SHARCO CONSORTIUM

Identification and evaluation of management options for Sharka disease[1]

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Scope of the document: Management options which can be applied to limit the risk of entry, establishment and spread of the Plum Pox Virus in the European Union. To be added to the SharCo ~~Risk Management System.~~[1] Revision, by the SharCo consortium, of the management options listed in the January 2011 PRA issued from the Dutch Plant Protection Services

[1]

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Appendix II: SharCo Cultivation Guidelines, (SharCo Deliverable DA1.2, http://www.sharco.eu)

Appendix III: ISPM 27 Annex 02; DP 2 (2012): Plum pox virus (IPPC hosted by FAO) (Published 10-05-2012). https://www.ippc.int/file_uploaded/1336641118_DP_02_2012_En_2012-05-08.pdf.

This document is based on the initial Dutch PRA document, which was then modified, adapted and completed accordingly to the SharCo state-of-the-art (FP7/2007-2013, Project n°204429) concerning the potential measures of sharka containment (http://www.sharco.eu).

Glossary

Areas: Unless stated, the term 'area' is used to define a territory which can be part of a country or of a region and in which the prevalence of PPV is relatively homogeneous, either absent, or present sporadically or highly endemic.

PRA area: corresponds to the zone of application of the European directive 2000/29

1. Introduction

PPV can be managed by multiple approaches such as certification programs for the production of PPV free propagation material, vector control, replacement of old orchards with PPV resistant cultivars and case by case eradication programs. Management strategies, which always rely on PPV detection and identification methods, can be specifically geared towards different circumstances, e.g. depending on the geographical areas in Europe, the prevalence of the virus strain(s) and the local agronomical situation (for example, small gardens versus large orchards). Here, we will discuss successively different methods and strategies to manage the disease as follows:

1) Detection and inspection methods
2) Eradication of infected trees (nurseries, orchards, public and private gardens, etc…)
3) Pre- and post-entry quarantine inspections
4) Vector control and interference with the transmission process
5) Resistant cultivars and rootstocks
6) Pest free production zones
7) Implementation of *Prunus* cultivation guidelines
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9) Relocation of the production sites for production of propagation material
10) Alternance of *Prunus* production sites with non-*Prunus* production blocks
11) Control of trade and/or traceability of the propagation material when marketable

Management options are only described for the main pathway of PPV spreading "Plants for planting and grafting of *Prunus* spp." because other pathways are highly uncertain or much less relevant (plants for planting of non *Prunus* spp., non-*Prunus* wild species, fruits of *Prunus* spp. or pollen flow) and since PPV is already present in many EU countries. The control of "Plants for planting and grafting of *Prunus* spp." involves three types of *Prunus* cultivation:

i) Mother plant collections or blocks

ii) Nurseries which are sites of production for propagation material (including seedlings, rootstocks and grafted plants).

iii) Orchards which are sites of stone fruit production.

However, because PPV is a quarantine pathogen and is regulated at the European level under the 2000/29 Directive, we will first discuss the current and basic PPV management measures that correspond to the application of the current phytosanitary legislation.

2. Current phytosanitary legislation (Council Directive 2000/29/EC)

In summary, the Council Directive 2000/29/EC requires that plants intended for planting of *Prunus* species susceptible to PPV have been: (a) raised under a certification scheme or derived from material maintained under appropriate conditions and tested for PPV at least once during the last 3 complete cycles of vegetation, and (b) prepared in a cultivation place in which (or in the vicinity of which) no symptoms of disease have been observed since the beginning of the last 3 vegetative cycles and (c) plants with virus symptoms have been rooted out (Annex IV, Part A, Section I, article 23.1 and Section II, article 16). The application of the current 2000/29/EC directive at the European level has been reviewed earlier by the SharCo project and the results of this review disseminated (see SharCo deliverable DA1.1, appendix I of this document).

Unfortunately, as verified in the sharka pest risk analysis, the current status of PPV spread in Europe remains very alarming since most of the European sites of production are fully contaminated and only some countries have areas[2] where either PPV is not present or where the disease is still under

[2] Definition of area: an officially defined country, part of a country or all or parts of several countries [ISPM No. 5 Glossary of phytosanitary terms 2007, International Plant Protection Convention, FAO]. In the case of this Sharka PRA, it concerns a territory which can be part of a country or of a region and in which the prevalence of PPV is relatively homogeneous.

control. This situation is partially due to the poor efficiency of the European regulation (2000/29) to control the virus for the following reasons:

i) unsatisfactory implementation of some requirements described in the 2000/29 Directive by some member states (MS) because these requirements are considered as non-realistic (for example 3 years suspension of the plant passport; measure extended in some countries to the whole "place of production");
ii) need to implement standard protocols and laboratory analysis for PPV detection, due to the poor reliability of the requested visual inspections,
iii) inefficient measures of control of the sanitary status of the mother plants mainly due to the imprecise definition in the 2000/29 Directive of the conditions of maintenance of mother plant trees (so called "appropriate conditions"),
iv) lack of harmonization of diagnostic protocols and methods,
v) Insufficient and imprecise definition of the so-called "immediate vicinity" of plant propagation locations, which is determinant for restriction of PPV spreading locally, especially in nurseries.

Other considerations have to be taken into account to guarantee pest freedom of the nursery or crop:

- Introductions in nurseries can occur by aphid transmission from the surroundings. Therefore, in areas where the virus and aphid vector species are present, testing of material once in a period of 3 years, which is the current minimum requirement, will not be sufficient.
- The "immediate vicinity" is interpreted differently by different MS, if this concept is applied at all. Since PPV may be spread by vectors over several hundreds of meters, a buffer zone of 250m without *Prunus* host plants is not sufficient to guarantee pest freedom of the crop especially in areas with high PPV prevalence. Moreover, PPV can be present in hosts without causing visible symptoms and removal of all *Prunus* host plants in the buffer zone should thus be recommended.
- The use of cultivars developing weak to no symptoms (so called "tolerant" varieties) will make frequent and intensive testing of individual trees even more necessary (however, all cultivar have to be sampled and tested, not only tolerant ones) .

For these reasons, the current EU requirements may be difficult, if not impossible, to implement in areas where the virus and vectors are prevalent or may not give high levels of guarantee that the crop is pest free. Bazzoni et al. (2008), have stated that the application of phytosanitary quarantine rules is the basic strategy to control and prevent the spread of Sharka but that this approach is ineffective in areas where Sharka is already endemic.

Examples of lax application of the 2000/29/EC directive can be found all over Europe. In areas where PPV is present at low prevalence, PPV infection cannot be fully excluded as indicated by incidental findings in nurseries or in propagation material, such as have been reported from the UK and the Netherlands (Mumford 2006a,b; Verhoeven et al., 2006, 2008). If PPV infection is found in mother plants, the official certification scheme in the UK requires that "…diseased trees are destroyed plus adjacent trees in the same row (for 5 m either side) and in the two adjoining rows (for 10 m). For other mother plants, only the diseased trees are destroyed. **Movement of material from infected sites can be licensed**, but certain criteria apply and the material can only be moved to a local market, e.g. within UK, not exported…" (Mumford 2006b).

Another example is the fact that in a few EU member states (the Netherlands, Poland), a shorter period than the 3 years virus-free observation period may be implemented depending on the situation, e.g. in cases where PPV is found in not more than 1 or 2 plants at the place of production. The procedure in such cases may include destruction of the infected plants and the host plants in the vicinity and intensive testing of other host plants at the production place.

In consequence, the proper application of the current 2000/29/EC directive is deeply questioned. Extra information on its application in different EU member states is available in the SharCo DA1.1

deliverable (see appendix I and www.sharco.eu). But in summary, the following conclusions can be drawn:

- ✓ Actions of PPV eradication in large areas of Europe where PPV is endemic are not realistic any more, and need to be substituted for by new and integrated control strategies. These must be aimed at reducing the impact of the sharka disease and at preventing the introduction of new, emergent or remergent PPV strains in those areas.

- ✓ Eradication still remains a primary objective in many areas of France, Italy, Spain and in some other European countries. These countries, with economically and socially important Prunus industries, run control or eradication programs developed at the national level; however an harmonized action, coordinated at the European level, is likely to be more effective.

- ✓ The production of PPV-free propagation material remains a major objective for every European country, in order to avoid the introduction and/or the spread of PPV (or of yet absent strains) in regions still clear of the virus. However, the current European rules, as described in the 2000/29 Directive, are not sufficient to ensure a satisfactory level of protection. In fact, these rules are mainly based on the stone fruit production in "areas known to be free from PPV", but this condition still exists or is verifiable only in very few places in Europe. In addition, the situation in terms of the implementation of these rules, was significantly degraded with the recent entry of novel MS in which Sharka is at the endemic stage.

The achievement of the objective (production of PPV-free propagating material) requires an important improvement of the present European policy concerning the control of sharka disease. Potential modification of the Directive should take into account the following points:

i) propose different levels of PPV containment depending on the situation in different MS or, within a MS, between specific and localized areas of the country,

ii) be more specific concerning the criteria and requirements for the establishment of nurseries and the production of stone fruit propagating material;

iii) include systematic and mandatory laboratory testing for PPV detection instead of rely only on visual inspections,

iv) require more severe conditions of maintenance, localization and inspections of the mother plants and limit their cultivation to specific plantations or blocks established and maintained under appropriate conditions to avoid PPV natural infection;

v) modify the present European regulation concerning the plant passport by introducing, for example, a rule that control (assessment through test certificates) or, better, forbids the circulation of potentially infected material from PPV-infected to PPV-free areas;

vi) reinforce and harmonize the eradication measures of PPV, or of specific PPV strains, in areas where such an objective is still realistic and achievable.

Some of those modifications should also apply to any area where *Prunus* plants are present (ornamentals, fruit production, wild species).

We are aware that it is quite difficult to plan such an ambitious program in a general Directive, as the 2000/29 is, so that the possibility to produce a 'PPV-specific' regulation could be considered.

3. Methods to manage the disease

Avant-propos: some herbaceous plant species have been reported as potential reservoirs of PPV from which aphids could transfer the virus to *Prunus* plants (see SharCo PRA document). Although it has been shown experimentally that PPV can be transmitted from infected herbaceous plants to *Prunus* hosts, the relevance of herbaceous plants in the epidemiology of PPV is presently questioned (see "Host range" in the SharCo PRA) and probably rather limited since the leaf surface of such plants is very limited as compared to the total leaf surface of infected Prunus hosts. Therefore, specific measures concerning the control, cultivation and/or eradication of such 'potential' hosts will not be considered in the following.

3.1 Detection and inspection methods

Visual inspection

Visual inspection does allow detection of PPV by its symptoms, especially during the period of active growth. Diagnostic symptoms range from mild to severe and vary with the climate conditions, virus strain, host species and cultivar (James and Thompson, 2006; Llácer and Cambra, 2006; Polák, 2006). Symptoms may disappear with the onset of hot weather and obviously during the winter period in which many nursery plants are commercialized. Almost all known apricot, plum and peach cultivars are susceptible to PPV, but may remain symptomless during a latent period of two or three years following initial contamination and could thus act as unseen reservoirs . Moreover, many trees fail to develop symptoms for several years following contamination (Quiot et al., 1995; 1978; Smith et al., 1997). Therefore, **even if the search of symptoms can be used to survey rapidly a large area and to get a first cleaning (this can be useful in some situations, at least to maintain the disease at a low rate), the lack of symptoms cannot be relied on as an absolute conclusive proof that a tree is not PPV-infected.** For this reason, absence of virus should be confirmed by large scale routine screening methods, especially in eradication programs, certification schemes and control of mother plant blocks for the production of virus free planting material. The sampling protocol and the detection method should be validated, so as to guarantee the accurate and reliable detection of limited amounts of viral targets during routine analysis.

Detection and diagnosis

An integrated approach, which includes biological indexing, serological and molecular assays, using validated reagents and methods, has been recommended in the EPPO protocol for PPV detection and characterization (OEPP/EPPO, 2004).

For biological indexing, budwood to be grafted must be collected from at least three different branches of each plant (this is critical because of the uneven distribution of PPV). The main indicator plants used for greenhouse indexing of PPV are seedlings of *P. persicae* cv. GF305, *P. tomentosa* or *P. persica* × *P. davidiana* cv. Nemaguard. The indicators must be graft-inoculated according to conventional methods such as bud grafting (Desvignes, 1999), using at least four replicates per indicator plant. The grafted indicator plants are maintained under standardized conditions and, after 3 weeks, are pruned to a few centimeters above the top graft (Gentit, 2006). Biological tests are sensitive but inapplicable for large scale testing.

Laboratory detection methods have been evaluated in order to produce and validate PPV testing (Cambra et al., 2006a, 2011; Olmos et al., 2008; Capote et al., 2009; Vidal et al., 2012). Immunological methods remain the most common approach for PPV detection and diagnosis (Cambra et al., 2006a, 2011). There are several broad spectrum antibodies for universal PPV detection, e.g. the reference 5B-IVIA PPV-specific validated monoclonal antibody (Cambra et

al.,1994) that has an extremely broad specificity towards all PPV strains (Candresse et al. 1998; Cambra et al.,2006a; García and Cambra, 2007; Barba et al., 2011; Candresse et al., 2011). Specific antibodies, e.g. strain specific monoclonal antibodies may be used for further characterization of PPV isolates and for PPV strain typing (Boscia et al., 1997; Myrta et al., 1998, 2000; Cambra et al., 2006a; Croft et al., 2008). Nevertheless, the PPV-M, PPV-Rec and PPV-T strains cannot be distinguished when using the PPV-M specific monoclonal antibody AL (Boscia et al., 1997) because the three strains behave as M-type as shown by Zagrai et al. (2009). In addition, some PPV isolates may not be recognized by monoclonal antibodies specific for the strain to which they belong (Candresse et al., 1998). Nucleic acid-based techniques can be used to characterize PPV isolates to the strain level, but partial or complete re-sequencing of the PPV genome is recommended and is more accurate to distinguish unambiguously PPV isolates (see SharCo Deliverable DE3.2 and DE1.4, see www.sharco.eu).

Conventional polymerase chain reaction (PCR) based assays (Wetzel et al. 1991, 1992; Levy and Hadidi, 1994; Olmos et al., 2002) are usually more sensitive than molecular hybridization or serological ELISA tests, but not practical for large scale surveys (López-Moya et al., 2000; García and Cambra, 2007). Currently, real-time RT-PCR assays for the universal detection of PPV (Schneider et al., 2004; Olmos et al., 2005) based on the use of spotted crude plant extracts on membranes or on the direct use of diluted plant extracts have demonstrated their efficiency in routine PPV detection and have been validated (Capote et al., 2009; Vidal et al., 2012). An IPPC (hosted by FAO) PPV protocol has been adopted after an international consultation (IPPC, 2012). This protocol for PPV-detection and characterization is an update of the OEPP/EPPO (2004) protocol, which includes, for the first time in international protocols, validated real-time RT-PCR methods for the sensitive, specific and accurate detection of PPV during all periods of the year, including latency in winter (Capote et al., 2009).

In addition, the combined use of ELISA and real-time RT-PCR detection methods increase the accuracy of PPV detection at any prevalence, because of coincidental results between both detection tests lead to a practical post-test probability of 100% in determining the disease status of the analyzed plants (Vidal et al. 2012).In general, is recommended the combination of two different detection methods (biological, serological and molecular) with the validated and recommended protocols and reagents, in order to officially support a critical positive PPV detection (OEPP/EPPO, 2004). Thus, the analysis by both laboratory diagnostic tests of critical material, such as mother plants, propagating plant material and plant material for exportation, is strongly recommended. At the same time it must be kept in mind that no method will allow the detection of a recent inoculation by aphid unless the sample contains the inoculated leaf (which of course is highly unlikely in real conditions).

Sampling and analysis

Appropriate sample selection is critical for PPV detection. Sampling should take into account the virus biology and local climatic conditions, in particular the weather conditions during the growing season. If typical symptoms are present, collect flowers, leaves or fruits showing symptoms. In symptomless plants, samples should be taken from at least one-year-old shoots with mature leaves or fully expanded leaves collected from the middle of each of the main branches (detection is not reliable in shoots less than one year old). Plant material should preferably be collected from the internal parts of the tree canopy. In springtime, samples can be flowers, shoots with fully expanded leaves or young fruits. In summer and autumn, mature leaves and the skin of mature fruits can be used for analysis.

Various sampling strategies can be used, according to the different steps/types of cultivation (mother plants blocks, nurseries, orchards, isolated trees) and the objectives (certification of virus-free plant

material, installation of a pest-free production zone, implementation of disease control measures aiming at limiting or eradicating PPV in production orchards…).

In orchards, hierarchical sampling has been widely used in the US and Canada for *Plum pox virus* surveillance. Leaf material is collected from groups of four trees (sample unit N) every 16 trees and tested by ELISA for PPV infection: thus, 25 % of all trees in an orchard are sampled and tested (Hughes et al., 2002). The sampling effort (N= the number of sampling units to be analyzed) may vary, depending on the desired level of precision of estimated disease prevalence and the actual disease prevalence. Thus, when disease prevalence is very low, the required N is very high (Madden and Hughes, 1999). In this situation and/or when all individual diseased trees have to be identified and removed, sampling 100 % of the trees, either individually or in composite of 4 trees will be preferred (Thompson, 2006). 100% of the isolated trees (cultivated, wild and ornamental PPV host Prunus) should be individually tested in eradication programs, in pest free protected production zones as well as in buffer zones. In nurseries, sampling strategies have been described elsewhere (see SharCo deliverable DE3.2 (www.sharco.eu).

The size of the sampling unit and the sampling scheme of each tree are critical to reduce the chance of missing an infection. PPV is known to be unevenly distributed within infected trees, and virus titer can fluctuate during a growth season. An adequate number of leaves must be sampled to overcome the irregular distribution of PPV within the tree and ensure that at least one positive leaf is selected. Leaves should be preferably selected around the canopy of each individual adult tree from the middle of each scaffold branch (see SharCo ML05). Re-sampling at intervals will reduce the chance of missing an infection.The number of individual leaves to be pooled will also depend on accuracy and sensitivity of the analytical (serological or molecular) method chosen. Preliminary experiments should be carried out to optimize the number of leaves to be pooled.

Analysis: The spot real-time RT-PCR technique can be successfully used throughout the year (at any vegetative or latency period) with composite samples of up to ten plants without compromising detection accuracy. By contrast, the ELISA procedure showed significant differences in detection accuracy depending on the period of year and the number of plants analyzed being the vegetative periods of spring and summer the best periods for PPV detection, followed by autumn (SharCo milestone ML5, www.sharco.eu). As a consequence, ELISA is not recommended for composite samples in winter during latency but it can be used in spring or summer, during the vegetative periods, on composite samples of 4 plants (SharCo milestone ML5, www.sharco.eu). The use of larger composite samples (up to 10 plants) can be used at any vegetative or latency period but only if using real-time RT-PCR. Single or composite samples should be constituted by 8-12 mature leaves or buds collected from adult tree or 3-4 mature leaves or buds per nursery plant.

PPV detection should be performed according to the following recommendations:

i) All mother plants, maintained or not in nurseries but not established under screenhouses or insect-proof nets or covers, must be individually tested every year. One third of the mother plants grown under insect-proof facilities should be sampled and analysed, individually or as pools of 4 plants, every year along 3 year-periods so that 100% of plants are analysed over a 3-year period. Testing should be performed using any recommended laboratory method (OEPP/EPPO, 2004 and IPPC, 2012), using at least 12 mature leaves or buds /adult plant.

ii) Any bud stick (about 10-12 buds) introduced in a nursery from an external origin (i.e. not originated in the nursery own mother plant block), should be analysed before grafting to ensure the PPV-free status. All budsticks are to be individually tested by ELISA using a portion of the apical and basal parts. Alternatively, if real-time RT-PCR is used, individual budsticks can be tested in pools of up to 10 budsticks. ELISA tests are recommended during the vegetative period and molecular tests at any period of the year. This recommendation is not necessary if the budsticks are originating from mother plants blocks established and controlled according to cultivation guidelines (see SharCo cultivation guidelines below)

iii) Nursery blocks of plants (rootstocks and grafted plants) cultivated in open field. To increase the efficiency of PPV detection in a nursery, it is recommended that the rootstocks and cultivars and combinations that are most susceptible to natural PPV infection (see SharCo deliverable DE3.1) be preferentially sampled and tested. 1) In nurseries located in PPV-free areas, annually test at least 1% of the rootstocks or grafted plants before commercialisation, using any recommended laboratory methods (OEPP/EPPO, 2004 and IPPC, 2012) during the vegetative period or using real-time RT-PCR during the dormant period[3]. ELISA tests have to be performed during the vegetative period using pools of up to 4 plants. Real-time RT-PCR analyses could be done at any period of the year, using groups of up to 10 plants. 2) In nurseries located in areas in which sporadic PPV outbreaks occur and a buffer zone of minimum 500 m is secured, same considerations apply to the testing strategy but at least 10% of the nursery plants should tested yearly. 3) In nurseries located in areas where PPV is endemic same considerations apply to the testing strategy as previous (point 2), but only if a buffer zone of minimum 1,000 m is secured. If such buffer zones cannot be secured, the alternatives are the production under screenhouse or relocation of the production site so that the above requirements are applicable. In any case the nursery plants should be tested before commercialization outside of the area of production However, we would recommend not establishing and maintaining nursery plots in open fields in such areas (see SharCo cultivation guidelines). If relocation of the nursery plots is not possible (see below point 9), we have to point out that testing even a significant percentage of nursery plants will never ensure at 100% trading of PPV-free *Prunus* plant material.

iv) Production orchards: 1) Estimation of PPV prevalence: All adult trees in an orchard could be analysed in pools of up to 4 plants, using any recommended laboratory methods (OEPP/EPPO, 2004 and IPPC, 2012) during the vegetative period or by real-time RT-PCR in the dormant period. Another option to estimate PPV prevalence is the analysis of the trees in an orchard using the hierarchical method (Hughes et al., 2002; Gottwald, 2006) (See comment above). 2) PPV eradication programs: If the purpose is the complete removal of infected trees in an orchard, sample 100% of the trees and analyse in pools of up to 4 plants, using by any recommended laboratory methods (OEPP/EPPO, 2004 and IPPC, 2012) during the vegetative period or by real-time RT-PCR in the dormant period. Collect at least 8 leaves or buds/tree.

v) Surveys to declare an extensive area PPV-free or free of a specific PPV strain: 1) clearly define the limits of the area with the help of aerial pictures based on geographical identification systems, 2) localize all susceptible Prunus spp. trees in the area, including regular orchards, wild, isolated, ornamental and backyard trees. Visual inspection during

[3] Dormant period means, here, winter or latency period, during the first year in nursery (grafted or not) and during the second year, before commercialization, when plants are remaining in the fields. Nevertheless, tests could be done on plants just before shipment.

blooming is recommended in order to locate isolated Prunus trees, 3) sample and analyse all PPV-susceptible hosts over a maximum period of four years. Analyse plants in pools of up to 4 plants, using any recommended laboratory methods (EPPO, 2004 and IPPC, 2012) during the vegetative period or using real-time RT-PCR in the dormant period. Collect 8-12 leaves or buds/tree.

Conclusions regarding the detection and inspection methods

- Visual inspection on its own is not sufficiently effective to guarantee PPV freedom of planting material.
- Biological, serological and molecular methods are available for detection, diagnosis and/or identification of any PPV isolate or of specific PPV strains in planting material.
- The probability of detection of an infected plant in an orchard or a nursery will largely depend on the percentage of infection, on the aggregation of cases and on the intensity of sampling (according to the defined objectives). Moreover, in a symptomless, but infected plant, detection probability will depend on the sampling intensity (because of the irregular distribution of PPV within a plant) and on the accuracy of the detection method (technique and reagents).
- No method, however sensitive, will allow the detection of a recent inoculation by aphid unless the sample contains the inoculated leaf itself (which of course is highly unlikely under real conditions).

3.2 Eradication programs

While present in the EU, PPV is currently considered a quarantine organism of mandatory eradication. Nevertheless, applying eradication measures in areas with a high PPV prevalence would mean the destruction of hundreds of orchards and millions of trees and consequently of the local or national Prunus industry. As a result, application of the European directive is dependent on each MS and of the situation faced by each MS. However, while eradication can appear unrealistic in areas where sharka is endemic, it can still be a solution of choice in other specific situations:

i) in case of entry in PPV-free area or in areas with sporadic outbreaks where the disease is still under control
ii) In case of entry of a new PPV strain in areas where this strain is not already present (i.e.: PPV-M in the Aragón region of Spain, Capote et al., 2010).
iii) Entry of a new PPV strain not present (yet) in the EU.

This management measure is indispensable in order to limit the establishment of new PPV strains or isolates in those new areas, especially in PPV-free protected zones (see below 'protected zones').

Eradication measures, of any PPV strain or of specific PPV strains, include:

i) Sampling and laboratory testing (serological and molecular tests following the EPPO and IPPC-FAO standards, see above) of any PPV host plants (including wild and ornamental hosts) or of specific hosts (i.e.: cherries in case of PPV-C) in an area from 500 m to several km around detected infection foci. The precise eradication perimeter should be decided by a specific scientific committee, who should take into account in its recommendation the specific situation of the outbreak (PPV strain, environment, ….).
ii) Prompt removal of any PPV infected plant (or plants infected with the specific PPV strain targeted) and, if recommended by the scientific committee, removal of all PPV host plants in the eradication zone.
iii) If individual orchards are infected at a significant rate, eradication of individual trees is often less efficient than removal of the whole plot. It is therefore recommended that the scientific committee should also make a recommendation about the threshold contamination rate dictating complete eradication of the orchard (rates of 5% or 10% are typically used).
iv) Three years of regular inspection surveys (sampling and laboratory testing) with no PPV

detection (or no detection of a specific PPV strain) before declaration of the success of the eradication program. Under some circumstances (particular host or PPV strain for example), the use of a longer period could be recommended upon analysis by the scientific committee.

3.3 Quarantine procedures

Pre-entry measures of quarantine

Following the 2000/29/EC directive, *Prunus* propagation material (as listed in the annexes of the directive but care should be taken to update the list of PPV host *Prunus* species, which is no longer in line with information from the scientific literature) can enter the EU area only under the preliminary agreement by the relevant National Plant Protection Service (PPS of entry of the material or PPS of the country where the material will be maintained in quarantine). If no phytosanitary certificate is produced by the third country, entry of the material requires a Letter of Authorization (LOA) produced by the PPS once guarantee is provided about the proper confinement of the imported material. In general, materials imported from third countries remain located in Centers of quarantine, which are acknowledged by the Ministry of Agriculture (quarantine stations, or research centers with adequate facilities) for all the time needed for the quarantine controls. This involves usually a limited amount of plant materials which, once proven healthy through negative testing results may be released to be propagated and disseminated in EU.

While this procedure was set up in order to prohibit the entry of any PPV infected material, thus limiting the introduction (entry, establishment and spread) of new PPV strains or isolates in EU, it is not fully satisfactory for the following reasons:

i. Some countries do not benefit from effective quarantine stations where the material can be properly confined (see SharCo deliverable DA1.1, appendix I) and in consequence cannot apply those quarantine measures
ii. In some specific cases, an exception of the directive 2000/29/EC (article 4, line 6) allows the entry and trading on the local market, without preliminary quarantine measures, of propagation material originating from neighboring third countries. However, once the new PPV strain/isolate is established in a EU MS, here considered as the 'local market', no quarantine measures are designed to prohibit/restrict the dissemination to other MS or areas. Moreover, some countries consider as 'local market' their whole national territory.
iii. In some cases, the procedure of sampling for quarantine testing does not reach 100% of the plant material introduced in EU and can be reduced to random sampling. Therefore it is not satisfactory especially in the case of latent (i.e.: recent and/or uneven distribution of the infection) or low titer infection (i.e.: dormant material trade in winter).

We thus recommend extra measures of post-entry or post-quarantine surveillance.

Post-entry and/or post-quarantine surveillance

Once the *Prunus* material entering EU from third countries, neighboring non-EU member states included, has been established, the first propagating plot(s) should be registered, sampled and all trees tested using reliable serological or molecular procedures. A regular inspection survey should be maintained, if possible, over 3 years. At the entry, the material should be properly labeled (see part 11 'traceability') so its origin, final use, propagation and destination must be recorded. Proper traceability of this material should be implemented also (see below 'Development of an European fruit tree traceability system' part 3.11 of this document).

Local quarantine measures

In some cases, when a PPV outbreak happens in a PPV-free area, a local quarantine measure should be applied, including i) delimitation of the foci, ii) establishment of an eradication program of infected trees, iii) surveys of any Prunus material present in the zone over at least 3 years, iv) restriction and control of movement of plant material out of this zone.

3.4 Vector control and interference with the transmission process

Legal and illegal transport of infected propagative plant material is the main pathway of spread of sharka disease over long distances. More locally, PPV is transmitted under natural conditions by a number of vector aphid species in a non-persistent manner, being generally *Myzus persicae* Sulzer and *Aphis spiraecola* Pagenstecher as the most efficient vectors (Labonne et al., 1995; Gildow et al., 2004; Cambra et al., 2006b). However, in Eastern and Central Europe, those aphid species are under-represented, therefore other vector aphid species (*Hyalopterus pruni, Rhopalosiphum padi* and *Phorodon humili*) are expected to play an important role in PPV spread (SharCo deliverable DE3.1 version N°2). The characteristics of Potyvirus transmission by aphids have long been known, including the virus–vector specificity and the existence of an auxiliary factor or helper component (HC) involved in the retention of the virus in the aphid stylet (Pirone and Blanc, 1996; Froissart et al., 2002; Moreno et al., 2009). Non-persistent aphid-transmission is a complex process which involves short acquisition, retention and inoculation periods. The number of PPV virions acquired and inoculated by a single *M. persicae* species has been estimated as well as the percentage of infected GF305 peach seedlings after a single probing (reported at 20%, Moreno et al., 2009). As for the other non-persistently transmitted viruses, it has been demonstrated that a large number of aphid species (more than 20), including many which are not hosted by *Prunus*, could act as PPV vectors (Labonne et al., 1995). It should be stressed that in this type of transmission, the infectivity is lost through the aphid's moults. Thus, the winged aphids are non-viruliferous when they take off for their first long distance emigration flight even if they were on an infected plant (and unless they have probed on their host after moulting and before departing, which is not reported as a normal behavior). This has two consequences on the virus dissemination: (i) the aphid species hosted by *Prunus* trees are not necessarily the main vectors, and (ii) the dissemination is mainly due to aphid visitors, i.e. aphids performing short range flights in their search for a suitable host plant. Consequently, long distance dissemination by viruliferous aphid species individuals is thought to be very unlikely at more than 1,000 meters from the source of inoculum. Due to these aphid transmission characteristics, it can be assumed that introduction of the disease into new territories more than a few kilometers away from an infection foci is the result of human trading activities in Prunus propagation material (that has escaped visual inspections or other inefficient control methods), while the local spread can be due to both aphid activity and local growing practices (i.e. top grafting). After its introduction, a key parameter for the spread of the virus is the quality of the inoculum sources available for aphids, the prevalence of the disease and the vector activity and propensity in a given ecological area.

The spread of the non-persistently transmitted viruses is not or only poorly affected by the use of insecticide treatments against aphid, as most of the vector aphids come from outside the crop and as the lethal action of the insecticides is not as quick as the transmission process. Moreover, in some instances, insecticides may increase, rather than suppress the spread of virus transmission by destruction of predators and parasitoids or by causing increased aphid activity. Another control measure, the biological control of the vector, is also not an effective strategy to reduce the spread of non-persistently transmitted plant viruses. Control strategies other than conventional aphicide treatments have thus to be identified and used.

Complete physical protection against aphids can be effective. Physical protection by cultivation under insect-proof facilities should be combined with intensive aphid species monitoring methods inside the protection screenhouse or tunnel and testing for PPV detection. Monitoring for PPV-viruliferous aphids could be a useful strategy to predict possible natural PPV infections (SharCo deliverable DE3.1). The use of horticultural mineral oils has been demonstrated as an alternative to conventional pesticides. Although the efficiency is not complete, and the strategy is inapplicable for orchard trees, the physical barrier an oil film covering plants represents has the potential to significantly reduce PPV spread in young nursery plants (Vidal et al., 2010).

Application of mineral oil has been shown to reduce significantly the level of PPV spreading (SharCo deliverable DE3.3; Vidal et al, 2010) in open-field nursery plots located in different European ecological areas with different PPV strains (SharCo WPE3, 2º Reporting Period; Vidal et al.,

(submitted)[4].

Physical protection may not be feasible or too costly for tree nurseries (except for the more basic material, see also below "Certification") but is highly recommended in case of mother plants blocks situated in PPV endemic regions or for the production of certified PPV free material in areas where PPV is established (See SharCo cultivation guidelines, deliverable DA1.2 appendix II).

3.5 Use of resistant cultivars and rootstocks

The use of PPV resistant *Prunus* material is a potentially very interesting strategy to limit the dissemination of the virus and the impact of the disease, both in nurseries and orchards. The complete resistance to PPV implies the inability of the virus to infect the plants, thus restricting the spread of the virus[5]. Unfortunately, the number of varieties identified as truly resistant to PPV is extremely limited (mainly for apricot) to non-existent (i.e. peach). In apricot, very few sources of resistance have been identified, for instance 'Stark Early Orange', 'Stella', 'Harlayne', 'OrangeRed', 'NJA2', 'NJA42' (Kegler et al., 1998; Martínez-Gómez et al., 2000; Badenes and Llácer, 2006; Bassi, 2006; Karayiannis, 2006; Krška et al., 2006). In plum, hypersensitivity to PPV has been described and few genitors are currently used to develop cultivars and rootstocks resistant to natural infection (Hartman and Neumuller, 2006). No true resistance to PPV has been identified in *Prunus persica* (peach) and the only solution lies in interspecific crosses with *P. davidiana* and *P. dulcis* (almond) (Decroocq et al., 2005; Pascal et al., 2002). Because of this limited number of sources of resistance together with problems linked to breeding of perennial plants (extended vegetative periods, labour-, space- and time- consuming experimentations), traditional breeding programs will not be able, in the short term, to provide the industry with enough resistant varieties.

New biotechnological strategies were assessed and validated in the last ten years (Scorza and Ravelonandro, 2006; SharCo deliverable DG2.3). It is now well established that ectopic expression of viral genomic sequences can confer specific antiviral resistance which is mediated by RNA silencing. More particularly for PPV, it was demonstrated efficient RNA silencing-mediated PPV resistance in 'HoneySweet' plum which was transformed with a PPV-CP construct (Scorza et al., 2001). In addition, new gene constructs based onto the use of intron-hairpin-RNA (ihRNA) strategy have been built and introduced into the plum genome. They confer a PPV resistance level equivalent to the 'Honeysweet' cultivar (SharCo deliverable DG3.3). Other strategies of interference with PPV RNA *via* the artificial miRNA approach (amiRNA) or the production by the host plant of PPV-specific scFv antibodies were also validated in the course of the SharCo project (SharCo deliverable DG2.3 and DG2.5). However, those strategies rely on the production of *Prunus* genetically modified plants which is i) not effective in every *Prunus* cultivated species (presently only European and Japanese plums are efficiently and reproducibly transformed, see (Scorza and Ravelonandro, 2006), ii) in many countries it is not accepted (yet). This might impede severely, in the near future, the implementation and the usefulness of biotechnology-derived PPV resistant stone fruit cultivars. In consequence, while resistance derived either from traditional breeding programs or from biotechnological approaches would be an excellent option (and will likely be in the future), it does unfortunately not fulfill the industry's needs at the moment.

Currently, partially resistant or tolerant cultivars are being deployed in Europe, especially in areas where sharka is endemic. Varieties presenting few, if any, symptoms on fruits are favored in those regions, in order to maintain feasible commercial fruit production. However, the risk of growing partially resistant cultivars is that the virus is still able to multiply in these hosts. Such trees can act as virus sources from which the virus can be spread by aphids (or by commercial movement of infected

[4] The use of oil treatments was recently approved and recommended by CFIA in Canada to prevent PPV spread in nurseries and young plantations

[5] As generally intended, the term "Tolerant" is used to define a germplasm that, following inoculation of PPV, get infected without significant virus restraint (as ascertained by laboratory assay) but remains symptomless on both leaves and/or fruits. Conversely, with the term "Resistant" is defined the germplasm in which the lack of symptoms is accomplished by the ability of the plant to restrict the multiplication and movement of the virus within the plant. When the resistance is absolute (i.e., the virus is not able to multiply in any kind of plant tissue) the germplasm is defined as "Immune".

planting material), thus increasing rather than lowering the impact of the disease for susceptible crops in the surroundings. As a consequence, those cultivars cannot be recommended in regions were control and/or eradication is still the target.

The behavior of the most common rootstock of Prunus grown under PPV natural inoculum pressure was reported (Vidal et al. 2010 and SharCo deliverable DE.3.1). Given the variability in susceptibility, the use of the less susceptible rootstocks in nurseries and plantation ('GF677', 'Garnem', 'Greenpac', 'Myrobolan BN4kr', 'Docera 6') is recommended as a means of reducing the likelyhood that young nursery plants could be contaminated through a rootstock source.

3.6 Pest free protected production zones

Pest free production area, sufficiently effective to guarantee pest freedom of the crop.

Intensive and frequent surveys, sampling and testing programs will be needed to confirm pest freedom. Such programs will especially be important in areas where *Prunus* host plants are regularly imported or where PPV is (still) not present and should be kept so for the production of PPV-free certified material or for the maintenance of mother plants blocks. PPV-free areas can be envisionned as representing part of a country, a region or a county, but strict rules will have to be applied depending on the area, the country. Given the pathogenicity of PPV and how it is difficult to fight, freedom of PPV is a big advantage for production and is possibly even more importantly for nurseries and the production of healthy planting material. The establishment of protected zones would restrict the circulation of Prunus material and thus prevent entry. Moreover, the existence of protected zones could allow the reduction of regulatory constraints on areas of endemicity by not imposing a general status that they cannot meet. Basically, feasibility is good because (1) protected zones have been implemented with success for other pathogens, (2) efficient detection techniques are available to ensure that testing of material at entry can be performed efficiently and effective quarantine measures implemented.

Recommendations for the establishment of PPV free zones

In addition to the above reported (3.1.vi- Detection and inspection methods) specific comments:

Selection of the zone: A bio-geographical area in which PPV does not occur on *Prunus* species (cultivated and wild material) and is officially declared free of PPV by the relevant National Plant Protection Service, based on appropriate sampling and testing protocols carried out during 3 consecutive years. We suggest using an intensive survey strategy, in order to secure the absence of PPV in the area. Visual inspections are not sufficient to detect low rate PPV outbreaks or latently infected material and will be complemented with both serological and molecular tests. In case of previous detection, to avoid problems associated with long latency periods, successful eradication is declared after 5 consecutive years of negative detection.

Conditions of establishment: A PPV-free area should have a minimum surface defined by the relevant Plant Protection Services and should be separated from PPV outbreaks under control by a "safety zone". This "safety zone" should be kept PPV-free. The areas respecting those conditions could apply for a "protected zone" status.

Consequently, when establishing new mother plants or nurseries blocks as well as orchards in the PPV-free protected zones, the only material allowed for planting or grafting will be certified PPV-free, produced either in PPV-free areas or under insect proof facilities.

Conditions of maintenance: Once a PPV-free zone has been declared and new propagation and/or production sites have been established, further introduction of *Prunus* material except seeds should be strictly limited, preferably only to certified "PPV-free" produced either in PPV free areas or under insect proof screen house . **Any plant material that can host PPV and can be used either for planting or grafting (budsticks, branches, rootstocks) but are originating from any non-PPV-free areas have to be forbidden of entry in the PPV-free protected zone**. The above conditions do not apply if the *Prunus* material is not addressed for planting or grafting in the protected zone but simply addressed for trading and only if it is entering and maintained temporally in the area in a dormant state.

Conditions of control and re-evaluation as PPV free: The status of the PPV-free areas has to be re-evaluated through a new survey every 3 years, by the relevant National Plant Protection Services.

Establishment and management of production areas to guarantee freedom from specific PPV strains

To some extent, the above measures also apply to areas free of specific PPV strains (ex: PPV-M in Spain) in order to prevent the introduction of propagation material infected with new PPV strain(s). In this case, this has to be based on appropriate sampling, testing and **strain typing protocols.**

3.7 *Prunus* cultivation guidelines

The formulation of cultivation guidelines aiming to better specify the actions needed to achieve the containment of sharka was one of the main goals of the WPA.1 workpackage of the EU project KBBE 204429-CE "SharCo". Those guidelines are a series of specific recommendations and agronomic strategies related to the establishment of nurseries and plantations and to their management, on how to handle the PPV threat and to overcome the risk of dispersal of new or emergent PPV isolates or strains (see annex below, appendix II). One major tool is the exclusive production and trade of PPV-free nursery plants and rootstocks, certified by a more 'credible' phytosanitary passport. This aspect will be discussed further on in part 3.8) Certification schemes. The full cultivation guidelines (SharCo deliverable DA1.2) are presented in the annexes, below (appendix II).

Scope of the document

Since different parts of the stone fruit production industry (nursery industry or fruit industry) play different roles in the spread of the Sharka, especially in case of long distance dissemination, different agronomical and cultivation approaches are necessary in order to have a rational control of the disease. Following the above consideration the SharCo guidelines have been divided in different sets, addressing the following productions:

- Mother Plant Blocks
- Nurseries, intended as sites of production of planting material
- Production orchards

Distinction was also made between areas presenting distinctive phytosanitary status (virus prevalence). Therefore, in each of the three proposed sets of guidelines [mother plants, nurseries, production orchards] a further differentiation has been made between areas i) PPV-free, ii) with PPV outbreaks under control, iii) with endemic presence of PPV.

The reason for this further differentiation is that in areas where the virus is endemic~~,~~ it is not realistic any more to insist on the eradication of the virus, therefore the actions must be aimed at (1) reducing the impact of sharka, (2) preventing the entry and spread of new PPV strains, (3) slowing down the contamination of new orchards, (4) preventing the spread of the pathogen to uncontaminated areas of the EU. In the areas where the virus is present with outbreaks under control~~,~~ the major objective remains disease containment or, even better, eradication, as well as the prevention of further introductions. Obviously, in uncontaminated areas the most critical objective is to avoid the introduction of PPV. Details on those *Prunus* cultivation guidelines are presented in the annex of this document.

3.8 Certification (Council Directive 2008/90/EC)

Council Directive 2008/90/EEC of 29 September 2008 is a recast version of Directive 92/34/EEC "on the marketing of fruit plant propagating material and fruit plants intended for fruit production" (http://ec.europa.eu/food/plant/propagation/index_en.htm; accessed November 2010). The directive contains the general requirements for the production of propagation material and fruit plants. Propagation material includes seeds and all plant material intended for the propagation and production of fruit plants. Fruit plants are defined as "plants intended to be planted or replanted, after marketing". The directive stipulates that plant propagation material and fruit plants of genera and species listed in the Annex, including *P. dulcis*, *P. armeniaca*, *P. avium*, *P. cerasus*, *P. domestica*

and *P. persica* may only be marketed if they are either CAC (Conformitas Agraria Communitatis), pre-basic, basic or certified material. The conditions to be met are more strict for pre-basic material and basic material and less strict for CAC material. Certified material should be produced directly from basic or pre-basic material.
More specific requirements which must be met for the production of the different types of material are laid down in Commission Directive 93/48/EEC ("setting out the schedule indicating the conditions to be met by fruit plant propagation material and fruit plants intended for fruit production, pursuant to Council Directive 92/34/EEC). The following is stated in relation to plant health:

Article 3
"...in the case of CAC material the material must, at least on visual inspection, be substantially free from any harmful organisms and diseases impairing quality, or any signs or symptoms thereof, which reduce the usefulness of the propagating material or fruit plant and in particular be free from those organisms and diseases listed in the Annex hereto in respect of the genus or species concerned.
3. Any material showing visible signs or symptoms of the harmful organisms or diseases referred to in paragraph 1 at the stage of the growing crop shall be properly treated immediately upon their appearance or, where appropriate, shall be removed."

Article 6
In the case of pre-basic, basic and certified material, the requirements set out in Articles 3, 4 (1) and 5 hereof are applicable in so far as the certification schemes referred to in Article 7 hereof do not impose more stringent conditions.

Article 7
Pending the establishment of a Community certification scheme, pre-basic, basic and certified material shall satisfy the conditions for each respective category as laid down in national schemes of certification provided that they comply, as far as possible, with existing international schemes of certification.

In the EU, national certification schemes have been implemented and certified planting material is on the market. The guarantee level that certified plants are completely free of PPV will, however, largely depend on the prevalence of PPV in the area of production, the frequency of aphid transmission, environmental conditions and the intensity (frequency and sample size) by which the material is tested. In the Netherlands, the production of virus free propagation material is based on the certification scheme published by EPPO (OEPP/EPPO, 2001) and has been described by Verhoeven et al., (2008). It includes testing of nuclear stock (grown in insect-proof screenhouses or facilities) twice a year and testing of basic material grade 1 plants individually once every year. The categories basic material grade 2, mother trees and stool beds are inspected and randomly tested every year. This system will give a high guarantee for PPV freedom in areas with a low PPV prevalence and where aphid transmission occurs only incidentally but will be difficult to implement in areas where PPV is present and aphid transmission occurs frequently: latent infections can remain undetected because not each plant is tested individually and testing is not 100% proof because of the irregular distribution of PPV in the plant.
For CAC material, testing for viruses is not required and freedom for PPV may be based on visual inspection only under the marketing directive (see also Verhoeven et al., 1998). Under the present phytosanitary legislation (Council Directive 2000/29/EC), there are specific requirements for all plants intended for planting of *Prunus* species susceptible to PPV which means, among others, that CAC-material has to be derived from material tested for PPV every three years (see further the paragraph below: "Current phytosanitary legislation" and Appendix III). However, studies (see SharCo deliverables WPE3 and data from the North-American phytosanitary services) have demonstrated that visual inspections are not satisfactory to comply with both the 2008/90/EC and the 2000/29/EC

directives.

Note that the lifetime of cultivars of peach in Europe is very short, so implementation of a certification scheme for the production of PPV-free propagation material of this species may be complicated (pers. comm. G.P. Jongedijk, Propagation Nurseries The Netherlands, December 2010).
The Council Directive 2008/90/EC does not include specific requirements for the production of ornamental *Prunus* plants in which PPV can also be spread. Council Directive 98/46/EC (http://ec.europa.eu/food/plant/propagation/index_en.htm) is in place for propagation material of ornamental plants. It requires (article 5 1) that "propagating material when marketed: shall at least on visual inspection, be substantially free from any harmful organisms impairing quality, or any signs or symptoms thereof, which reduce its usefulness". Because PPV can be symptomlessly present and because information is lacking about symptomatology in at least some of the ornamental Prunus hosts of PPV, this requirement does not guarantee the PPV-free-status of the plants.
Based on the biology of PPV (existence of latent infection phase and natural transmission by many vector species) the requirements for the production of fruit plants in the marketing directive on their own are not sufficient to guarantee freedom of PPV of fruit plants and certainly not of *Prunus* ornamentals because they are not included in the Directive. Fruit plants raised under certification schemes including strict testing regimes for PPV (e.g. based on the EPPO certification scheme) will, however, have a low chance of being infected.

3.9 Relocation of the production sites devoted to propagation material (mother plants and nurseries)

* Mother plant blocks: Taking also into account the lifespan of the mother plants, it is not recommended to locate mother plant blocks in open fields in endemic areas. The best strategy is the use of insect-proof green houses. If this protection cannot be used, relocation of the production sites devoted to mother plants is necessary. They should be re-located to PPV-free protected zones (as defined previously) or in areas isolated from possible aphid contamination from identified infection foci. In areas with disease outbreaks under control, it is recommended to either use insect-proof green-houses or to protect mother plant blocks with appropriate buffer zones. The size of the buffer zone should be decided by a specific scientific committee, who should take into account in its recommendation the specific situation of the area where the mother plant blocks are established (PPV strain, environment, ….). If such requirements are not applicable, mother plant blocks should be re-located in a pest free protected zone.

* Nurseries: Following the SharCo *Prunus* cultivation guidelines, the production of PPV-free planting material outside insect-proof facilities is acceptable only if the recommended buffer zone is free of any PPV-infected *Prunus* species and if the nursery plants are treated with mineral oil during the more active flights of aphid species. Since the level of inoculum in endemic areas is supposed to be high, the probability of disease spread events exceeding 500 m is increased; therefore the recommendation in endemic areas is to increase the radius of the buffer zone up to a minimum of 1 km. If such requirements are not applicable and screen-houses cannot be used, the production sites should be re-located in a more appropriate area, where the above conditions can be obtained. No plant material (budsticks, branches, seedlings) except seeds and certified PPV-free materials should be transferred to the re-located production sites from orchards, public or private gardens or other nurseries located outside the re-location site.

3.10 Alternance of *Prunus* and non-*Prunus* production sites and/or blocks

In order to limit aphid transmission from a stone fruit orchard to another, or from a nursery production site to another, a strategy complementary to the above measures (see 'virus control') would be the plantation of alternating *Prunus* and non-*Prunus* production plots or sites.

3.11 Traceability of the Prunus propagation material

Conditions of delivery of the current phytosanitary passport are stated in the 2000/29/EC directive. However, significant variability in the interpretation and implementation of the 2000/29 Directive as applied to PPV has been observed in different EU member states. A survey performed during the first step of the SharCo program (deliverable DA 1.1: Overview on the current implementation of European directives, appendix I) has shown that the directive is often implemented in a lax manner; moreover, some of its requirements have been criticized by various stakeholders as appearing in some cases too weak (i.e.: control of propagation materials limited to visual inspections) or, alternatively, as too severe or unrealistic (i.e.: 3 years of suspension of the Phytosanitary passport of a whole nursery, even if a small contamination occurs in one single site of production, distant from the others). As consequence of this situation, the conditions of delivery of the phytosanitary passport do not guarantee the absence of PPV from propagating material and, therefore, need reviewing and improvement.

One possibility would be the implementation of at least **two different types of phytosanitary passports, one for propagation material devoted to the local market** (thus permitting the marketing on the local market, in areas where sharka is endemic, of material with a lower level of garanty of PPV freedom (such a material originating from a nursery in which a minor outbreak has occurred) **and another one, for long-distance trading of PPV-free propagation material in EU and import of material into protected zones**. However, the term 'local market' should be refined since some EU countries are considering as local market, the whole National territory. This requirement for two distinct phytosanitary passports is, in fact, reflecting the need for a more efficient fruit tree traceability system. It should included clear labels stating the origin of the plant material, the country and the region of production as well as its true-to-type identity (species, cultivar, accession, collection of origin etc…). The use of a proper label should be implemented and obligatory, whatever is the destination of the plant material (local open market, supermarket, nurseries etc…).

4- Conclusion

A large Prunus industry is, on the long term, only viable in areas where PPV is under control. In areas where PPV is endemic, fruit production can be maintained to some extent through the use of cultivars presenting a lower economic impact of the disease (few to no symptoms on the fruits). However, plant propagation and maintenance of mother plants blocks in such areas are not recommended, except under appropriate conditions (screenhouse, insect proof facilities). Of importance is the presence of more aggressive PPV strains (such as PPV-M) that could limit the expansion of one of the pillars of the Prunus industry worldwide, peach cultivars. In such context, the combination of the different strategies and approaches above mentioned could significantly reduce the PPV spread in nurseries and orchards and contribute to PPV containment.

Propagation of plants and Prunus cultivation (nurseries and orchards) in PPV-free zones is essential and has to be supported, EU-wide. The nursery plants produced in such areas can guarantee the PPV-free status of the propagation material. In other areas, strict PPV management as described above can guarantee a relatively low level of contamination, thus maintaining the PPV spread as low as possible. Currently, the systematic use of the available powerful detection methods with validated reagents and protocols make easier PPV control. Given the pathogenicity of PPV and the existence of latent outbreaks, visual inspection is not sufficient to ensure the production of PPV-free material and is thus not recommended as a sole control measure. In order to limit the spread of the virus, sampling and testing by laboratory methods have to be complemented with the exclusive propagation of PPV-free material certified according to the EPPO standards. The cultivation of more PPV-resistant rootstocks and cultivars combined with oil treatments and appropriate distance (or officially declared buffer zones) to PPV infection foci, will improve the sanitary status of plant material.

The “quality” of the EU phytosanitary passport, concerning PPV, as well as the traceability of the propagation material have to be considerably improved for limiting the long distance spread of the virus and the export of PPV-free material. Indeed, the multiplication of PPV-free materials in isolated and inspected nurseries located in PPV-free areas or in appropriate conditions (screenhouse, in vitro culture) is the only way to produce healthy plants. The trade of tested and certified plants, supported by a proper phytosanitary passport, is equally crucial. The new plantations could then be protected in the most susceptible initial periods by oil treatments and other PPV management procedures, as described above, if PPV foci are present in the area. Obviously, the use of PPV-resistant material will be a solution in the future. While unrealistic in areas where PPV is endemic, eradication of the new infections is still highly recommended for the containment of PPV. This would limit the spread of the virus, the impact of the disease and the establishment of new strains or isolates. Accurate methods and protocols are available making this task technically feasible. The exclusive eradication of specific strains is also a possible option to preserve extensive areas free of, in example, PPV-M or PPV-C.

The combination of the different strategies described in this document will certainly contribute to control or limit PPV spread and to manage sharka disease.

5- Feasibility of those management measures

	Areas where PPV is endemic	Areas where PPV is under control	PPV-free areas
1) Detection and inspection methods	Applicable. However methods that allow typing of the strains should be strongly promoted.	Applicable but difficult to maintain the measure at a large scale and for a long period (over several years)	Applicable for regular control surveys
2) Eradication of infected trees (nurseries, orchards, public and private gardens, etc...)	Unrealistic except for emergence of new isolates or the establishement of PPV protected zones.	Applicable but difficult to implement the eradication measures to public and private gardens	Applicable
3) Pre- and post-entry quarantine inspections	Applicable but only with the right quarantine facilities available in the MS and with the proper traceability system	Applicable with a proper traceability system	Applicable with a proper traceability system
4) Vector control and interference with the transmission process	Oil treatment: applicable and efficient to some extent. Use of insectproof facilities for mother plant blocks: applicable.	Oil treatment: applicable and efficient to some extent. Use of insectproof facilities for mother plant blocks: applicable.	Oil treatment: not necessary. Use of insectproof facilities for mother plant blocks: not necessary.
5) Resistant cultivars and rootstocks	Applicable but not in the short term, because insufficient number of resistant varieties available to fullfill the industry's needs	Applicable but not in the short term, because insufficient number of resistant varieties available to fullfill the industry's needs	Applicable but not necessary
6) Pest free production zones	Unrealistic because large eradication programs would be required and large number of tests needed [1]	Applicable	Not relevant in those PPV-free areas
7) Implementation of *Prunus* cultivation guidelines	Applicable	Applicable	Applicable
8) Certification schemes	Applicable but the production of PPV-free material is conditionned by the availibility of confined facilities (mother plant blocks under insectproof facilities, in vitro propagation, multiplication under insectproof facilities)	Applicable but the production of PPV-free material is conditionned by the availibility of confined facilities (mother plant blocks under insectproof facilities, in vitro propagation, multiplication under insectproof facilities)	Applicable
9) Relocation of the production sites for production of propagation material	Moderatly applicable since it would be dependent on the availibility of isolated, PPV-free areas/zones.	Applicable	Not necessary
10) Alternance of *Prunus* production sites with non-*Prunus* production blocks	Moderatly applicable since dependent on the size of the production sites available. The effect of this measure is also dependent on the presence of wild /cultivated Prunus plants in the vicinity.	Moderatly applicable since dependent on the size of the production sites available. The effect of this measure is also dependent on the presence of wild /cultivated Prunus plants in the vicinity.	Not necessary
11) Control of trade and/or traceability of the propagation material when marketable	Applicable	Applicable	Applicable

[1] This measure could still be applicable when promoting the establishment of areas free of specific PPV isolates.

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7- ANNEXES

Appendix I: Overview on the current implementation of the 2000/29/EC European directive

See SharCo deliverable DA1.1

Appendix II: Cultivation Guidelines

See SharCo deliverable DA1.2

Appendix III: ISPM 27 Annex 02; DP 2 (2012): Plum pox virus (IPPC hosted by FAO)

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