EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES

# Pest Risk Analysis for

## Rose rosette emaravirus and its vector Phyllocoptes fructiphilus



# September 2018

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This risk assessment follows the EPPO Standard PM 5/5(1) *Decision-Support Scheme for an Express Pest Risk Analysis* (available at http://archives.eppo.int/EPPOStandards/pra.htm) and uses the terminology defined in ISPM 5 *Glossary of Phytosanitary Terms* (available at https://www.ippc.int/index.php). This document was first elaborated by an Expert Working Group and then reviewed by the Panel on Phytosanitary Measures and if relevant other EPPO bodies.

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Photo: Rose Rosette Virus: Reddened shoots on an infected rose). Courtesy: Patrick Di Bello, Oregon State University (US)

# Based on this PRA, *Rose rosette emaravirus* and its vector *Phyllocoptes fructiphilus* were added to the EPPO A1 Lists of pests recommended for regulation as quarantine pests in 2018.

# Pest Risk Analysis for *Rose rosette virus* (*Emaravirus*) and its vector *Phyllocoptes fructiphilus* (Acari: Eriophyoidae)

#### **PRA area:** EPPO region **Prepared by:** EWG on *Rose rosette virus* **Pate:** 2017/00/18/21 Eurther reviewed of

**Date:** 2017-09-18/21 Further reviewed and amended by EPPO core members and Panel on Phytosanitary Measures (see below).

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This PRA follows EPPO Standard PM 5/5 *Decision-Support Scheme for an Express Pest Risk Analysis*. For the determination of ratings of likelihoods and uncertainties, experts were asked to provide a rating and level of uncertainty individually during the meeting, based on the evidence provided in the PRA and on the discussions in the group. Each EWG member provided anonymously a rating and level of uncertainty, and proposals were then discussed together in order to reach a final decision.

All personal communications in this PRA were obtained in 2017 from the following experts: J.W. Amrine Jr. (Emeritus Professor of Entomology, West Virginia University, Morgantown, USA), D. Byrne (Texas A&M University, College Station, USA), P. Chakraborty (Department of Botany, University of North Bengal, Siliguri, India), P. Di Bello (EWG member - Oregon State University), T. Druciarek (EWG member - University of Arkansas), A. Katsiani (Department of Plant Pathology, University of Arkansas, Fayetteville, USA), S.X. Shan (Laboratory Services Division, University of Guelph, Guelph, Canada), N. Yardimci (Süleyman Demirel University, Isparta, Turkey).

Following the EWG, the PRA was further reviewed in November-December 2017 by the following PRA core members: Nuria Avendaño Garcia (Spain), Niklas Björklund (Sweden), Jose Maria Guitian Castrillon (Spain), Alan MacLeod (UK), Lucio Montecchio (Italy), Robert Steffek (Austria), Nursen Urstun (Turkey), as well as by EPPO Staff (Françoise Petter).

The Panel on Phytosanitary Measures considered the management options in 2018-03. EPPO Working Party on Phytosanitary Regulation and Council agreed that *Rose rosette emaravirus* and its vector *Phyllocoptes fructiphilus* should be added to the A1 Lists of pests recommended for regulation as quarantine pests in 2018.

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### Summary of the Pest Risk Analysis for *Rose rosette virus* (Emaravirus) and its vector *Phyllocoptes fructiphilus* (Acari: Eriophyoidae)

## **PRA area:** EPPO region

**Describe the endangered area:** Potentially, any area where *Rosa* spp. are grown in the EPPO region is endangered. Areas most at risk would be areas of high densities of rose, and where *Phyllocoptes fructiphilus* can maintain high populations outdoors (the threshold for climatic conditions that would be favourable to *P. fructiphilus* has not been defined – see section 9.2), or areas of commercial rose production in glasshouses. The climatic requirements of *P. fructiphilus* are not known in detail, and there are uncertainties concerning its ability to establish in very northern regions or where climates are more arid.

## Main conclusions

#### Overall assessment of risk:

*Rose rosette virus* (RRV) and its vector, the eriophyoid mite *P. fructiphilus*, have had high economic and social impacts in the USA. All species and cultivars of *Rosa* are considered at risk from the virus and vector, as no known tolerant or resistant species or varieties have been identified. The virus causes witches' broom, flower abortion or flower malformation, distorted leaf growth and reduction in cold hardiness, leading to mortality of roses.

Current measures in the EPPO region do not significantly reduce the probability of entry. Risk of entry on *Rosa* plants for planting (except seeds and pollen) is considered to be high with moderate uncertainty, and on cut flowers of *Rosa* it is considered to be low to moderate with moderate uncertainty. The likelihood of establishment in the EPPO region is considered very high. If introduced, the magnitude of spread would be moderate to high, due to the extensive trade in *Rosa* and because of the aerial dispersal of *P. fructiphilus*, with a moderate uncertainty.

As for the USA, potential impacts in the EPPO region could be high, and locally may be very high. The highest economic impacts are expected to be incurred by nurseries and areas producing rose products such as rose oil. Potential environmental impacts are expected to occur if native (especially endangered) *Rosa* species in the EPPO region are susceptible hosts. Social impacts would occur through the loss of employment and income in the production and transformation industry (especially for rose flowers for oil) and in those countries where *Rosa* has significant cultural importance.

The EWG considers RRV to be a high risk to the EPPO region. *P. fructiphilus* is considered to be a potential pest for the EPPO region, as vector of RRV and possibly through direct feeding damage (see section 4.1.1). Establishment of *P. fructiphilus* in the EPPO region in the absence of RRV would also increase the risk of the virus, as the vector is very unlikely to be eradicated if found in the wider environment and would spread quickly. Measures to prevent the introduction of *P. fructiphilus* irrespective of the virus should also be considered.

*Phytosanitary measures to reduce the probability of entry:* Risk management options are considered for *Rosa* plants for planting (except seeds and pollen) and for *Rosa* cut flowers.

<b>Phytosanitary risk for the</b> <u>endangered area</u> (Individual ratings for likelihood of entry and establishment, and for magnitude of spread and impact are provided in the document)	High		Moderate		Low	
<b>Level of uncertainty of assessment</b> (see <i>Q</i> 17 for the justification of the rating. Individual ratings of uncertainty of entry, establishment, spread and impact are provided in the document)	High		Moderate		Low	
<i>Other recommendations:</i> The EWG made recommendations (detailed in section 18) relating to the need for surveys on <i>P. fructiphilus</i> and of awareness campaigns targeting travellers.						

## **Stage 1. Initiation**

#### **Reason for performing the PRA:**

Rose rosette disease has been observed in North America since the 1940s on wild and cultivated roses (*Rosa* spp.). During the last decades, the disease has become widespread in North-Central, South-Central and South-East USA, and its incidence has grown exponentially in cultivated roses. In the USA, rose rosette disease has caused substantial losses and is considered as a major threat to the rose industry and rose cultivation. Rose rosette is transmitted by an eriophyoid mite (*Phyllocoptes fructiphilus*, Acari: Eriophyoidae; Keifer 1940). In 2011, a virus called *Rose rosette virus* (*Emaravirus*, RRV) could be consistently identified in symptomatic plants. RRV was later confirmed as the sole causal agent of rose rosette disease. RRV is still spreading in the USA, and it was reported for the first time in India in 2017. Potential entry of the vector independently of the virus has also been considered.

Considering the severity of damage caused by RRV and its current spread in the USA, the EPPO Secretariat considered that this virus should be added to the EPPO Alert List. The Panel on Phytosanitary Measures suggested *Rose rosette virus* as a priority for PRA, which was confirmed by the Working Party on Phytosanitary Measures in June 2017. This PRA covers both RRV and its vector *P. fructiphilus*.

This PRA follows EPPO standard PM 5/5 <u>Decision-Support Scheme for an Express Pest Risk Analysis</u>, as recommended by the Panel on Phytosanitary Measures. Pest risk management was conducted according to the EPPO Decision-support scheme for quarantine pests PM 5/3(5) (detailed in Annex 1).

The information given in a UK PRA was extensively used when preparing this PRA (Tuffen, 2016).

PRA area: EPPO region (map at <u>www.eppo.org</u>).

#### Stage 2. Pest risk assessment

#### 1. Taxonomy

**Taxonomic classification.** Kingdom: Virus / Order: Bunyavirales / Family: Fimoviridae / Genus: *Emaravirus* / Species: *Rose rosette emaravirus* syn. *Rose rosette virus*. (ICTV, 2016). Acronym. RRV (used from here onwards).

Disease. rose rosette disease (RRD), rose witches' broom.

Laney *et al.* (2011) characterized RRV and identified it "most probably" as the causal agent of rose rosette disease. Di Bello *et al.* (2015) confirmed that RRV is transmitted by *P. fructiphilus* and is the sole causal agent of rose rosette (i.e. it causes the disease and is not part of a virus complex).

For records of rose rosette disease in North America pre-dating the description of the virus in 2011, it cannot be entirely certain that the causal agent was RRV. However, for the purposes of this PRA, these records are assumed to be caused by RRV unless there is evidence to suggest otherwise.

#### 2. Pest overview

The biology and epidemiology of RRV is closely linked to that of *P. fructiphilus*, and *P. fructiphilus* is assessed with RRV in this PRA. This section focuses on RRV. Information on *P. fructiphilus* is given in section 4.1 (incl. biology and detection).

It should be noted that the US literature often separates *R. multiflora* and other roses, and this is also reflected below. This is because rose rosette disease was originally studied mostly on *R. multiflora*, and then later on other *Rosa* species. *R. multiflora* is a species of Asian origin that is considered an invasive species in the USA, where it has been classified in some states as a noxious weed because of its aggressive habit that excludes native plants (Amrine, 2002) (it is not considered invasive in the EPPO region).

#### 2.1 Biology and epidemiology of RRV

#### 2.1.1 Symptoms and damage

Initially, symptoms appear on leaves, then on stems and branches. Symptoms of rose rosette include unusual reddening and distortion of leaves, rapid shoot elongation, red shoots, witches' broom, excessive thorn

production, dieback, reduced flowering and flower malformation. Symptoms and disease development are detailed in Annex 2.

Symptoms of rose rosette are highly variable, depending on the rose species or cultivar affected, the age or growth stage of the plants, the stages of the disease and climatic conditions. Plants of the same cultivar may show different symptoms (such as red shoots or excessive thorn production) (Windham *et al.*, 2016; Ong *et al.* 2014, Allington *et al.*, 1968). Excessive thorniness is broadly considered a good indicator for the presence of the virus in cultivated roses (Hong *et al.*, 2012); however, this may not be the case outside the USA, as other pathogens have been found to cause excessive thorniness (see below). Infected plants may show only some of the symptoms, especially in the early stages of the disease, and symptoms may evolve over time (e.g. the reddish colour disappears as leaves mature on healthy plants) (Babu *et al.*, 2015). Hong *et al.* (2012) notes that by the time symptoms may be less visible in some types of rose plantings. For example, in mass-plantings of one cultivar, infected plants are easier to recognize in winter when witches' brooms are not masked by healthy foliage (Windham *et al.*, 2016).

Infected plants lose their aesthetic value and gradually display a general decline leading to plant death (Di Bello, 2015; Windham *et al.*, 2016). Mortality is usually due to enhanced susceptibility to frost injury and winter kill (Epstein and Hill, 1995). Most sources indicate that infected roses die within 1-5 years (after symptom appearance; Hong *et al.*, 2012; Baker *et al.*, 2014; Babu *et al.*, 2015; Di Bello, 2015; Windham *et al.*, 2016). The disease progresses faster in smaller plants. It is mentioned that infected seedlings rarely survive past 1 year, single crowned plants usually die within 2-3 years and parts of some multi-crowned plants may survive up to 5 years (Missouri Botanical Garden, no date; MPI, 2013). The incidence may remain low in a large bed of newly planted roses for 1-2 years and all plants may become symptomatic rapidly in the following year (Windham *et al.*, 2016).

Increased susceptibility of RRV-infected plants to powdery mildew (species not specified) has been observed (Hong *et al.*, 2012).

'Reversion', 'recovery' or 'regression' is mentioned in older literature (e.g. Epstein and Hill, 1995; Amrine, 1996; Illinois, 1999). It is believed to be due to an infested plant appearing to have recovered after the symptomatic portions die out or are removed, while the virus is still present in other parts.

Finally, symptoms similar to rose rosette may have other causes. On roses, herbicides can cause similar symptoms (e.g glyphosate can cause witches' broom; 2, 4-D can cause leaf distortion); however, roses normally recover from the herbicide injuries in the following year (unless plants are injured again by herbicides). Nutrient deficiency may also cause similar symptoms, but those generally affect the whole plant, while symptoms of RRV may be more localized (Hong *et al.*, 2012; Babu *et al.*, 2015). Symptoms due to insect damage or non-biological environmental conditions (such as wind, temperature and sun) may also resemble rose rosette symptoms (Ong *et al.*, 2014; Sim *et al.*, 2017; Singh and Owings, 2014). In other parts of the world, a phytoplasma in the subgroup 16SrI-B (aster yellows group) causes excessive thorniness on rose (MPI, 2016, citing Kamínska *et al.*, 2006), a new phytoplasma causing witches' broom on *Rosa* x *damascena* was recently described from India (Saeed *et al.*, 2016) and a new closterovirus was associated with symptoms of leaf rosette on rose (He *et al.*, 2015).

## 2.1.2 Transmission

RRV is transmitted by *P. fructiphilus* and by grafting (Di Bello, 2015; Di Bello *et al.*, 2017). Regarding other transmission modes:

- Vegetative propagation was suggested as a means of transmission of RRV by Baker *et al.* (2014). However, infected cuttings are less likely to root. Doudrick (1984) did not obtain rooting of cuttings taken from symptomatic rose plants. Cuttings are nevertheless considered because it has not been proven that infected cutting will never root.
- Mechanical transmission to other roses has been investigated. Between two studies (Doudrick, 1984; Epstein and Hill, 1999), 5 out of 123 inoculated plants developed symptoms during mechanical transmission trials with crude extracts were prepared by grinding tissue in chilled (4°C) buffer with antioxidants. These conditions are not met under typical pruning conditions, and it is therefore considered here that pruning would not transmit the virus. A precautionary approach is however recommended in the USA in relation to pruning tools (see *Control* in section 12).
- Transmission of RRV to adjacent plants through root grafting has been hypothesized (e.g. Allington *et al.*, 1968) but is not demonstrated. It is not clear whether root grafting happens in roses (Ong *et al.*, 2014)

- RRV is not known to be transmitted by soil or seed. No evidence of seed and soil transmission was found (Di *et al.* 1990; Epstein and Hill, 1995; Windham *et al.*, 2016). Seed transmission has been reported for another emaravirus, i.e. High Plains wheat mosaic virus, which has also been intercepted on seeds (Forster *et al.*, 2001, Bothelo *et al.*, 2016); however there is uncertainty about whether this relates to true seed transmission, as the vector *Aceria tosichella* is directly associated with seeds, and the virus may be carried and transmitted by the mites present on the seeds. *P. fructiphilus* does not have a similar association with seeds.
- RRV is not known to be transmitted by pollen. No specific reference to pollen was found. For the other emaraviruses described to date, no transmission by pollen has been reported (Mielke-Ehret and Muhlbar, 2012).
- Transferring *Tetranychus urticae* (Acari: Tetranychidae) from infected roses to uninfected roses failed to transmit the virus (Allington *et al.*, 1968, Amrine *et al.*, 1988). The methodology used in these experiments was favourable to transmission, and *T. urticae* is therefore not considered a potential vector in this PRA.
- Attempts to transmit the disease by dodder (*Cuscuta campestris, C. gronovii* and *C. pentagona*) failed due to the dodder not producing haustoria on rose (Doudrick 1984; Epstein and Hill, 1995; Epstein and Hill, 1999).
- Attempts to transmit the disease by powdery mildew were not successful (Epstein and Hill, 1999).
- No other vector has been identified (see section 4.2).

## 2.1.3 Incubation period

Following transmission by grafting in *R. multiflora* and cultivated roses, the incubation period reported in the literature varies from a few weeks to over one year (Epstein and Hill, 1999; Roebuck, 2001; Di Bello *et al.*, 2017; Tipping and Sindermann, 2000). While, in experiments on mite transmission, once a plant is exposed to the virus, symptoms developed within 17 to 146 days in *R. multiflora* and ornamental rose cultivars (Allington *et al.*, 1968; Amrine *et al.*, 1988; Di Bello *et al.*, 2013; Di Bello *et al.*, 2017). No information is available for other *Rosa* species.

Epstein and Hill (1995) have noted an apparently lower symptom expression of rose rosette disease in *R*. *multiflora* plants growing in shaded areas as an understory plant under a tree canopy.

## 2.1.4 Susceptibility

The susceptibility of Rosa species and cultivars varies.

#### Rosa multiflora is highly susceptible.

Many cultivars of **ornamental roses** are known to be susceptible, and all are considered to be potentially susceptible. Tolerant and resistant genotypes have yet to be identified (Hand, 2014; Singh and Owings, 2014; Windham, 2014; 2016, Baker *et al.*, 2014; Di Bello *et al.*, 2017). In resistance screening experiments (Di Bello *et al.*, 2017), all cultivars but one (out of 20) developed symptoms and tested positive for the virus; the authors noted that the possible resistance of this cultivar (Stormy Weather) should be tested under field conditions. A larger trial of 191 cultivars has identified 84 potential resistant cultivars with further work required to confirm that they are indeed resistant (Windham *et al.*, 2017b). Other factors influence the susceptibility in the field. For example, RRV is seldom reported in miniature roses, even though these are susceptible to RRV (Windham *et al.*, 2016).

**Other** *Rosa* **species**. *Rosa* **woodsii** is reported to only show mild symptoms (Epstein and Hill, 1999). Some other species were reported to be "resistant" to rose rosette disease, such as *R. setigera* (Epstein and Hill, 1999), *R. aricularis, R. arkansana, R. blanda, R. palustris, R. carolina* and *R. pimpinifolia* [=*R. spinosissima*] (Windham *et al.*, 2016, citing others). However, the majority of this work took place prior to a diagnostic test for RRV, and these species may have been only asymptomatic. Of those, *R. setigera* and *R. palustris* are currently studied for their possible use in breeding programmes due to their apparent resistance to RRV (Zlesak *et al.*, 2017; Roundey *et al.*, 2017).

Consequently, this PRA considers that there are no known resistant species or cultivars to date. Extensive research is being conducted on resistance in the USA (including on field resistance and development of molecular markers or genes that confer resistance to RRV – Byrne *et al.*, 2017; Windham *et al.*, 2017b), and more information on resistance and tolerance will become available in the future.

#### 2.1.5 RRV is systemic

RRV rapidly moves systemically and is usually present throughout the plant even in asymptomatic parts. Grafting of budless shields have shown that the virus moves from infected scion to the rootstock within 1-2 weeks, down to lower nodes within 2 weeks, and into the roots within 3 weeks (Doudrick, 1984). Further

systemic movement to the roots of the infected plants was demonstrated by testing the roots of 20 infected roses three months after disease onset, with 50% testing positive for RRV (Di Bello *et al.*, 2017).

## 2.2 Detection and identification

Detection of rose rosette disease on rose plants in the USA has generally relied on symptoms. The presence of symptoms may be a good indicator of the presence of the virus, but symptoms are not always reliable. Checking for a combination of symptoms is nevertheless an important part of the diagnosis. In the presence of symptoms that may also have other causes (see 2.1.1), the presence of *P. fructiphilus* can help discriminate between RRV and other factors (Hoy *et al.*, 2013); however, its presence is also not easy to determine (see section 4.1.4).

Molecular tests are now available to detect and identify RRV. Although systemic, RRV is not evenly distributed and may be present at low concentration. If infection is suspected, additional sampling and continued monitoring for symptoms and the vector is advised in case of negative results. Testing is most reliable when samples are taken from fully emerged new shoots. The methods include:

• *RT-PCR* (reverse-transcription – Polymerase chain reaction): Laney *et al.* (2011) was the first to publish an RT-PCR assay but a more sensitive assay with an internal control was later designed (Di Bello *et al.* 2015, 2017). Dobhal *et al.* (2016) independently developed a RT-PCR assay. To date, the sensitivity of these two assays has not been compared.

RRV shows low genetic diversity among all RNAs, especially RNAs 2 and 3 (Tzanetakis *et al.*, 2017), thus assays designed on these RNAs should be able to reliably detect a large number of isolates.

• *RTR-PA* (Isothermal reverse transcription recombinase - polymerase amplification). Babu *et al.* (2017a and b) developed this assay based on multiple gene targets. It was cheaper and quicker than PCR (20 min.), and can be used with no need for a thermocycler. The sensitivity of this assay compared to PCR is not known.

To date, kits allowing detection in the field are not yet available.

#### 3. Is the pest a vector?

Yes 🗌 No

RRV is not a vector. Its vector *Phyllocoptes fructiphilus* is considered below.

## 4. Is a vector needed for pest entry or spread?

Without an arthropod vector, the virus can enter and spread through vegetative propagation and grafting, but is not expected to result in significant epidemics. *P. fructiphilus* is to date the only known vector of RRV in North America (see section 4.1). Because *P. fructiphilus* plays an important role in the epidemiology of the disease, it is also covered in this PRA.

#### 4.1 Phyllocoptes fructiphilus

*P. fructiphilus* was shown to transmit RRV (Di Bello, 2015; Di Bello *et al.*, 2017). Its hosts belong to the genus *Rosa* (see section 7).

#### 4.1.1 Morphology and biology

*P. fructiphilus* is a tiny eriophyoid mite (140-170  $\mu$ m long, 43  $\mu$ m wide; Hoy *et al.*, 2013), with a spindleshaped structure and is typically yellow to brown (Babu *et al.*, 2015). It has four life stages: egg, larva, nymph and adult (Allington *et al.*, 1968). Two types of females occur during the year (Fig. 1). Deutogyne females overwinter in protected places on the host (e.g. under bud scales). After overwintering they move onto developing shoots to lay eggs. Protogyne females and males will develop from those eggs, building-up Spring and Summer mite populations. Later in the year deutogyne females will start to develop from eggs laid by protogynes. Only deutogyne females are able to survive harsh winter conditions. Eggs hatch in 3-4 days [4.3 days at 23°C in Tuffen, 2016 citing others]. The two immature stages (larva and nymph) each develop in about 2 days (Hoy *et al.*, 2013 citing others). There is no direct copulation in case of eriophyoids, and they reproduce through arrhenotoky (females are diploid and males are haploid and develop from unfertilized eggs). An uninseminated female can deposit a haploid (male) egg and become inseminated by picking up the spermatophore laid by her male offspring after he becomes an adult or any other male. A mated female may produce both fertilized (female) eggs and unfertilized haploid eggs that become males (Hoy *et al.*, 2013). A single female is sufficient to start a new colony.

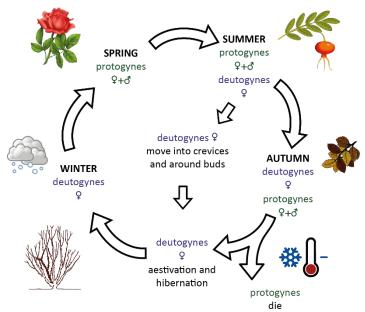


Figure 1. Life cycle of *P. fructiphilus*; modified from Manson and Oldfield (1996) and Druciarek (2016)

Development from egg to adult takes approximately 11 days at 23°C (Kassar and Amrine, 1990). Multiple generations are produced until the weather turns cold in the autumn; in areas where weather is mild, development may occur into the winter months (Tuffen, 2016 citing Amrine, 1996, Hoy, 2013). Under greenhouse conditions, generations are continuously produced without overwintering (deutogyne) females (T. Druciarek, pers. comm.).

During the growing season, *P. fructiphilus* can be found under bud scales and on petals (T. Druciarek, pers. comm.). It is also found on growing shoot tips (Hoy *et al.*, 2013), within leaf folds of new shoots or at petiole bases (Babu *et al.*, 2015, citing others). Keifer (1940) reports *P. fructiphilus* as an inhabitant of rose fruits, but this seems to be debated in the literature. Females overwinter until early spring in protected places such as beneath the bark or bud scales, on living host tissue (Babu *et al.*, 2015, citing others).

Mites need living green tissue to survive (Cloyd, 2013). Feeding during winter is believed to not be necessary (T. Druciarek, pers. comm.). One publication states that the mite can only survive about 8 hours without being on a host plant (Missouri Botanical Garden, no date). However, there is no data to support this figure, and survival in the absence of a host is much longer (two weeks) when individuals are kept at a low temperature with sufficient moisture (T. Druciarek, pers. comm.).

Epstein and Hill (1999) reported that populations were observed to increase during extended periods of high temperatures, and there was a reduced spread of rose rosette in shaded areas. Daily temperature fluctuations of 17°C or more resulted in the collapse of overwintering populations in Iowa (Epstein *et al.*, 1997). *P. fructiphilus* may also be sensitive to low relative humidity, with periods of drought associated with low population densities (Missouri Botanical Garden, no date). In transmission trials, Amrine *et al.* (1988) noted an effect of lower than normal temperatures and humidity in the decreased transmission (which may be due to effects on the mite's development and feeding, or to plant stress making the plants less suitable for the mite).

No study has been conducted on the lower temperature threshold for the survival of *P. fructiphilus*. *P. fructiphilus* collected during the summer and stored at  $4^{\circ}$ C in plastic bags on host plant material in complete darkness were still alive even after 2 months (the limit of survival at low temperature being the decay of the plant material). It is expected to survive also at  $2^{\circ}$ C (T. Druciarek, pers. comm.).

Feeding damage to rose plants has been observed for high populations of the closely-related species *Phyllocoptes adalius* and *P. resovius* (leaf discoloration and malformation as well as severely delayed bud development and stunting of the plants) (Druciarek and Lewandowski, 2016). Damage was serious for *P. adalius* in glasshouses in Poland (Druciarek *et al.*, 2014). High populations of *P. fructiphilus* cause similar feeding damage to the plant as its closely related species, and it was also noted that there is a very close relationship between these *Phyllocoptes* species of rose (T. Druciarek, pers. comm.). Therefore, it is considered in this PRA that *P. fructiphilus*, in addition to its major role as vector of RRV, has the potential to cause damage as a pest on its own in some conditions.

## 4.1.2 Dispersal

Passive aerial dispersal has been suggested as the major way that eriophyoids spread (Michalska *et al.*, 2010; Sabelis and Bruin, 1996), and this mode of spread is also characteristic for *P. fructiphilus*.

Amrine (1996) states that "mites are thought to disperse by actively entering the air column on warm, sunny days". The migration of eriophyoid mites may increase as resources diminish and the condition of the host plant deteriorates. It has also been hypothesised that eriophyoid mites respond to wind and other dispersal cues by adopting specific behaviours (such as standing erect on the host), but this is still debated (Kiedrowicz *et al.*, 2017; Melo *et al.*, 2014).

There is little information on the dispersal of *P. fructiphilus* in the literature:

- Cultivated roses planted downwind of infected *R. multiflora* are generally considered more at risk. Some growers have observed symptoms on previously healthy plants within four weeks of being planted downwind from infected *R. multiflora* (Hong *et al.*, 2012). A pronounced edge effect was observed in Tennessee rose beds close to a source of RRV (the roses nearest to the source were more likely to become infected than those on the opposite side of the bed).
- In contradiction to the above, Epstein *et al.* (1997) studied the spread of the disease, and observations indicated no aerial dispersal of *P. fructiphilus* over 100 m from rose rosette inoculated plots to healthy plots of roses, as no plants developed symptoms at 100 m or more from the infected ones. Spore traps were used to detect airborne movement of *P. fructiphilus*, but no mites were detected at 10 m from the infested site. However, this study used only one spore trap for a short period of 6 weeks (mid-June through July) and did not recover any eriophyoid species, which is unusual (T. Druciarek, pers. comm.).

It is thought that wind can carry the vector over long distances, however the maximum distance *P. fructiphilus* can spread by wind is not known. Some studies were conducted on others eriophyid species. Pady (1955) captured dispersing *Aceria tosichella* (wheat curl mite) individuals 3.2 km from the nearest wheat field. In the study of Zhao and Amrine (1997) the presence of 88 individuals representing 51 eriophyoid species in the top and mid-layers of heavy snow strongly indicates that these mites came down with the snow formed at high altitude (i.e. they had been transported by air currents at high altitudes and at long distances).

Eriophyoid mites are limited in ambulatory dispersal, mostly due to their extremely small size. However, when green parts of plants are in direct contact, mites can crawl from plant to plant (Sabelis and Bruin, 1996).

Other transport hypotheses have been made in the literature but have not been demonstrated to date:

- on tools, equipment, clothes. It is suspected that the microscopic eriophyoid mites may also move on pruning shears, gloves or similar equipment (Duffner *et al.*, 2001; Singh and Owings, 2014).
- on other arthropods (phoresy). In some publications, it has been suggested that *P. fructiphilus* may disperse by travelling on the body of other arthropods (Cloyd, 2013, Hand, 2014, Roebuck, 2001). However, Skoracka *et al.* (2010 citing others) mentions that while phoresy happens in eriophyoid mites, it is usually rare and accidental.
- Splash dispersal or dispersal by rain was reported for some eriophyoid species (Jeppson *et al.*, 1975; Schliesske, 1977, 1990). However, it probably has the lowest frequency among the different modes of dispersal (Michalska *et al.*, 2010).

#### 4.1.3 Transmission of RRV

From the literature, *P. fructiphilus* appears to be an effective vector of RRV and able to develop large populations on *Rosa* plants. Di Bello (2015) and Di Bello *et al.* (2017) (on cultivar Julia Child) showed an acquisition access period of 5 days and an inoculation access period of one hour. The acquisition period may indicate that RRV probably needs to propagate in the mite (as suggested in the EMARaV/ *Eriophyes pyri* complex - Mielke-Ehret *et al.*, 2010). Symptoms were more prominent and developed sooner when virus-carrying mites were allowed to feed for 14 days. The infection rate increased with the inoculation access period, and was 5% and 60% for 1 h and 14 days inoculation access period. Infection reached 100% in control plants, with mites allowed to feed for 30 days (Di Bello, 2015; Di Bello *et al.*, 2017).

Amrine *et al.* (1988) found that mites kept for two weeks at  $4^{\circ}$ C (a temperature at which the mite is inactive and does not feed) were then still able to transmit RRV to 20% of plants (for 2 experiments, symptoms showing on 4 plants out of 10 after 29 and 47 days, with at least 30 mites allowed to feed for 14 days). This, along with the findings of Di Bello (2015) and Di Bello *et al.* (2017) that infection reached 100% when mites were allowed to feed for 30 days, demonstrate a long retention time in the mites.

It is unlikely that transovarial virus transmission exists for emaraviruses, a conclusion consistent with observations done by Mielke-Ehret *et al.* (2010) for *Pigeonpea sterility mosaic emaravirus* (PPSMV) and *Maize red stripe emaravirus* (MRSV).

## 4.1.4 Detection and identification

Detection and identification of eriophyoid mites is difficult due to their tiny size, and the possible coexistence on the same rose plant of several species of eriophyoids or other mites (Ochoa *et al.*, 2016; Bauchan *et al.*, 2017). Collins *et al.* (2017) has developed a kit for isolating *P. fructiphilus* by means of washing rose material and catching them on pollen sieves, which may prove a more rapid way to check non-dormant host material for mites than visual inspection.

*P. fructiphilus* is difficult to see even with a hand lens (Hoy *et al.*, 2013), and detection and identification require magnification by a stereoscope/microscope. It can be identified as an eriophyoid by the characteristic elongated-vermiform body shape (T. Druciarek, pers. comm.). Identification to the species level can be based on morphological characters, which requires taxonomic expertise (mite specialist). For example, the structure of the prodorsal shield allows *P. fructiphilus* to be differentiated from *P. adalius* (present in both Europe and the USA, see section 4.2 and Annex 3) and *P. resovius* (present in Europe). Details on morphological characters are given in Druciarek *et al.* (2016). Figures 2 and 3 illustrate the morphology of *P. fructiphilus*, *P. adalius* and *P. resovius*, and additional pictures are given in Annex 3.

*P. fructiphilus* can now also be identified based on ITS sequence (available in GenBank, accession number: AJ251692) using the primers developed by Kumar *et al.* (2001).



Figure 2. SEM picture of Phyllocoptes fructiphilus (courtesy of R. Ochoa)

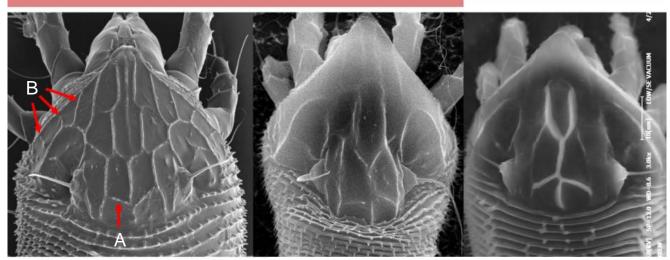
# **North America**



Phyllocoptes fructiphilus

Phyllocoptes adalius

Phyllocoptes resovius



Confirmed vector of RRV

Not known to be vectors of RRV

**Figure 3.** Differences in shield ornamentation allowing for differentiation of *P. fructiphilus* against other closely-related *Phyllocoptes* species on roses (SEM pictures courtesy of R. Ochoa, T. Druciarek and M. Lewandowski) Key diagnostic features to distinguish *P. fructiphilus* from *P. adalius* and *P. resovius* are the short lines at about 2/3 of median line length (A) which are joining with admedian lines. These lines are directed towards the anterior end of the

shield in *P. fructiphilus*, whereas in *P. adalius* and *P. resovius* they are perpendicular or directed towards the rear margin. Also, three additional short lines (B) located on each lateral side of the prodorsal shield are characteristic only for *P. fructiphilus* (Druciarek and Lewandowski 2016; Druciarek *et al.*, 2016).

#### 4.2 Other potential vectors

No other vector of RRV has been identified to date. All recognized and tentative emaraviruses have been shown or are hypothesized to be transmitted by eriophyoid mites (Di Bello 2015, citing others). Tuffen (2016) notes that only one vector was found per emaravirus (citing others). No transmission was obtained in trials with other arthropods (incl. spider mites, aphids, leafhoppers, plant hoppers and thrips) (Allington *et al.*, 1968; Amrine *et al.*, 1988). Nevertheless, eriophyoid mites are little studied, and information on known species is only partial. It is not excluded that other species may be a vector, especially other *Phyllocoptes* species of rose. JW Amrine (pers. comm.) mentioned that *P. adalius* has been thoroughly tested as a vector, but cannot transmit RRV; however, Druciarek *et al.* (2014) note that the morphological similarity of *P. fructiphilus* and *P. adalius*, and the symptoms observed in *P. adalius*-infested roses, suggested that *P. adalius* may transmit the virus (see also Druciarek, 2016). *P. adalius* is present both in the USA and the EPPO region, and has emerged in recent years as a pest of roses in glasshouses in Poland.

If other *Phyllocoptes* species can act as vectors, this will increase the risk of establishment, spread and potential impact. Information on *Phyllocoptes* species and *P. adalius* in particular are given in Annex 3.

#### 5. Regulatory status of the pest

RRV and *P. fructiphilus* are not listed as quarantine pests by any EPPO country according to EPPO Global Database (EPPO GD, 2017). It was added to the EPPO Alert List in 2016 (EPPO, 2016).

Regarding non-EPPO countries, RRV was recommended as a regulated pest in New Zealand in 2016 (MPI, 2016). RRV or *P. fructiphilus* were not found in the countries' regulations available from the IPPC website. No further information was sought.

# 6. Distribution 6.1 RRV

Until recently, RRV was known only from Canada and the USA. In 2017, it was reported from India (Chakraborty *et al.*, 2017). In the USA, RRV is believed to be native to the eastern Rocky Mountains, where it occurs on the native *R. woodsii* (Tuffen, 2016, citing Martin, 2013). RRV is considered as endemic to areas with extensive *R. multiflora* populations, the bulk of which exist east of the Rocky Mountains. From the 1930s to the 1940s *R. multiflora* was promoted as a living fence and for erosion control and was extensively planted in the east (Amrine, 1996).

**Table 1. Known distribution of RRV** (records are in EPPO GD, 2017, which provides references, except additional references cited below).

Continent	Distribution
North America	Canada: Manitoba (Connors, 1940), Ontario
	USA: Alabama, Arkansas, California, Connecticut, Delaware, Florida, Georgia, Illinois,
	Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Michigan, Minnesota, Mississippi,
	Missouri, Nebraska, New Jersey, New York, North Carolina, Ohio, Oklahoma, Pennsylvania,
	South Carolina, Tennessee, Texas, Utah (Brown, 1994), Virginia, West Virginia (Brown,
	1994), Wisconsin, Wyoming.
Asia	India (West Bengal).

**History of the distribution in North America**: rose rosette disease was first observed at the start of the 1940s in Manitoba (Canada), California and Wyoming (USA). In 1960-1990s, it spread to the Midwest and the South, and was found in Texas in 1990 (Baker *et al.*, 2014, giving original references). RRV is now widespread in north-central, south-central, southeast, and mid-west portions of the Northeast and in a few western states (Babu *et al.*, 2015 citing others; Windham *et al.*, 2016). RRV is apparently still spreading (for example, first found in Florida in 2013 - Babu *et al.*, 2014; Louisiana, 2015; Morgan *et al.*, 2015) and Windham *et al.* (2016) stated that it 'will continue to spread into new areas providing the climates in those areas are conducive for supporting populations of eriophyoid mites'. It is not known if this spread is natural or if the nursery trade plays a role. No details were found on the current distribution in Canada; in Ontario, it was found for the first time in a rose garden in 2014.

**India** (first record published in Chakraborty et al., 2017, in a *Rosa multiflora* hybrid breeding line (P. Chakraborty pers. comm., 2017)). The sequences of the Indian isolates were found to be highly identical to the American ones (A. Katsiani, pers. comm., 2017), and the virus might have been introduced from the USA.

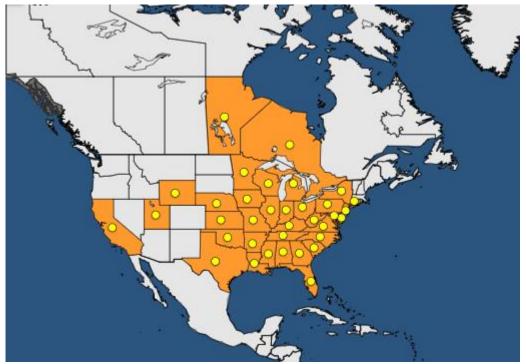


Figure 4. Distribution of RRV in the USA and Canada (from EPPO Global Database, 2018. Whole States are marked and not the distribution within states)

# Uncertain records:

- USA: New Mexico, Canada: Alberta, Saskatchewan (American Rose Society, no date). The author mentions records arising from «published literature as well as my evaluation of images sent to me». In addition, Colorado is mentioned in some Internet sources, without references. No confirmed records were found in the literature corresponding to the 4 records above. All published records are in Table 1.
- **Mexico:** Symptomatic plants were observed in the North of the country (J. A. Acuña-Soto, pers. comm.). The presence of RRV is not confirmed to date.
- There are some uncertainties on the distribution of RRV as rosette symptoms on rose have been described from several other countries, but the causal agent(s) may be different. This is the case for China (He *et al.* 2015) and Iran (Kamali *et al.*, 2015). In addition, Yardimci and Culal Kilic (2009) surveyed viruses of *R. x damascena* in the Isparta region (southwestern Turkey) and they mention symptoms of rosette in rose plantations, including excessive thorniness; mites were also observed. No detailed study has been carried out to date to determine the cause of the symptoms (N. Yardimci, pers. comm.). Regarding mites, the related species *P. adalius* is known to be present in Turkey (Denizhan *et al.*, 2015).

## Absent:

- Germany, Finland. Chakraborty *et al.* (2017) mentions German and Finnish isolates, citing Laney *et al.* (2011), but this referred to isolates of European mountain ash ringspot-associated emaravirus (EMARaV) and not RRV.
- New Zealand. MPI (2013) states that RRV and rose rosette are not reported from New Zealand: Epstein and Hill (1999) refers to a statement that 'graft transmission and development of rose rosette disease symptoms occurred on *R. eglanteria* (*R. rubiginosa*) from New Zealand', but it is uncertain whether Epstein received infected samples from New Zealand or if he obtained plants from New Zealand that he infected with the disease in the USA. No later information showing the presence of RRV in New Zealand was found when preparing this PRA, and RRV is considered absent.

## **6.2** *Phyllocoptes fructiphilus*

*P. fructiphilus* is known only from North America (Druciarek *et al.*, 2014) and is believed to be native to the Rocky Mountains (Martin, 2013; Amrine, 2014). Its known distribution in the USA does not exactly correspond to that of RRV: there is no information about its presence in some areas where RRV has been reported, and it is present in areas where RRV is not present (T. Druciarek and P. Di Bello, pers. comm.). No record was found for Canada. Finally, it is not known if *P. fructiphilus* is present in India (the vector is not mentioned in the first report of RRV - Chakraborty *et al.*, 2017).

There is uncertainty about the global distribution of *P. fructiphilus* (including for the EPPO region), because eriophyoids are poorly studied, they are difficult to detect and identify, they are known to have moved between continents (Navajas *et al.*, 2010; 40% of 77 alien phytophagous mites in Europe are Eriophyoidae), and they may therefore be present unnoticed. The EWG also recommended that NPPOs of EPPO countries conduct surveys for *P. fructiphilus* (see section 18 *Remarks*).

In the USA, *P. fructiphilus* was originally described from *R. californica* in California in 1940 (Kiefer, 1940). Its distribution has not been fully studied (and it is probably present in some other States where RRV was found), but records exist for: Alabama, California, Delaware, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Maryland, Missouri, Nebraska, New York, North Carolina, Ohio, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Utah, Virginia, West Virginia (E. de Lillo and J. Amrine, unpublished databases), Arkansas, Oklahoma (P. Di Bello, T. Druciarek, pers. comm.). As of 2017, it was not known to occur in Florida (even if RRV was found there).



Figure 5. Presence of *P. fructiphilus* in US States (from records above)

# 7. Host plants and their distribution in the PRA area 7.1 RRV

RRV has only been found on *Rosa* spp. (see Table 2), on a wide range of wild and cultivated species and varieties. All known cultivars are considered susceptible or potentially susceptible (see Section 2.1.4). In India, RRV was reported in a *Rosa multiflora* hybrid breeding line (P. Chakraborty pers. comm., 2017; Chakraborty *et al.*, 2017). Many hybrids have been identified as hosts in the USA, including hybrids with parents that are not known as hosts (e.g. *R. rugosa, R. x damascena*); some hybrids are listed in Annex 4. Limited information was found in the literature on rose rosette on species other than *R. multiflora* and 'cultivated roses'.

Laney *et al.* (2010, cited in MPI, 2013) surveyed 34 plant species around symptomatic roses in different US States but found no evidence of alternative hosts. In field observations, rose rosette disease did not affect other Rosaceae or other families (Epstein and Hill, 1999).

Di *et al.* (1990) and Rohozinski *et al.* (2001) worked on RRD-infected roses and mentioned some experimental hosts based on their symptomatology. However, these roses were likely infected by other viruses, and not RRV. Di *et al.* 1990 used the presence of double stranded RNAs at specific molecular weights as a diagnostic for the presence of RRV. However the double stranded RNAs of the same molecular weights as described in Di *et al.* (1990) were later found in asymptomatic roses (Hill *et al.*, 1994) and are not the correct size of the RNAs of RRV (Laney *et al.* 2011; Di Bello *et al.* 2015). While in the study by Rohozinski *et al.* (2001), the tobacco artificially inoculated with RRD displayed virus like particles (VLPs) much smaller than the VLPs of RRV and other emaraviruses (20 nm versus 110-180 nm) (Gergerich *et al.* 1983; Kim *et al.* 1994; Silvestro and Chapman, 2004).

Species	Common name	[Origin] Presence in EPPO region	Reference	
R. multiflora	Japanese rose	[E Asia] Cultivated and naturalized (not considered invasive in the EPPO region, unlike in the USA).	Allington <i>et al.</i> , 1968	
R. arkansana var. suffulta [R. suffulta]	sunshine rose	[N America] not known	Allington <i>et al.</i> , 1968	
R. banksiae	Lady Banks' rose	[China] cultivated	Amrine, 2002	
R. bracteata	Macartney rose	[E Asia] Cultivated	Windham, 2014. Note: infected by grafting, not host of <i>P. fructiphilus</i> (Epstein and Hill, 1999)	
R. canina	dog rose	[Europe, W Asia, N Africa] Wild and cultivated	Allington <i>et al.</i> , 1968; Amrine, 2002	
R. corymbifera (= R. dumetorum)	corymb rose	[Europe] Wild and cultivated	Amrine, 2002	
R. gallica	French rose	[C and S Europe, S-W. Asia]. Wild and cultivated	Allington <i>et al.</i> , 1968 (observed to be apparently infected with rose rosette)	
R. glauca [=R. rubrifolia]	blue-leaved rose	[C and S Europe] wild and cultivated	Allington <i>et al.</i> , 1968	
R. hugonis	golden rose of China	[E Asia] cultivated	Allington <i>et al.</i> , 1968 (observed to be apparently infected with rose rosette)	
R. nutkana	Nootka rose	[N America] not known	Allington <i>et al.</i> , 1968	
R. odorata		[China] cultivated	Allington <i>et al.</i> , 1968	
R. pimpinellifolia [R. spinossisima altaica]	Scottish rose, Burnet rose	[Europe to Far-E Asia, N. Africa]. Wild and cultivated	Allington <i>et al.</i> , 1968 (observed to be apparently infected with rose rosette)	
R. pisocarpa	cluster rose	[N America] not known	Allington <i>et al.</i> , 1968	
R. rubiginosa [=R. eglanteria]	sweet briar	[Europe, W Asia] Wild and cultivated	Allington et al., 1968, Windham, 2014	
R. soulieana	tea rose	[E Asia] not known	Allington <i>et al.</i> , 1968 (observed to be apparently infected with rose rosette)	
R. villosa	apple rose	[Europe and Asia] wild and cultivated	Amrine, 2002	
R. woodsii	Wood's rose	[N America] cultivated	Allington et al., 1968	
Cultivars of Rosa			Allington et al., 1968 (observed to be	

Species	Common name	[Origin] Presence in EPPO region	Reference
hybrids Teas, Floribundas and Grandifloras			apparently infected with rose rosette)

# 7.2 Phyllocoptes fructiphilus

Most eriophyoid mites are highly host-specific (80% to one host species, 95% to one genus, and 99% to one family) (Skoracka *et al.*, 2010). *P. fructiphilus* has been found on *Rosa* species and on many cultivars. To date, host range testing on other Rosaceae species have not found other hosts (Epstein and Hill, 1999, Amrine 2002).

Hosts of P. fructiphilus include: R. arkansana, R. banksiae, R. canina, R. dumetorum (= R. corymbifera), R. eglanteria, R. gallica, R. hugonis, R. banksiae, R. montezumae, R. multiflora, R. nutkana, R. odorata, R. pisocarpa, R. rubrifolia, R. soulieana, R. spinosissima var. altaica, R. villosa (= R. pomifera), R. wichurana, R. woodsii, R. woodsii var. ultramontana (= R. gratissima) (E. de Lillo and J. Amrine, unpubl. databases), as well as R. carolina, R. clinophylla, R. foliolosa, R. mushata (probably a misspelling of R. moschata), R. nitida, R. palustris, R. roxburghii, R. rugosa, R. setigera, R. virginiana (Solo et al., 2017).

There is limited evidence that *P. fructiphilus* cannot reproduce on *R. bracteata* (Amrine, 2002), but the EWG considered that it is not sufficient evidence to exclude *R. bracteata* as a potential host. In this PRA, all rose species (and hybrids) are considered as hosts or potential hosts.

## 8. Pathways for entry

The following pathways for entry of RRV and *P. fructiphilus* are discussed in this PRA. All pathways are considered from areas where the pest occurs to the EPPO region. Pathways in bold are studied in section 8.1; other pathways were considered very unlikely and are briefly discussed in section 8.2.

- *Rosa* plants for planting (except seeds and pollen)
- Rosa cut flowers
- *Rosa* seeds and pollen
- Natural spread into the EPPO region
- Rose hips

RRV and *P. fructiphilus* are considered to be potentially associated with all *Rosa* species and cultivars.

## **8.1** Consideration of pathways

Rosa plants for planting are studied in detail in Table 3 and Rosa cut flowers in Table 4.

For all pathways and at the scale of the PRA area, it is considered that the current phytosanitary requirements in place are not sufficient to prevent the introduction of RRV and *P. fructiphilus* (see individual pathways). Examples of prohibition or inspection are given for some EPPO countries (it was not possible in this express PRA to fully analyse the regulations of all EPPO countries). Similarly, the current phytosanitary requirements of EPPO countries in place on the different pathways are not detailed in this PRA (although some were taken into account when looking at management options). EPPO countries would have to check whether their current requirements are appropriate to help prevent the introduction of the pest.

Table 5. <i>Rosa</i> plants for planting (except seeds and pollen)						
Pathway	Rosa plants for planting (except seeds and pollen)					
Coverage	• Commodities such as bare-rooted plants, pot plants, cuttings/budwood, rootstock, tissue					
	culture.					
	• All <i>Rosa</i> species and cultivars					
Pathway	Partly.					
prohibited in the	Plants of Rosa, intended for planting, other than dormant plants free from leaves, flowers and					
PRA area?	fruit from non-European countries are prohibited in the EU. This is not effective to prevent					
	entry, because both RRV and its vector can be associated with dormant plants.					
	Not searched for other EPPO countries.					
Pathway subject to	Presumed in most EPPO countries.					
<mark>a plant health</mark>	In the EU, there are general requirements for plants for planting.					
inspection at						
import?						

# Table 3. Rosa plants for planting (except seeds and pollen)

Pathway	Rosa plants for planting (except seeds and pollen)				
Pest already	Not known.				
intercepted?					
• 0	RRV may be present in any plant part, including roots, and could be associated to tissue				
	cultures.				
	Non-dormant plants may carry all life stages of <i>P. fructiphilus</i> , and dormant plants may carry overwintering females. <i>P. fructiphilus</i> is very unlikely to be associated with tissue cultures.				
	RRV is recorded in nurseries in the USA, and there is no official certification scheme for rose				
	(NCPN, 2017). However, it is not known from which areas plants are exported to the EPPO				
	region.				
	Symptomatic rose plants are likely to be discarded, and propagating material not taken from them. However, infected plants may be asymptomatic (the duration of the incubation period is not known for some <i>Rosa</i> species) (see section 2.1.3).				
	There is an uncertainty on whether RRV is associated with species mentioned as 'resistant' in the literature (see section 2.1.4).				
	The production of tissue culture may not be subject to systematic testing of material prior to tissue culture in the USA. There is no information on tissue culture imports, nor on whether such material is subject to inspections or tests if it is traded.				
	<i>P. fructiphilus</i> is potentially associated with all rose species and cultivars. <i>P. fructiphilus</i> due to its size cannot be seen with the naked eye. It is also difficult to detect it with a hand lens (Hoy, 2013). In the context of inspection at import, detection of overwintering females is not practical (requiring the use of a stereomicroscope and opening buds). On plants with leaves, symptoms of direct mite feeding might not be visible at low population densities.				
	Pesticide treatments would not fully eliminate the vector.				
	The virus and the vector would survive in and on <i>Rosa</i> plants respectively.				
	Plants are transported in conditions that allow them to survive and such conditions will also				
	allow survival of the associated vector.				
Trade	Trade data are available for the EU, and no data were found for other EPPO countries.				
	For the EU (see Annex 5), Eurostat indicates minor imports of rose plants for planting from the USA and India (about 0.5 t in 2016, less than 1.5 t per year in 2010-2015), and minor imports from Canada (< 100 kg per importing EU country). Corresponding quantities in 'pieces' in 2016 are given in Eurostat as ca. 11 000 from the USA, 4600 from India, and 2400 from Canada.				
	The species traded are not known. <i>R. multiflora</i> is regulated in 13 US states where its importation, distribution, trade and sale have been banned (nyis.info, 2017).				
	Some <i>Rosa</i> plants may be brought to the EPPO region for exhibitions and contests, but no details are available.				
	Roses are popular and some material may be imported through internet trading (Tuffen, 2016) and travellers may also bring back plants in their luggage. Such material may escape from import controls.				
Transfer to a host	The host plants will be planted or further propagated. If there is no vector, the virus would be limited to the infected plant, and could pass onto other plants through propagation, or be transient and disappear when the plant dies (see section 9). If the vector is present, it may move to other rose plants and transmit RRV.				
	It is noted that a single female of <i>P. fructiphilus</i> is sufficient to initiate a population and nymph and adult stage of the vector that may be present at arrival are suitable for movement through the air (unlike for e.g. a moth that usually arrives as egg or larvae which first have to develop into an adult before transfer may occur).				
Likelihood of entry and	<b>Tissue cultures</b> . Low with a low uncertainty if the plant material is tested and found free for RRV before being reproduced <i>in vitro</i> ; moderate with moderate uncertainty if the material is not tested (condition of production, whether there are imports).				
	<b>Other plants for planting.</b> High [due to the very high probability of association] with a moderate uncertainty (no data on imports to non-EU countries, whether plants are imported from infected nurseries, duration of the incubation period of the virus (i.e. increasing the chance of infected asymptomatic plants)).				

Table 4. Rosa cut fle	owers
Pathway	Rosa cut flowers
Coverage	• All <i>Rosa</i> species and cultivars
Pathway	No for EU countries.
<b>-</b>	Not searched for other countries.
PRA area?	
	Probably in some EPPO countries.
a plant health inspection at	Yes in the EU, with specific requirements targeting <i>Bemisia tabaci</i> .
import?	
	Not known.
intercepted?	
	RRV and all stages of <i>P. fructiphilus</i> may be associated with cut flowers.
that may be	
associated	
	RRV reduces production and the quality of flowers. Flowers from symptomatic plants are not
	likely to be marketed, but if flowers are taken from asymptomatic portions of infected plants,
with the pathway	they are very likely to carry the virus and the mite.
	Tuffen (2016) notes that quality standards for cut roses are very high and plants are likely to
	be treated with high levels of pesticides, some of which may lower the populations but not
	completely eliminate <i>P. fructiphilus</i> .
	There are no data on the abundance of <i>P. fructiphilus</i> in glasshouses (where the majority of
g • 1 1 •	cut flowers would be produced) in the USA (T. Druciarek, pers. comm.).
0	RRV would survive in <i>Rosa</i> cut flowers.
storage	Regarding <i>P. fructiphilus</i> , there is no specific data on the lower threshold for survival of <i>P.</i>
storage	<i>fructiphilus</i> , but populations collected during the summer are known to survive storage at 2-4°C (see section 4.1.1). The EPPO PRA on <i>Thaumatotibia leucotreta</i> (EPPO, 2013) mentions
	that <i>Rosa</i> cut flowers should be precooled at $2^{\circ}$ C to preserve quality and extend the vase life.
	It is expected that the vector would survive even at 2°C.
Trade	Data was available for the EU and not for other EPPO countries.
	For the EU, Eurostat (see Annex 5) shows a minor trade of rose cut flowers from the USA
	(200  kg in  2016 - 100-700  in  2012-2015), and no trade from Canada. The trade of cut roses
	from India appears to have increased continuously in 2012-2016 (from >300 t to >900 t,
	mostly to the Netherlands and the UK); it is not known if these flowers come from areas in
	West Bengal where RRV was recently found. Corresponding quantities in 'pieces' in 2016
	are given as ca 30 million from India, and ca. 4000 from the USA.
Transfer to a host	Transfer is very unlikely to occur if the virus is present without the vector on cut flowers.
	Transfer of a single infectious female of P. fructiphilus can be sufficient to initiate a
	population and transmit the disease. P. fructiphilus is transported by wind, but it would need
	to land on a rose plant by chance. Roses are widespread in the EPPO region but still cover a
	relatively small percentage of the total land area. P. fructiphilus may also spread by contact. It
	will not likely reach a rose plant by crawling, however, if there is no contact between the cut
	flowers and the plant.
	<i>Rosa</i> cut flowers have a limited shelf-life (about 2 weeks) and are mostly used indoors. If
	other <i>Rosa</i> plants are also kept indoors, the mite could transfer whilst cut flowers are still fresh enough to support visble populations, but this supposes that the supposes are kept peop
	fresh enough to support viable populations, but this supposes that the cut roses are kept near an indoor rose plant (Tuffen, 2016).
	Cut flowers would remain in a state of freshness suitable for RRV and <i>P. fructiphilus</i> for a limited period. Such flowers, or <i>Rosa</i> waste carrying the vector, may be discarded outdoors in
	the open, e.g. in gardens, or compost, or when retailers or florists discard damaged or unsold
	flowers without proper disposal of waste (this may also be the case when roses are repacked
	in packaging stations located in the vicinity of host plants). If still alive when rose material is
	discarded outdoors, P. fructiphilus would need to move to a rose plant (see above). The cut
	flowers would have to be imported and discarded at a time of the year suitable for P.
	fructiphilus to transfer.
	Cut flowers may also be used outdoors (such as in graveyards), which would facilitate
	transport of the mite to hosts by the wind.

Pathway		Rosa cut flowers
		Finally, cut flowers may also be used (illegally) for propagation, and the probability of
		transfer is then similar to that for plants for planting.
Likelihood of		Low to moderate with moderate uncertainty (uncertainties on the probability of transfer, the
entry and		trade volume from areas where the virus and the vector are present, duration of the incubation
uncertainty		period of the virus (i.e. increasing the chance of infected asymptomatic plants).
		Note: cut flowers is an unlikely pathway for the virus on its own (i.e. without the vector, only
		RRV in the cut flowers)

#### **Overall rating of the likelihood of entry** (based on the highest rating from all pathways)

				0		,	
	Rating of the likelihood of entry	Very	low	Low	Moderate	High	Very high
						$\checkmark$	
	Rating of uncertainty				Low	Moderate	High
						$\checkmark$	

## 8.2 Very unlikely pathways

The following pathways are considered to be very unlikely:

- Seeds, pollen. Neither RRV nor *P. fructiphilus* are associated with seeds or pollen.
  - Likelihood of entry: very low with a low uncertainty
- Natural spread to the EPPO region: RRV cannot spread naturally on its own. *P. fructiphilus* may spread by wind (see Section 4.1.2), but entry on its own into the EPPO region from countries where RRV and *P. fructiphilus* naturally occur is not possible due to the large distance between the EPPO region and the countries where the virus and vector are known to be present.

Likelihood of entry: very low with a low uncertainty.

• **Rosehips.** *P. fructiphilus* may be present on rosehips. Rosehips are collected from several *Rosa* species. They are mostly processed or used for domestic consumption and are therefore very unlikely to act as a pathway.

Likelihood of entry: very low with a low uncertainty

## 9. Likelihood of establishment outdoors in the PRA area

## 9.1 Persistence of RRV

Rose plants may be imported as final consumer goods or for further propagation. If *P. fructiphilus* is associated with the plants at import (or if there is any other vector at destination), RRV could be transmitted to other rose plants, therefore ensuring establishment.

The only case where RRV is likely to be transient and would not eventually establish, is if there is no vector and the plants are not used for further propagation. RRV would disappear when the plant dies (of the virus or other causes). Symptoms may also be noticed and the plant destroyed before it dies naturally.

The climatic conditions will influence the establishment of *P. fructiphilus*, and the presence of rose will affect the establishment of both RRV and its vector.

## 9.2 Climatic suitability

RRV has been reported from many US States and from Canada (Figure 4) in subtropical, temperate, or Mediterranean climates. The climatic requirements of *P. fructiphilus* are not known in detail, but it is present in many US States (Figure 5). It was found in New York State, e.g. in Ithaca where the annual extreme minimal temperature reaches  $-23^{\circ}$ C (USDA hardiness zone 6a). In the EPPO region, this corresponds to the south of Finland and Sweden (see Annex 6). There is an uncertainty on the presence of the vector in northernmost locations where rose rosette was found, i.e. Manitoba and Ontario. The closely-related *P. adalius*, which has a similar biology, is present outdoors in Finland (Druciarek *et al.*, 2016). The EWG considered that *P. fructiphilus* would not be restricted by the low temperatures of the EPPO region, not even in the northern countries.

In the south of the USA, *P. fructiphilus* is present in Texas in areas where average monthly temperatures in July and August reach ca.  $30^{\circ}$ C, i.e. similar to Ouarzazate in Morocco (one of the southernmost locations of the EPPO region where roses are produced). High temperatures may therefore not be a limiting factor for the establishment of *P. fructiphilus* in the southern part of the EPPO region.

There is contradictory information in the literature on the effect of low relative humidity. Some authors note that *P. fructiphilus* is sensitive to low relative humidity, and that periods of drought are associated with low population densities. *P. fructiphilus* and RRV are not found on cultivated roses grown in large scale nurseries in dry areas of California and Arizona (Hoy *et al.*, 2013). However, the American Rose Society (no date) note that hot and dry summers seem to have increased the speed of spread, and Hong *et al.* (2012) note that spraying against the vector needs to be more frequent in periods with hot and dry weather when the vector is most active.

According to this contradictory information, it is not known if the vector could establish in the EPPO regions with low relative humidity e.g. in Turkey, where roses for rose oil are grown in the dry area of Isparta, and in Morocco in the very dry/hot pre-Saharian area of the Daddes Valley near Ouarzazate (rose-office.com). The vector is not observed in similar conditions in the USA, and consequently it is not known if the vector could establish.

In conclusion, it is considered that establishment of RRV and its vector is likely in most of the EPPO region. Because the exact climatic requirements of *P. fructiphilus* are not known, there is, however, uncertainty about the potential of establishment of *P. fructiphilus* in very northern regions and where climates are more arid.

## 9.3 Host plants

Roses are present throughout the EPPO region, including the highly susceptible host *R. multiflora*, as well as many other different *Rosa* species and cultivated roses. Given the wide host range of RRV and *P. fructiphilus* amongst *Rosa* species in North America, which includes non-North American species, it is considered very likely that *Rosa* species present in the EPPO region and not yet known as hosts could become hosts for RRV and *P. fructiphilus*.

**Known hosts:** *Rosa multiflora* has been used for breeding purposes and as a rootstock for ornamental roses in the USA (Epstein and Hill, 1995) and in Europe (T. Druciarek, pers. comm.). It is grown as an ornamental, and is also a naturalised garden escapee in several EPPO countries (e.g. Tuffen, 2016). *R. canina, R. corymbifera, R. gallica, R. glauca, R. pimpinellifolia, R. rubiginosa,* and *R. villosa* are native in the EPPO region and widespread both in the wild and cultivated as ornamentals or for other purposes (e.g. hedge plants). Some *Rosa* species that are parents to susceptible hybrids are also widespread in the EPPO region: *R. x damascena,* an economic crop for the production of rose oil, and *R. rugosa,* an ornamental plant considered invasive in part of the EPPO region (Germany and Poland to Scandinavia - Weidema, 2006).

**Other Rosa species in the EPPO region, currently not known as hosts**: In addition to *R*. x *damascena*, *R*. *alba* and *R. centifolia* are economic crops for the production of rose oil. There are also many native rose species in the EPPO region. No complete search was made for this PRA, but Tuffen (2016) analysed the situation for the UK and listed the following species (which may also be native in some other parts of the EPPO region): *R. agrestis*, *R. arvensis*, *R. caesia*, *R. mollis*, *R. micrantha*, *R. obtusifolia*, *R. sherardii*, *R. stylosa* and *R. tomentosa*. There are also threatened or endangered *Rosa* species in the EPPO region, such as: *R. zakatalensis*, *R. abutalybovii*, *R. komarovii*, *R. jaroshenkoi*, *R. isaevii* (all from Azerbaidjan), *R. acicularis* (incl. Finland, Kazakhstan, Kyrgyzstan, Russian Federation, Sweden), *R. dolichocarpa* (Russian Federation) and *R. pendulina* (Central and Southern Europe) (IUCN, 2017 http://www.iucnredlist.org/details/203449/0).

#### 9.4 Other elements relevant for establishment

- A single female of *P. fructiphilus* may establish a population.
- It is not known if *P. adalius*, or other eriophyoid mite species in the EPPO region, might be able to act as vectors, which would help establishment of RRV even in the absence of *P. fructiphilus* on the imported material.

Rating of the likelihood of establishment outdoors	Very	low	Low	Moderate	High	Very high ✓
Rating of uncertainty				Low ✓	Moderate	High 🗆

Uncertainty: limits of the potential area of establishment in the EPPO region.

#### 10. Likelihood of establishment in protected conditions in the PRA area

No specific records of RRV or P. fructiphilus in protected conditions in the USA or Canada were found,

except a mention in Illinois (1999) that RRV infects roses 'grown outdoors and in greenhouses'. However, populations of RRV and *P. fructiphilus* have been established successfully in glasshouses for experimental purposes in the USA (Di *et al.*, 1990; Di Bello *et al.*, 2016). In Europe, the related species *P. adalius* is an economic pest on glasshouse roses, and can reach densities as high as 340/cm<sup>2</sup> on leaves and petals in glasshouse rose production (Druciarek *et al.*, 2014). *P. resovius* was also found in a rose glasshouse in Poland (Druciarek *et al.*, 2016). *P. fructiphilus* could spread in glasshouses by air movement inside the facility, by crawling from plant to plant and possibly on tools, equipment or clothes, and establish populations. Temperature and relative humidity in protected conditions would presumably be favourable. Cropping practices are not expected to prevent its establishment.

Rating of the likelihood of establishment in protected conditions	Very	low	Low	Moderate	High	Very high ✓
Rating of uncertainty				Low	Moderate ✓	High 🗆

Moderate uncertainty due to absence of specific records in glasshouse production in the USA or Canada.

## 11. Spread in the PRA area

Spread of RRV and *P. fructiphilus* has occurred in the USA. This may have been due to their natural spread, and favoured by the widespread distribution of unmanaged stands of *R. multiflora* (which is considered an invasive species in the USA), and through human-assisted pathway, e.g. its use as a rootstock for ornamental roses (Epstein and Hill, 1995).

Both RRV and *P. fructiphilus* are spread through human-assisted pathways, in particular the trade of infected asymptomatic plants. Infection of healthy plants through root grafts is a hypothesis, but generally the presence of a vector would be necessary for further spread. Rose plants in gardens, parks, the urban environment and in the wild may serve as reservoirs for RRV, including native rose species if shown to be hosts.

There is a large trade of rose within the EPPO region that would facilitate spread of RRV and *P. fructiphilus* if established. Within the EU, Eurostat indicates imports by EU28 countries from EU28 partners in 2016 of over 29000 t and 58 million 'pieces' of plants for planting (commodity code 06024000 - roses grafted or not) and over 197000 t and 4 billion 'pieces' of *Rosa* cut flowers (commodity code 06031100) [note: data in Eurostat is not consistent for import from, and export to, EU28, but give an indication of the volume of trade]. If *P. fructiphilus* was introduced, it could ensure local spread naturally from individual foci and spread through human-assisted pathways, especially plants for planting (see section 8.1) (it is not known if *P. adalius* or other Eriophyoidae species could play the same role). As *P. fructiphilus* is tiny and may be confused with other species that are present, this may delay detection of new outbreaks of the vector. Vegetative propagation is common for rose, which would increase the risk of spread from one infested mother plant (although they are less likely to root, see section 2.1.2). RRV is transmitted by grafting. Certification schemes are not known to be used for rose in the EPPO region.

The dispersal of *P. fructiphilus* is analysed in section 4.1.2. Natural spread will be higher in areas where the climatic conditions are favourable to *P. fructiphilus* producing high populations and many generations per year.

The presence of hosts will favour spread. No information was found on the distribution of *R. multiflora* in the EPPO region, although it may be present in a large part of the region at least as an ornamental (see section 9.3). Tuffen (2016) states that *R. multiflora* is unlikely to play as important a role in the natural spread of the pest in the UK as in North America, but that there are many other widespread native rose species that could play a similar role. For example, in the EPPO region, *R. canina* and *R. rubiginosa* are widespread. Natural spread rates will be affected by the density of *Rosa* spp. in the wider environment.

*P. fructiphilus* could also move locally on clothes, tools and equipment (even if not demonstrated to date – see section 4.1.2), for example between plants, fields or facilities. RRV is not readily mechanically-transmitted, and is very unlikely to be transmitted with tools etc. (although a precautionary approach is recommended in the USA – see section 12.3).

Rating of the magnitude of spread	$\bigcup^{Very \ low}$	Low	Moderate	High	Very high
Rating of uncertainty			Low 🗆	Moderate	High □ ✓

*Uncertainties:* duration of the incubation period of the virus (long incubation periods increase the chance that infected plant material will be moved), natural spread of *P. fructiphilus* in areas with low rose densities and the frequency by and distance over which the vector species is carried by wind; whether there are species already present in the EPPO region that can act as a vector of RRV.

## 12. Impact in the current area of distribution

## **12.1 Nature of the damage**

RRV decreases the aesthetic value of rose plants, affects flower production and quality, and kills plants. Susceptible rose plants die within 1-5 years after symptom appearance. The damage is so severe on *R. multiflora* that rose rosette disease was envisaged as a biocontrol agent against this invasive plant in the USA (until it was found infecting other roses).

## 12.2 Impact in countries where it occurs

**India**. RRV is reported to be an 'emerging pest of rose gardens' (Chakraborty *et al.*, 2017). Testing was done on 20 symptomatic plants in two rose gardens. No details are given on the presence of the vector (in that area nor in India), nor on the overall situation in India.

**Canada**. No details were found regarding impact of RRV in commercial production, private gardens, landscaping or in the wild. The original record in Manitoba (Connors, 1940) does not mention impact, and the recent record from Ontario relates to a rose garden on a campus. In Ontario, the vector was not found on the samples submitted for identification (S. Shan, pers. comm., 2017).

**USA**. RRV has grown exponentially in cultivated roses in the mid-south USA due to increased use of mass plantings of shrub roses in residential and commercial landscapes (Windham, 2014), and impacts have increased. Current impacts in the USA are analysed in Tuffen (2016) and point to high impact, both economic (commercial rose production) and social (gardens, aesthetic value, landscape, rose gardens, etc.):

« In Alabama, containerised rose production is largely reported as affected (Conner and Hagan, 2012). The University of Kentucky reported that losses can occur in home and commercial landscapes, nurseries and botanical gardens (Ward and Kaiser, 2012). One rose production business in North Texas reported at a 2015 conference they had seen a 25% reduction in gross revenue as a result of the disease (recording of presentation available online: https://www.youtube.com/watch?v=2YUoSOxKnCw&list=PLRyqQJrldHJE0T-4XVZjYUlxfSJNH7fTu&index=5). The situation in the USA has been described as "an epidemic", with impacts particularly bad in northern Texas (Bahari, 2015). In Collin County, Texas, the Rose Rosette Eradication Alliance was established to help spread word and reduce incidence of the disease at a community level (Cook, 2015). Both Knock Out<sup>®</sup> roses and Drift roses, some of the most popular roses in the USA, are susceptible and are being significantly impacted by RRV (Bender, 2013, Sheridan, 2014). The seriousness of the impacts seen in the USA led to the USDA funding a \$4.6 million-dollar project to help devise solutions to combatting the disease (UDaily, 2014). »

In addition, references in Tuffen (2016) also indicate that several botanical gardens and old gardens were forced to remove roses and to replace them because of RRV (citing e.g. Fort Worth Botanic Garden, 2016; Brooklyn Botanic Garden, 2016; Aspinwall, 2014; Shaner, 2006; Fox 23 News, 2016; Holloway, 2015). «Southlake, Texas, was reported to be removing and replacing 5400 rosebushes in medians (central reservations) and parks due to RRV at an estimated cost of \$500 000 (Bahari, 2015). A news article reported in North Texas the destruction of 1200 roses from a business park, and 300 at a church, with the use of roses in landscaping apparently now decreasing and some owners destroying roses assuming that disease is inevitable (Holloway, 2015). There are numerous other examples of botanic gardens and public parks which have had rose gardens or collections destroyed or significantly depleted by RRV. » (Tuffen, 2016).

Economic studies to assess the impact of the disease are in progress (Byrne et al., 2017).

One publication mentions that RRV infects roses 'grown outdoors and in greenhouses' (Illinois, 1999), but no specific information was found on damage to glasshouse production.

No mention of environmental impacts was found in the US literature, apart from the beneficial effect due to the mortality of the invasive *R. multiflora*.

The disease is thought to be under-reported in the USA (historical reports and disease maps gave only a partial picture of the distribution; only recently have extensive surveys started, but not in all areas of the US (P. Di Bello, pers. comm.)).

## 12.3 Control [note: this reflects what is done in countries where the pest occurs]

Detailed information on control is available only from the USA (in the recent findings in India and in Ontario, Canada, the plants found infected were removed). Control relies on a combination of methods to reduce the spread of RRV and *P. fructiphilus*. Early detection is essential for the success of the control, but is difficult. Current recommendations for managing the disease in the USA are as follows:

## **Removal and destruction of infected plants**

- Monitoring plants throughout the season for symptoms, with quick action when symptoms are observed. Weekly monitoring is mentioned (Babu *et al.*, 2015; MGS, no date).
- Plants showing any level of symptoms should be quickly removed and destroyed, including roots, to prevent further spread of the vector and virus (Di Bello *et al.*, 2017). When removing plants, precautions should be taken to avoid the spread of *P. fructiphilus* and RRV (e.g. bagging plants, not transporting them away, not leaving them on the site; Windham *et al.*, 2016). Removal of symptomatic plants is however not considered fully effective as there would also be asymptomatic plants.
- Applying herbicides at removal sites to prevent regrowth from root fragments.
- Removing Rosa plants adjacent to infected plants (Hoy et al., 2013).
- Monitoring plants around infested plants.

## Sanitation measures

- Removing *R. multiflora* in the vicinity of cultivated roses (MGS, no date, Tuffen, 2016 citing Bolques *et al.*, 2014, Conner and Hagan, 2012, Hand, 2014). Hong *et al.* (2012) states that this should be done in a 100 m radius around rose nurseries and gardens, and locations of removed plants should be monitored for regrowth for up to one year, with any regrowth removed and destroyed (Hand, 2014).
- Starting any operation in a rose plantation from healthy areas towards infested areas (to avoid spreading the mite) (Olson *et al.*, no date). After relevant operations, tools should be cleaned and fresh clothes should be put on before moving to a disease-free plant or area (Missouri Botanical Gardens, no date). This would avoid spreading the mite. Cleaning tools is a general phytosanitary measure, although mechanical transmission of RRV is not considered to be effective (see section 2.1.2).
- Not using leaf blowers around rose plants, to avoid spreading the mite (Missouri Botanical Garden, no date).

## Establishment of rose plants

- Starting from good quality planting material from nurseries with a history of selling high quality plants (Di Bello *et al.*, 2014) (there is no certification scheme to guarantee they are pest-free)
- Spacing plants so that canes and leaves do not touch each other, to make it more difficult for *P. fructiphilus* to crawl from plant to plant (Hand, 2014).
- Establishing a barrier between a rose planting and a possible source of *P. fructiphilus* and RRV. Experiments at the University of Tennessee have shown that a barrier of *Miscanthus sinensis* reduces the initial incidence of rose rosette in rose test plots (although once the disease became established, the disease progressed as in plots without barriers) (Windham *et al.*, 2016, 2017a).
- Planting ornamental roses, commercial rose and landscape roses as far away as possible from known stands of *R. multiflora*. This should be at least 100 m, but a greater distance is preferred if the ornamental roses are downwind from *R. multiflora* (Missouri Botanical Gardens, no date; Baker *et al.*, 2014; Hong *et al.*, 2012).

## Mite control

Not all authors agreed on the efficacy of measures aimed at controlling the mite, especially the use of acaricides. Mites tend to shelter in crevices where it is difficult for products to reach (Cloyd, 2013, Hand, 2014, Roebuck, 2001). In addition, treatments will not entirely prevent transmission of the virus to healthy roses as the vector has a short inoculation access period (1h) (see 4.1.3). Preliminary research indicates that some acaricide treatments may be effective to reduce impact (see below) (Windham *et al.*, 2017a). Applying acaricides during the growing season has been recommended by some authors (Tuffen, 2016 cites Amrine, 1996, Baker *et al.*, 2014, Singh and Owings, 2014) to decrease populations and therefore reduce spread.

Acaricides should be used in rotation to avoid the build-up of resistance (Baker *et al.*, 2014). Growers who apply abamectin, fenpyroximate and spiromesifen in rotation every 5-7 days from bud break throughout the growing season report significantly reduced incidence of RRV (Tuffen, 2016 citing AmericanHort, 2013, a group of professional growers). Acaricides may also be used on plants surrounding spots where infected plants

have been removed. Sprays can be applied every two weeks from April until September, with additional sprays in hot, dry weather when eriophyoid mites are most active (Hong *et al.*, 2012). In recent experiments (results not fully published yet), plants sprayed at 14 day intervals with fenpyroximate, spiromesifen, spirotetramat or bifenthrin did not develop symptoms, and the use of acaricides for reducing the impact of rose rosette is considered promising (Windham *et al.*, 2017a). More research is needed to determine parameters for spraying (trigger, number of sprays, spray intervals) (Windham *et al.*, 2017a).

The following products are mentioned as being used in the literature (in bold, allowed in the EU – although not in all countries and not for all uses): horticultural oil (e.g. neem oil, mineral oil) or insecticidal soap (Missouri Botanical Garden, no date, MGS, no date, Baker *et al.*, 2014, Babu *et al.*, 2015), **fenpyroximate**, **spiromesifen**, **spirotetramat**, **bifenthrin** (Windham *et al.*, 2017a), carbaryl (Hong *et al.*, 2012, noting that carbaryl against eriophyoid mites can lead to outbreaks of spider mites), endosulfan (Babu *et al.*, 2015), **abamectin**, **deltamethrin**, **imidacloprid**, **malathion**, permethrin, **pyrethrin** (Di Bello *et al.*, 2014), dicofol, dienochlor, fenbutatin-oxyde (Illinois, 1999). Note: in recent experiments, Windham *et al.* (2017a) found that abamectin + horticultural oil and carbaryl sprays were ineffective.

Acaricide treatments should be combined with cultural control methods (Hong *et al.*, 2012). Natural enemies of eriophyoids (Hoy *et al.*, 2013), such as predatory mites, should be favoured (e.g. using selective pesticides). Other control methods are mentioned to reduce populations of *P. fructiphilus*. However, it is not clear if they reduce the incidence of the disease: pruning roses hard in late winter (by 2/3) to remove overwintering mites (Missouri Botanical Garden, no date); removing and destroying any fallen foliar materials that may harbour the mite and destroying them before replanting healthy rose plants (Babu *et al.*, 2015), removing mature fruits from all plants to reduce overwintering mite populations (Bauchan *et al.*, 2017).

## Other methods mentioned in the literature

Pruning of plants has sometimes been erroneously recommended as a means to eliminate infected plant parts and RRV. However, RRV is now known to be systemic and moves to the roots thus pruning of symptomatic plant parts is not considered effective or recommended (Di Bello *et al.*, 2017). Windham *et al.* (2016) hypothesized that the apparent success of pruning out symptomatic parts may be due to a long incubation period which allows the rose to appear virus free for a long time. In recent experiments [results not fully published yet], removing symptomatic canes at first detection of symptoms was ineffective (Windham *et al.*, 2017a).

There is currently no commercial biocontrol agent marketed against *P. fructiphilus*. Ochoa et al (2016) note that predatory mites (Phytoseiidae, Tydeidae and Bdellidae) were found to be associated with eriophyoid mites on rose and could possibly be used as biocontrol agents. However, biological control would only contribute to reducing the incidence of RRV (Hoy, 2013).

## Methods known to be under investigation:

Intensive research is being conducted in the USA on impact and control, including a project involving scientists from 6 states, private rose breeders, the American Rose Society, AmericanHort, and the rose industry (Byrne *et al.*, 2017). Topics of current research include:

- management practices and educational material (Byrne *et al.*, 2017).
- resistance, investigated by breeders and researchers (Byrne et al., 2017, Windham et al., 2017b).
- detection methods that are user-friendly and allow early detection prior to the appearance of symptoms (see section 2.2)
- effectiveness of debated control methods, such as pruning infected canes to remove RRV infection and acaricides to reduce the impact of RRV (Windham *et al.*, 2017a).
- user-friendly cellphone-based kit for counting eriophyoid mites on rose, in order to help professionals and homeowners better time the application of acaricides (Collins *et al.*, 2017).
- predatory mites and competing eriophyoid mites (D. Byrne, pers. comm.)
- an antiviral compound is being tested on plants (Byrne project information, 2017).

The rating below is based on the situation in the USA. The EWG rated the magnitude of impact as high. In epidemic areas like in northern Texas, impact could be rated as very high.

Rating of the magnitude of impact in the current area of distribution	Very low	Low	Moderate	High ✓	Very high □
Rating of uncertainty	Low 🗸	Moderate	High $\Box$		

#### 13. Potential impact in the PRA area

### Will impacts be largely the same as in the current area of distribution? Yes / No

RRV may have high economic, environmental and social impacts. The potential impact is assessed to be similar, but specific impacts would be partly different due to different uses of rose plants and rose products in the EPPO region as compared to those in the US. Potential impacts would be highest where conditions are conducive to high populations and many generations of *P. fructiphilus* (climate, wind), and areas of high densities of rose.

As in the USA, control strategies would involve destruction of rose plants. Early detection is essential, but difficult, and the control methods currently available are unlikely to completely eliminate the disease. Research would be needed on control methods.

It was noted that rose is generally not subject to certification schemes in the EPPO region, under which symptoms of viruses in general would be checked. RRV is mentioned in the EPPO Certification scheme (PM 4/21; EPPO, 2002) as rose rosette disease. Symptoms of RRV would render plants unmarketable, and such plants would not be used to produce propagating material. However, the incubation period that can last many months or tolerance of some cultivars would likely hinder early detection.

## **Economic impacts**

The following economic impacts are expected if RRV and its vector would become established in the EPPOregion:

- breeders, nurseries and retailers: Symptomatic rose plants will be unmarketable and will have to be destroyed.
- producers of cut roses: Royalfloraholland.com (2016) report that the growing area in Europe [probably referring to the EU only] has decreased by 27% in the past 5 years, with currently ca. 1700 ha (presumably referring only to the production for cut flowers). Rose is still grown on about 200 ha in Germany, Spain and Italy. In the Netherlands, the area of *Rosa* cut flowers grown in glasshouses decreased from ca. 932 ha in 2000 to 257 ha in 2016 (Statline, 2017).
- Industry: The potential impact could be high on rose oil production and processing industry, as well as on other minor products from that industry, such as rose concrete, rose water and rose essence. Rose for oil is a high value crop in some EPPO countries as rose oil is a valuable export commodity intended for the cosmetic and perfume industry. Bulgaria and Turkey are the largest producers worldwide. The production of rose oil relies primarily on R. x damascena [a hybrid known to be a RRV host], to a lesser extent R. centifolia (Kovacheva et al., 2010; Gunes, 2005), as well as R. alba and R. gallica [known host] (Prance and Nesbitt, 2012) or a hybrid of R. gallica and R. centifolia (http://www.rose-office.com/). The production covers over 3500 ha in Bulgaria (Kovacheva et al., 2010, BNAEOPC, 2016) and ca. 2300 ha in Turkey (Gunes, 2005). In both countries, the production is concentrated in a few areas. Bulgaria and Turkey supply 80-90% of the world's yearly consumption of rose oil. In 2001-2008, the annual export of rose oil from Bulgaria increased from 1020 to 1800 kg. In the same period, the average price of Bulgarian rose oil increased from 3217 € to 4600 € per kg (Kovacheva et al., 2010). Market Insider (2016) reports prices of 10 000 USD per kg for Bulgarian rose oil. Morocco is also a major producer, and to a lesser extent France, Italy and Russia (Kovacheva et al., 2010). Production is also registered in Uzbekistan and Ukraine, and appears to be starting countries Azerbaijan Georgia (http://www.rose-office.com). in some other such as and The economic impact will depend on the susceptibility of the main species (R. x damascena and R. centifolia). Impact on rose oil is not reported in India, which is a producer, but the virus has only recently been reported there.

Impacts would occur even before plants are killed, as the disease affects flower production. Organic production has increased to answer the demand for organic high value rose products (Gunes, 2005; <u>https://ec.europa.eu/budget/euprojects/supporting-organic-rose-oil-production-bulgaria\_en</u>). Even if effective chemical treatments were found, control of the vector would be more difficult in such systems.

- the popularity of roses may decrease, which would have impacts on the rose industry (Tuffen, 2016, noting that this has been seen previously with diseases of ornamentals (Impatiens downy mildew led to many garden centres ceasing to stock Impatiens)).
- costs associated with the replacement of rose plants in private and public landscaping, or gardens open as a business (Tuffen, 2016).
- additional control costs to control the vector and the virus.
- impact on commercial production of other rose products (such as rose water, teas, jams).

*Uncertainty relating to economic impact*: whether places where rose is economically important (especially rose oil production) are favourable to the vector.

## **Environmental impacts**

Environmental impacts would occur if wild roses are affected and killed by the disease. Several wild species in the EPPO region are known to be susceptible (see section 9.3), such as *R. canina* and *R. rubiginosa* (=*R. eglanteria*), though no mention of mortality due to RRV-infection was found in the literature. Possible negative impacts would be:

- death of plants in habitats where rose is used in landscaping, hedges, game cover, slope stabilization or erosion control. In the UK, countryside hedgerows are considered to be a priority habitat (Tuffen, 2016).
- on native rose species, through killing of plants, including endangered species (see section 9.3).
- on animals : invertebrates that rely on *Rosa* spp., sometimes in a specific manner (e.g. the gall forming wasp *Diplolepis spinosissimae* is a specialist on *R. spinosissima*), species feeding on rosehips, and the impact on pollinators (from Tuffen, 2016). Apart from the case of a specific relationship, there would probably be alternative sources in the environment.

*Uncertainty*: susceptibility of native rose species in the EPPO region, role of roses in the environment and whether some native animal species depend on native rose species in the EPPO region.

## **Social impacts**

- loss of employment and income for people working in companies producing roses (e.g. nurseries, cut flowers), or in historical places with rose gardens etc.
- loss of employment and income in the production and transformation industry of rose flowers for oil. In Bulgaria, this industry involves ca. 65 000 people, mostly seasonal workers (Koracheva *et al.*, 2010). In Turkey, 8200 families grew oil roses in 2005 (Gunes, 2005). Yalcin-Heckmann (2016) indicates that 10000 farming households are involved in the production of rose in the Isparta area of Turkey (in addition to other types of agricultural products and animal husbandry).
- loss to rose germplasm repositories. Europe hosts some unique rose collections, such as the 'Europa-Rosarium Sangerhausen' (Germany), which is the largest rose collection in the world, and plays an important role as a budwood source and in research.
- loss of aesthetic value by damage and death of rose plants in parks, cities and gardens. Rose gardens would suffer losses. As in the USA, RRV has the potential to entirely destroy collections of roses as well as kill those grown in private gardens (Tuffen, 2016).
- roses have a significant cultural value in some EPPO countries, and are a national flower for several EPPO countries. In the UK, rose is one of the national emblems of England, and *R. spinosissima*, also known as Scots rose, is considered a symbol of Scotland (Tuffen, 2016, citing others).
- decrease of availability of rose products with cultural importance (such as jam of wild *R. canina*, rosehips of various species as a source of food and traditional medicine; rose water, heavily used in cooking in some regions). Rose petals or flower buds are sometimes used in tea and syrup, to flavour food or as candy. *Uncertainty*: extent to which those would be affected.

Given the above, the potential impact is rated as being similar as for the USA (high with a low uncertainty). The potential impact in countries producing rose oil (e.g. Bulgaria, Turkey, Morocco, France, Italy, Russia, Ukraine, Azerbaijan and Georgia) could be very high, with a moderate uncertainty relating to climatic suitability and susceptibility of the *Rosa* species used.

Rating of the magnitude of potential impact	Very low	Low	Moderate	High ✓	Very high
Rating of uncertainty			Low ✓	Moderate	High $\Box$

## 14. Identification of the endangered area

Potentially, any area where *Rosa* spp. are grown in the EPPO region is endangered. Areas most at risk would be areas of high densities of rose, and where *P. fructiphilus* can maintain high populations outdoors (the threshold for climatic conditions that would be favourable to *P. fructiphilus* has not been defined – see section 9.2), or areas of commercial rose production in glasshouses. The climatic requirements of *P. fructiphilus* are not known in details, and there are uncertainties concerning its ability to establish in very northern regions or where climates are more arid.

## 15. Overall assessment of risk

Rose rosette virus and its vector, the eriophyoid mite *P. fructiphilus*, have had high economic and social impacts in the USA. All species and cultivars of *Rosa* are considered at risk from the virus and vector, as no known tolerant or resistant species or varieties have been identified. The virus causes witches' broom, flower abortion or flower malformation, distorted leaf growth and reduction in cold hardiness, leading to mortality of roses.

Current measures in the EPPO region do not significantly reduce the probability of entry. Risk of entry on *Rosa* plants for planting (except seeds and pollen) is considered to be high with moderate uncertainty, and on cut flowers of *Rosa* it is considered to be low to moderate with moderate uncertainty. The likelihood of establishment in the EPPO region is considered very high. If introduced, the magnitude of spread would be moderate to high, due to the extensive trade in *Rosa* and because of the aerial dispersal of *P. fructiphilus*, with a moderate uncertainty.

As for the USA, potential impacts in the EPPO region could be high, and locally may be very high. The highest economic impacts are expected to be incurred by nurseries and areas producing rose products such as rose oil. Potential environmental impacts are expected to occur if native (especially endangered) *Rosa* species in the EPPO region are susceptible hosts. Social impacts would occur through the loss of employment and income in the production and transformation industry (especially for rose flowers for oil) and in those countries where *Rosa* has significant cultural importance. Potentially, the whole of the EPPO region where roses are grown is endangered by the pest.

The EWG considers RRV to be a high risk to the EPPO region. *P. fructiphilus* is considered to be a potential pest for the EPPO region, as vector of RRV and possibly through direct feeding damage (see section 4.1.1). Establishment of *P. fructiphilus* in the EPPO region in the absence of RRV would also increase the risk of the virus, as the vector is very unlikely to be eradicated if found in the wider environment and would spread quickly. The EPPO Panel on Phytosanitary Measures agreed with the EWG that measures to prevent the introduction of *P. fructiphilus* irrespective of the virus should also be considered.

## Stage 3. Pest risk management

#### **16.** Phytosanitary measures

#### 16.1 Measures on individual pathways to prevent entry into the EPPO region

Measures were studied for *Rosa* plants for planting (except seeds and pollen) (Annex 1) and measures for cut flowers were adapted from those. It is noted that cut flowers may be used for propagation, and this practice should be discouraged and prohibited.

The EWG recommended that measures should apply to all rose species: RRV has a wide host range amongst *Rosa* species, and there are no known resistant species or cultivars to date.

The situation where only RRV (and not *P. fructiphilus*) occurs in an area is considered highly unlikely, and the measures apply to situations where the vector may also be present on the plants.

Possil	ole pa	thwa	vs (in	Measures identified (see Annex 1 for details)
	of impo			
Rosa		for	planting	Pest-Free area (PFA) for RRV and <i>P. fructiphilus</i> + Plants packed in conditions preventing infestation by <i>P. fructiphilus</i> during transport.
				Plants grown under complete physical isolation (following EPPO Standard PM 5/8, see Annex 7 for specification) + Plants should be packed in conditions preventing infestation by <i>P. fructiphilus</i> during transport. [This may be possible only for small quantities of high value material]
				Pre- or Post-entry quarantine (at least one growing period) with visual inspection for RRV and <i>P. fructiphilus</i> and testing for RRV [This may be possible only for small quantities of high value material]. in the framework of bilateral agreements
				For tissue cultures: produced from mother plants tested and found-free from RRV and free from <i>P. fructiphilus</i>
Rosa o	cut flow	ers		PFA for RRV and <i>P. fructiphilus</i> + Cut flowers packed in conditions preventing infestation by <i>P. fructiphilus</i> during transport.
				Originating from plants grown under complete physical isolation (following EPPO Standard PM $5/8$ ) + Cut flowers packed in conditions preventing infestation by <i>P</i> . <i>fructiphilus</i> during transport.

## **16.2 Eradication and containment**

Eradication of *P. fructiphilus* (with or without the virus) would be very difficult if it spreads into the wider environment. It can be spread by wind over large distances and is difficult to eradicate by spraying insecticide and difficult to identify by visual inspection (microscopic size and may not cause symptoms on infested plants without RRV). Eradication of RRV may be more likely to be achieved, as the virus would not remain in the progeny of the vector (transovarial transmission is not known) and RRV-infected plants are more likely to be detected and destroyed. However, as the virus may be spread with the vector, eradication of RRV may only be possible if detected at an early stage, for example plants found infected that were recently imported. Public awareness would be useful for early detection of the virus (e.g. via pest alerts or communication via stakeholder groups) (Tuffen, 2016).

The following factors would make eradication and containment difficult:

- difficulty of detection (RRV and *P. fructiphilus*), linked to incubation period, highly variable and non-specific symptoms, tiny size of *P. fructiphilus*, cryptic life cycle (refuge-seeking), and mode of dispersal.
- wide presence of *Rosa* species in various environments (nurseries, production of cut flowers, retailers, wild, landscape, hedges, parks, gardens, rose gardens, etc.).
- whole plants need to be removed and monitored to ensure that regrowth from root fragments cannot serve as a source of inoculum.

The EWG underlined that there are no precise data on the dispersal capacity of the vector, which prevents an accurate determination of the size of an infested area and buffer zone (including in relation to destruction of plants and surveys). However, Epstein *et al.* (1997) showed that 12.5% of plants (on average) became symptomatic at 100 m, over 3 years after the introduction of infected plants. The EWG considered 200 m around the infested plants to be an appropriate precautionary minimum to take into account of the fact that absence of symptomatic plants does not mean absence of the virus. This distance may need to be adapted to local conditions (e.g. areas with strong wind regimes).

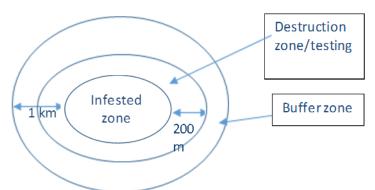


Figure 6: Suggested zones for an eradication programme

## An eradication programme should include:

- ascertaining the presence of RRV and destroying infested plants
- Testing suspect plants to confirm the presence of RRV and inspecting them for the presence of the vector.
- Prohibiting movement of rose plants at least from the site where the virus was found.
- Destroying any plant infected with the virus or infested by the vector, including its roots (to prevent regeneration from suckers). Precautions should be taken to avoid the spread of the vector (e.g. bagging plants before any manipulation to avoid dispersing the vector, destroying plants on site, rapid destruction of the plants after detection, acaricide treatments). Nearby host plants should be treated with acaricides.

## - delimitation and measures in the infested area:

- Delimiting an area around infected plants (with a minimum radius see above and figure 6) and conducting visual surveys. The delimited area should be extended as new findings are made. Trace-forward and trace-back studies should also be conducted to identify possible areas where infected plants might be present and to trace-back the possible origin of the outbreak.
- If eradication is deemed possible, destroying infected rose plants and other rose plants within a certain radius (e.g. 200m, see above) (including wild and cultivated roses, and including roots). Preferably all removed plants, or a representative sample of plants, should be tested and inspected for the vector before destruction, and if found, the delimitated area should be redefined.
- In a glasshouse, the whole glasshouse should be disinfested.
- Hygiene measures should be implemented to avoid spread (clothes, sanitation of tools, etc.).

- *delimitation and measures in the buffer zone*. The buffer zone (see figure 6) should initially extend at least 1 km from the infested area and may be modified based on subsequent surveys. Surveys of *Rosa* plants in late spring and summer should be carried out each year for at least 2 years for virus symptoms (due to the long incubation period) and for vector infestations, before the outbreak may be declared eradicated. Cases where surveys may need to be continued after these 2 years include large outbreaks, when the pest introduction is estimated to date back for several months, or in areas where plant density is high.

No *Rosa* spp. should be moved out of the demarcated area (infested area and buffer zone) until the eradication attempt has been declared successful. On a case-by-case basis, movement of tissue culture plants and plants grown throughout their lives under complete physical isolation (starting with virus-free material), in both cases proved to be free of the RRV and its vector, may be allowed. Awareness campaigns could be useful for additional surveillance.

The measures above are general measures. They would need to be adjusted depending on the specific outbreak situation (e.g. wild, nursery, garden etc.).

## **17. Uncertainty**

The overall uncertainty in the PRA is moderate, and more data on the following would be useful:

- biology and dispersal capacity of *P. fructiphilus*, and subsequent impact on virus spread and incidence, as well as on the global distribution of *P. fructiphilus*.
- whether there are other vectors, especially species present in the EPPO region, incl. *P. adalius* and *P. resovius*.
- other transmission modes
- host range and susceptibility of rose species in the EPPO region (to RRV and *P. fructiphilus*), in particular roses used for rose oil, as well as wild and endangered rose species.
- duration of the incubation period.

- sensitivities of existing assays and how soon after transmission they can detect RRV.
- trade into the EPPO region.
- situation in Canada, Mexico and India (presence of the vector, distribution within the country, impact).
- confirmation that records of rose rosette disease in North America pre-dating the description of the virus in 2011 were caused by RRV.

#### 18. Remarks

The EWG recommended that NPPOs of EPPO countries should conduct surveys for *P. fructiphilus* on rose on their territories, because *P. fructiphilus* may have a wider distribution than known so far (eriophyoids are difficult to detect and identify, are known to have moved between continents and may be present unnoticed). Although detection and identification are complicated, they are possible (detection should best imply the participation of a mite specialist, and molecular identification is possible, see section 4.1.4).

As part of wider biosecurity awareness raising, information on the risk of transporting rose plants and cut flowers can usefully be given at ports of entry, targeting passengers who may carry rose plants or cut roses in their luggage.

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## Annex 1. Consideration of pest risk management options

The table below summarizes the consideration of possible measures for plants for planting (based on EPPO Standard PM 5/3). Cut flowers were taken into account and a note made when the measures would differ from plants for planting. When a measure is considered appropriate, it is noted "yes", or "yes, in combination" if it should be combined with other measures in a systems approach. "No" indicates that a measure is not considered appropriate. A short justification is included.

Option	Rosa plants for planting (except seeds, pollen) / Rosa cut flowers									
e	The current phytosanitary measures do not significantly reduce the probability of introduction									
	of RRV or its vector.									
	See some current measures in Table 3 in the main text of the present PRA.									
Options at the place	Yes in combination* (for measures marked with *, see after the table).									
	This would require inspection of the plants over a sufficiently long period. Symptoms of RRV would not be visible during the incubation period, and some symptoms are not characteristic of the virus at early stages. Infected plants may look healthy after symptomatic parts have been removed. <i>P. fructiphilus</i> may not be detected, due to its small size and due to it being hidden in protected places on the plants.									
<b>č</b> 1	Yes in combination*.									
production	Reliable tests exist (see section 2.2 Detection and identification). However, testing is not very effective on its own. Depending on the stage of infection, the virus may not be detected as it takes some weeks for the virus to move throughout the plant (see section 2.1.5) and can be present at low concentrations. Testing is most reliable when samples are taken from fully emerged new shoots.									
	This is a measure recommended by MPI (2016) in the framework of a post-entry quarantine, with testing done on leaf material collected during spring or spring-like conditions.									
Treatment of crop	Yes in combination*.									
	Acaricide sprays may eliminate part of the vector population, but not all, and not the virus.									
Resistant cultivars	No. There is currently no known resistant ornamental cultivar or species.									
plant, growth stage	For tissue culture, yes in combination. Tissue culture plants should only be derived from mother plants that have been inspected and found free from <i>P. fructiphilus</i> and tested (preferably twice during appropriate times of the year) and found free of the virus.									
harvest										
	No for other types of plants: the virus and the vector may be in the plants at any stage.									
	A certification scheme could include measures to exclude the virus, but the EWG considered that no combination of measures can effectively exclude the vector.									
Growing under complete physical isolation	Yes. In principle yes, but in practice very difficult due to the size of <i>P. fructiphilus</i> . This may be feasible only for small quantities of high value material. This would imply the use of healthy planting material and other conditions as defined in EPPO Standard PM 5/8 (EPPO, 2016b) for airborne pests (details are given in Annex 7).									
	This is also theoretically possible for cut flowers, but unlikely to be used in practice.									
Pest free production site and pest free place of production	Only by growing under complete physical isolation, see above.									
Pest-free area	Yes, following ISPM 4. The pest free area (PFA) should be established on the basis of surveillance. The exporting country should provide surveillance data to demonstrate that RRV and <i>P. fructiphilus</i> is absent from all or part of its territory and information on how pest freedom is maintained.									
	For a country where RRV and <i>P. fructiphilus</i> are present in part of the country, measures should be in place to prevent the movement of infested plants to the PFA. Delimiting surveys should be conducted to determine the exact distribution of both the virus and the vector.									
	There should be restrictions on the movement of rose material into the PFA, as well as on tools that may carry <i>P. fructiphilus</i> .									
	The Panel on Phytosanitary Measures did not agree with the alternative suggested by the EWG (PFA only for RRV, with visual inspection for RRV where treatments are applied to control the vector). This option would allow trade from areas where the vector is widespread but the virus									

Option	Rosa plants for planting (except seeds, pollen) / Rosa cut flowers
	is absent but it would have a lower risk reduction level. The Panel considered that acaricide
	treatments would not provide complete control of the mite.
<b>Options after harve</b>	est, at pre-clearance or during transport
Visual inspection of	Yes in combination* for RRV, only on non-dormant material, but early infections may not be
consignment	observed.
	No for <i>P. fructiphilus</i> , because the mite is hidden and may not be detected (it would be practically impossible).
U	Yes for tissue culture. However, the reliability is limited by the sample size that can be taken
commodity	in practice.
	Yes in combination for <b>other plants for planting</b>
consignment	No. No phytosanitary treatment has been developed for RRV and <i>P. fructiphilus</i> to date. Acaricides may be applied, but they may not control the vector completely, and would not control the virus.
	No. RRV may be in different parts of the plant (including the roots), and the vector may be as
	well (except for the roots).
product, which can	
be removed	
	Yes, in combination*, for some measures. Plants should be packed in conditions preventing
	infestation by <i>P. fructiphilus</i> during transport.
method	<i>P. fructiphilus</i> or RRV would not remain associated with packaging material on its own.
	e implemented after entry of consignments
Post-entry	Yes.
quarantine (or pre- entry quarantine)	The plants should be kept in post-quarantine (or in pre-entry quarantine) for a sufficient period. Given the reported incubation periods, at least one growing period would be appropriate, with visual inspection for RRV and <i>P. fructiphilus</i> , and testing for RRV.
	This may be feasible only for small quantities of high value material. These options should only be implemented in the framework of bilateral agreements.
Limited distribution	No. This is not suitable for plants for planting.
of consignments in	
time and/or space	
or limited use	
	No. This option will reduce the probability of introduction (entry and establishment) to only a
	very limited extent. Early detection is difficult, and natural spread would complicate
	eradication. Import of infested plants would present a risk for any part of the EPPO region.
	Roses are widespread in the EPPO region in many environments.

\*The EWG considered whether the measures identified above as 'Yes in combination' (listed below) could be combined but concluded that no combination can effectively prevent entry of the vector.

'Yes in combination' measures:

- Visual inspection at place of production for RRV and P. fructiphilus
- Testing at the place of production for RRV
- Treatment of the crop against *P. fructiphilus*
- [For non-dormant material only], visual inspection of the consignment for RRV
- Testing of the commodity for RRV (plants for planting other than tissue culture)

## Annex 2. Symptoms of RRV and development of the disease

Symptoms include:

- on leaves (Fig. 1, 2, 3)
  - Unusual red colour. The new leaves of many rose cultivars have reddish pigments; however, on healthy plants, the reddish colour disappears as leaves mature, while it remains on infected plants (Babu *et al.*, 2015). Some plants show only a less striking reddish-pink colour at the underside or margins of leaves (Hong *et al.*, 2012).
  - Distortion (Babu *et al.*, 2015), e.g. enation (Di Bello *et al.*, 2017), strapped (unusually long, thin) leaves (Windham *et al.*, 2016), rough texture (Hong *et al.*, 2012).
  - o Leaf mosaic (Babu et al., 2015).



Fig. 1. Leaf mosaic and mottling on a rose infected with RRV (P. Di Bello)



Fig. 2. Leaf patterns in an early stage of RRV infection (P. Di Bello)



Fig. 3. Leaf distortion and reddened shoots on an infected rose (P. Di Bello)

- on stems and branches (Fig. 3, 4, 5)
  - Rapid elongation of new vertical and lateral shoots with pronounced red pigmentation (Crowe, 1983; Baker *et al.*, 2014), followed by breaking of axillary buds, leading to witches' broom, which is often associated with plants symptomatic for more than one year (Babu *et al.*, 2015; Windham *et al.*, 2016).
  - $\circ~$  Shortening of internodes (Ward and Kaiser, 2012), leading to stunting.
  - Thorn proliferation: excessive amount of pliable thorns (Baker et al., 2014).
  - Dieback of shoots and blackening and death of the canes (Hong *et al.*, 2012) due to increased susceptibility to cold injury (-2°C) and increased winter kill of whole plants (Epstein and Hill, 1995).
  - Uneven thickening of stems (Babu *et al.*, 2015), flattening of stems (fasciation) (Windham *et al.*, 2016). Canes may grow in a spiral pattern (Hong *et al.*, 2012). Succulent stems (Olson *et al.*, no date; Epstein and Hill, 1999).



Fig. 4. Reddened shoots on an infected rose (P. Di Bello).



Fig.5. Multiple witches' broom on a RRV infected rose (P. Di Bello).

- on flowers
  - Reduced flowering (Baker *et al.*, 2014), as distorted buds do not open in most cases (Windham *et al.*, 2016).
     Flowers that do form are distorted, with fewer petals. In some cases, petals and sepals are converted to leaf like tissue (phyllody) (Baker *et al.*, 2014).
  - Abnormal flower colour (Babu et al., 2015), for example mottling (Hong et al., 2012).

The development of the disease was described from *R. multiflora* as occurring in three stages (from Tuffen, 2016, citing Epstein and Hill, 1997, 1999):

- 1. In stage 1, symptoms are largely foliar, with leaves showing reddening as well as deformation such as elongation or crinkling. Shoots of affected canes are light pink to deep magenta and generally appear vigorous, though maybe more succulent than unaffected canes. Flowers are reduced and may be distorted.
- 2. Stage 2 is also known as the early rosette stage. Leaves will continue to be red in appearance and distorted. Lateral buds break dormancy and begin to grow which is the start of typical witches' brooming. Petioles are shortened giving a rosette appearance to symptomatic shoots. Flower formation is rare. Epstein and Hill (1997) states that at this stage light frosts (-2°C or lower) will result in visible damage to the affected leaves.
- 3. In stage 3 infected plants show intense rosetting, reduced leaves often hair-like and red in colour, witches' brooming with weak apical growth and chlorotic canes. Plants at this stage will seldom survive the winter.



Fig. 6. Various stages of *Phyllocoptes fructiphilus* feeding on an unopened flower. (P. Di Bello).

## Annex 3. Information on Phyllocoptes spp., morphology of P. fructiphilus and details on P. adalius

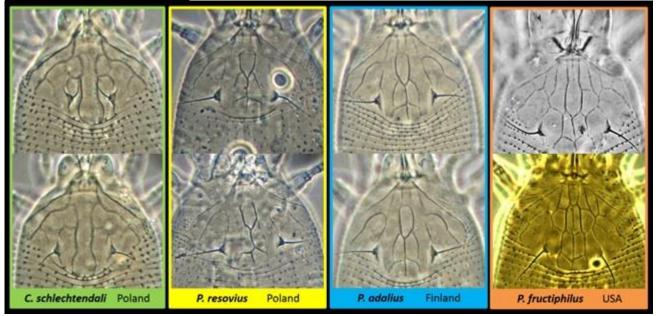
## Phyllocoptes spp.

The genus *Phyllocoptes* occurs worldwide and includes over 190 species (Vacante, 2015) (De Jong *et al.*, 2014 - Fauna Europaea lists 69 species for Europe), which have a narrow host range. Only 5 species are known to be associated with with *Rosa* in the world: *P. adalius* (syn. *P. rosarum*), *P. fructiphilus* (syn. *P. slinkardensis*), *P. chorites*, *P. linegranulatus*, and the newly described *P. resovius* (Druciarek and Lewandowski, 2016).

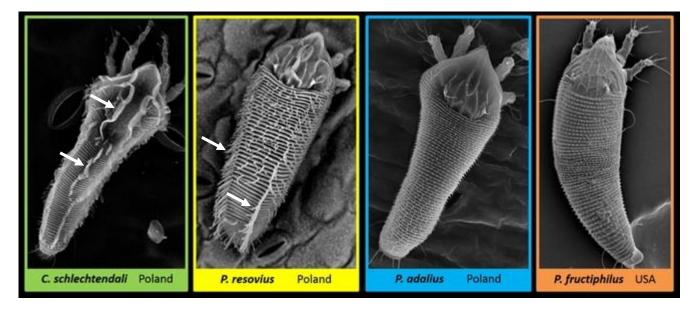
- *P. fructiphilus* and *P. adalius* are economically important, the former as a vector of RRV, and the latter in Poland due to feeding damage on roses in glasshouses (Druciarek *et al.*, 2014).
- *P. resovius* was described from glasshouse roses in Poland (rest of distribution unknown) and causes similar damage as *P. adalius*.
- *P. chorites* and *P. linegranulatus* are not known as pests and they are not known to be present in Europe (De Jong *et al.*, 2014).

Eriophyoids are poorly studied, their taxonomy and identification to the species level are complicated, and it cannot be excluded that there may be other species associated with *Rosa*, not yet collected or reported (Tuffen, 2016). The geographical distribution data in particular seem patchy (e.g. see *P. adalius* below).

#### Morphology of P. fructiphilus



**Fig. 1.** Light micrographs of prodorsal shield ornamentation for *Callyntrotus schlechtendali*, *P. resovius*, *P. adalius* and *P. fructiphilus* (T. Druciarek)



**Fig. 2.** SEM pictures of *Callyntrotus schlechtendali*, *Phyllocoptes resovius*, *P. adalius* and *P. fructiphilus*. Note the characteristic waxy secretions present in case of *C. schlechtendali* and *P. resovius* (arrows). Such structures are absent in case of *P. adalius* and *P. fructiphilus*. (T. Druciarek)

## Information on *P. adalius*

**Distribution.** Originally reported in the USA (California), and also reported from Finland, Sweden, Poland, China (Druciarek *et al.*, 2014, 2016, citing others), and Turkey (Denizhan *et al.*, 2015). This species probably has a wider distribution than currently known (poor knowledge of eriophyoids, small size, cryptic life cycle, etc.).

Hosts. At least Rosa canina, R. tomentosa, R. villosa (Ellis, 2007), R. bracteata, R. carolina, R. fendleri, R. multiflora, R. palustris, R. setigera, R. woodsii (Amrine 2002), cultivated rose (Druciarek et al., 2014).

#### **Detection and identification**. As for *P. fructiphilus*, see section 4.1.4.

**Biology.** The demographic parameters of *P. adalius* are studied in Druciarek *et al.* (2014). *P. adalius* is parthenogenetic (arrhenotokous), which is typical for Eriophyoidae. The maximum longevity was ca. 30 days for females. Each female produced about 28 eggs. The mean generation time was about 16 days, and doubling time was 3.3 days. *P. adalius* has a high capacity for rapid population increase on leaves and petals (up to  $340/\text{cm}^2$ ) in glasshouse rose production. Such high population density can lead to the aggregation of mites, leading to migration to other roses.

**Impact.** In Poland, *P. adalius* is one of the most important pests of rose production. In recent years, it has emerged as a serious problem in greenhouses, where mites can rapidly establish populations at high density on leaves and petals. *P. adalius* causes feeding damage, and symptoms range from simple mosaic-red discoloration and deformation of leaves to severely delayed bud development and stunting of the whole plant. The initial symptoms of leaf discoloration and malformation are especially evident on newly developed leaves that may already harbour hundreds of mites (Druciarek et al 2016, citing Labanowski 2009; Druciarek *et al.* 2014). It is not known if *P. adalius* is a pest in countries other than Poland (which may be the case due to a lack of surveys and studies).

**Control.** Given the high populations reported, control methods may be applied in glasshouses in Poland (but are not detailed in the publications consulted). Druciarek *et al.* (2014) noted that a few species of predatory mites have been studied for their potential for eriophyoid control, and that *Amblyseius swirskii* and *A. andersoni* may be promising candidates for the biocontrol of *P. adalius*.

# Annex 4. Some rose hybrids found to be hosts of RRV

Hybrids		
Hybrids with R. eglanteria		Allington et al., 1968 (observed to be apparently
, ,		infected with rose rosette)
Hybrids with R. multiflora		Allington et al., 1968 (observed to be apparently
,		infected with rose rosette)
R. rugosa x R. odorata		MPI, 2013 citing Epstein and Hill, 1999
R. x damascena x R. spinosissima		Windham et al. (2016) citing pers. comm-
Don Juan x (Rosa soulieana	All Ablaze Cl	Di Bello et al. (2015, 2017)
seedling x Trumpeter)		
Fragrant Cloud x Tradition	America Cl	Di Bello et al. (2015, 2017)
Jersey Beauty x Tiffany	Bellind's Dream	Di Bello et al. (2015, 2017)
Paul's Scarlet Climber x Gruss an	Blaze improved Cl	Di Bello et al. (2015, 2017)
Teplitz		
Rosa sempervirens ×	Bonica	Di Bello et al. (2015, 2017)
Mademoiselle Marthe Carron		
(Red Max Graf x seedling) x (Pink	Carefree Spirit	Di Bello et al. (2015, 2017)
Meidiland x Immensee)	·	
Seedling of Carefree Beauty x	Double Knock-Out	Di Bello et al. (2015, 2017)
seedling of Razzle Dazzle		
(Queen Charlotte x Della Balfour) x	Easy Does it	Di Bello et al. (2015, 2017)
Baby Love		
(Wimi x Rouge Meilland) x	Francis Melliand	Di Bello et al. (2015, 2017)
Margaret Merril		
(City of San Francisco x Baby	Home Run	Di Bello et al. (2015, 2017)
Love) x Knock Out		
Robin Hood x Virgo	Iceberg	Di Bello et al. (2015, 2017)
[(Voodoo x Rosa soulieana	Julia Child	Di Bello et al. (2015, 2017)
derivative) x Summerwine] x Top		
Notch		
Carefree Beauty seedling x Razzle	Knock Out	Di Bello et al. (2015, 2017)
Dazzle seedling		
(Tamango x Tchin-Tchin) x Patricia	Marmalade Skies	Di Bello et al. (2015, 2017)
Sport of Double Knock Out	Pink Double Knock Out	Di Bello et al. (2015, 2017)
Sport of Knock Out	Pink Knock Out	Di Bello et al. (2015, 2017)
Charlotte Armstrong x Floradora	Queen Elizabeth	Di Bello <i>et al.</i> (2015, 2017)
Brite Eyes x Alaska	Sunny Knock Out	Di Bello <i>et al.</i> (2015, 2017)
(Carefree Beauty x Yakimour) x	Sunshine Daydream	Di Bello <i>et al.</i> (2015, 2017)
Christopher Columbus		
Showstopper x seedling	Veterans Honor	Di Bello et al. (2015, 2017)
Ruby Ruby x (Neon Cowboy x	Yabba Dabba Doo	Di Bello <i>et al.</i> (2015, 2017)
Flower Carpet)		

## Annex 5. Imports of rose plants for planting and cut flowers into the EU (from Eurostat)

- 0 indicates quantities below 100 kg
- EU countries without imports were deleted from the tables below

# Plants for planting (commodity code 06024000 - roses grafted or not) (in 100 kg)

<b>^</b>	USA					Canada				India					
	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016
Austria	:	:	:	:	:	:	:	:	:	:	:	:	:	2	:
Belgium	:	:	:	0	:	:	:	:	:	:	:	:	:	:	:
Bulgaria	:	:	:	:	:	:	:	:	:	:	1	:	:	:	:
Germany	0	:	:	:	0	:	0	:	:	:	:	:	:	:	:
Denmark	:	1	:	:	:	:	:	:	:	:	:	:	:	:	:
Finland	11	0	1	:	0	0	0	0	0	0	:	:	:	:	:
France	2	0	0	5	3	:	:	:	0	:	:	:	:	11	4
Hungary	:	:	:	:	0	:	:	:	:	:	:	:	:	:	:
Italy	:	2	3	2	2	:	:	:	:	:	:	:	:	:	:
Netherlands	:	:	:	:	0	:	:	:	:	:	:	:	:	:	:
Portugal	:	:	:	:	:	:	:	:	:	:	:	3	:	:	:
Sweden	0	8	7	24	0	:	:	:	:	:	:	:	:	:	:
EU28	13	11	11	31	5	0	0	0	0	0	1	3	:	13	4

# Plants for planting (commodity code 06024000 - roses grafted or not) (as 'supplementary quantity')

	USA						Canada					India				
	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016	
Austria	•••	• •	•••	:	•••	•••	• •	:	• •	:	:	:	:	30	:	
Belgium	•••	• •	•••	200	•••	•••	• •	:	• •	:	:	:	:	:	:	
Bulgaria	•••	• •	•••	:	•••	•••	• •	:	• •	:	5	:	:	:	:	
Germany	57	:.	•••	:	69	•••	16	:	•••	:	:	:	:	:	:	
Denmark	:	113	•••	:	•••	•••	•••	:	•••	:	:	:	:	:	:	
Finland	7 900	1 400	6 225	:	400	4 082	3 982	3 130	2 297	2 369	:	:	:	:	:	
France	607	146	582	4 641	2 976	:	:	:	12	:	:	:	:	14 225	4 600	
Hungary	:	:.	•••	:	3	•••	•••	:	•••	:	:	:	:	:	:	
Italy	:	1 010	1 229	825	695	:	:	:	:	:	:	:	:	:	:	
Netherlands	:	:	:	:	136	:	:	:	:	:	:	:	:	:	:	
Portugal	:	:	:	:	:	:	:	:	:	:	:	10 000	:	:	:	
Sweden	2 567	3 160	6 246	12 920	6 520	:	:	:	:	:	:	:	:	:	:	
Total	11131	5829	14282	18586	10799	4082	3998	3130	2309	2369	5	10000	0	14255	4600	

# Internal EU trade (rose plants for planting). Reported by EU 28 with partners EU28, imports and export)

	2012	2012	2013	2013	2014	2014	2015	2015	2016	2016	
	in 100 kg	Supplementary quantity	in_100kg	Supplementary quantity	in_100kg	Supplementary quantity	in_100kg	supplementary_quantity	in_100kg	supplementary_quantity	
Import	200 800	46 782 178	203 172	50 722 319	219 761	52 355 588	235 414	54 705 302	292 875	58 193 05	
Export	215 531	71 351 275	225 098	71 044 725	215 086	67 329 512	201 914	60 631 093	214 993	63 579 38	

## Rose cut flowers (commodity code 06031100) (in 100 kg)

	USA	USA					Canada					India				
	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016	
Austria	:	:	:	:	:	:	:	:	:	:	:	:	0	:	:	
Belgium	:	:	:	:	:	:	:	:	:	:	:	:	1	:	:	
Cyprus	:	:	:	:	:	:	:	:	:	:	20	50	83	39	25	
Germany	:	0	:	:	:	:	:	:	:	:	:	0	:	:	4	
Spain	:	:	:	:	:	:	:	:	:	:	:	:	0	:	:	
France	0	2	0	0	:	:	:	:	:	:	:	0	:	:	:	
UK	:	:	:	5	:	:	:	:	:	:	2 232	3 623	4 665	6 981	8 672	
Greece	:	:	:	:	:	:	:	:	:	:	118	60	193	174	86	
Croatia	0	:	:	:	:	:	:	:	:	:	:	:	:	:	:	
Hungary	:	:	:	0	:	:	:	:	:	:	:	:	:	:	:	
Italy	:	:	:	:	:	:	:	:	:	:	18	:	11	:	10	
Latvia	:	:	:	:	:	:	:	:	:	:	4	:	:	:	:	
Malta	:	:	:	:	:	:	:	:	:	:	2	:	:	:	:	
Netherlands	:	:	4	2	2	:	:	:	:	:	721	736	1 072	140	958	
Poland	:	:	:		0	:	:	:		:	:	:	:	:	:	
Sweden	1	:	:	0	0	:	:	:	:	:	13	0	:	:	:	
EU28	1	2	4	7	2	:	:	:	:	:	3 128	4 469	6 025	7 334	9 755	

#### Rose cut nowers (commodity code 06031100) (as 'supplementary quantity')

	USA					Canada					India				
	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016
Austria	:	:	•	:	:	:	:	:	:	:	:	:	480	:	:
Belgium	:	:	•	:	:	:	:	:	:	:	:	:	3 000	:	:
Cyprus	:	:	:	:	:	:	:	:	:	:	46 580	97 480	107 120	80 520	57 3
Germany	:	477	:	:	:	:	:	:	:	:	:	400	:	:	17 5
Spain	:	:	:	•	:	:	:	:	:	:	:	:	1 800	:	:
France	1 000	3 600	1 980	50	:	:	:	:	:	:	:	368	:	:	:
UK	:	:	:	8 800	:	:	:	:	:	:	7 939 530	12 466 331	15 546 388	21 886 721	27 478 (
Greece	:	:	:	:	:	:	:	:	:	:	428 440	219 260	690 540	596 840	330 6
Croatia	200	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Hungary	:	:	:	1 000	:	:	:	:	:	:	:	:	:	:	:
Italy	:	:	:	:	:	:	:	:	:	:	59 700	:	40 320	:	33 5
Latvia	:	:	:	:	:	:	:	:	:	:	4 700	:	:	:	:
Malta	:	:	:	:	:	:	:	:	:	:	6 000	:	:	:	:
Netherlands	:	:	7 850	4 650	3 976	:	:	:	:	:	2 839 653	1 440 405	2 104 024	272 830	1 950 2
Poland	:	:	:	:	16	:	:	:	:	:	:	:	:	:	:
Sweden	4 000	:	:	0	9	:	:	:	:	:	57 100	1 000	:	:	:
Total			9830	14500	4001	0	0	0	0	0	11381703	14225244	18493672	22836911	298673
Internal trad	o (roso au	t flowors	) (commo	dity ondo	0603110	D Donos	tod by F	II 28 with	nortnor	G FU28 in	nnorts and av	nort	•	•	·

#### Internal trade (rose cut flowers) (commodity code 06031100). Reported by EU 28 with partners EU28, imports and export

	2012	2012	2013	2013 2014		2014	2015	2015 2015 2		2016	
	in 100 kg	supplementary_quantity	in_100kg	supplementary_quantity	in_100kg	supplementary_quantity	in_100kg	supplementary_quantity	in_100kg	supplementary_quantity	
Import	1 386 973	2 645 863 863	1 454 043	2 838 981 629	1 470 228	3 083 733 660	1 930 555	3 955 546 187	1 975 173	4 303 370 3	
Export	1 397 210	4 002 911 075	1 348 348	3 726 112 300	1 395 891	4 160 933 325	1 579 668	3 196 312 095	1 601 117	3 244 381 1	

#### Annex 6. Basic comparison of climate between the area where RRV and P. fructiphilus are present and the EPPO region

P. fructiphilus has been reported from climates Dfa, Dfb, Cfb (see Fig 1a and Fig 2).

According to the published literature, rose rosette disease is found in the following climates: Csb, Cfb, Cfc, Dfb, Dfa, Bsk (Fig 1b and Fig 2)



Fig 1a Distribution of P. fructiphilus in USA according to E. de Lillo and J. Amrine, unpubl. databases, and Fig 1b Distribution of RRV in the USA and Canada according to EPPO GD (from EPPO Global Database, 2017. Whole States are marked and not the distribution within states)

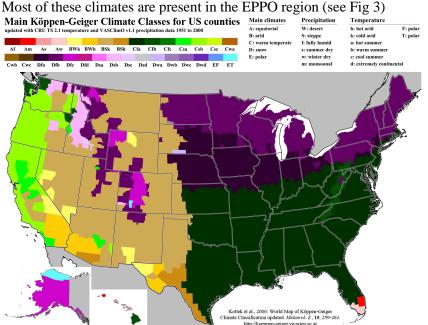
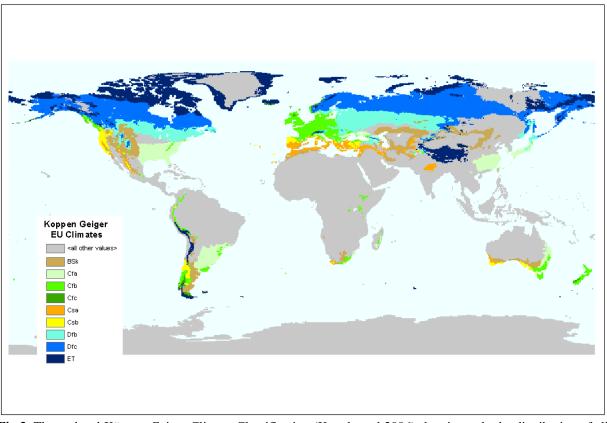


Fig 2. Map of Köppen-Geiger climate classes in the USA (from Kottek et al., 2006)



**Fig 3.** The updated Köppen-Geiger Climate Classification (Kottek et al 2006) showing only the distribution of climates that occur in the EU (note: colours different from those in fig. 2)

## **Comparison of plant hardiness zones**

To assess the potential northern limit of the area of potential establishment of *P. fructiphilus*, the presence of the pest in plant hardiness zones was checked. *P. fructiphilus* is present in zones above 6a in the USA (e.g. Ithaca, marked by a star on Fig 4. (and rose rosette disease has also been reported in plant hardiness zones 5). Plant hardiness zones 6 correspond to the South of Finland and Norway in the EPPO region (see Fig 5).

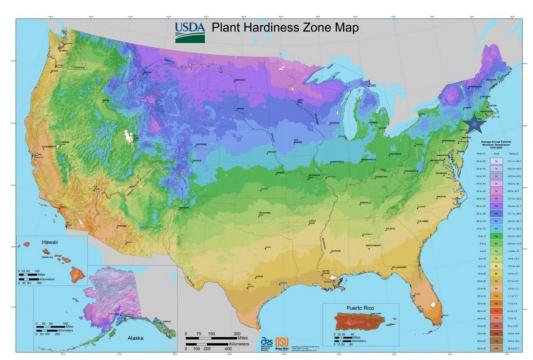


Fig 4: USA hardiness zones (source USDA, 2012)

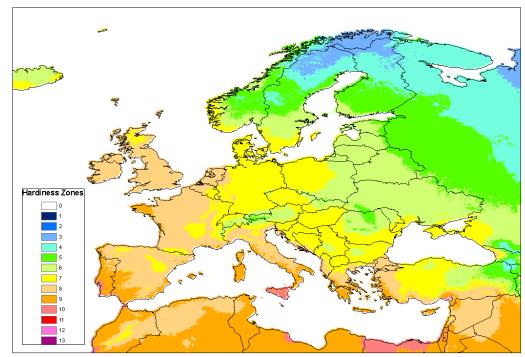


Fig 5. Hardiness Zones in Europe updated by Magarey et al. (2008)

# Annex 7. Specific requirements for Growing plants under physical isolation

Facilities where Rosa plants are grown should implement requirements as defined in PM 5/8 *Guidelines on the phytosanitary measure 'Plants grown under complete physical isolation'* 

Facilities should be approved by the NPPO according to the criteria detailed in this Standard.

The following general measures need to be implemented to guarantee and maintain pest freedom status, and to ensure that the measure 'complete physical isolation' will be effective:

- The structure should be free from *Phyllocoptes fructiphilus* before starting production.
- Access to the structure should be limited to trained and authorized personnel.
- All Rosa plants for planting for production that enter the structure should be free of the pest concerned and/or its vectors, and freedom should be verified prior to introduction.
- Other plants or plant products that could potentially carry *Phyllocoptes fructiphilus* should not be introduced into the structure.
- Growing media or any material (e.g. plant containers and boxes) likely to carry *Phyllocoptes fructiphilus* which are introduced into the structure should also be free from *Phyllocoptes fructiphilus*
- Traceability of any plant for planting that is introduced should be guaranteed.
- The risk of entry and movement of RRV and *Phyllocoptes fructiphilus* with the personnel working in the structure should be evaluated and mitigation measures taken if necessary (e.g. use of different working clothes in different areas).
- The entire structure should be inspected regularly to ensure physical integrity, in particular following meteorological events. These inspections should be recorded.
- Regular inspections of all plants for signs and symptoms of pest infestation being produced under complete physical isolation should be carried out during the growing period to monitor any possible breach in the system. This should include trapping and laboratory testing of plants showing suspicious symptoms and/or where appropriate of asymptomatic plants. Inspections should be recorded.

Good production practices such as regular sanitation of the site of production (e.g. absence of weeds and cleaning or disinfection of the whole site of production at the end of production period) are also recommended. Establishment of a footbath or a foot mat at the entrance is also recommended. Establishment of a buffer zone surrounding the structure may be appropriate, for example a hostfree zone or taking control measures to reduce pest or vector prevalence.

Specific measures to be requested for RRV and Phyllocoptes fructiphilus:

- Glass structure (or equivalent solid material) or Plastic structure (such as polyethylene)
- Windows locked shut
- Double doors
- Positive airflow at entry points
- Cleaning and disinfection of footwear before entering the structure, or the use of dedicated footwear
- Cleaning and disinfection of machinery before entering the structure, or the use of dedicated machinery
- Cleaning and disinfection of tools before entering the structure, or use of dedicated working tools
- Dedicated clothes

#### Consequences of a breach

In the event of a breach (e.g. if RRV or *Phyllocoptes fructiphilus* are detected within the structure or there is physical damage to the integrity of the structure), plants grown within the structure should no longer be considered as free from the RRV and *Phyllocoptes fructiphilus*. The NPPO should be notified. It is the responsibility of the NPPO to decide on the appropriate corrective action.