SHARCO
SHARKA CONTAINMENT IN VIEW OF EU-EXPANSION

SEVENTH FRAMEWORK PROGRAMME

Plum Pox Virus (Sharka) *on Stone Fruits*

L E A F L E T

Introduction

Sharka, caused by plum pox is the most devastating viral disease worldwide of stone fruits including peaches, apricots, plums, nectarines, almonds, sweet and sour cherries. Plum pox symptoms were first observed in plums by plum growers in Bulgaria between 1915 and 1918. However, the paper describing the viral nature of the disease appeared in 1932, when prof. D. Atanassov named it "Sharka po slivite" meaning "Pox of plum". Nowadays, sharka is widely used common name of the disease around the world.

Distribution

The disease is spread to a large part of **Europe** – Albania, Austria, Belgium, Bosnia & Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, France, Germany, Greece, Hungary, Italy, Lithuania, Luxembourg, Moldova, Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Switzerland, Ukraine, United kingdom, former USSR, in the **Mediterranean**, the **Middle East** (Egypt and Syria), Turkey, Cyprus, India, Argentina, Chile, **Asia** (China), **North America** (United States, Canada).

Economic impact

The costs associated with sharka disease involve not only direct losses in stone fruit production, commercialization, eradication, compensatory measures, but also indirect costs including those from preventive measures such as quarantine surveys, inspections, control of nurseries, diagnostics and the impact on foreign and domestic trade. An evaluation of the global cost associated with sharka management worldwide, excluding indirect trade losses, could exceed 10 000 million euros over the last 30 years (Cambra et al., 2004).

Hosts

Host range depends on virus strain. PPV infects all *Prunus* species including:

- *Prunus armeniaca* - Apricot
- *P. persica* - Peach
- P. persica var.*nectarine* - Nectarine
- *P. domestica* - Plum
- *P. cerasifera* - Myrobalan plum
- *P. insititia* - Damson plum
- *P. salicina* - Japanese plum
- *P. glandilosa* - Cherry almond
- *P. avium* - Sweet cherry
- *P. cerasus* - Sour cherry
- *P. amygdalis* - Almond

Important secondary hosts serving as reservoirs of the virus are several wild *Prunus* species as:

- P. *spinosa* - Blackthorn
- *P. americana* - American plum
- *P. bessey* - Mahaleb,
- *P. mume* - Japanese apricot

- *P. pumila* - Sand cherry
- *P. hortulana* - Hotulan plum
- P. *davidana* – Chinese wild peach
- P. *tomentosa* – Nanking cherry
- P. *nigra* – Cnada plum
- P. *martime* – Beach plum
- P. *laurocerasus* – English cherry – laurel

In addition some herbaceous plants such as *Chenopodium foetidum, Nicotiana benthamiana, N. clevelandii, N. occidentalis* #37B, *N. megalosiphon, N. bigelowii, Nicandra physaloides, Pisum sativum* cv. "Colmo" are also hosts and are often used as indicator plants to detect virus infection from diseased stone fruit plants.

Symptoms

PPV symptoms on stone fruits depend on host species and cultivar and the strain of the virus. The symptoms may vary considerably with the age, the temperature and nutrient status of the plant. The different strains of PPV may cause different degrees of disease severity. Often, many trees fail to show symptoms short after the infection (latent infection). The lack of symptoms, however, is not a proof that a plant is healthy and does not have the disease. In some cases the disease symptoms may be observed on only a few leaves or fruits along on few branches, or may be expressed throughout the entire tree. The symptoms may appear in spring and early summer and disappear later during the periods of hot summer. When symptoms do occur, they are very diagnostic and easily recognized.

Leaf symptoms on infected trees include:

- chlorotic (yellowing) and nectrotic (browning) spots, diffuse spots, bands, rings or blothes (Fig. 1A and B)
- light green discoloration along the nerves (vein clearing) (Fig. 2)
- leaf distortion and deformation
- discolorations along the nerves of red coloured leaves of some *Prunus* ornamentals (Fig. 3)

Some sensitive plum varieties may also show bark splitting.

Fig1 A **Fig1B** **Fig. 2** **Fig. 3**




***Flower symptoms*:**

- spots on petals or color-breaking may occur on some sensitive peach varieties and on the petals of some sensitive plum cultivars (Fig. 4A and B)

Fig. 4A

Fig. 4B

***Fruit symptoms*:**

Generally, plum fruits are more severely affected and show more severe symptoms than other stone fruits.

The symptoms on ***plum fruits*** consists:

- darker rings or spots on the skin- often accompanied with gum (Fig.5 A and B)
- reddish discoloration of the flesh
- severe deformation and premature dropping, 20 – 30 days before normal maturity date (Fig. 6)
- the fruits are pulpy, have less sugar content, they are tasteless and unmarketable

Fig. 5A **Fig. 5B** **Fig. 6**

***On peach and apricot fruits*:**

- lightly pigmented yellow rings or line patterns (Fig.7)
- deformation, the fruits may be irregular in shape and develop necrotic or brown dead areas
- rings and spots on the surface of apricot seed (stone) (Fig. 8)

Fig. 7

Fig. 8

***On cherry fruits*:**

- chlorotic and necrotic rings, notched marks and premature drop (Nemchinov et al., 1998)

Strains

Plum pox virus is comprised of several strains based on biology, serological reactions and molecular and biological properties. The most common strains, which differ in their symptoms severity among host species and in patterns of spread by aphids are PPV-M and PPV-D.

PPV-D (Dideron**)** strain – first isolated from apricot in France. It can also infect peaches, nectarines and plums. PPV-D is the most spread strain of the virus in western Europe and occurs in Chile and the US (Pennsylvania). This strain is known not to be seed-transmitted, can be difficult transmitted to experimental hosts and is less efficiently aphid - vectored. PPV-D strain is the non-epidemic form of the virus.

PPV-M (Markus) strain - first identified on peaches in Greece. Found also on infected plums, apricots and cherries. PPV-M is the most common strain of the virus in southern, eastern and central Europe. Once established in a region, the M strain can spread quickly and is very difficult to eliminate. PPV-M strain spreads rapidly by aphids and is considered as the epidemic form of the virus.

PPV-Rec (Recombinant) strain **–** up to now isolated from apricots, plums, myrobalan plums, blackthorns. Identified also in cherry trees in Czech Republic. Most probably PPV-Rec strain has arouse by homologous recombination between PPV-M and PPV-D strains (Glasa et al., 2001, 2002). It is already detected in several countries as Albania, Bulgaria, Czech Republic, Germany, Hungary, Slovakia, Bosnia and Herzegovina, Pakistan. PPV-Rec strain has a high virulence and aphid transmissibility (Glasa et al., 2002).

PPV-EA (El Amar) strain – originally isolated from apricot in Egypt and so far is only found in this North African country. It can also infect plums, nectarines and peaches. Still little information is available for the EA strain, but in some characteristics it is similar to PPV-M strain.

PPV-C (Cherry) **strain –** originally isolated from sour cherry from Moldova (Kalashyan at al., 1994) but can infect also sweet cherry. Experimentally PPV-C can also be transmitted to other *Prunus* species. PPV-C has been reported from several countries in eastern Europe, and Italy and is still not found in North America.

PPV-W (Winona) - isolated from a plum in Ontario, Canada during the course of a homeowner surveys. Still not found in some other regions and countries. It is genetically distinct from all other viral strains known to date (James and Varga, 2005).

Surveys and Detection

Visual inspection of trees, especially during the period of active growth, allows detection of PPV on the basis of symptoms. Often infected trees do mot show symptoms for up to 3 years after infection. For that reason the lack of symptoms is not a good indicator that the plant is healthy. Symptoms alone cannot be relied upon to determine the incidence or range of sharka

disease. Laboratory testing should back up any visual inspection of the trees. Bioassays on herbaceous indicator plants (*Nicotiana* species, *Ch. foetidum*) by mechanical inoculations or by the use of woody indicator plants (GF 305, GF 31 peach, *P. tomentosa*) are recommendable.

Other laboratory tests as the Enzyme Linked Immunosorbent Assay (ELISA) and the Polymerase Chain Reaction (PCR) are now widely used to confirm the presence of the virus even in low concentrations in roots, bark, flowers, leaves, fruits or seeds. Polyclonal and monoclonal antibody (5B-IVIA) (Cambra et al., 1994) have been developed for the universal detection of all PPV strains, and for the selective and specific detection of PPV-D (Cambra et al., 1994), PPV-M (Boscia et al., 1997), PPV-EA (Myrta et al., 1998) and PPV-C (Myrta et al., 2000). Different variants of RT-PCR test (immunocapture, print and squash capture) (Wetzel at al. 1992, Olmos et al., 1996) have been developed to universally or specifically amplify PPV strains. Molecular hybridization test based on nuclear acid sequences specifically complementary to virus RNA can be applied, too. An EPPO protocol for PPV detection has been published (Bulletin OEPPO/EPPO Buletin, 2004) and is available at http://eppo.org/quarantine/virus/Plum_pox_virus.

Nevertheless, what of the mentioned above methods will be used for PPV identification, the critical point of its detection is the way of ***sampling*** of infected trees. When the typical symptoms are present, symptomatic flowers, leaves or fruits could be used. When the trees are symptomless, the standard sample should include at least 10 fully expanded leaves, taken around the canopy.

Transmission and Spread

Long distance spread - it is through the movement and use of infected propagative materials (grafting and budding). The use of infected seedling, root stocks or budding material and even infected flowers is the main pathway for PPV introduction to a new country or region.

Seed transmission – No PPV isolate is currently recognized to be seed transmitted, so vertical transmission of PPV from infected mother plants to their progeny does not occur (Pasquini and Barba, 2004).

Short distance spread – once introduced in a new territory, PPV can be spread secondary by at least 20 aphid species, although only 4-6 are considered important PPV vectors. The capacity for vector transmission varies considerably between the strains and is dependent also on the host cultivar, the age of hosts, aphid species, the time of the year and the environmental conditions. The most important aphid vectors of PPV are *Brachicaudus cardui, B. helichrysi, Myzus persicae, Phoron humili,* Aphis spiraecola, A. craccivora, A. gossypii, A. fabae. PPV transmission by aphids usually occurs during their flight in the spring and autumn. The mechanism by which aphids transmit PPV is called nonpersistent transmission. It means that once the aphids probe into an infected plant, they acquire the virus within a few seconds. The virus remains infectious (not more than one hour) and when the winged migratory aphids move from the infected plant onto a healthy plant they transmit the virus. Actually the virus can be transmitted by the aphids only for a short time (a matter of minutes). Aphids make two kinds of probes on the leaf surface, **test probes** and feeding probes. Rapid transmission of PPV occurs specifically during aphid test probes and not during the longer-lasting feeding probes.

Control

Plum pox virus has proven to be very difficult disease to control. There is no anti-virus treatment that can be applied to infected trees or orchards.

The best method for controlling PPV is **to prevent** the introduction of the virus to new fruit-growing areas. PPV prevention includes the following:

- Production of virus-free trees through the indexing of mother trees and the selection of virus-free budwood and rootstocks. Commercial growers and nursery propagators should purchase only certified virus-free planting stock.
- Strict regulations and inspections of imported propagative materials and it further movement.
- Annual visual inspections and surveys in nurseries and orchards. Regular virus testing of *Prunus* nursery stock is necessary to prevent the long range spread of PPV.

Having failed to prevent PPV spread from a growing area, the next **control** measure is:

- **Removal** and **destruction** of infected trees as quickly as possible. A single infected tree in an orchard would serve as a virus source and an infection foci for all surrounding trees and for closely adjacent orchards. It is also important to eradicate sucker shoots developing from the stumps because they are known to be a good source of PPV. Suckers can be treated with herbicides. Once trees are removed, they should be destroyed promptly so that aphids on them do not have the opportunity to move to neighboring trees. It is recommended to growers to wait three years before replacing an orchard with stone-fruit trees.
- Although the use of insecticides against aphid populations do not completely protect trees from aphid inoculation with PPV because it may only take one or few aphids to test probe and inoculate a tree, **chemical control** of migratory or over-wintering aphids may slow dawn the spread of PPV.
- **Weed control** (including wild *Prunus* species) in and around susceptible orchards may slow dawn infection rate of sharka disease since it will eliminate the reservoirs of infection.
- Use **of resistant cultivars and rootstocks**.
- **Development of resistant cultivars** through commercial breeding and/or genetic engineering. Few naturally occurring resistant genes are available for plant breeders to use in developing highly resistant fruit varieties. Hybrid plum cultivars have been identified that respond to PPV by a hypersensitive response thus preventing the spread of the virus from the dead infection site to the other parts of the tree. The transfer of resistant genes to stone fruit crops is challenging and time consuming using the conventional breeding techniques. Genetic engineering and the use of biotechnology can help development of sharka resistant cultivars.

References

- Cambra, M., M. Asencio, M.T. Gorris, E. Perez, E. Camarosa, J.A. Garcia, J.J. Moya, D. Lopez-Abella, C. Vela, A. Sanz, 1994. Detection of plum pox potyvirus using monoclonal antibodies to structural and non-structural proteins. Bull. OEPP/EPPO, 24: 569-577.
- Boscia, D., H. Zeramdini, M. Cambra, O. Potere, M.T. Gorris, A. Myrta, B Diterlizzi, V. Savino, 1997. Productriojn and characetization of a monoclonal antibody specific to the M serotype of plum pox potyvirus. Eur. Journal Plant Pathol. 103: 477-480.
- Bulletin OEPP/EPPO 2004. Plum pox potyvirs. 34, 247-256.
- Glasa, M., O. Kudela, V. Maria-Jeanne, JB. Quiot, 2001. Evidence of a naturally occurring recombinant isolate of plum pox virus from Slovakia. Plant Dis. 85, 920.
- Glasa, M. V. Maria-Jeanne, G. Labonne, Z. Subr, O. Kudela and JB. Quiot, 2002. A natural population of recombinant Plum pox virus is viable and competitive under field conditions.
- James D., A. Varga. Nucleotide sequence analyses of Plum pox virus isolates W3174: Evidence of a new strain. Virus Research110, 143-150.

- Kalayshan, J.A., N.D Bilkey, T.D. Verderevskaya, E.V. Rubina, 1994. Plum pox virus in sour cherry in Moldova. Bull. OEPP/EPPO, 24, 645-649.
- Myrta A., O. Potere , D. Boscia, T. Candresse, M. Cambra & V. Savino. 1998. Production of a monoclonal specific to the EL Amar strain of Plum pox virus. Acta Virologiac 42: 248-250.
- Myrta, A., O. Potere, A. Creszcenzi, M. Nuzzaci, & D. Boscia, 2000. Production of two monoclonal antibodies specific to cherry strain of plum pox virus (PPV-C). Journal of Plant Pathology 82, 95-103.
- Nemchinov, L., A. Crescenzi, A. Hadidi, P. Piazzolla, and T. Verderevskaya, 1998. Present status of the new cherry subgroup of plum pox virus (PPV-C). In: Hadidi, A., Khetarpal, R.K., and H. Koganezawa, eds. Plant Virus Disease Control. Pp. 629-638. APS Press, St. Paul, MN.
- Olmos, A., M. A. Dasi, T. Candresse, and M. cambra. 1996. Print-capture PCR: A simple and highly and sensitive method for the detection of plum pox virus (PPV) in plant tissues. Nucleic Acids Research, 24& 2192-2193.
- Paequini, G. and M. Barba, 2004. The question of seed transmissibility of plum pox virus. Bulletin OEPP/EPPO 36, 287-292.
- Wetzel, T., T. Candresse, G. Macquaire, M. Ravelonandro, and J. Dunez. 1992. A highly sensitive immunocaptute polymerase chain reaction method for plum pox potyvirus detection. J. Virological Methods, 39: 27-37.

Prepared by:

More information of SharCo project can be found at the following website*:*
http://www.sharco.eu