPO A2 list; A2 pests are locally present in the EPPO region).

<u>Likelihood of entry and spread by trade of plants</u>
BlScV can be spread over large distances and enter new areas by import and trade of infected plants for planting, other than seeds, of *Vaccinium* spp. Findings of BlScV in Italy, the Netherlands and Poland during the last 10 years indicate BlScV has been introduced with import of plants for planting.

Likelihood of establishment and spread

BlScV can probably establish anywhere where host plants are present. However, the likelihood of spread and thus transfer from an infected plant to other plants depends on the presence of aphid vector species. The most relevant vector species known today, *Ericaphis* (*Fimbriaphis*) *fimbriata,* is associated with highbush blueberry in the Netherlands. Therefore, transfer and natural spread is likely to occur in the Netherlands. The virus can also transfer and spread by vegetative propagation from an infected plant.

Potential impact

BlScV can cause high yield losses in highbush blueberry in its current area of distribution in North America. Infected plants of sensitive cultivars produce less fruit with up to more than 80% yield reduction. Infected plants can finally die after some years depending on the cultivar and virus strain. In the Netherlands, temperatures are very similar to those in parts of the current distribution area and cultivars are grown that are sensitive to BlScV. In addition, *Ericaphis* (*Fimbriaphis*) *fimbriata* - the most relevant vector species - is commonly occurring in highbush blueberry crops. Therefore, we assess a major impact for the PRA area comparable to that in its current area of distribution in North America with a medium uncertainty. There is especially uncertainty about the rate of natural spread under Dutch conditions. The disease will be more difficult to control, e.g. by the use on virus free planting material, when natural spread occurs more frequently.

Control options (by growers)

Options to control the disease are:

- Use of virus free (certified) planting material
- Aphid control
- Immediate removal of infected plants

At present, certified planting material is not available in the Netherlands, but may be expected within a few years.

Aphids can be well controlled by an insecticide (Calypso: thiacloprid) in highbush blueberries in the Netherlands. However, since this is the only insecticide registered against aphids in highbush blueberry at the moment, withdrawal of registration of this insecticide could make control of BlScV much more difficult.

Immediate removal of infected plants would be most effective when combined with testing because plants may already be infected before symptoms appear. Tolerant cultivars can act as a important source of infection without being noticed.

Main uncertainties in the risk assessment

- The likelihood of association of BlScV with plants for planting imported from North America. Plants are (usually) not tested for viruses at import.
- The host range of BlScV, especially if the commonly occurring *Vaccinium myrtillus* is a host plant and could act as a reservoir for BlScV.
- The rate of natural spread through aphids in the Netherlands
- The distance over which BlScV can spread naturally by aphids
- Differences in natural spreading between BlScV-strains

Regulatory options to reduce the risk (by official authorities)

Possible regulatory options to reduce the risk of entry of BlScV with import of plants for planting of *Vaccinium* spp.:

I. The plants should be officially certified under a certification scheme requiring them to be derived in direct line from material which has been maintained under appropriate conditions and found free from BlScV by obligatory testing and have been grown in an area free from BlScV or at a production place that is free from BlScV surrounded by a buffer zone of at least 500 m free of *Vaccinium* spp.

II. Plants should have been grown in an aphid-proof greenhouse and originate from material that have been tested and found free of BlScV.

III. After import, the plants should be grown under post-entry quarantine conditions free of potential vectors and subjected to official visual inspections and found free from BlScV by obligatory testing.
IV. At import, the plants should be subjected to official testing and found free from BlScV.

Option I will be most effective because the effectiveness of testing (options II and III) is greatly limited by sample size. Also tolerant cultivars and irregular distribution in the plants hamper virus detection. The advantage of option II is that also other pests and diseases may be detected during the post-entry quarantine period which are invisible at import.

Inventory of highbush blueberry pests and diseases present in North America
Many highbush blueberry pests have been identified which originate in North America. Several of these pests have been introduced into Europe: *Blueberry scorch virus* and probably several other viruses (*Peach mosaic rosette virus*, *Tobacco ringspot virus*, *Blueberry shoestring virus* en *Blueberry red ringspot virus*), the aphid *Ericaphis fimbriata* (sy, *E. scammeli*) which is the main vector of BlScV, an insect (*Dasineura oxycoccana*) and two fungi (*Monilinia vaccinii-corymbosi* and *Phomopsis vaccinii*). Two highbush blueberry pests were identified originating in Asia and introduced into both North America and Europe (the brown marmorated stink bug *Halyomorpha halys* and the spotted-wing drosophila *Drosophila suzukii*). Many of these pests have most likely been introduced with an import of plants for planting of *Vaccinium* spp. In addition, there is a risk of introductions of other pests and diseases with imports of plants for planting especially viruses that are not yet present in Europe and the bud mite *Acalitus vaccinii.*

Specific import requirements for plants for planting of highbush blueberries and other *Vaccinium* spp. from North America (and other continents) may be considered to reduce the risk of further introductions. Possible risk reduction options for import of plants for planting of *Vaccinium* spp. are:

I. Post-entry quarantine period including visual inspections and testing against viruses and phytoplasmas
II. Restricted allowance by admitting only plants if they are:
    a in a dormant stage, and
    b have been officially certified under a certification scheme requiring them to be derived in direct line from material which has been maintained under appropriate conditions and subjected to official testing for at least the relevant harmful organisms and has been found free, in these tests, from those harmful organisms, and
    c have been grown at a production place that is free from at least the relevant harmful organisms (in areas where one or more of the relevant organisms are present a buffer zone free from *Vaccinium* spp. may be needed depending on the biology of the relevant organisms).

Several viruses have already been introduced into Europe and may be more widespread than currently known. Implementation of certification schemes for highbush blueberry in Europe could also be considered to reduce the risk of spread of these viruses with trade of plants for planting.

Fresh (blueberry) fruit can also be a pathway for highbush blueberry pests, especially for fruit-invading insects. Also for this pathway specific risk reduction options may be considered. Possible risk reduction options are:

I. Pest free area or pest free production place for the relevant organisms,
II. Methyl bromide fumigation (or irradiation) in the exporting country (marketing of fresh fruit which has been irradiated is currently prohibited in the Netherlands),
III. Import only for processing under certain conditions,
IV. Fruit grown under a certification program.

From the evaluation it becomes clear that besides BlScV many other pests and diseases are associated with *Vaccinium corymbosum*, which may pose a need for further analysis.

Introduction (Pest Risk Initiation)

1. What is the reason for performing the PRA?

In 2001 and 2003 two *Vaccinium corymbosum* samples with *Blueberry scorch virus* (BlScV) - like symptoms were sent to the NPPO of the Netherlands. The presence of BlScV in the samples was confirmed in 2003, once reliable tests had been developed. However, the origin of the samples was unknown. The detection of BlScV in the samples was reason for performing a PRA in 2004, which was finalized in January 2005 (with a minor update in 2007) and to conduct surveys in the Netherlands. The pest was not detected during surveys in 2004 and 2005. However, the virus was found during surveys in 2008 at a production site in the southern part of the Netherlands. This first report of BlScV in the Netherlands and the availability of new scientific information on the biology and epidemiology of BlScV since the last PRA were the reasons to perform an update of the PRA from January 2005. Note that BlScV was found for the first time (in 2004) in the EU in Piedmont in Italy (Ciuffo et al., 2005). In 2008, BlScV was found for the first time in a field in the Netherlands and in Poland (Paduch-Cichal et al., 2011). BlScV has, thus far, not been reported from other EU-countries.

In addition to the more detailed pest risk analysis for BlScV, a brief inventory was made of highbush blueberry pests in North America which are not (yet) present in the Netherlands but which could be introduced with imports from North America. This inventory is discussed after the risk analysis for BlScV.

2. Scientific names and taxonomy

Family:	*Flexiviridae*
Genus:	*Carlavirus*
Species:	*Blueberry scorch virus*
Acronym:	BlScV (before BBScV has been used)
Common names:	blauwe-bessenverdorringsvirus (Dutch)

Different strains of BlScV are distinguished. Initially, two groups of strains have been distinguished, the New Jersey or East Coast strains and the Northwest or West Coast strains based on differences in symptom expression and cultivar response (Martin et al., 1992; Bristow et al., 2000; Wegener et al., 2006). More recently, variability in symptom expression and molecular variation have suggested that multiple strains of BlScV are present in British Columbia (Wegener et al., 2006). Also, molecular characterization of BlScV isolates in Italy suggested the presence of a new BlScV-strain present in Piedmont (Moretti et al., 2011).

3. PRA-area

The Netherlands.

4. Does a relevant earlier PRA exist?

Yes, a PRA has been made by the Netherlands in 2005 with a minor update in 2007 (Lammers et al., 2007). This PRA has been reviewed by the EPPO Panel on Phytosanitary Measures in 2007. Information from this PRA will be used in the present PRA where relevant. The present PRA focuses on the risk for the Netherlands and especially on the risk of spread and the potential impact under Dutch conditions.

Pest Risk Assessment

5. Host plant range (Worldwide)

Natural

BlScV main host is *Vaccinium corymbosum* (highbush blueberry) (MacDonald & Martin, 1990; Bristow et al., 2000). Other natural hosts are *Vaccinium macrocarpon* L. (cranberry) and *Vaccinium membranaceum* (black huckleberry), although only symptomless infections have been reported (Wegener et al., 2004; 2006). In a fact sheet, Catlin & Schloemann (2004), have indicated that samples of *V. ashei* (syn. *V. virgatum*; rabbiteye blueberry) tested positive for BlScV. Moretti et al. (2011) found *V. ashei* to be a probable host in the Trentino area in Italy. It is unknown if other *Vaccinium* species such as *V. myrtillus* (European blueberry) are (natural) host plants of BlScV.

Experimental

Chenopodium quinoa proved to be a susceptible host plant under experimental conditions (ICTV, 2002). Another experimental host plant recently described is *Nicotiana occidentalis* (Lowery et al., 2008). Mechanical inoculation with a BlScV isolate on C. quinoa and N. occidentalis-P1 resulted in symptoms on inoculated plants and infection was confirmed by testing in PCR (pers.comm. A.W. Werkman, July 2012).

6. Host plant range (PRA area, including acreage)

<u>Area of commercially grown crops</u>

Vaccinium corymbosum (highbush blueberry)

The production area of highbush blueberry has increased in the Netherlands during about the last 10 years (Fig. 1). In 2010, 535 hectares of highbush blueberries were grown, compared to 173 hectares in 2000. Highbush blueberry is now, together with blackcurrant (*Ribes nigrum*), the main berry crop in the Netherlands. Because of the soil preferences of *V. corymbosum* (acid soil with high organic matter content), production is concentrated in the provinces Drenthe, Limburg and Noord-Brabant (Fig. 2).

Vaccinium macrocarpon (cranberry).

On the Wadden islands Terschelling and Vlieland, the natural populations of *V. macrocarpon* are used for professional production of juice, jelly etc. (Natuurinformatie, 2011).

Vaccinium ashei/virgatum (rabbiteye blueberry)

No information on the production of *V. virgatum* in the Netherlands is available. The crop may not be grown or only at a very small scale in the Netherlands.

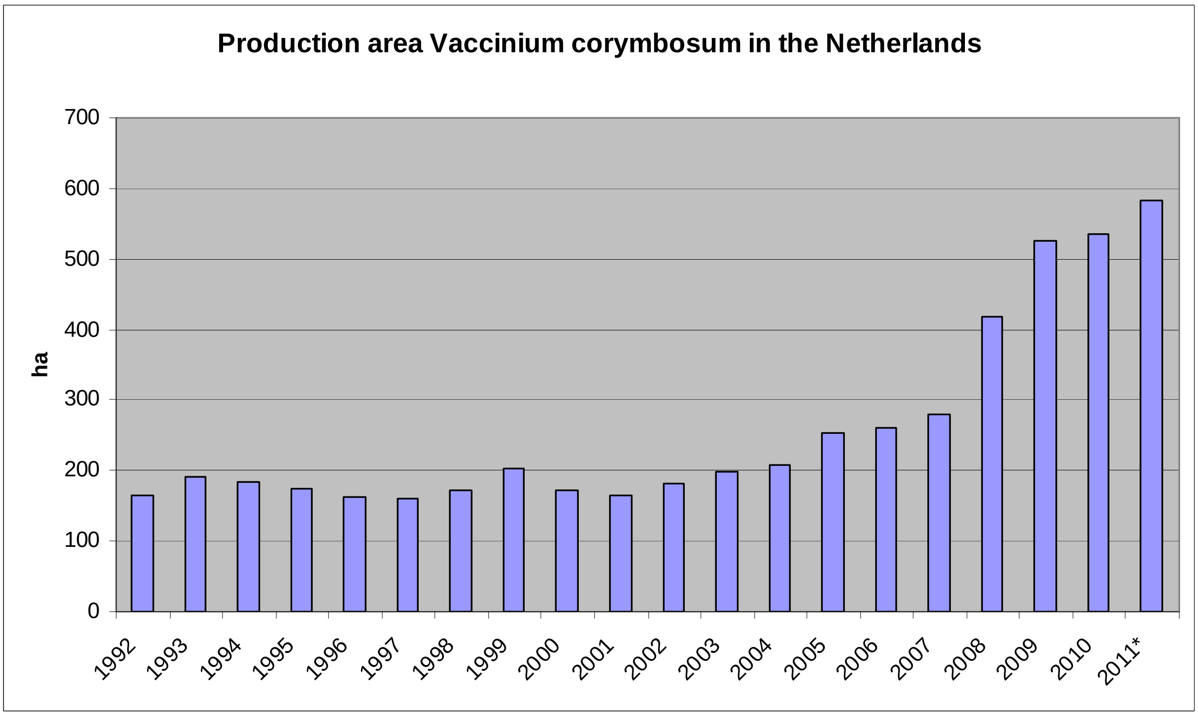

Figure 1. Production area of *Vaccinium corymbosum* in the Netherlands (statline.cbs.nl; data extracted 5/5/2012; * 2011 provisonal figures).

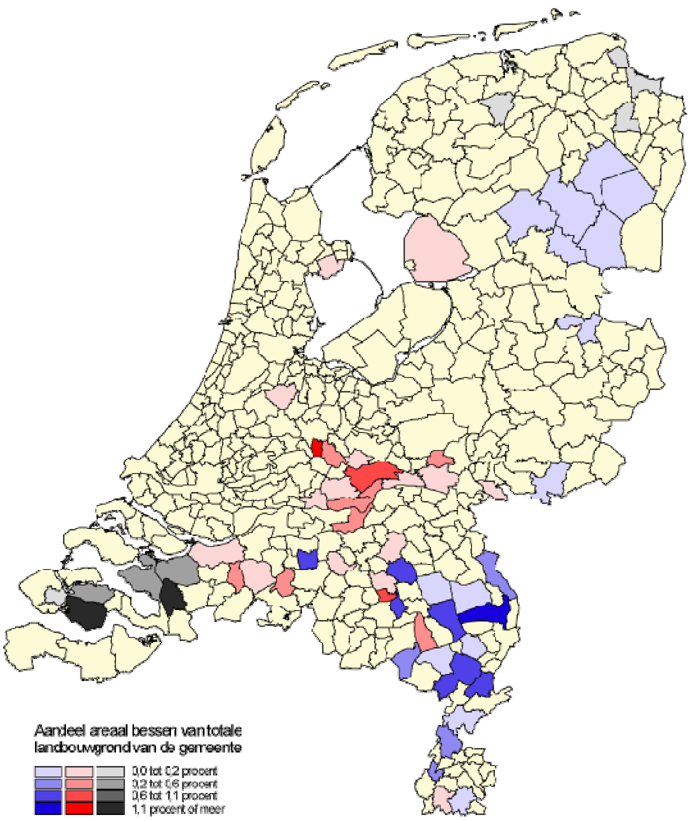

Figure 2. Areas where *Vaccinium* sp. production is concentrated in the Netherlands. Blue: highbush blueberry; red: redcurrant; black: blackcurrant. Areas indicated in dark blue are municipalities with highest concentration of blueberry production (Source: http://www.cbs.nl/nl-NL/menu/themas/landbouw/publicaties/artikelen/archief/2010/2010-bessen-2009.htm; last access 2nd February 2012).

Area of non-commercially grown plants

According to Soortenbank.nl (last access 24th February 2012), *V. corymbosum* is a rare species in nature areas in the Netherlands. However, the species has been spreading rapidly in recent years and has become a more common species in some nature areas (pers. comm. A. Klimkowska, Radboud universiteit Nijmegen, February 2012).

V. macrocarpon (syn. for *Oxycoccus macrocarpos* (Aiton) Pursh) is present along the coast of the Wadden islands and on the mainland coastline. The natural populations of *V. macrocarpon* are used for commercial purposes (see above). *V. macrocarpon* is also present in the eastern part of the Netherlands but rare (Soortenbank.nl, last access 24th February 2012). *V. myrtillus* is generally occurring in forests and heathland in the Netherlands but it is not known if this species can be infected by BlScV.

7. What is the current area of distribution of the pest?

At present the known distribution is limited to five countries (Table 1). BlScV probably originates in North America. In Poland, ELISA tests indicated the presence of BlSCV in hihgbush blueberry and cranberry crops (Paduch-Cichal et al., 2011).

Table 1. Known distribution of *Blueberry scorch virus* (BlScV)

Country	Year of first finding	Status	Source
Canada	2000	Present	Bristow et al., 2000
USA	1980	Present	Martin & Bristow, 1988
Italy	2004	Present, few occurrences	Ciuffo et al., 2005; Moretti et al., 2011 EPPO-PQR, January 2012
The Netherlands	2008	Transient, under eradication	EPPO-PQR, January 2012
Poland	2008	No official status	Paduch-Cichal et al., 2011

8. What is the international phytosanitary status?

Presently not a quarantine pest in the EU. It is recommended by EPPO for regulation (A2 List) (http://www.eppo.org/QUARANTINE/listA2.htm; last access 27th July 2012).

9. Does it occur in the Netherlands?

As stated above, two *Vaccinium corymbosum* samples with BlScV-like symptoms were sent to the Dutch NPPO in 2001 and 2003. The presence of BlScV in the samples was confirmed in 2003, once reliable tests had been developed. However, the origin of the samples was unknown. These results were reason to make a PRA and conduct surveys in commercial *V. corymbosum* fields:

- In 2004: 38 fields were visited and 2 samples with suspicious symptoms were taken for RT-PCR-testing. No findings of BlScV.
- In 2005: 46 fields were visited. Inspectors looked for symptomatic plants to take samples. If no symptoms were present, in each field one leaf sample from 5 randomly chosen plants was taken and tested by RT-PCR. No findings of BlScV.
- 2006-2007: no surveys
- In 2008: 47 fields were visited and 26 samples were taken. One plant, cv. Dixi, was found positive for BlScV . The whole plant was removed.
- In 2009: 5 plants with suspicious symptoms were sampled from the field in which BlScV had been found in 2008. Two plants, cv. Dixi, were found positive for BlScV. The three other plants, cv. Bluecrop (2x) and Elliot, tested negative. No plants were removed.

- In 2012, the field was visited again and a few plants of cv. Dixi showed BlScV-like symptoms (blighted branches and blossoms). Several plants of cultivar Bluecrop which was present next to the two rows of "Dixi-plants" showed chlorosis and a low fruit production which resembled a description of BlScV-symptoms on this cultivar (http://www.agf.gov.bc.ca/cropprot/blsv.htm, last accessed 20th June 2012). One "Dixi-plant" and two "Bluecrop-plants" with symtoms were sampled and tested positive for BlScV.

The origin of the infections in the field is unknown. The cultivar Dixi originates from the USA but has been cultivated in the Netherlands for more than 40 years. The crops, both "Dixi" and "Bluecrop", in which BlScV was found had been planted in 1987 according to the grower and BlScV might have been present in the field for many years before it was actually detected. The grower, however, only suffered serious losses in "Bluecrop" (losses in "Dixi" were limited to a few plants and considered minor) since about 2012 and, therefore, the origin of the infection remains uncertain. No other fields with highbush blueberry were observed near the infected field and according to the grower the nearest field was about 2 km away.

10. Likelihood of entry: pathway analysis

BlScV can be transmitted by aphids and by infected propagation material (Bristow et al., 2000; Lowery et al., 2008; Oudemans et al., 2011). There is no indication for seed transmission. Possibly the virus can spread through root contact but transmission by mechanical means does not occur according to Schilder (2012).

For entry of the virus into new areas, only trade of infected propagation material (plants for planting, other than seeds) is relevant. Trade of blueberry fruit might be a pathway because fruits can be infected. However, the likelihood of transfer from fruit to a host plant is assessed as "very low". Under experimental conditions, transfer from fruit to plants has been shown for *Plum pox virus*, another non-persistent virus, (Labonne & Quiot, 2001; Gildow et al., 2004). However, under practical conditions transfer seems very unlikely: it only might happen if infected fruit would be placed in the vicinity of a host plant. Because aphids usually do not feed on harvested fruit and, the transmission efficiency of BlScV is low, the pathway of infected fruit is not considered further in this PRA.

Natural spread only occurs over short distances, probably less than 1 km (see questions 11 and 12).

Below the pathway "trade of plants for planting of *Vaccinium* spp. other than seeds from areas where BlScV is present" is discussed in detail. All *Vaccinium* species are included in this pathway. At least four *Vaccinium* species are known to be host plant and it is uncertain if other *Vaccinium* species can be excluded as host plants (question 5).

Probability of association

Highbush blueberry propagation or planting material is produced from soft- or hardwood cuttings of selected clones and from seed obtained by pollinating flowers (in vivo). Oudemans et al. (2011) have shown that BlScV can be transmitted from infected mother plants into cuttings. However, transmission from mother plants to cuttings was not 100%, possibly due to variations in virus titres throughout the plant. Testing of cuttings from 8 mother plants showed transmission percentages from 0 – 100. There was a significant lower survival rate of cuttings when derived from infected mother plants compared to uninfected plants. In total, 40% of the surviving plants propagated from BlScV-infected mother plants were found infected.

Propagation material can also be produced *in vitro* and a distinction should be made between *in vitro* and *in vivo* produced propagation material because they pose different risks (Lammers et al., 2007). The probability of association with *in vitro* produced propagation material is lower than for *in vivo* produced propagation material because of differences in production conditions, as discussed in the former PRA (Lammers et al., 2007).

BlScV is known to be present in 6 states in the USA, Washington, Oregon, Michigan, New Jersey, Massachusetts and Connecticut and in British Columbia and Quebec in Canada (EPPO, 2011a). In several states of the USA, BlScV is under official control. In Washington, Michigan and Oregon, state quarantine measures are in force and specific requirements are in place for movement of susceptible plants of *Vaccinium sp* (Michigan, 2011; Oregon, 2011; Washington, 2012). In New Jersey, "All blueberry growers selling or offering for sale, propagating wood, rooted cuttings or plants must be certified"; mother plants are tested yearly for BlScV (NJ, 2010). The probability of association with plant for planting grown under these official measure will be largely reduced. The probability of association will also be influenced by the prevalence of the virus. BlScV appears for example to be more widespread in British Columbia (Canada) than in Oregon and Washington (Martin et al., 2006b). In Europe, BlScV has been reported from three countries, Italy, Netherlands and Poland but only from fruit orchards and not from nurseries producing planting material.

Import volumes

Plants for planting of *Vaccinium* spp. are imported into the Netherlands in particular from the USA, a country where BlScV is present (Tables 1,2). The database does not indicate the type of plants for planting that is imported but according to information obtained from growers in 2004 only *in vitro* plants are imported from North America (Lammers et al., 2007). In the past other types of planting material may have been imported but no figures are available. Import volumes from Italy or Poland are not known.

Table 2. Import of plants for planting, other than seeds, of Vaccinium spp. into the Netherlands from non-EU countries during 2007-2010 (source: import database National Plant Protection Organization)

Year	**Month**	**Country of origin**	**Number of plants**
2007[1]	April	USA	1
	November	USA	26800
2008[1]	April	USA	1
2009[1]	February	USA	7000
2010[2]	January	South Africa[3]	15
2011	-	-	0

[1] *Vaccinium corymbosum*

[2] *Vaccinium* species not indicated in database

[3] BlScV not present

Survival during transport

BlScV can very likely survive transport conditions. Conditions that are suitable for transport of plants will also allow for survival of the virus. Finally, the risk of entry with import of plants for planting, other than seeds, will mainly depend on cultivation conditions, the prevalence of the virus and its vectors and the implementation and intensity of testing and/or certification schemes (including the use of aphid-screened greenhouses) in the country of origin and import volumes.

Examples of introductions

In the USA, it has been suggested that it is likely that BlScV found in the Pacific Northwest was introduced via infected propagation material from New Jersey. The argument for this is that BlScV was not detected in the native vegetation or weeds in and around infected highbush blueberry fields in Oregon and Washington. Additionally, the disease appeared in several highbush blueberry fields at the same time, suggesting introduction with infected propagation material (Martin et al., 1992).

In Italy, BlScV has been found in Piedmont and Trentino, the two major areas of blueberry production in Italy (Ciuffo et al., 2005; Moretti et al., 2011). Isolates from both areas were genetically different and also other observations suggest that the introduction in the two areas occurred independent from each other. In Trentino, the initially infected plants seemed to be *V. ashei* imported from Oregon in 2002. Symptom expression in *V. ashei* is less obvious than in *V. corymbosum* and long latency periods could be the reason that the virus was not detected before 2009 despite surveys in previous years (Moretti et al., 2011). The origin of the infection in Piedmont was less obvious. The isolates formed a distinct clade from the North American strains. One of the infected orchards was established more than 25 years ago in a remote area and the introduction may have originated many years ago. As stated above (question 9), the origin of the infection in the Netherlands is unknown. The origin of BlScV in Poland is also unknown. BlScV may have been introduced with imports of plants from North America in the seventies of the 20^{th} century (Paduch-Cichal et al., 2011).

<u>Conclusion on the likelihood of entry from third countries (USA and Canada)</u>:
BlScV can enter the Netherlands with import of plants for planting of *Vaccinium* spp. from infested areas in the USA and Canada. The risk of entry will very much depend on the cultivation conditions (*in vivo*/*in vitro*), prevalence of the virus and its vectors and the implementation and intensity of certification schemes and/or testing (including the use of aphid-screened greenhouses) in the country of origin. When strict certification schemes are implemented the likelihood of entry will be low. However, no quantitative data are available on the association of BlScV with plants for planting from North America to provide a meaningful rating for the actual likelihood of entry. Findings in Italy, the Netherlands and Poland suggest that BlScV has been introduced more than once (maybe at least four times) from North America into Europe.

<u>Conclusion on the likelihood of entry from EU-countries</u>:
In Europe, BlScV has only been reported from Italy (Ciuffo et al., 2005; Moretti et al., 2011) and the Netherlands. The volume of plants for planting, other than seeds, of *Vaccinium* spp. entering the Netherlands from Italy is not known to the assessors. The virus has thus far only been reported from fruit orchards and not from nurseries.

11. Likelihood of establishment?

The presence of host plants, the climate, the pest's biology and the presence of aphid vectors will affect the likelihood of establishment after the pest has entered the PRA area with propagation material. Each of these factors is discussed below.

<u>Host plants (crop plants and wild plants)</u>
The main host plant of BlScV, *V. corymbosum,* and some other host plants are present in the Netherlands (see questions 5 and 6). BlScV can probably establish anywhere where host plants are present.

So far, wild *Vaccinium* plants or weeds have not been playing a significant role in dispersing and maintaining BlScV in North America. In Oregon and Washington (USA), BlScV was not detected in wild *Vaccinium* spp. or in weeds in and around infected highbush blueberry fields (MacDonald & Martin, 1990). Wegener et al. (2006) also could not detect BlScV in *Vaccinium* spp. or weeds in and around highbush blueberry fields in British Columbia (Canada). They detected, however, BlScV in a cranberry field (*V. macrocarpon*) adjacent to a BlScV-infected highbush blueberry crop and in black huckleberry (*V. membranaceum*) located at least 30 km away from the nearest highbush blueberry field. Although wild *Vaccinium* spp. do not seem to play a significant role in the epidemiology of the virus in North America, *Vaccinium* spp. that are hosts and are located close to a commercial field are considered a potential source for BlScV. In the Netherlands, different *Vaccinium* species including *V. myrtillus* (bilberries) and *V.*

macrocarpon (cranberry; see question 6) occur in nature. Especially, *V. myrtillus* is a common species in areas where *V. corymbosum* is grown commercially (http://www.soortenbank.nl/ ; last access 1st February 2012) but it is unknown if this species is a host of BlScV (see also question 6). However, *V. corymbosum* seems to become a more common species in nature areas (see above question 6) from which it could act as a virus source for commercial fields.

Climate

Average temperatures in parts of the current area of distribution are very similar to temperatures in the PRA region (Table 3). Both the Netherlands and parts of the current area of distribution have a 'Cf' climate according to the Köppen-Geiger climate classification system. 'C' climates have an average temperature above 10 °C in their warmest months, and a coldest month average between -3 °C and 18 °C. The 'f' stands for 'constantly moist: rainfall throughout year' (e.g. Kottek et al., 2006).

Table 3. Average maximum (T-max) and minimum temperature (T-min) (°C) at two locations in the *Blueberry scorch virus* distribution area in North America (www.usatoday.com) and Maastricht (http://www.klimaatatlas.nl/klimaatatlas.php)

Month	**Distribution area of *Blueberry scorch virus* in North America**				**PRA-area**	
	Vancouver, British Columbia (Canada)		**Trenton, New Jersey (USA)**		**Maastricht (the Netherlands)**	
	T - max	**T - min**	**T - max**	**T - min**	**T – max**	**T - min**
January	6	1	3	-4	5	0
February	8	2	4	-4	6	0
March	10	3	10	1	10	3
April	13	6	16	6	14	5
May	16	9	22	12	18	9
June	19	12	27	17	21	11
July	21	13	29	19	23	14
August	21	13	28	19	23	13
September	18	11	24	15	19	11
October	13	7	18	9	15	7
November	8	3	12	4	9	4
December	6	1	6	-1	6	1

Aspects of the pest's biology that would favour establishment

Symptomless infection

Field infections in sensitive cultivars may be detected by visual inspections. However, it can take some years after infection before symptoms start to develop (incubation period). Moreover, several tolerant cultivars have been identified, which might be related to the virus strain. Tolerant cultivars that become infected, serve as a symptomless virus reservoirs (Martin & Bristow, 1988; 1995; Oudemans et al., 2011). Similar rates of spreading have been found for a tolerant and sensitive cultivar, respectively (Bristow et al., 2000). Both long incubation periods and complete absence of symptoms, make it possible that BlScV will be first detected many years after its introduction.

Transmission
Transmission of the virus to other plants will be essential for establishment on the long term. Without transmission the virus will stay in the plant with which it was introduced and finally be eliminated when the crop is removed (which can nevertheless take many years after its introduction). The virus might be transmitted through root contact but for long term establishment transmission to other fields or plants in the natural environment is necessary. Natural spread through aphids and the presence of vector species in the Netherlands is discussed in detail below (question 12).

Adaptation
Generally, viruses can mutate and/or recombinate which increase their genetic variability and, thereby, favour establishment in new environments.

<u>Conclusion on establishment</u>
BlScV has been found in the Netherlands in one field with *Vaccinium corymbosum* in different years (see question 9). These findings indicate that the virus can survive under Dutch conditions, although no information is available on the origin of infection and possible spread by aphids in that particular field. How likely the virus will establish for the long term will depend on the likelihood that it will spread from the plant(s) on which it has been introduced to other plants. The likelihood of spread is discussed below.

12. How likely and how rapidly will the pest spread in the PRA-area? (*by human assistance and naturally*)

<u>Human assisted spread</u>
Spread by trade or movement of infected plant material could be rapid. The absence of symptoms in tolerant cultivars and a latency period in sensitive cultivars will increase the risk of spread with infected plant material. Seed transmission has never been reported (Martin, 2006). According to Schilder (2012), "the virus is not transmitted by contact between plants or mechanical means".

<u>Natural spread</u>
Aphids transmit BlScV in a non-persistent way (Bristow et al*.,* 2000; Raworth, 2004). Several aphid species have been reported as vector species under experimental conditions: *Aphis pomi, Ericaphis fimbriata, Ericaphis scammeli, Myzus ornatus, Myzus persicae, Illinoia pepperi* and *Rhopalosiphum padi* (Table 4). *E. fimbriata* and *E.scammeli* can be considered as the same species (Pansa & Tavella, 2008). *E. fimbriata* is a highbush blueberry-colonizing aphid species. The other aphid species are migrants.

Table 4. Aphid species reported to transmit *Blueberry scorch virus*

Aphid species	**Source and test species**	**Source**
Aphis pomi	*Nicotiana occidentalis > N. occidentalis*	Lowery et al., 2008
Aphis spiraecola	*Vaccinium corymbosum > V. corymbosum*	Lowery et al., 2008
Ericaphis fimbriata[1]	*Nicotiana occidentalis > N. occidentalis* *Vaccinium corymbosum > V. corymbosum* *V. corymbosum > N. occidentalis*	Bristow et al., 2000; Raworth, 2004; Lowery et al., 2008
Ericaphis scammeli[1]	*Vaccinium corymbosum > V. corymbosum*	Pansa & Tavella, 2008
Myzus ornatus	*Nicotiana occidentalis > N. occidentalis* *Vaccinium corymbosum > V. corymbosum*	Lowery et al., 2008
Myzus persicae	*V. corymbosum > Beta vulgaris*	French et al., 2003
Illinoia pepperi	*Vaccinium corymbosum > V. corymbosum*	Environmental Protection Agency , 1999
Rhopalosiphum padi	*Nicotiana occidentalis > N. occidentalis*	Lowery et al., 2008

[1] *E. fimbriata* and *E. scammeli* can be considered the same species (Pansa & Tavella, 2008)

The efficiency of aphid transmission is low (Bristow et al., 2000; Pansa & Tavella, 2008; Lowery et al., 2008; Raworth et al., 2008). The highest reported transmission efficiency in highbush blueberry is 20% for *E. fimbriata* (Lowery et al., 2008). This species seems to be the main vector based on transmission efficiency and being a colonizer of highbush blueberry. Despite the low transmission efficiency, the percentage of infected plants can rapidly increase through natural spread. In a commercial plantation in Washington state (USA), the percentage of infected plants as determined by ELISA increased 16.8 and 21.2 percentage points in one year time in cultivars Pemberton and Stanley, respectively (Bristow et al., 2000). Bristow et al. (2000) also studied the increase in number of diseased plants (based on scorch symptoms) and found an increase from nearly 0 to 100% in about 5 and 10 years time in cultivars Berkely and Pemberton, respectively. In one year the disease incidence in "Berkely" even increased from about 5 to 75%. Because the latency period of BlScV varies and can take several years these data may not exactly indicate the increase in percentage of infected plants. Wegener et al. (2006) determined increases in percentages of infected plants using ELISA in 3 commercial fields with different levels of control measures against BlScV in British Columbia (Canada). Increases in percentages of infected plants varied from 4.4 – 5.2 from 2001 to 2002 and from 4.2 to 9.6 from 2002 to 2004.

Besides the colonizing aphid species *E. fimbriata,* migratory aphid species might also transmit the virus during probing. Migrant aphid species were for example able to transmit the virus under laboratory conditions but the role of migrant aphid species in the epidemiology of the virus it is not (yet) known (Lowery et al., 2008; Sweeney et al., 2009). However, the spread patterns observed by Bristow et al. (2000) and Wegener et al. (2006) and the fact that no infection source was found around blueberry fields in the study of Wegener et al*.* (2006) suggest that aphid species colonizing the highbush blueberry fields rather than migratory species are most relevant for natural spread. Bristow et al. (2000) considered transmission by the aphid *Fimbriaphis fimbriata* (syn. *E. fimbriatia*) the most important means by which highbush blueberry plants became infected.

No published studies are known on the range aphids can spread the disease within one season. Bristow et al. (2000) and Wegener et al. (2006) studied spreading within fields; data suggested that the virus is transmitted mainly to adjacent or nearby plants. Wegener et al. (2006) have stated that natural spread is limited to less than 1 km but that the virus can readily spread between fields 5 -10 m apart. Like for *Plum pox virus* which is also transmitted by aphids in a non-persistent way, spreading of BlScV within a season may generally be limited to 100 m from the source plant. However, spreading over distances of several hundreds of meters cannot be excluded (Wijkamp & Van der Gaag, 2011).

Raworth (2004) found that *E. fimbriata* overwinters as egg on highbush blueberry in British Columbia and emerges during bud break in late February and March. He suggested that the rate of transmission of BlScV by this aphid may vary considerably with peak aphid densities in late June and early July varying from 300 to 9,000 aphids per plant in different fields. In Italy it was also found that *E. scammeli* overwinters as egg on the host plants and hatches starting from bud break (Barbagallo et al., 1999). Aphid populations in Italy peak in the period late May-beginning of July (Pansa & Tavella, 2008).

The observations on blueberry scorch disease in Italy (Moretti et al., 2011) suggest that BlScV has, thus far, spread slowly since its introduction. In Piedmont, the virus may only have been detected many years after its introduction. It was found in six orchards of which in two the virus was only found in one sample. In Trentino, three orchards were found infected which had one *V. ashei* cultivar in common, the possible origin of the infection (see also question 10 above "Likelihood of entry").

In Italy, four fields where BlScV was found have been monitored for several years (M. Turina, pers. comm. to A.W. Werkman, 2012). During this period newly symptomatic/infected plants were observed every year. However, healthy potted plants that were placed in the field did not become infected, even if aphids were transferred on them. Therefore, the exact way of transmission in these fields remains unclear. Raworth et al. (2008) did similar experiments and placed trap plants in infected fields for 2 weeks (experiments performed in different fields and during 3 growing seasons). Trap plants were tested for BlScV for up to 5 years after exposure in the field. The percentage of trap plants that had become infected during the 2 weeks of exposure in the field ranged from 0 – 4% depending on year and field plot.

The potential rate of natural spread of BlScV in the Netherlands will largely be influenced by the number of aphids present in the crop and control measures applied against aphids. In the Netherlands, there are no published data on the presence of aphids in highbush blueberry. However, based on field observations the situation has been described as follows (pers. comm. J.J.M. Bal, senior consultant fruit crops, ZLTO, the Netherlands, 19th April 2012): "Nearly 100% of the aphids in highbush blueberry are *E. fimbriata*. Incidentally, other aphid species are observed (*Aphis fabae* and *Myzus persicae*). Large numbers of *E. fimbriata* can occur, i.e. presence on about 60-70% of young shoots. In protected cultivation, the population can be very large with hundreds of aphids per shrub. Aphids are controlled by spray applications with thiacloprid (Calypso). Usually, one application is sufficient. Pirimicarb (Pirimor) has never worked against *E. fimbriata*. After the registration of Calypso, aphids have become a minor problem in highbush blueberry." Based on this information on the prevalence of aphids in highbush blueberry fields it seems likely that BlScV can be spread by aphids in highbush blueberry in the Netherlands. The vector is present and climatic condition are similar to those in for example western Canada where BlScV is present (Table 3). More difficult is to assess the rate of spread and if BlScV will spread with similar rates as reported from the USA and Canada (see above) because quantitative data to compare aphid densities are not available. Potentially, it may reach similar rates as observed in the USA and Canada.

Natural spread of the virus over larger distances (i.e. between fields or production places) will very much be influenced by the distances between production places and host plant densities in between commercial fields. The cultivation of highbush blueberry is concentrated in certain areas in the Netherlands (Fig. 2) which increases the likelihood of natural spread between fields. This can be illustrated with the following observation in Canada: the presence of BlScV in British Columbia was first observed in 2000, when 20 highbush blueberry fields were infected. In 2001 and 2002, the number of infected fields increased to 60 and 77, respectively. Initial mapping of these fields indicated that BlScV spread had followed the wind direction (Anonymous, 2002). Thus, because of the presence of *E. fimbriata* in highbush blueberry and the concentration of production fields in certain areas in the Netherlands, natural spread within fields and between fields can be expected although there will also be fields quite isolated from other fields and presence of BlScV in such a field will generally pose a low risk to other fields. However, host plants of BlScV present in private gardens, public green or nature areas may also facilitate spread over larger distances. *V. corymbosum* seems for example to become a more general plant species in nature areas (question 6) where it could act as a virus reservoir for commercial fields. *Vaccinium myrtillus* (bilberries) is generally occurring in nature areas in the eastern and southern part of the country where also highbush blueberry is commercially grown (www.soortenbank.nl; last access 1st February 2012). So far, this plant species has not been reported as a host. However, if *V. myrtillus* can be infected and act as a natural source of BlScV, the likelihood of natural spread over larger areas will increase.

Conclusion on spread
BlScV can be spread throughout the PRA area by movement or trade of infected plant material. Thus, when BlScV is introduced on plants which are used for vegetative propagation the virus may be spread with the material retrieved from these plants.

The aphid species *E. fimbriatia,* the main natural vector of BlScV in North America, is present in highbush blueberry fields in the Netherlands and it is likely that natural spread of BlScV can occur under Dutch growing conditions. The rate of natural spread will affect the potential impact to a great extent which is discussed below (question 13). Natural spread may generally be limited to 100 m but spread over distances of several hundreds of meters cannot be excluded. This assessment is, however, uncertain because very limited data are available on natural spread distances. Host plants present outside commercial fields might act as a virus reservoir and facilitate spread between commercial fields.

13. What is the potential damage when the pest would become introduced? (*without the use of control measures*)

Impact in its current area of distribution
Symptoms caused by BlScV have been extensively reported (Bristow et al., 2000; Catlin & Schloemann, 2004; French et al., 2003; MacDonald & Martin, 1990; Martin and Bristow, 1988, 1995; Martin et al., 1992; Wegener et al., 2004). In tolerant cultivars symptoms might be completely absent, whereas in some sensitive cultivars complete necrosis of flowers and young leaves and twig dieback has been observed. Severely blighted bushes bare little fruit. Twig dieback causes lateral buds below the point of necrosis to grow and produce branches later in season. Over a period of several years, infected bushes become very twiggy and the fruit load is markedly reduced compared to healthy plants. The productivity of diseased plants that show symptoms is declining every following year and plants of some cultivars eventually die (Bristow et al., 2000). The severity of the symptoms can also vary from year to year (Catlin & Schloemann, 2004) and depends on cultivar and virus strain (Bristow et al., 2000).

Bristow et al. (2000) carried out a field study over a period of three years to compare the yield of healthy blueberry plants (*V. corymbosum*) with diseased plants. The study was carried out with healthy field bushes and adjacent bushes exhibiting symptoms of Blueberry scorch disease. The plants belonged to the (sensitive) cultivar Pemberton. All ripe berries were always harvested on three dates (16th July, 8th and 25th August). Total yields of healthy bushes were compared with bushes that showed symptoms for one, two and three years. Compared to healthy bushes, bushes showing symptoms for the first, second and third year carried 31%, 72% and 83% less fruit, respectively. In another study by Bristow et al. (2000), six cultivars (out of 59 tested) showed no symptoms and no significant reduction in yield, 30 cultivars showed dieback of twigs and blighted leaves and flowers.

The epidemiological studies from the USA and Canada (Bristow et al., 2000; Wegener et al., 2006) and more qualitative descriptions of the disease (e.g. PCM, 2009) indicate that BlScV can cause a serious economic impact due loss of yield and premature death of plants. It is considered an important disease of highbush blueberry in North America and has been included in the certification scheme for the production of planting material (testing being required). In Piedmont (Italy), economic damage (reduced production and/or dead plants) has been observed, varying with the cultivar (Lammers et al., 2007).

Potential impact in the Netherlands
Climate and growing conditions in the Netherlands are not expected to restrict the development of disease symptoms once a plant becomes infected with BlScV. The potential impact will vary among cultivars: most cultivars are sensitive to the New Jersey

strains of BlScV, while several cultivars are tolerant to the Northwest strains. In the PRA area, cultivars sensitive to both groups of strains are grown (e.g. Dixi, Northland) and cultivars sensitive to only the New Jersey strains (e.g. Bluecrop) (Wegener et al., 2006; Lammers et al., 2007; www.schrijnwerkers.nl/www.esveld.nl/www.oude-aa.nl). Uncertainty about the natural spread rate under Dutch conditions (see question 12), however, hampers the assessment of the potential impact for blueberry production in the Netherlands. BlScV-infections will reduce the yield (depending on cultivar and virus strain) but total impact in a sensitive cultivar will largely depend on the percentage of plants that becomes infected during the lifetime of a crop.

As already discussed above, the observations in the single field in the Netherlands where BlScV has been found are difficult to interpret because it is not known how and when BlScV was introduced into the field (see also question 9). BlScV might have been present for many years in the field without causing much impact (question 9). However, the recent symptoms an losses in "Bluecrop" may be caused by BlScV. Also note that BlScV has only become a serious problem in the northeastern USA during the 1990's, after being a disease of minor importance for nearly 20 years (Martin et al., 2006b).

Conclusion on impact

The potential impact is major because cultivars sensitive to BlScV are grown in the Netherlands and the main vector species is present. Infected bushes produce less fruit (up to more than 80% reduction), and can finally die-back after some years depending on cultivar and virus strain. However, there is an uncertainty about the natural spread rate under Dutch conditions which hampers the assessment of the potential impact for blueberry production in the Netherlands. Potentially, it may be as high as observed in Canada and the USA with increases in disease incidences of up to or even more than 20% points per year.

14. Which control measures are available?

(*indicate efficacy of available pesticides and non-chemical methods; also discuss the availability of control measures in the future taking into account the possibility of resistance development against pesticides and possible withdrawal of pesticides*)

Virus-free planting material

Since propagation material is an important pathway for introduction and spread of BlScV, virus-free planting material should be used. This should be based on testing since symptoms are not reliable for detection of the virus. It can take several years before symptoms develop and many cultivars do not show symptoms at all (Schilder, 2012).

In the EPPO region, a certification scheme for *Vaccinium* spp. has been published (www.eppo.org, PM 4/18(1)) but certification schemes for BlScV have not been implemented in the EU yet.

For testing, ELISA and RT-PCR protocols are available. However different strains of BlScV exist that do not consistently test positive in ELISA (Sweeney et al., 2009). This is of concern since testing programs relying solely on ELISA or which only use RT-PCR after a positive ELISA therefore may not always detect the virus.

Control of aphids and removal of infected/diseased plants

In British Columbia, aphid control is recommended as part of an area wide management strategy (Raworth, 2004; Sweeney et al., 2009). For aphid management, the insecticide pymetrozine is used which has an antifeedant effect and may, therefore, interfere with virus transmission. Margaritopoulus et al. (2010) indeed found that pymetrozine both reduced acquisition and inoculation of *Potato virus Y,* another non-persistently transmitted virus. In British Columbia, it is recommended to apply pymetrozine in highbush blueberry in early spring, after egg hatch, but before development of winged forms.

In the Netherlands, pymetrozine does not have an official registration in highbush blueberry crops. The only pesticide that is effective against aphids and is registered in highbush blueberry is thiacloprid (Calypso), a neonicotinoid (http://www.ctgb.nl/, last access 1st March 2012). This insecticide is highly effective against *E. fimbriata* and since its registration aphids became a minor problem in highbush blueberry (see above).

Wegener et al. (2006) studied the extent of spread of BlScV in three commercial fields in British Columbia (Canada). He found a decreased rate of spread of the virus in one field where infected plants were immediately removed upon detection (using ELISA) and imidacloprid was applied repeatedly against aphids. The observations suggest that removal of infected plants and/or application of imidacloprid contributed to the disease control. However, in this intensively managed field the percentage of infected plants still increased from 10.2 to 19.6% over three years. In two other less intensively managed fields the percentage of infected plants increased from 5.7 to 19.7 and from 29.4 to 41.7% in the same period. Aphicides should be applied before removal of the bushes because removal will encourage movement of the aphids (Martin et al., 2006b).

Schilder (2012) recommends to remove and burn all plants if the percentage of infected plants in a field is high. Testing and subsequent removal of infected plants with combined with aphid control can be implemented if the percentage of infected plants is low. Because roots can also be infected, plants should be removed with roots. Otherwise herbicides should be applied to kill the roots and to prevent emergence of infected suckers from roots that have been left behind (Schilder, 2012).

<u>Resistance</u>
No cultivars of *Vaccinium corymbosum* are known that are resistant to BlScV. Several tolerant cultivars have been identified depending on strain and blueberry cultivar (See also question 13). However, these tolerant cultivars can act as an inoculum source (Martin & Bristow, 1988; 1995; Oudemans et al., 2011). Bristow et al. (2000) found for example a similar rate of spread in symptomless cv. Stanley as in symptomatic cv. Pemberton. Therefore, tolerant cultivars are not suitable as control measure when both tolerant and sensitive cultivars are grown.

<u>Conclusions control measures</u>
Once a plant is infected, removal and destruction of the plant is the only control option.
The use of virus-free planting material, disease monitoring and immediate removal of infected plants and aphid control will be the most important tools to control the disease.
It should, however, be noted that presently aphid control in highbush blueberry depends on one insecticide (thiacloprid) only. Disease problems may be enhanced by the use of tolerant cultivars which may act as an unseen virus reservoir for sensitive cultivars. Control/elimination in an isolated field will be possible but once established in a highbush blueberry production area control will be difficult, especially when sensitive cultivars are grown next to tolerant ones.

15. What is the expected damage when the pest would become introduced?
(*with the use of available control measures*)

If all possible control measure are applied (use of virus-free starting material, scouting, testing, immediate removal of infected plants, control of aphids) the damage level may be limited. However, incidentally, damage may still be major. In a field in Canada for example, the percentage of diseased plants still increased with about 2% per year during a 2-years period, despite testing of every plant, immediate removal of infected plants and repeated application of aphicides (Wegener et al., 2006). Testing of every plant every year is not feasible for a grower. Moreover, when only symptomatic plant are removed, the percentage of diseased plants may increase more rapidly, because infected plants can remain symptomless for up to two years or only express marginal symptoms (Bristow et al*.,* 2000). Note that in the Netherlands control of aphids in highbush blueberry depends on only one insecticide, which in the case of withdrawal would make it much more difficult to control BlScV. In addition, once the virus has established, it may be difficult to raise plants that are 100% free of the virus as a result of e.g. introductions from infected *V. corymbosum* plants in private gardens or nature areas. Control will also be more difficult in areas with high densities of highbush blueberry crops because of the risk of spread of BlScV between nearby fields.

In the Netherlands, the potential impact but also the possibilities to control the virus will largely depend on the rate of natural transmission as also discussed above (question 13). If natural transmission is rare and virus free planting material is being produced within a certification scheme or equivalent program the impact will be minor.

16. To which extent will the costs of control measures increase when the pest would become introduced?

Production costs may rise due to increased costs for planting material, labour for scouting, removal of infected plants and application of insecticides. Since visual detection of the virus is not always possible, testing of planting material could also add to the producer's costs.

17. To which extent will the introduction of the pest affect export markets

The presence of the virus may effect the export of planting material of *V. corymbosum* because BlScV is for example regulated for planting material (plants for planting moving in trade) in several states in the USA (question 10)*.*It is not expected to have any effect on export markets of blueberry fruit. Quarantine measures in for example Oregon - USA are (Oregon, 2011):

"(a) The blueberry plants must originate from a pest free area.
(b) The blueberry plants are certified in accordance with the regulations of an official certification program in the state or province of origin that includes testing and inspection for blueberry viruses and is approved by the director.
(c) The blueberry plants are free of blueberry scorch virus based on an official laboratory test using a protocol approved by the director.
(d) The blueberry plants are micropropagated and/or grown in an insect-proof greenhouse or screenhouse and originate from mother plants that have been tested and found free of blueberry scorch virus.
(e) Blueberry fruit must be free of leaf tissue and other plant debris before being imported into the control area. Notification and phytosanitary certificates are not required for shipments of blueberry fruit."

18. Conclusion on the pest risk of BlScV

- BlScV probably originates in North America where it spreads naturally by at least one aphid species, *E. fimbriata*. This aphid species is also present in highbush blueberry in the Netherlands.
- Findings of BlScV in Italy, the Netherlands and Poland during the last 10 years indicate that there is a risk of entry of BlScV from North America into Europe with import of plants for planting of *Vaccinium* sp. The risk of entry may change over time depending on measures taken in the countries of origin, type of plant material and import volumes.

- BlScV can cause high yield losses in highbush blueberry in its current area of distribution in North America. Infected plants of sensitive cultivars produce less fruit with up to more than 80% yield reduction. Infected plants can finally die after some years depending on the cultivar and virus strain. Temperatures in parts of North America where the virus has a major impact are very silimar to those in the Netherlands. In the Netherlands, cultivars are grown that are sensitive to BlScV and the most relevant vector species is commonly occurring in highbush blueberry crops. Therefore, BlScV may potentially have a similar impact in the Netherlands as it has in its current area of distribution in North America. However, there is uncertainty about rate of natural spread under Dutch conditions. The disease will be more difficult to control, e.g. by the use on virus free planting material, when natural spread occurs more frequently

19.Uncertainties

The main uncertainties in the present PRA are:

- The likelihood of association of BlScV with plants for planting imported from North America,
- The host range of BlScV, especially if the generally occurring *Vaccinium myrtillus* can act as a reservoir for BlScV.
- The rate of natural spread through aphids in the Netherlands
- The distance over which BlScV can spread naturally by aphids,

Identification and evaluation of risk reduction options

20. Indicate the pathway. The pathway is "import of plants for planting of *Vaccinium* spp."

21. Identification and evaluation of options to reduce the risk of entry or spread by import or trade

Table 5. Overview of possible risk reduction options for the pathway "import or trade of planting material of *Vaccinium* sp."

Risk Reduction Option	Risk reduction	Justification[1]
I. Options at the place of production		
Detection of the pest at the place of production by visual inspection and/or testing	yes	Visual inspection: not sufficient because of latent infections as a result of reletively long incubation period and use of tolerant cultivars. Testing: detection threshold level depends on sampling intensity and performance of the testing method. Also irregular distribution of the virus in the hampers detection. Certification scheme will provide a high level of protection until plants are grown in the open field (without aphid screens).
Prevention of infestation of the commodity at the place of production: - use of resistant cultivars - growing the crop in specified conditions (e.g. physical protection) - crop treatments - harvest at certain times of the year or growth stages	yes: physical protection	Resistant cultivars are not available, nor crop protection agents that can protect the crop from infections. Physical protection could be used to prevent infection through aphids but will probably not be feasible except for more basic material.
Establishment and maintenance of a pest-free production site, pest-free place or pest-free production area	yes	Pest-free production place: possible but a buffer zone without host plants will be needed to prevent introduction from the virus by aphids. The size of the buffer zone is difficult to assess because of limited data. Like for *Plum pox virus* a buffer zone of 500 m or even less may be sufficient (Wijkamp & Van der Gaag, 2011).
II. Options after harvest, at pre-clearance or during transport		
Detection of the pest in consignments by inspection or testing	yes	Risk reduction level depends on sampling and testing intensity
Removal of the pest from the consignment by treatment or other phytosanitary procedures (remove certain parts of the plant or plant product, handling and packing methods)	no	No treatments available: plants have to be destroyed to kill the virus,
III. Options that can be implemented after entry of consignments		
Detection during post-entry quarantine	yes	Visual inspection + testing during a post-entry quarantine period. Risk reduction level depends on sampling intensity and duration of the post-entry quarantine period. Reduction of risk higher than with testing at

Risk Reduction Option	Risk reduction	Justification[1]
		import alone.
Consider whether 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	no	Pathway is plants for planting.
Effective measures that could be taken in the importing country (surveillance, eradication, containment) to prevent establishment and/or economic or other impacts	yes	Eradication is possible when detected at an early stage: once the virus has spread to natural vegetation, eradication will be difficult. In addition, latent infections can make it difficult to determine the size of the area infested. In the case of the finding of an infection, whole plots may need to be destroyed to obtain a high degree of certainty that the virus has been eliminated.

[1] a more detailed justification for those options which reduce the risk is given below.

Ad I. Options at the place of production

Production of virus-free plants is possible under a certification scheme or equivalent system. Plants should have been derived in direct line from plants tested and found free of BlScV) and raised at a production place that is free of the virus and where no host plants are grown in a 500 m buffer zone or the plants have been raised in an aphid-proof greenhouse (Table 5). The latter option (aphid-proof greenhouse) can be feasible for basic propagation material but probably not for the production of large number of plants. Note that the buffer zone size needed is highly uncertain and smaller buffer zones might already be appropriate.

EPPO (no year) has published a certification scheme for plants for planting of *Vaccinium* spp. As far as known, no certification schemes have been implemented in the EU for *Vaccinium* spp. and all plants for planting have CAC-quality (*Conformitas Agraria Communitatis*) which means that plants, at least on visual inspection, should be substantially free of pest and diseases (Council directive, 2008). These requirements are insufficient to guarantee virus-free planting material. The requirements for certified material are much more strict and such material may be expected within some years (information from Naktuinbouw, the Netherlands, March 2012). A list of organisms for which testing methods are being developed by Naktuinbouw is included in Annex I.

Visual inspection will not be sufficient to guarantee pest freedom because of the possibility of latent infections as a result of relatively long incubation period and use of tolerant cultivars (Oudemans et al., 2011; Schilder, 2012). For testing, it has been advised to take several samples from different branches because of the uneven distribution of the virus in the plant (Schilder, 2012).

Ad II Options after harvest, at pre-clearance or during transport

See also above for limitations of visual inspections and testing. Plants could be tested for presence of the virus. The sample size will largely determine the detection level. Especially with low infection incidences and irregular distribution of the virus, the probability of not detecting the virus, despite its presence, can be high.

Ad III Options that can be implemented after entry of consignments

Post-entry quarantine

A post-entry quarantine period during which plants are grown, inspected and tested for BlScV will decrease the likelihood of entry of BLScV but also other pests and diseases (see below "Inventory of pests and diseases in North America"). New Zealand for example has specific requirements for the import of *Vaccinium* nursery stock. Import is only allowed for dormant cuttings, plants in tissue culture and specifically described tissue culture-derived plants from a few specific origins (Biosecurity New Zealand, 2010). These requirements are: "In summary, an import permit is required and a phytosanitary certificate must accompany all consignments certifying that the nursery stock has been inspected and found to be free of any visually detectable regulated pests, and has been treated for regulated insects and mites (cuttings only). On arrival in New Zealand, the nursery stock must be grown for a minimum period of 9 months (tissue culture) or 16 months (cuttings) in a Level 3 post-entry quarantine facility where it will be inspected, treated and/or tested for regulated pests." *Vaccinium* nursery stock can also be imported into New Zealand from offshore accredited facilities with less strict post-entry quarantine conditions. In that case *Vaccinium* nursery stock must be grown for a minimum period of 6 months in a Level 2 post-entry quarantine facility where it will be inspected, treated and/or tested for regulated pests.

Eradication measures

Eradication measures could be more or less strict with a decreasing degree of certainty that the virus has been eradicated but with lower impact for the producer(s) concerned. More strict measures could be implemented for propagation material than for plants grown for fruit production because there is a much higher risk of spread to other plots when propagation material is infected. Possible options in the case of finding an infection are:

a) Only plants with visible symptoms and plants directly neighbouring infected plants are removed and destroyed. Neighbouring plants have an increased chance of being infected as infected older highbush blueberry plantings show a clustered distribution of diseased plants (Oudemans et al., 2011). Plants from the infected plot may not be moved unless tested and found free of BlScV. Aphicides needs to be applied to minimize natural spread of the virus within the plot and to the environment. Fruits may still be harvested.
b) Like (a) and, in addition, plants are tested randomly to detect latent infections and when more plants are found infected parts or the whole plot need to be destroyed. Still this option does not give a full guarantee that the virus will be eliminated from the plot because only a sample of plants is being tested. This is especially the case when tolerant cultivars are present at the plot.
c) This option includes destruction of the whole plot even when only one plant has been found infected. In this way also latent infections will be removed. In addition, all host plants within 500 m from the infected plant(s) should be removed and destroyed. This is a more stringent option than options a and b, with a higher chance of eradication

In any situation, a trace-back survey and if relevant a trace-forward survey will be needed to detect any other infection related to the first finding.

Inventory of pests and diseases in North America

Introduction

Highbush blueberry (*V. corymbosum*) is native to North America and has been introduced into Europe around 1930 for its fruit production (Naumann, 1993). Since the introduction of the highbush blueberry crop several highbush blueberry pests have been (unintentionally) introduced from North America into Europe: *Dasineura oxycoccana* and *Blueberry scorch virus* (BlScV), both reported for the first time from Italy in respectively 1996 and 2004 (EPPO, 2011a), the aphid *Ericaphis fimbriata* (sy, *E. scammeli*) which is the main vector of BlScV (Coeur d'acier A, 2010), *Monilinia vaccinii-corymbosi,* reported for the first time in Austria (Gosch, 2003) and *Phomopsis vaccinii*, reported for the first time in Lithuania (Gabler et al., 2004; Kacergius & Jovaisiene, 2010). Recently, other viruses have been reported in highbush blueberry in Europe which probably also originate from North America (Paduch-Cichal et al., 2011). It is unknown how these pests were introduced but import of plants for planting from North America seems to be the most likely pathway of introduction for at least BlScV and other viruses. More pests may be introduced in future through trade from North America. Therefore, a brief inventory was made of highbush blueberry pests present in North America which are not (yet) present in the Netherlands. The goal was not to make a complete lists of pests but to list at least (most of) the pests which are considered most important in North America. Some generic management options are discussed to reduce the risk of further introductions.

Data and methodology

Four main sources were used to list highbush blueberry pests in North America:

- PCM (2009): a list of "All pests: disorders, diseases, and insects for blackberries, blueberries, raspberries, and strawberries" in Oregon and Washington (Northwest UDSA) from Peerbold Crop Management available on internet.
- DeFrancesco J & Murray K (2011); pest management strategic plan for blueberries in Oregon and Washington. Summary of a revision workshop held on April 18, 2011 in Troutdale, Oregon. Oregon State University, Cordley Hall, OR, USA ().
- Fitzpatrick (2009): Insect life histories in fruit, shoot and root environments of cranberry and blueberry. Proceedings of the IXth International Symposium on *Vaccinium*. Acta Horticulturae 810, 231-250.
- EPPO (no year): the EPPO certification scheme for pathogen-tested material of *Vaccinium* spp. PM 4/18(1)

In addition, other highbush blueberry pests reported/listed in papers studied to obtain more information on the biology and impact of listed pests were included. The EPPO database for quarantine pests (EPPO, 2011a) was used for data on the geographical distribution of the pests and if not present in this database other sources were used (Table 6). The type of damage that each of the pest can cause was summarized using information from the above mentioned sources, existing datasheets from EPPO and CABI and some additional papers but no detailed impact assessment was made.

Results

Table 6 lists pests present in North America and which are not (yet) present in the Netherlands. Some of the pests are also present on other continents including Europe. Those pests which are already present in Europe will generally have a higher likelihood of introduction through trade or natural spread than those which are not (yet) present on the European continent because of absence of border inspections and large EU internal trade volumes. Note that some pests do not originate from North America but have been introduced from another continent, e.g. *Halyomorpha halys* and *Drosophila suzukii* which also have been introduced into Europe.

Pests already present in Europe

Blueberry scorch virus. See the pest risk analysis above.

Blueberry red ringspot virus. In Europe, this virus has been reported from Poland, Czech Republic and Slovenia. A natural vector may be involved in spread of the disease in USA (Table 6).

Recently, the presence of *Blueberry scorch virus* (BlScV), *Blueberry shoestring virus* (BSSV)*, Peach Rosette Mosaic Virus* (PRMV) and *Tobacco ringspot virus* (TRSV) have been indicated by ELISA tests in Poland (Paduch-Cichal et al., 2011). The viruses may have been introduced by import of plants for planting of *Vaccinium* sp. from USA or Canada in the seventies of the 20th century (Paduch-Cichal et al., 2011). Thus, several viruses have already been introduced into Europe with import of plants for planting. Infected plants may have only been used for fruit plantations and not for further propagation and, therefore, the viruses may only be locally present. However, this is uncertain. The main vector of BlScV, *Ericaphis fimbriata*, is present in Europe (see the PRA for BlScV above). The vector of BSSV, the aphid species *Illinoia pepperi* is only known from North America (pers. comm. P. Chen, NVWA, the Netherlands, 18-04-2012). The vector of PRMV and TRSV, nematode vector species of the *Xiphinema americanum* group are locally present in the EU (Van der Gaag et al., 2010). If the nematode vector would become infected with TRSV or PRMV, the viruses will become difficult to control.

Ericaphis fimbriata (syn. *E. scammeli*). This species occurs widespread in highbush blueberry in the Netherlands. It is considered the main vector of BlScV in North America (see Pest Risk Analysis of BlScV above).

Dasineura oxycoccana. This gall midge species was probably introduced in 1996 in Italy by import of plants for planting from Austria and Germany but initially originated from the USA (EPPO, 2001). In Europe, this gall midge does not seem to be an important pest (EPPO, 2001; Table 6).

Drosophila suzukii. This fruit fly species originates in Asia and has been introduced into North America and Europe. High yield losses have been reported from North America (up to 40%) and Japan (up to 77%) (EPPO, 2011b). It seems especially a risk for late ripening cultivars (Table 6). The pest is spreading rapidly in Europe. The Netherlands Food and Consumer Product Safety Authority (NVWA) has recently published a paper on the pest in a trade journal to inform fruit growers about the risk (Dijkstra, 2012).

Halyomorpha halys. This bug species originates in Asia and has been introduced into North America and Europe (EPPO, 2008). It is unknown how the pest was introduced into Europe. In Europe it is only known to have a limited distribution in Switzerland and limited damage (damage to ornamentals in one garden) has been reported thus far (Meijer et al., 2008). It may, however, become a problem for agriculture in the future, especially for fruit growers because it has been reported as an increasing problem for fruit growers in the USA (EPPO, 2008; Palmer & Lurvey, 2011). The number of generations per year may greatly affect the damage potential of *H. halys*. Summers in the Netherlands are relatively cool compared to those in USA states where *H. halys* has been reported to cause problems in fruit crops and damage potential may be lower than in the USA. A detailed analysis would be needed to assess the impact potential of *H. halys* for fruit production in the Netherlands.

Monilinia vaccinii-corymbosi. This fungus, originating in North America (Batra, 1991), has thus far been reported from 4 European countries: Austria, Switzerland, Spain and Slovenia (Gosch, 2003; Barrau et al., 2006; Munda, 2011) but may already have a wider distribution in Europe. The fungus affects emerging shoots and leaves and later in the season also the flowers and fruits; it has potentially a major impact but cultivars differ in susceptibility (Ehlenfeldt et al., 2010; DeFrancesco & Murray, 2011). It is unknown how the fungus arrived in Europe. It may have been introduced with imports of plants for planting or fresh fruit because it infects both shoots and fruits.

Phomopsis vaccinii. P. vaccinii is an EU-quarantine pest and is regulated for plants of *Vaccinium* spp., intended for planting, other than seeds. It has been present in North- and South-America since long time (Farr et al., 2002). There has been one notification of interception in the EU in 1996 by Italy on plants originating from the USA (Europhyt, last access 29th March 2012). EPPO (1997c) has reported findings of the pathogen in Romania and UK (already in 1956) on plants imported from the USA and the Netherlands but the pathogen did not establish in these countries. In 2004, the fungal pathogen was reported in Lithuania (Gabler et al., 2004; Kacergius & Jovaisiene, 2010). *Phomopsis vaccinii* was later also found in Germany and the Netherlands (pers. comm. G. v. Leeuwen, Netherlands Food and Consumer Product Safety Authority. March 2012). The official pest status in the Netherlands is "absent, only isolated finding, confirmed by survey" (nVWA, 2011). However, because of the finding in the past and uncertainty about its presence in Western Europe, it is not listed in Table 6. *P. vaccinii* causes twig blight but does not seem to be an important pest of *V. corymbosum*. PCM (2009) has stated the following about the impact in Northwestern USA: "Twig blight is not common in Oregon and Washington and growers usually don't treat for it." Weingartner & Klos (1975, referred to by EPPO, 1997c) reported on an epidemic of *P. vaccinii* in Indiana and southern Michigan and the fungus was considered a serious pathogen of highbush blueberry under favourable conditions. In the Netherlands, various *Phomopsis* spp. can infect highbush blueberry but do not seem to cause much impact (pers. comm. G. v. Leeuwen, Netherlands Food and Consumer Product Safety Authority. March 2012). Note that identification of *Phomopsis* spp. on morphological criteria is generally very difficult and identification of species found on *Vaccinium* sp. is further complicated by the fact that *V. corymbosum* can be infected by various *Phomopsis* spp. Taxonomic studies of various species are, however, underway.

*Blueberry stunt phytoplasma.*The present distribution of *Blueberry stunt phytoplasma* is not clear. The phytoplasma is according to EPPO (no year) present in Europe. However, not other information has been found about its occurrence in Europe. In the USA, it is considered a serious pest of highbush blueberry. It is present in cultivated fields in New Jersey, North Carolina, Massachusetts, New York, Michigan, Maryland, and eastern Canada. It also occurs in wild highbush blueberries in New Jersey. Entire fields may become affected with little or no crop produced (Goheen, 2010). It is naturally transmitted by the leafhopper, *Scaphytopius magdalensis* (Table 6).

Pests not (yet) present in Europe

Bacteria. No important bacterial diseases of highbush blueberry present in North America has been found. Vaccinium spp. might carry the quarantine organism *Xylella fastidiosa,* a serious pest of especially grape but not highbush blueberry. New Zealand has, however, included this bacterium in their testing program for import of Vaccinium nursery stock Biosecurity New Zealand, 2010).

Viruses. Various viruses have already been introduced into Europe but still several have been identified that are present in North America but not (yet) in Europe (Tables 6, 7). Viruses which can spread rather rapidly in a field through vector(s) or pollen pose the highest risk because they are generally more difficult to control than those (mainly) spreading by contact and/or propagation material. There is a potential risk of introduction of these viruses because they can pass undetected (symptomless) during import of plants for planting. Besides *Blueberry scorch virus*, which is naturally spread by aphids and locally present in Europe, *Blueberry shock virus* (BlShV) and *Blueberry leaf mottle* virus (BLMV)which are naturally spread by infected pollen (Boylan-Pett et al., 1991; Childress & Ramsdell, 1987; Sandoval et al., 1995) may be among the most damaging ones for Europe Table 6). For example, the presence of BlShV has made it necessary to move the *Vaccinium* clonal collection in Oregon from a field planting to a protected potted collection (Postman et al., 2009). *Blueberry shoestring virus* has been reported as causing several millions of losses annually in Michigan (Isaacs et al., 2008). Infected bushes can yield 25% less than healthy noninfected bushes (Mercure, no year). The natural vector is the aphid species *Illinoia pepperi* is not present in Europe and, therefore, introduction of the virus will probably not have much impact because in absence of the vector, the virus can be well controlled by the use of virus free planting material. However, introduction of the vector will increase the potential impact of the virus and more than 100 species of alien Aphididae have been introduced into Europe already (Coeur d'ácier, 2010). A few phenomena/diseases have been described in highbush blueberry in North America which may be caused by viruses but which have not been identified yet. They could be qualified as relatively unknown risks (Tables 6,7).

Insect and mites. Several berry-feeding insects were identified: *Grapholita packardi* (cherry fruit worm), *Acrobasis vaccinii* (cranberry fruitworm), *Rhagoletis mendax* and *Sparganothis sulphureana* (Table 6). The moth *G. packardi,* which has an EU-quarantine status, is especially known as a pest of sweet and sour cherry (common name: cherry fruit worm) and does generally not seem a major pest of blueberry although incidentally major losses may occur (Table 6). There is a risk of introduction with imports of fresh fruits because the larvae of *G. packardi* bore into fruits. The pest has for example been intercepted on fresh fruit of peaches and nectarines imported from the USA into New Zealand in 1999 (Biosecurity New Zealand, 2009). The cranberry fruitworm *Acrobasis vaccinii* has no quarantine status but has been described as a serious pest of highbush blueberry and the primary direct fruit pest of cranberries (Fitzpatrick, 2009). The blueberry maggot *Rhagoletis mendax* is regulated for all plants and products (EU directive 2000/29/EC). *Sparganothis sulphureana* has no EU quarantine status. It is especially a pest of cranberry but also attacks highbush blueberry in eastern United States (Fitzpatrick, 2009). Trade of dormant blueberry plants without soil, leaves and fruits do not appear to be an (important) pathway of these berry-feeding pests. *G. packardi* can attack above ground shoots of apple trees but attack of shoots of blueberry shoots has not been reported (CABI, 2011). However, two major fruit boring pests, *Tuta absoluta* and *Drosophila suzukii,* have been introduced into Europe most likely by import of fresh fruit (Potting, 2010; EPPO, 2011b) and other fruit-boring insects may be introduced in a similar way. Note that *G. packardi* has an EU quarantine status but is not regulated for plants (including fresh fruit) of *Vaccinium* spp. (Table 6). Other pests attacking fruit or blossom are *Anthonomus musculus*, *Conotrachelus nenuphar* and *Popillia japonica*, the latter one originating in eastern Asia (Table 6). Both *Conotrachelus nenuphar* and *Popillia japonica* are listed on the EU-quarantine list (Table 6). The bud mite *Acalitus vaccinii* has been reported as a problem especially from Michigan and eastern United States. The mite is hidden in the buds and difficult to control by pesticides (Weibelzahl & Liburd, 2010). Note that several other insect pests have been mentioned by Fitzpatrick (2009), e.g. various foliage feeding insects, which are not listed in table 6. They seem to be of less importance as compared to the fruit feeding insects and/or their introduction with plants for planting in a dormant stage without soil or with import of fresh fruit less likely. As already indicated, it was also not the intention to present a complete list of highbush blueberry pests in North America but include at least the most important ones.

Fungi. One new fungal species (new to Europe) was identified: blueberry leaf rust (*Thekopsora minima*). This rust species has been reported from North America, Asia and South Africa. Not much had been reported about its impact (Table 6). Possibly, the two most important fungal pathogens originating in North America have already been introduced into Europe (*Monilinia vaccinii-corymbosi* and *Phomopsis vaccinii*)

Nematodes. The only nematode species identified was *Xiphinema americanum s.l.* The species or species complex is the vector of several nepoviruses. In the EU, non-European populations are regulated because of the possibility to act as a vector of these viruses.

However, vector species of the Xiphinema americanum complex are already present in Europe (Table 6; Van der Gaag et al., 2010).

Conclusions and risk reduction options

Many highbush blueberry pests have been identified which originate in North America. Several of these pests have been introduced into Europe: *Blueberry scorch virus* and probably several other viruses (*Peach rosette mosaic virus*, *Tobacco ringspot virus*, *Blueberry shoestring virus* en *Blueberry red ringspot virus*), an insect (*Dasineura oxycoccana*) and two fungi (*Monilinia vaccinii-corymbosi* and *Phomopsis vaccinii*). Two highbush blueberry pests were identified originating in Asia and introduced into both North America and Europe (the brown marmorated stink bug *Halyomorpha halys* and the spotted-wing drosophila *Drosophila suzukii*). *Blueberry scorch virus* which has up to now only locally been reported from Italy, the Netherlands and Poland and *Drosophila suzukii* which is spreading rapidly in Europe appear to be among the most serious emerging risks for blueberry production in the Netherlands. In addition to these pests which are already present in Europe, many other pests of highbush blueberry have been identified that are present in North America, but not (yet) in Europe.

A detailed pathway-analysis has not been conducted but import of plants for planting is likely to be an important pathway for several of the pests identified in this paper. Import of plants for planting especially poses a risk for the introduction of viruses that are not yet present in Europe and insects present in buds (the bud mite *Acalitus vaccinii*). Therefore, generic risk-mitigation measures may be considered to reduce the risk of new introductions with imports of plants for planting of *Vaccinium* spp. New Zealand has for example special requirements for the import of highbush blueberry plants including specific testing for various viruses and phytoplasmas and a post-entry quarantine period to detect any pests which could have been symptomlessly present at import (Biosecurity New Zealand, 2010). Another less strict alternative could be the requirement that only dormant plants may be imported and only when they have been produced according to a certification scheme including testing for absence of harmful viruses and phytoplasmas and visual inspection for absence of other pests and diseases (which could include a specific list of pests and diseases). Thus, possible risk reduction options (by regulation) for import of plants for planting of *Vaccinium* spp. are:

I. Post-entry quarantine period including visual inspections and testing against viruses and phytoplasmas
II. The plants may only be imported if they are:
    a in a dormant stage, and
    b have been officially certified under a certification scheme requiring them to be derived in direct line from material which has been maintained under appropriate conditions and subjected to official testing for at least the relevant harmful organisms using appropriate indicators or equivalent methods and has been found free, in these tests, from those harmful organisms, and
    c have been grown at a production place that is free of at least the relevant harmful organisms.

A pest free area on its own is in principle sufficient to guarantee freedom of the concerned organisms. However, for viruses and other pathogens that can have long latency periods and/or for which tolerant cultivars are available, testing of the (candidate-mother) plants will increase the guarantee that the plants are free of these pests. Also note that several viruses have been detected in Europe in recent years and may be more widespread than currently known. Therefore, implementation of certification schemes for highbush blueberry in Europe could also be considered to reduce the risk of spread of the viruses with trade of plants for planting.

Besides plants for planting, blueberry (and other kinds of) fruits can act as pathway especially for various fruit-invading pests. General requirements may be considered for exporting countries where relevant pests are present to guarantee freedom of fruit-

feeding or fruit-boring insects. Canada for example has specific requirements for the import of fresh fruit of *Vaccinium* spp. from certain states in the USA (CFIA, 2012) because of the presence of the blueberry maggot *Rhagoletix mendax*. These requirements may also be applied to other fruit-invading pests. The Canadian import requirements in relation to *R. mendax* are that fruits must comply with one of the three following requirements:
1. Blueberry Certification Program (BCP): The blueberries must originate from the operation of an approved grower under the BCP and be accompanied by a certificate in the form of a label called a Movement Certification Label. The BCP is based on approval of growers, pest monitoring and control procedures, grading, fruit sampling and testing.
2. Fumigation: The blueberries must be fumigated with methyl bromide.
3. Processing plant: The blueberries must be destined to a CFIA approved processing plant that is authorized to receive blueberries from regulated areas.

The provinces British Columbia and Newfoundland require that blueberries originating from infested areas must be fumigated with methyl bromide regardless if they were produced under the Blueberry Certification Program or if they are destined to an approved processing plant.

Methyl bromide has, however, adverse effects on the ozone layer and will be phased out. According to EPPO (1997d), methyl bromide damages many fruits and reduces their shelf life. Irradiation may be an alternative for fumigation using methyl bromide. The generic dose of 150 Gy for all tephritid fruit flies was approved and annexed by the International Plant Protection Convention in 2009. Dosages < 150 Gy may even be sufficient for *Rhagoletis* fruit flies (Follett, 2007); a dosage of 60 Gy for *R. pomonella* has been approved by the USA and the IPPC (Follett, 2009; IPPC, 2009). Lower dosages may even be sufficient in combination with cold treatments, e.g. exposure to 1°C for 2 days (Follett, 2009). These irradiation dosages do not kill the insects but render them sterile. which makes it difficult for the importing country to check that the treatment has been effective. Certification of the irradiation facility and accompanying papers demonstrating that the proper doses has been achieved will, therefore, be important. Marketing of fresh fruit which has been irradiated is currently prohibited in the Netherlands (VWA, 2008; http://www.voedingscentrum.nl/encyclopedie/doorstralen.aspx; last access 28th September 2012). Other fumigants and physical treatments like heat and cold are not viable options for fresh blueberries (Garland & Watler, 1998). High CO_2 concentrations do not provide a 100% mortality (Prange & Lidster, 1992).

Thus, possible risk reduction options for import of fresh fruit (of *Vaccinium* spp.) are:
I. Pest free area or pest free production place of the relevant organisms,
II. Methyl bromide fumigation (or irradiation) in the exporting country (marketing of fresh fruit which has been irradiated is currently prohibited in the Netherlands),
III. Import only for processing under certain conditions,
IV. Fruit is grown under a certification program.

Table 6. Highbush blueberry (*Vaccinium corymbosum*) pests present in North America (and other continents) and not (yet) present in the Netherlands

Pest name	**Geographical distribution**	**Comments on impact and distribution**	**EU Q-status**	**References**
INSECTS AND MITES				
Acalitus vaccinii (Acari)	North America (from Canada to Florida and Texas)	"The mites remain almost continuously in the protective confines of the bud." (Weibelzahl & Liburd, 2010). Infestation can lead to growth retardation, small flowers, small fruits, buds may not open.	no	Isaacs & Gajek, 2003; Fitzpatrick, 2009; Weibelzahl & Liburd, 2010
Acrobasis vaccinii (Lepidoptera)	North America	Larvae feed inside berries. "Cranberry fruitworm, *Acrobasis vaccinii* Riley (Lepidoptera: Pyralidae), is the primary direct fruit pest of cranberries and a serious pest of highbush blueberries" (Fitzpratick, 2009)	no	Mallampalli & Isaacs, 2002; Fitzpatrick, 2009
Anthonomus musculus (Coleoptera)	North America	Cranberry weevil/blueberry blossom weevil. Insect of concern in New York highbush blueberry. Only pest that uses blossoms as a hosts for developing larvae.	no	Fitzpratick, 2009
Argyrotaenia franciscana (Lepidoptera)	North America	Minor pest (PCM, 2009)	no	PCM, 2009
Choristoneura rosaceana (Lepidoptera)	North America	Feeds on developing buds and leaves (PCM, 2009). Leafrollers minor problem in Oregon, more common in Washington (DeFrancesco & Murray, 2011)	no	PCM, 2009; DeFrancesco & Murray, 2011
Conotrachelus nenuphar (Coleoptera)	North America	Plum curcilo. Presently minor pest in highbush blueberry in central and eastern USA. Adults oviposit in the fruits and the larvae enters the berry.	IAI	Fitzpatrick, 2009; EPPO, 2011a
Dasineura oxycoccana (Diptera)	North America (origin), Europe: Italy, Slovenia, United Kingdom	Extent of its damage in northwest USA not well known (PCM, 2009). Removed from EPPO alert list in 2001: pest not considered important (EPPO, 2001).	no	EPPO, 2001; PCM, 2009; EPPO, 2011a
Drosophila suzukii (Diptera)	Asia (origin), North America, Europe	Recently introduced into Europe, spreading quickly (EPPO, 2011b). DeFrancesco & Murray (2011): "In the 2 years that the pest has been present in Oregon and Washington agricultural crops, the most damage from the fly appears to be on late maturing fruit. In blueberries, this would be cultivars that ripen in August and September."	no	PCM, 2009; DeFrancesco & Murray, 2011; EPPO, 2011ab
Eriococcus azaleae (Hemiptera)	North America.	Recently identified pest in Oregon. Young crawlers penetrate the bark where they feed. Reports on its presence in Germany, Belgium and Russia (a.o. Scalenet) are probably based on misidentifications/ misinterpretations	no	PCM, 2009; Scalenet on http://www.sel.barc.usda.gov/scalenet/scalenet.htm (last access 6th March 2012)

Pest name	Geographical distribution	Comments on impact and distribution	EU Q-status	References
Grapholita packardi (Lepidoptera)	North America	Lays eggs on fruit. Described as an occasional pest on blueberry in North Carolina, Michigan and New Jersey by EPPO (1997a). PCM (2009) has stated "While a rare pest in most regions, Cherry fruitworm larvae have been reported to cause crop loss of 50% or more." Regulated in the EU for "plants of *Cydonia* Mill., *Malus* Mill., *Prunus* L. and *Pyrus* L., other than seeds, originating in non-European countries". Note that in the EU-legislation plants includes any living (parts of) plants including fresh fruit.	IIAI (as *Enarmonia packardi*)	EPPO, 1997a; PCM, 2009
Halyomorpha halys (Heteroptera)	Asia (origin), North America, Europe: Switzerland (limited distribution)	DeFrancesco & Murray, 2011: "The brown marmorated stink bug is not currently a pest in Oregon and Washington blueberries, but it is a potentially serious pest due to its growing presence in these states and its wide host range."	no	Palmer & Lurvey, 2011; PCM, 2009; DeFrancesco & Murray, 2011; EPPO, 2011a
Illinoia pepperi (Hemiptera)	North America, other continents?	Vector of *Blueberry shoestring virus;* see below	no	See below: *Blueberry shoestring virus*
Popillia japonica (Coleoptera)	Asia (origin), North America, Europa: Azores	Beetles feed on fruits. Polyphagous. Pest of highbush blueberry in central and eastern USA.	IAII	Fitzpatrick, 2009; EPPO, 2011a
Rhagoletis mendax (Diptera)	North America	Described as the most serious pest of highbush blueberry in eastern and midwestern North America; eggs are deposited into the berries and at high populations densities all berries can become infested.	IAI	Fitzpatrick, 2009
Sparganothis sulphureana (Lepidoptera)	North America (from Maine to Florida)	Damages fruit and leaves	no	Turner & Liburd, 2007
<u>FUNGI</u>				
Monilinia vaccinii-corymbosi	North America (origin); Europe: Austria, Switzerland, Spain and Slovenia	"Mummy berry is one of the more serious diseases to affect blueberries and can cause nearly 100 percent yield loss if infection is widespread" (DeFrancesco & Murray, 2011).	no	Batra, 1991; Barrau et al., 2006; Gosch, 2003; PCM, 2009; DeFrancesco & Murray, 2011; Munda, 2011
Thekopsora minima	North America, Asia, Africa	No information in impact. Schilder & Miles (2011) have stated the following after an outbreak in Michigan (USA): "The severity of the outbreak in 2010 warrants further research into economic losses, epidemiology, and management of the disease."	no	Schilder & Miles, 2011; Farr & Rossman, 2012

Pest name	Geographical distribution	Comments on impact and distribution	EU Q-status	References
VIRUSES				
? Blueberry bronze leaf curl?	North America (Michigan, USA)	Causal agent suspected to be a virus but not identified (yet).	no	DeFrancesco & Murray, 2011
Blueberry fruit drop	North America	Phenomenon only observed in cultivar Bluecrop. Causal agent unknown, may be a virus. Observations indicates spread within a field and to adjacent fields (Martin, 2011)	no	Martin et al., 2006a, Martin, 2011
Blueberry leaf mottle virus (BLMV)	North America; Europe: Bulgaria, Hungary, Portugal (see comments)	Spread naturally by pollen. EPPO (no year): "Grapevine Bulgarian latent nepovirus is a distantly related strain of blueberry leaf mottle nepovirus and occurs in Bulgaria, Hungary and Portugal. It has never been reported to infect *Vaccinium*." EPPO (1997): "In Michigan commercial highbush blueberry plantings, BLMV causes virtually 100% crop loss within 4-5 years after infection. Within 10 years, the virus will kill blueberry cv. Rubel. Infected blueberry cv. Jersey does not usually die, but growers remove diseased bushes when symptoms are evident."	IAI	EPPO, no year; EPPO (1997b)
Blueberry necrotic ring blotch virus (BNRBV)	North America (southeastern USA)	DeFrancesco & Murray (2011): "Blueberry necrotic ring blotch virus in the southeastern United States can lead to complete defoliation of bushes and has been reported in southern highbush and rabbiteye blueberries. There is a virus consistently associated with this disease. (Fifty symptomatic plants were infected with the virus, and it was not detected in symptomless plants.) It should be noted that symptoms develop in late summer, and it would be very difficult or impossible to identify infected plants based on symptoms early in the growing season. There is a good test available for blueberry necrotic ring blotch virus (the virus that has been associated with this disease). No information on spread mechanism but rapid movement suggests an aerial vector (Tzanetakis & Martin, 2010). "Disease progresses to complete defoliation" (Martin, 2011)	no	DeFrancesco & Murray, 2011; Tzanetakis & Martin, 2010; Martin, 2011
Blueberry red ringspot virus (BRRV)	North America, Asia (Japan), Europe (Czech Republic, Poland, Slovenia)	No known vector. In a study by Scherm et al. (2008), effects of BRRV on berry yield were inconsistent. The results indicate a potential for reduced overall yield on severely infected shoots but also a potential for a higher yield in the first handharvest, i.e., an advance in ripening. Plesko et al. (2010) describe red ringspots or red blotches on stems, leaves and on some cultivars also	no	EPPO (no year); Scherm et al., 2008; Viruses on line (http://www.agls.uidaho.edu/ebi/vdie/descr103.htm#Range; last access 5th March 2012); Plesko et al., 2010; Pribylova et al.,

Pest name	Geographical distribution	Comments on impact and distribution	EU Q-status	References
		symptoms on fruits. Martin (2011) report natural spread in the USA and a mealybug may be involved.		2010; Martin, 2011; Paduch-Cichal et al., 2011; Spak et al., 2012
Blueberry scorch virus (BlScV)	North America (origin), Europe (Italy, NL (one field), Poland)	Vectored by aphids. Main vector: *Ericaphis fimbriata.* Considered as the most serious disease threat to blueberry in northwest USA (PCM, 2009).	no	PCM, 2009; EPPO, 2011a; Paduch-Cichal et al., 2011
Blueberry shock virus (BlShV)	North America	Spread by pollen. In infected plants flowers and young vegetative leaf shoots suddenly die when flowers are just about to open. The entire bush may be blighted but, more common, only a portion of the branches will show symptoms. Plants recover after one or two years with low fruit yields and the recovered pants produce a full crop; recovered plants are still infected and a source of infection of new plantings (Martin et al., 2006).	no	PCM, 2009; Martin et al, 2006
Blueberry shoestring virus (BSSV)	North America, Europe (Poland)	Vectored by the aphid species *Illinoia pepperi.* Losses estimated at several million dollars in Michigan (Isaacs et al., 2008). 25% yield loss in infected bushes (Mercure, no year). In nortwestern USA, the vector is not present and the virus has not been reported (DeFrancesco & Murray, 2011). The vector is neither present in Europe (pers. comm. P. Chen, NVWA, the Netherlands, 18-04-2012). Presence in Poland indicated by ELISA (Paduch-Cichal et al., 2011)	no	DeFrancesco & Murray, 2011; Isaacs et al., 2008; Paduch-Cichal et al., 2011; Mercure, no year.
Nepoviruses: *Tomato ringspot virus* (ToRSV), *Tobacco ringspot virus* (TRSV), *Peach rosette mosaic virus* (PRMV)	ToRSV and TRSV: North America, South America, Africa, Asia, Europe, Oceania; PRMV: Canada, USA, Turkey, Egypt, Poland	Vectored by certain species of *Xiphinema americanum* s.l. From the PRA on *X. americanum* s.l. (Van der Gaag et al., 2010): in the USA, ToRSV can be a major problem, in particular in eastern and western states. It is a major pathogen of blueberry in New York. TRSV is not a major problem in the USA, except in blueberry in eastern States (New York, New Jersey, etc) (pers. comm. M. Fuchs, Cornell University, USA, 2009). The impact and PRMV has been assessed as low in its the current area of distribution (Van der Gaag et al., 2010) but this might (partly) due to its limited distribution in North America. PRMV caused leaf strapping and leaf malformation in cvs Jersey and Berkley; effects on fruits yields were not determined (Ramsdell & Gillett, 1981). Presence of TRSV and PRMV in highbush blueberry in Poland indicated by ELISA (Paduch-Cichal et al., 2011). ToRSV not reported form highbush blueberry in Europe.	IAI	Ramsdell & Gillett, 1981; Van der Gaag et al., 2010; Paduch-Cichal et al., 2011

Pest name	Geographical distribution	Comments on impact and distribution	EU Q-status	References
NEMATODES				
Xiphinema americanum s.l.	North America, South America, Asia , Africa, Europe	The risk concerns the vector species of *X. americanum* s.l. Direct impact minor but transmits nepoviruses ToRSV, TRSV and PMRV (Van der Gaag et al., 2010; see also ToRSV, TRSV and PMRV in the present table).	Non-European populations: IAI	PCM, 2009; Van der Gaag et al., 2010
PHYTOPLASMAS				
Blueberry stunt phytoplasma	North America, Europe? (not reported from NL)	According to Miles & Schilder (2007) a serious and widespread disease of blueberry in Michigan; also reported from New Jersey, North Carolina, Massachusetts and New York. Entire fields may become affected (Goheen, 2010). Naturally transmitted by the leafhopper species *Scaphytopius magdalensis*. Indicated by EPPO (no year) as present in Europe but no other information found about its presence in Europe.	no	Chen, 1970; EPPO (no year); Miles & Schilder, 2007; Goheen, 2010.

Table 7. Overview of known phytoplasma's, viruses and phenomena/diseases possibly caused by viruses in highbush blueberry (*Vaccinium corymbosum*) reported from North America and their presence and quarantine status in the EU (see Table 6 for references).

Full name	**Vector**	**Presence and status in the EU**		
		Phytoplasma/ virus[1]	**Vector**	**Q-status**[2]
Blueberry stunt phytoplasma	*Scaphytopius magdalensis*	absent?	absent	No
Blueberry scorch virus (BlScV)	*Ericaphis fimbriati* and possibly other aphids	IT, NL, PL	various countries, exact distribution not known	No
Blueberry shock virus (BlShV)	pollen	absent[3]	not relevant	No
Blueberry shoestring virus (BSSV)	*Illinoia pepperi*	PL	absent	No
Blueberry leaf mottle virus (BLMV)	pollen	absent	not relevant	IAI
Tobacco ringspot virus (TRSV)	Vector species of *Xiphinema americanum s.l.*	PL	Bulgaria, France, Germany, Italy, Portugal, Spain,Slovenia[5].	IAI
Tomato ringspot virus (ToRSV)	Vector species of *Xiphinema americanum s.l.*	absent/present[4]		IAI
Peach rosette mosaic virus (PRMV)	Vector species of *Xiphinema americanum s.l.*	PL		IAI
Blueberry red ringspot virus (BRRV)	Vector may be involved	CZ,PL,SL	not known	No
Blueberry necrotic ring blotch virus (BNRBV)	Vector may be involved	absent	not known	No
Blueberry bronze leaf curl – unknown agent	Not known	absent	not known	No
Blueberry fruit drop – unknown agent	Vector may be involved	absent	not known	No

[1] Note that the Blueberry virus species mentioned in the table and reported from one or more EU-countries have only locally been detected. In the Netherlands, BlScV has for example been found in one production field.
[2] EU quarantine status. Note that no specific measures are in place for import of *Vaccinium* spp. (directive 2000/29/EC)
[3] Absent means absent or not reported (not known to occur).
[4] Absent or not reported from *V. corymbosum*; locally present on other host plants in Europe (ToRSV has a wide host range).
[5]The identifications in Bulgaria, Portugal and Italy may need confirmation (Van der Gaag et al., 2010).

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Annex I

List of organisms for which testing methods are being developed by Naktuinbouw, the Netherlands (March 2012)

****Vaccinium**

Pathogeen	Toetsmethode
Virussen/op virusgelijkende ziekten	
Blueberry mosaic agent	(b), (c)
Bleuberry red ringspot virus (BBRSV)	(b), (g)
Blueberry shoestring virus (BSSV)	(f)
Cranberry ringspot agent	(h)
Blueberry leaf mottle virus (BLMoV)	(a), (f)
Tobacco ringspot virus (TRSV)	(a), (f)
Peach rosette mosaic virus (PMRV)	(a), (f)
Tomato ringspot virus (ToRSV)	(a), (f)
Blueberry scorch virus (BlScV)	(f), (g)
Blueberry shock virus (BlShV)	(f), (g)
Phytoplasmas	
Blueberry stunt phytoplasma	(b), (d), (g)
Blueberry witches broom phytoplasma	(e), (g)
Cranberry false blossom phytoplasma	(g), (h)
Schimmels	
Phytophthora ramorum	(f), (g)

(a) mechanische inoculatie op Chenopodium quinoa, Nicotiana occidentalis 37B en Cucumis sativus.
(b) Inoculatie door enten op Vaccinium corymbosum 'Cabot'.
(c) Inoculatie door enten op Vaccinium corymbosum 'Stanley'.
(d) Inoculatie door enten op Vaccinium corymbosum 'Jersey'.
(e) Inoculatie door enten op Vaccinium myrtillus
(f) Elisa
(g) PCR
(h) Visuele controle