PLUM POX VIRUS: A POTENTIAL QUARANTINE PEST OF MEXICO

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SUMMARY

Plum pox or sharka, a viral disease induced by plum pox potyvirus, severely affects the production of *Prunus* species in Europe. This disease was detected in 1993 in Chile and so far, it has not been reported in any other American country. Due to the economic impact of this disease in countries where it is prevalent as well as to the commercial trade with regions that have, or may potentially have this pathogen, it is of great concern for the Plant Regulatory Mexican System. Latest developments concerning the biology, epidemiology, and disease management are provided in this paper to assure awareness among growers and professionals involved with *Prunus* production. Knowledge and understanding of this virus will be fundamental to guaranty success in any attempt to detect and eradicate it if eventually arrives to México.

KEY WORDS: PPV, potyvirus, *Prunus, S*harka disease.

EL VIRUS DE LA VIRUELA DEL CIRUELO: UNA ENFERMEDAD POTENCIAL CUARENTENARIA DE MÉXICO

RESUMEN

La viruela del ciruelo o sharka es una enfermedad inducida por el virus “plum pox potyvirus” que afecta severamente la producción de varias especies del género *Prunus* en Europa. Esta enfermedad fue detectada en 1993 en Chile sin que a la fecha se tengan reportes de su presencia en otros países de América. Por los daños económicos que la sharka causa en países en que es prevalente, así como por las relaciones comerciales frutícolas con países que poseen o pueden potencialmente poseer al patógeno, esta enfermedad es de importancia cuarentenaria para México. Datos actualizados concernientes a la biología, epidemiología y manejo de la enfermedad son proporcionados en este artículo con el propósito de informar y sensibilizar a productores y profesionales relacionados con la producción de diversas especies de *Prunus* para una adecuada toma de decisiones ante una eventual deteccion del plum pox potyvirus en México.

PALABRAS CLAVE: PPV, potyvirus, *Prunus,* enfermedad de la Sharka.

INTRODUCTION

Sharka disease, caused by plum pox potyvirus (PPV), induces extended yield losses in plums (*Prunus domestica* L.), apricots (*P. armeniaca* L.), and peaches (*P. persica* L.) because of reduced fruit quality, premature fruit drop and rapid natural virus spread by aphid vectors (Roy and Smith, 1994). On these stone fruits, economic losses reach high proportions in those areas where the disease is broadly spread, particularly in Europe. In the former Yugoslavia, for example, of 51’556,000 plum trees 58% are currently infected. A quick decline may occur when the tree is also infected with other viruses. Since the first report of PPV in the 1910s in Bulgaria (Atanasoff, 1932), between 1984 and 1992 this virus has spread to nearly every European country, various eastern Mediterranean regions, and Northern India (Roy and Smith, 1994; Gottwald, 1995). Due to the wide spreading nature of sharka disease in Europe and the recent report of PPV in Chile (Acuña, 1993), this disease is of great concern to the quarantine efforts of several American countries, including Argentina, Canada, and the USA (Levy and Hadidi, 1994; Levy, L. 1996, Personal Communication, USDA APHIS).

Although, México has a general quarantine program for budwood of *Prunus* species, neither serological nor indexing tests are performed to detect PVP, thus no specific regulation is currently underway on sharka disease. Even though the inclusion of PVP into the Mexican quarantine system may require appropriate economical and technical studies, a general knowledge of the disease and

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proficiency on effective and quick PVP detection appears to be a necessity. The current status of this disease in México as well as studies of sharka risk assessment are as fundamental issues to support any decision making regarding a quarantine program.

This paper was outlined during a training of the first author at L. Levy's laboratory in the Animal & Plant Health Inspection Service, Plant Protection & Quarantine facilities, Belsville, MD, USA and has the purpose of providing general information of the status of this disease worldwide with emphasis on strains, detection methods and epidemiological factors involved in disease spread. The quarantine programs on PVP in the United States and Europe are also discussed. A preliminary Spanish version of this paper was previously published (Mora *et al*., 1998).

Symptoms in Comercial Trees and Indicators

Apricot trees infected with PPV produce fruit with irregular ringspots and malformations thus reducing its commercial value; chlorotic ringspots usually appear on the fruits shell and stone turning necrotic in some cultivars (Figure 1). Plum fruits show similar symptoms, plus deeply engraved rings, irregular lines and pox lessions on the surface (Figure 1). The cv. Besztercei exhibits brownish-red patches saturated with gum in the flesh and brownish-red spots on the stone. Fruit may drop prematurely. Peach fruit are usually less affected and only show slight chlorotic ringspots, bands, white flesh and light colored skin (Figure 1), although the importance of symptoms depends on the strain and the variety. Engraved rings and brown necrotic tissues have been reported in some peach hybrids. In general, fruit quality is reduced and becomes unsuitable for direct consumption and industrial processing. Most of the susceptible apricot, plum, peach and nectarine show leaf symptoms which appear as pale or yellowish-green rings, spots or leaf mottling. In autumn, some cultivars, such as 'Cambridge Gage', have chlorotic rings or spots surrounded by reddish-brown margins. Highly sensitive plum cultivars may exhibit bark splitting and cancers on shoots and break down. Infected trees decline in a few years. In general, most wild and ornamental species of *Prunus* are susceptible, infected almonds, however, are symptomless.

In peach seedlings of GF 305, an indicator commonly used in quarantine programs, leaves show vein clearing and some curling (Figure 2), whereas in GF 31 bark necrosis and cracking appear in the lower part of one-year-old shoots. Typical symptoms on *Nicotiana benthamiana* includes mosaic and severe leaves distortion (Figure 2).

The Pathogen

Plum pox potyvirus is a member of the *Potyvirus* genus in the Potyviridae family. The virus is a flexuous particle 760-780 nm lengh and 20-24 nm in diameter, with helicoidal symmetry; the protein coat is 36kDa; RNA is single chained and positive, 3.5 kDa (Ward and Shukla, 1991). Pinwheel-shaped inclusion bodies in the cytoplasm of parenchymatal host tissue as well as some special cytopathological structures have been reported (Golinowski and Garbaczewska, 1980).

Plum pox potyvirus seems to be highly variable, with two main types: M (Marcus), a very aggresive, and D (Dideron), a less aggresive type (Marenaud, 1979). Another isolate, El Amar, was further reported (Wetzel *et al*., 1992). Some authors claim it belongs to type M and others place it as type D. Recently, another isolate has been reported from cherry in Moldavia (PPV-SC), which has been sequenced, characterized and clasified as a new type C (Nemchinov & Hadidi, 1996). These new types show the dangerous capacity of the virus to mute and change.

Aphid Vectors and other Transmission Mechanisms

Aphids transmit the virus in a non-persistent manner, probably responsible for short range virus spreading. At least ten vector species have been reported in Europe: *Aphis gossypii* Glover, *A. craccivora* Koch, *A. fabae* Scopoli, *A. spiraecola* Path, *Brachycaudus helichrysi* (Kaltenbach), *B. cardui* (Linnaeus), *Myzus persicae* (Sulzer), *M. variaus, Hyalopterus pruni* (Geoffroy), and *Phorodon humuli* (Kunze & Krczal, 1971, Avinent *et al*., 1994). Long distance dissemination occurs by vegetative propagation and use of infected varieties and rootstocks. These are the mecanisms that should be targeted on prevention and quarantine programs. Seed transmission has been reported only once (Nemeth & Kölber, 1982) but aparently the most accepted hypothesis now is that PPV is not seed transmitted (Triolo *et al*., 1993). Variability of the virus and the increasing number of growing cultivars suggest that this transmission mechanism should not be rule out.

Detection Methods

Observation of field symptoms is the oldest and cheapest diagnostic method, but it is the least reliable for the purpose of quarantine programs due to the confusion with symptoms produced by other causes. In addition, PPV is irregularly distributed on infected stone fruit trees making difficult the visual inspection, which should be carry out during the growing season. Indexing on GF305 peach seedlings (Bernhard *et al*., 1969) is a reliable diagnostic method for routine assays. This system is currently used as part of the quarantine program at Agricultural Research Service of the United States Department of Agriculture (ARS-USDA). GF305 indicator seedlings show vein clearing and leaf deformation from one to three weeks after grafting (Figure 2). This method has the drawback of requiring high investments in insect-proof glasshouses and in the maintenance of large number of plants. Testing can also be done with herbaceous plants such as *Nicotiana benthamiana* (Figure 2) and *Chenopodium foetidum* improving the response time. In any case, PPV infection must be confirmed with serological methods or other techniques.

Figure 1. Symptoms of plum pox potyvirus on different *Prunus* species. Apricot fruits: A) Chlorotic ringspots; B) Severe distortion; and C) Chlorotic rings in the stone. D) Peach with slight chlorotic ringspots. Plum: Irregular and chlorotic ringspots in fruits (E) and leaves of cv. Italian Prune (F).

Figure 2. Symptoms of plum pox potyvirus on differential hosts. A). Vein clearing and severe leaf distortion in peach GF-305. B). Mottle and leaf distortion in *Nicotiana benthamiana*. Left leaf represents healthy tissue. C) Autoradiograph of dot-blot hybridization analysis of 3' NCR RT-PCR amplified products of infected peach with PPV-D (samples 2 and 5) and tissue of GF-305 graft inoculated with accession 24821 (samples 3 and 6). Amplification products presents in samples 2,3, 5 and 6. Comparison of radioactive and non-radioactive hybridization (bottom picture). D). Acrylamide gel electrophoretic analysis of PPV 3' NCR RT-PCR amplified products of infected *Prunus tomentosa* with PPV-D (sample 3) and GF-305 / accession 24821 (sample 4). Samples 1 and 2 are healthy tissues. Ladder with molecular weight markers of 1000 bp, 700 bp, 500 bp, 400 bp, 300 bp, 200 bp and 100 bp.

The most common method now used is ELISA (Adams, 1978; Polák, 1988; Tobias *et al*., 1992), with the advantage of allowing the analysis of large number of samples at a low cost. More recent molecular techniques for detection, like the amplification of viral RNA by the polymerase chain reaction (RT-PCR) (Figure 2) (Korschineck *et al*., 1991; Wetzel *et al*., 1991; Levy & Hadidi, 1994) and dot-blot hybridization using the 220 bp PPV-D amplification product obtained with RT-PCR (Figure 2) (Levy *et al*., 1995) have been developed. They are more sensitive but also more expensive and difficult to use for routine purposes.

The application of reliable PVP detection methods in combination with extensive sampling is fundamental for the success of any quarantine program. Two techniques appear to be suitable for detection of PPV: RT-PCR (Korschinec *et al.,* 1991; Wetzel *et al.,* 1991; Levy and Hadidi, 1994) and DAS-ELISA with polyclonal and/or monoclonal antisera (Malinowski and Zawadzka, 1992; Cambra *et al.,* 1994). Although most extensive detection studies are carried out with ELISA, problems concerning sensitivity makes RT-PCR an optional test for corroborative tests. In México, we conducted a directed sampling on a few peach trees based on putative symptoms of plum pox. ELISA and RT-PCR tests indicated that such samples were negative to PPV. However, a more extensive sampling at the national level should be conducted to determine the current situation with respect to this disease.

The Quarantine Program at ARS-USDA

The quarantine program on PVP in the United States is conducted by the Agricultural Research Service (ARS) under the regulation of the Animal and Plant Health Inspection Service-Plant Protection and Quarantine (APHIS-PPQ) of the Department of Agriculture of the United States (USDA) (Foster, 1991). Imported budwood and seeds of plums, apricots, and peaches (cherry is excluded) entering the USA may entail years of isolation and testing before their release. The current system for PVP testing in this program include the following stages:

a) Input of information pertaining date of introduction, origin, recipients, accession coding, etc., into an internal data base named QIS 2.0 / for MS-DOS developed and operated by J. Bowman and G. Enberland. Results obtained during the testing process and the final quarantine action are inputed to the data base (J. Bowman, 1996, personal communication).

b) Chip budding onto 6-wk old *Prunus* species GF-305, a seedling selection of peach cv. Elberta. It takes one growing season to produce enough parent-plant budwood to begin quarantine pathogen testing and three years to complete the tests. This test is conducted under glasshouse conditions.

c) DAS-ELISA is performed on grafted symptomatic GF-305 to verify the presence of PVP. The RT-PCR is performed ocasionaly with high-risk accessions testing negative by both grafting and DAS-ELISA.

d) If accessions are free of PVP and other quarantine pathogens, they are released and offered for distribution to the recipient, the National Clonal Germoplasm Repository, and other interested people if patented issues allow it. Otherwise accesions are destroyed with the recipients approval.

The Quarantine Program in Europe

In Europe, PPV is considered a plant pest against which a permanent campaign is established. Plant protection services in different countries take preventative actions in the field based on pulling up and destroying diseased trees and replanting them with healthy certified plants. Also, an extension service program is carried out to inform growers about the danger of this virus.

In Spain, detection of the disease is based in periodic field samplings in affected regions such as Murcia and Valencia. Symptoms are more evident in young shoots and in mature fruits. Leaf, shoot and fruit samples are taken from symptomatic trees to be analyzed by ELISA. Verified infected trees are marked and pulled out. Since the official declaration of the PPV presence in Murcia, 10,000 apricot trees and 95,000 plum trees have been destroyed, with an investment of approximately one million dollars; growers receive around 10 dollars per destroyed tree.

Genetic control

In spite of the fact that a quarantine program is the classical approach for preventing the introduction and establishment of a foreign pest to an specific region, the international experience indicates that, in general, eventually such a pest may finally be established. The cost and effort involved in any quarantine program is greatly justified when other eradicative or protective approaches are simultaneously investigated to be prepared when the former approach eventually fails. On this regard, the cheapest and most effective protective mean to control plant viruses is host resistance. With PPV, the search for resistant cultivars is intense in different countries (Polák, 1994; Dosba *et al*., 1994; Audergon and Morban 1990; Karayiannis and Mainou, 1994), so far, however, none of the resistant materials have the quality of the present commercial varieties. Resistance levels of progenies from susceptible, productive and high quality varieties crossed with resistant, less commercial plants, are measured by the method described by Audergon & Morvan (1990), based in the use of GF305 indicator. The resistance inheritance has not been completely elucidated. Dosba *et al*. (1989), detected a complex segregation in the F_1 progeny. Another problem inherent to this classical breeding approach is that *Prunus* species such as peach (*P. persica*) and plum (*P. domestica*) have a juvenility period of 3 to 5 years (Sherman and Lyrene 1983) and, due to the time necessary to propagate and evaluate bree-

der selections, cultivar release has generally required 20 years from the initial hybridization. Further more, genes for biotic and abiotic stress resistance are, in many cases, found in native genotypes or in noncultivated species that generally produce poor-quality fruits. Using such nonimproved germplasm requires additional cycles of hybridization and selection, thereby lengthening the time to cultivar release.

New technologies of ADN recombination and introduction of resistant genes are promising. Abundant information has been developed about the molecular sequence of the virus (Garcia *et al*., 1994), which might allow resistant sources to be introduced in plants. Tobacco plants into which capsid coding genes have been inserted have shown some resistance (Ravelonandro *et al*., 1994). Insertion of capsid coding genes into plum and apricot has also been successful (Camara-Machado *et al*., 1994; Scorza *et al*., 1994), but *in vitro* regeneration of the shoots is still a limiting factor (Laimer *et al*., 1992). So far, genetic transformation has relied on the use of seed-derived explants including hypocotyls of plum (Mante *et al.,* 1989, Scorza *et al.,* 1995) and immature embryos of peach (Scorza *et al*., 1995, Hammerschlag *et al.,* 1989a, 1989b, Smigocki and Hammerschlag, 1991). This last technique is the only success, to date, in producing transgenic peach plants. In México, as well as in most of the other PPV free countries, breeders should be aware of this disease to avoid an accidental introduction of the pest in non-certified material and should include the search for host resistance to PPV in their current fruit breeding programs.

ADDENDUM

Procedure for growing and running GF 305 peach seedling test at the USDA, ARS, NGRL. Beltsville, Maryland, USA as outlined by R. Mok, technician in charge.

Growing GF 305

1. Crack the peach pit and remove the seed. Either use a hammer and crack on the suture or use a pair of vise grip pliers to force open the seed, being careful not to damage the seed.

2. Soak peach seeds in a fungicide solution for 1 hour. I use 'Banrot 40% WP' at a rate of 1 teaspoon per gallon of water. Several hundred seeds can soak in a 500 ml or 600 ml beaker.

3. Prepare a medium of vermiculite and coarse perlite, approximately 3:1 in proportion. Place medium in a plastic bag.

4. Remove seeds from fungicide mixture and place in the medium in plastic bag. Pour some of the fungicide solution that was used in the 1 hour soak into the bag containing the seeds and medium. Close the top of the bag and shake vigorously to thoroughly wet the medium. Add fungicide solution so that the medium is wet but there is no water standing in the bottom of the bag (or only a small amount). After again shaking the bag to wet the medium, seal the bag and place in cold at 4 ^{o}C. Check the bag weekly. Some of the moisture will settle out. Rotate the bag so that none of the seeds continually are sitting in moisture. If excess moisture is in the bag, open and drain away, then reseal.

5. Stratify seeds in this manner for 90 days at 4 ^{o}C.

6. Bring seeds out of cold. Open the plastic bag and place on lab bench at room temperature (RT). At this time some of the seeds may have already germinated. After 1 or 2 days at RT, the seeds should begin to uniformly germinate and the radicles will begin to emerge and elongate.

7. Plant seeds in 4 inch plastic pots using a soil-less medium containing mainly peat/vermiculite/perelite (we use 'Pro-Mix BX'). Pots are filled and pressed down to make a very firm growing medium. Make a hole in the center of the pot approx. 1.5 inches deep. Place the seed in the hole with the radicle pointing down or with the seed aligned sideways. Cover the seed and press firmly. This is important, to give the seedling a good strong base. Without this the plant will not stand upright but will fall over and be difficult to work with. A plant growing horizontally will also tend to send out side-branches low on the stem prematurely. This would make the plant not as good for testing purposes.

8. Let the plant grow approx. 6 weeks until it is ready to inoculate. When plants are approx. 10 in tall, begin fertilizing on a biweekly basis with a liquid complete fertilizer such as Peters brand 20-20-20. Plants are ready to inoculate when they are approx. 18 to 20 in tall and the main stem diameter is 3/16ths to 1/4 in.

Inoculating GF 305

1. The inoculum is taken from several branches of the accession or sample tree, sampling from all quadrants of the canopy. Cut branches that are similar in size to the diameter of the GF 305 seedling.

2. Each sample is graft inoculated to a set of 5 trees, with 2 chips placed on each indicator plant.

3. Clean away leaves and any small side branches that will interfere with the inoculation process (approx. the bottom 4-6 in of the plant).

4. Using a single edge razor blade or grafting knife, make 2 cuts in the GF 305 stem as low as is comfortable. Cut a corresponding chip from 2 pieces of your inoculum to fit into these cuts in the GF 305. If any length of time

greater than 15-20 passes before wrapping, wet down the cut area on GF 305 with water using a squeeze bottle. Insert the inoculum chips and make as secure as possible. Wrap with pieces of stretched parafilm (cut approx. 10-15 mm wide). Wrap tightly from the bottom up until all cut surfaces are covered.

5. Continue until all 5 trees are inoculated, using chips from all branch pieces of inoculum.

6. Include one set of positive controls (PPV, or PDV, or PNRSV, etc.) and one set of uninoculated controls for every 10-15 sets of inoculated test samples.

7. Ten days to 2 weeks after inoculation, cut back the top of GF 305 to 2-3 nodes above the top of the inoculum graft. The plant will now send out side branches from which the disease symptoms can be evaluated.

8. After emergence, remove all but one of the side branches from each plant and observe for disease symptoms.

9. After 4 weeks of growth, cut back the side branch so that only 2 or 3 nodes remain. The seedling will again push out new growth from which to observe for another 4 weeks. Discard the plants at this time.

10. If PPV serology is to be run from leaf samples of the GF 305, collect the mat the time of the first 4 weeks cutback. We collect the newest fully emerged leaves from each of the 5 plants and pool them together for the sample.

11. For best growth of the plants continue to fertilize every 2 weeks throughout the process.

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