Sharka Disease in Bulgaria: Past, Present and Future

I. Kamenova & S. Milusheva


To link to this article: https://doi.org/10.1080/13102818.2005.10817283

© 2005 Taylor and Francis Group, LLC

Published online: 15 Apr 2014.

Submit your article to this journal

Article views: 487

View related articles

Citing articles: 5 View citing articles
SHARKA DISEASE IN BULGARIA: PAST, PRESENT AND FUTURE

I. Kamenova¹, S. Milusheva²
AgroBioInstitute, Sofia, Bulgaria¹
Fruit Growing Institute, Plovdiv, Bulgaria²

ABSTRACT
This review is an attempt to synthesize almost 70 years investigations of Sharka disease in Bulgaria. The results obtained from first virus discovery, disease dissemination throughout stone fruit growing regions, present virus incidence on different Prunus species, natural hosts of the virus, virus detection, strain characterization, susceptibility of plum, peach and apricot cultivars to Sharka disease and virus control measures applied in the past and today are presented.

Discovery and dissemination
“Plum pox. A new virus disease” published at Annuaire de L’Universite de Sofia, Faculte D’Agronomie et de Sylviculture, vol. XI 1932-1933 by professor D. Atanasov, is the earliest documentation of the disease: “Towards the end of the world war in the Kyustendil plum growing region, lying in the south western corner of Bulgaria, was noticed for the first time a new disease on the plum tree, characterized by the premature ripening and dropping of the fruit. The disease gradually spread in all directions, so that at present it is very common in all western districts of Bulgaria along the Yugoslavs boarder. It has spread on the east to Philippolis, in southern Bulgaria, but does not occur in the extensive plum growing region of north Bulgaria (Trojan, Teteven, Drjanovo, Gabrovo, Trevena)” (5).

Actually the first sharka symptoms, on plum trees have been observed by some plum growers from Zemen district after the World War 1, between 1915 and 1918, while Atanassov has seen the diseased tree around 1926 in some villages close to the mentioned Zemen village (5).

Atanassov (5, 6) is not only the first who described the disease but also the first phytopathologist who demonstrated that the causal agent is a virus, since the disease is transmitted by grafting and by the aphid Brachicaudus helichrisi. Atanassov (6) has proposed the name Sharka, which is the Bulgarian name of plum pox. The disease is still known under this name although alternatives are suggested: Plum pox potyvirus (PPV) (syn. Sharka virus, syn. Prunus virus 7, Christoff, syn. Prunus broad streak and ringspot and variegation virus Christoff, syn Annulus pruni Christoff, syn. Prunus virus 5 Smith. At the EPPO conference on Sharka in 1968 it was decided to call the disease preferably Sharka (Plum pox).

Being the most spread local variety in the country Kyustendil’skà sliva has been and is still the most susceptible one. In the early days of sharka discovery more than 20 000 infected trees of Kyustendil’skà sliva have been counted (5). Nowadays, PPV could use as a classical example of an invasive plant pathogen who has spread fast throughout Bulgarian stone fruit growing regions. In recent investigations for PPV incidence on plum in different regions of Bulgaria, Sofia region has been recorded with the highest rate of PPV infection (77%), followed by Drjanovo (73%), Kyustendil (63%) and Plovdiv (39%) (49).
It could be noticed, however, that some of the main plum growing regions of the country, as Trojan and Lovech were not included in that investigation and for that reason the presented results can not be final. According to (21) in the region of Central Balkan Mountains and particularly in Trojan district highly infected are the orchards near the settlement and industrial plantations of Kyustendilska sinja sliva, Stanley and Teteyanka cultivars. The authors have emphasized that in some 30-40 years old orchards planted by local people no PPV infection has been found or only single trees have been noted. Still there are also no investigations and data concerning Sharka distribution in the regions around Rodopa Mountain, where according to the announcements of some local producers sharka infection is is rarely noticed.

In respect to PPV incidence on the different Prunus species in Bulgaria (51) have reported the sour cherry as the most infected (31.03%), followed by plum (24.34%), peach (15.72%), sweet cherry (11.74%), apricot (10.02%) and almond (1.50%). Unlike these results (44) have shown the plum as the most infected stone fruit specie (62.2%), followed by apricot (P. armeniaca) (23.4%) and peach (P. persica) (19.5%). An increase of Sharka infection levels in plum (26.54%), apricot (15.55%) and peach (21.15%) in one of the main stone fruit growing regions as Plovdiv region, compared to that of 24.5%, 11.7% and 15.7% for plum, apricot and peach, respectively (95) for a period less than 10 years has been noticed very recently (67).

At present, more than 70 years after sharka discovery the disease is present in most European countries (80), in the Middle East (Egypt) (63), India (88) and Chile (1), as well. In 1999 PPV was detected in Pennsylvania (USA) (58) and immediately after in Canada (90).

PPV has a great economical importance, due to both, the severity of the damages caused and the wide distribution of the virus. As Atanassov (5) has noticed “The plum pox is a very destructive disease which threatens the very existence of plum culture of Bulgaria, since the affected trees never yield marketable fruit”. According to (11) yield losses are caused by the premature fruit ripping and dropping, the rapid natural spread by aphid vectors and rapid decline and even death of the trees when co-infected with other viruses. Due to the decrease sugar content the plum fruits are unsuitable for direct consumption or for dried fruit, jam or brandy production.

The losses caused by PPV in each affected country depend on many factors, including the susceptibility of the grown species and cultivars, strain presence and the control measures applied. In Bulgaria, Sharka disease is the most economically important disease on plum, and still less important for peach and apricot productions. The great economical importance of sharka in Bulgaria and Yugoslavia due to the great susceptibility of Kustendilska sinja sliva, (Pozegace), which is the native and most distributed cultivar in both countries (10). Because of the premature dropping of the fruits which begin 30-40 days before their ripening, very small number of the fruits remains to full ripeness on the trees. Most of these fruits are with poxy appearance, under which the pulp is discolored and gummy. Generally these fruits are gummy and contain more organic acids, but less sugar and, especially, less sucrose. The fruits of the susceptible varieties of all hosts are smaller in size and have high stone-pulp ratio (10). Severe (80 to 100%) premature fruit dropping and losses up to 120 000 tones of fresh fruits have been reported for Kustendilska sinja sliva (101). Decrease of 19 and 25% of the fruit mass of two plum cultivars, Stanley and Stri- nava, respectively infected with sharka have been reported by (62). Fifteen and 36% reductions of the yield of Stanley and
Strinava, respectively for over three years of observations have been recorded by the same authors.

**Symptoms**

What does sharka disease cause on infected stone fruit species and how the disease can be recognized? According to (5) "At first the infected trees are apparently normal, only their leaves show the presence of very characteristic light green or yellowish-green spots, blots, streaks, arches or complete rings, resembling somewhat the ring spot disease of tobacco". Although the symptoms of sharka disease are well known among the virologist and when do occur they are specific and diagnostic the symptoms alone cannot relied upon to determine sharka infection. The expression of the symptoms varies depending on the host (species and cultivars), the age of infected plant, the strain and environmental conditions. Generally, the plums are more severely affected and show more severe symptoms than the other stone fruits. Usually at the climatic conditions in Bulgaria the first symptoms on diseased plum trees appear on the leaves of the cultivars Karadjeki at the beginning of May. Several days later the symptoms appear on the leaves of all other plum cultivars (101).

According to the author the typical sharka symptoms consist of light green chlorotic spots, rings, blotsches and lines, sometimes bordered by the nerves, sometimes along the nerves. These symptoms may be obvious or barely visible to the eye and depending to the cultivar the leaf symptoms may disappear, or become less distinct during the summer. Some plum cultivars may show so called "reverse sharka" when the rings and the spots become green, while the other part of the leaf blade turns yellow (101).

The name sharka of the disease is coming actually from the symptoms PPV induces on plum fruits. Plum fruit develops normally until it reaches its normal size and than begins to ripen from 10 to 15 and more days ahead of the fruit of normal trees (5). In this respect this disease resembles peach yellows. At this time and even before, on the fruit appears one to more blue spots, which often have the form of arches or rings with normal centers. Gradually the spots become sunken and the surface of the fruit begins to resemble the faces of people who have had small pox (variola). Under the spots and depressions the fruit tissues are necrotic, rusty, dry and shrunken, or may be quite normal, but have a red to purple color. The necrotic tissues extend often down to the seed. In such tissues pockets are often formed with gum. The diseased fruit is poor on sugar, worthless, and has an unpleasant taste. It shrivels up and falls prematurely (5).

Distinct, highly sensitive plum cultivars, like *Grose Grüne Reneklode* aside from fruit and leaf symptoms may show depressed growth of single shoots and smaller fruits on these shoots (101). No correlation between the intensity of leaf and fruit symptoms has been noticed for some plum cultivars as *Kratunka, Pink plum from Razgrad, Godovka, Petrovka, Pumpalka, Plovdivska* (101).

Strong effects of sharka disease on the vigor of some plum cultivars (*Czar, Moldovska rana, Pacific, Jelta edra, Reine-Claude verte, De Marie, Pop Hariton, Soperniza, Rodunta and Pazelt*) and later a dye of single trees has been reported (29).

Foliar symptoms observed on *P. cerasifera* Ehrh. cultivars have resembled those observed on plum (*P. domestica* L.) cultivars concerning their shape, color and disposal on the leaf blade (10). According to the author the symptoms can be single but when they are numerous often coalesce thus resulting in choroses of the whole leaf. Ring spots and arches on the surface of the fruits without internal damages of the flesh have been reported by the same author.

The symptoms on peach leaf include small spots, rings, vein clearing and ban-
Since the symptoms appear in the first leaves to expand, later with their growth they twist and distort (94, 112). Light green to yellow blotches and veinal chlorosis together with chlorotic rings, like the symptoms sharka disease cause on plum leaves have been reported for the peach cultivar Sun Crest, while lack of any leaf symptoms on the infected cultivar Cacamas has been reported (112). As both authors (94, 112) have noticed the most distinctive PPV symptoms have been developed on the leaves from the middle part of the shoots, while the younger leaves, close to the tip part have not shown any symptoms. Twice higher virus concentration in the leaves from the base and middle parts of peach shoots corresponding to severity of symptoms observed compared to that in the leaves from the tip part of the shoots has been found in several peach cultivars (27, 112). According (94), the symptoms on peach fruits are dependent from the cultivar and very often they are localized only on the fruit surface. When the fruits are yellow colored the symptoms are difficult to be observed but when the fruits are reddish colored the symptoms are visible and consist of ring spots and irregular line patterns often coalescing together and covering almost the all fruit surface. For some peach cultivars, premature fruits drop from the infected trees, as in the case of susceptible plum cultivars has been noticed (94).

Compared to symptoms on peach and especially the symptoms on plum the apricot leaf symptoms are less conspicuous. Single leaves may show light green diffuse spots around the veins early in the spring but later the symptoms usually disappear. The fruits of some cultivars may be severely marked and misshapen with poor flavour. According to (10) infected with sharka apricot trees are more susceptible to winter injury. Very characteristic, however, are the symptoms on the internal stone of the infected apricot fruit consisting of distinct rings, spots and arches (94).

The symptoms PPV induces on the leaves of infected cherry trees consist of pale-green rings, spots and irregular mottling around the nerves and tip part of the leaf blade. Some of the spots later become necrotic (95). Slightly sunken in the fruit flesh necrotic ring spot on the mature cherry fruits have been described (95).

Irregular ring spots and chlorotic mottled areas, affecting also the nerves on separate developed leaves of sour cherry trees from Bigarreau Burlat, Compact Stella, Van, Vega and in M-15 cultivars infected with sharka disease have been described (94). Tiny rings (2-3 mm in diameter), some of its slightly sunken in the fruit flesh have been observed on the matured fruits of Van and Vega cultivars. A weaker growth and development of infected trees, presence of flowers, green (deformed) and mature fruits simultaneously on infected trees and even dead of crown branches in infected trees of Northern Star sour cherry cultivar have been noticed (94).

**Hosts**

PPV can infect cultivated, ornamentals and wild species of Prunus, such as apricot (P. armeniaca), sweet cherry (P. avium), myrobalan plum (P. cerasifera), sour cherry (P. cerasus), common plum (P. domestica), dwarf flowering almond (P. glandulosa), damson plum (P. insititia), peach (P. persica), nectarine (P. persica var. nectyarina), Japanese plum (P. salicina), Nanking cherry (P. tomentosa), P. brigandine, P. holoserica, P. hortulana, Greengage (P. italica), Mahaleb (P. mahaleb), P. mandschurica, Beach plum (P. maritima), Japanese apricot (P. mume), Siberian apricot (P. sibirica), apricot plum (P. siomonii), flowering almond (P. triloba), P. bireana, P. bireana cv. Moseri, P. japonica, P. cerasifera “Nifgra”, P. cerasifera “Pseudula” and Persica x davidopersica “Atropurpurea”.

---

**20th Anniversary AgroBioInstitute** - R&D 
**Biotechnol. & Biotechnol. Eq. 19/2005** 
**Special Issue**
Prunus species proven to be natural hosts of sharka disease in Bulgaria include: common plum (Prunus domestica L.) (5), apricot (Prunus armeniaca) (6, 8), sweet cherry (Prunus avium) (6, 91, 92), P. cerasifera Ehrh. (6, 8), P. insititia L. and P. triloba (10), Japanese plum (P. salicina Lind. (98), peach (Prunus persica) (26, 112), sour cherry (Prunus cerasus) (91, 92) and almond (Prunus amygdalus) (95). Blackthorn (Prunus spinosa) has been found to be a symptomless host of PPV (Kamenova, unpublished).

According to (4) the wide distribution of the virus in nature is realized by weeds. The weed species, Lamium purpureum L., Lamium amplexicaule L., Vicia angustifolia L., Selene vulgaris L., Trifolium repens L., Rumenculus acer L., Solanum dulcamara L., Rumex acetosella L. and Songhus arvensis L. have been reported to be infected by sharka virus under field conditions or susceptible to artificial inoculation (70, 108). In Bulgaria, (54) have reported (Lamium album), Ranunculus acer, Selene vulgaris, Solanum dulcamara as natural hosts of PPV. Recently, large scale DAS-ELISA tests of weed species collected from plum and apricot orchards (885 and 810 plants, respectively) have revealed sharka infection of Capsella bursa-pastories L., Veronica hederefolia L., Rumex crispus L., Lactuca serriola L., and Lithospermum arvense L. The last three species are found as PPV hosts in respect to their speed of infection. The trees of highly susceptible on fruits cultivars as Kyustendilska sinja, Large Reine Claude verte and Tuleo Gras grown in comparatively wind protected orchards have been basically infected up to the fifth year post planting, while the tree of the less susceptible on the fruits cultivars as Belle de Louvain, Violette Reine Clande and Sakarka several years later. The infection of highly susceptible cultivars grown, however, in orchards not protected from the wind has occurred several years later. In studies for the reaction of some plum cultivars to field infection with PPV, Kyustendiliska sinja sliva is the cultivar reported long-distance spread of the disease is usually through the use of infected plants and the subsequent distribution of PPV contaminated materials as bud wood, rootstocks or seedlings. The secondary spread (short-distance spread) of the disease in the orchards is accomplished in non-persistent manner by aphid vectors. Brachicaudus helichrisi Kalt. is the first aphid species described as a vector of PPV (6, 10). Later, Brachicaudus cardui L. (55), Phorodon humuli (37, 56) and Myzus persicae Silz. (50, 55) have been reported as virus vectors. Several aphid species are able to transmit PPV in a non-persistent manner, as Myzus persicae playing the most significant role in sharka spread (60). The efficiency of aphid transmission largely depends upon the virus strain, the host species, the cultivar and the aphid species. Since M strains are transmitted more efficiently by aphids than D strains they are considered as the "epidemic" form of the virus. Investigations for the speed and rates of natural sharka spread in four plum orchards in highly infected region (Sofia district) made during the period of 1961-1979 have revealed the presence of high populations of B. helihrysi and Phorodon humuli species (106). The author has established a difference among the grown plum cultivars in respect to their speed of infection. The trees of highly susceptible on fruits cultivars as Kyustendilska sinja sliva, Large Reine Claude verte and Tuleo Gras grown in comparatively wind protected orchards have been basically infected up to the fifth year post planting, while the tree of the less susceptible on the fruits cultivars as Belle de Louvain, Violette Reine Clande and Sakarka several years later. The infection of highly susceptible cultivars grown, however, in orchards not protected from the wind has occurred several years later. In studies for the reaction of some plum cultivars to field infection with PPV, Kyustendiliska sinja sliva is the cultivar reported...
to be infected most rapidly in nature, since the first symptoms on the leaves have been noticed in the second year and up to the 7th year 30% of the trees have become infected (22). Subsequently, however, the speed of the disease spread has slowed down and in the 15th year 60% of the trees have been infected (22).

In comparative study for transmission of one peach isolate (S-20) to several woody and herbaceous test plants with the aphid species, *Muzus persicae, Myzodes varians, Brahiacaudus persicae, Br. helichrysi, Br. cardui, Phorodon humuli* and *Hyalopteris amygdale* (28) have found that the highest rate of transmission (86%) has occurred with *M. persicae* and the lowest one with *Br. cardui*. On that base the authors have concluded that *M. persicae* is practically the vector playing a determinate role for virus spread on the peach cultivars in Bulgaria.

Two viral proteins, CP and HCPro, have been shown to be involved in aphid transmission of number of potyviruses, and defects in either protein have been proved to abolish it (76). Aphid transmission of seven PPV isolates, including four Bulgarian isolates (one plum, one plum and two peach) with *M. persicae* to *N. benthamiana* plants has been studied (45). Transmission rates of 30 and 26% have been found for the apricot (PPV-A) and the plum (PPV-P) isolates, respectively, while both peach isolates (PPV-P45 and PPV-P48) and the other three isolates have failed to be aphid transmitted. Helper components with Mr. of 52 kDa have been successfully purified from *N. benthamiana* plants inoculated with both types of isolates (aphid and non-aphid transmitted). The authors have shown that the helper components of two of non-transmissible isolates with defective CPs (deletions and DAG substitutions to DAL and NAG) have mediated aphid transmission of non CP defective isolates, thus proving that the CP defects rather than the HPs have been the reason for their non transmission by aphids (45).

At present, there are no investigations concerning the presence of aphid species and their role in transmission and natural spread of Sharka disease in Bulgaria. However, having in mind the prevalence of PPV-M strains in several investigated stone growing regions in our country and the fact that basically M strains as ease transmitted by aphids, it could be expected further efficient implication of aphid vectors in Sharka spread.

Seed transmission of sharka disease is still not completely cleared and often the available information is contradictory. For the first time (87) in Hungary and (17, 81) in Romania have reported for seed transmission by the seeds of apricot, plum and peach, respectively. Seed transmission of PPV has been demonstrated experimentally (73), but according to (83) it is not known to occur in practice. In tests of several plum varieties the virus has not been transmitted by seeds (38). No seed transmission of PPV with seeds from peach and apricot seeds has been also reported (24). nemeth (74) has summarized various researches on seed and pollen transmission studies and has concluded that seed and pollen transmission very depend on the host. It is assumed that seed transmission in *Prunus* is known not to occur with D strain, but there are some reports of seed transmission with M strain.

In Bulgaria, the presence of PPV in the pollen of infected trees from *Green Reine-Claude* and *Kyustendiliska sinja sliva* has been proven after inoculation of *C. foetidium* plants (97). Based on the local lesions obtained the author has suggested the possibility of virus transfer by the pollen. Whether the transfer of the virus through the pollen, from infected to healthy plants could induce sharka infection, however, is a question which has not been studied and elucidated further experimentally. Virus presence in the pollen of flowers from sharka infected plum, peach and apricot...
trees has been established also by DAS-ELISA and IC-RT-PCR analyses by Kamenova (unpublished data). In a study for PPV transmission through the seeds of infected trees of Stanley cultivar the virus has been determined in 32-35% of seed coats and in 8% of cotyledons and not found in the samples formed from embryonic axis (65). After the germination of the seeds in vitro PPV has been detected in 8.3% of cotyledon segments but not in the roots and the pluemes of the seedlings.

The potential role of the wild Prunus species infected with sharka disease and the role of the weeds in PPV transmission and survival is still poorly studied and understood. Whether or not, the weeds distributed in fruit growing orchards worldwide and particularly in Bulgaria could play a significant role in sharka transmission and spread in the nature is not determined and more efforts in that direction are required.

**Virus detection**

The symptoms caused by PPV on the different stone fruit species are diagnostic and the visual inspection of the plants during the growing season is an essential measure for evaluating the state of health. Symptom inspection of infected trees alone, however, is not reliable method for sharka detection having in mind the irregular PPV distribution or the presence of symptomless infections in infected trees. Biological, serological and molecular techniques are used for PPV detection and identification.

**Biological testing.** The simplest method for detection of PPV is by mechanical inoculations of herbaceous plants and inoculation by chip budding or grafting of woody indicator plants. More than 60 herbaceous plants are known as host of PPV but the most used ones are C. foetidium, several Nicotiana species and Pisum sativum. The virus can be reliably detected also on woody indicator plants as peach seedlings (GF 305, Elberta), Myrobalan (GF 31) and Prunus tomentosa.

In comparative investigation of the sensitivity of the species N. benthamiana, N. clevelandii and C. foetidum to PPV with a view to be used for PPV detection in Bulgaria, (41) has found that N. benthamiana is the most suitable one because of its high susceptibility (60% infected plants) and the clear symptoms, consisting of dark-green mottling around the veins of systemically developed leaves, while N. clevelandii has been symptomless host of the tested isolates. At present, biological tests by grafting or chip budding of woody indicators are routinely and wide applied for PPV identification at Fruit Growing Institute, Plovdiv (96).

Basically, biological indexing is labor-intensive and time consuming since it provides results within 2 weeks with herbaceous plants and at least 3 to 6 months with woody plants and often can be limited by the potential of uneven distribution of the virus.

**Serological testing.** Early (around the seventies) serological detection of PPV has been based on precipitation, latex or radial gel diffusion test. By the use of PALLAS method (protein A coated latex linked antisera) (39) has identified PPV in leaves and fruits from several infected plum varieties. With the development of PPV in leaves and fruits of several infected plum varieties. With the development of PPV techniques, the possibility of fast, reliable and accurate detection and identification of PPV in fruit trees has considerable increased. In fact PPV and Arabis mosaic virus are the first plant viruses, for which ELISA test was successfully applied (2, 16).

In comparative study for PPV detection by visual observations and DAS-ELISA method a disagreement between ELISA values and the visual observations has been found (40). The observed pox symptoms on the leaves and fruits of several plum cultivars and the negative ELISA results have suggested the presence of "pseudo-pox" symptoms, while the lack of visual symp-
Symptoms on leaves and fruits accompanied with positive results of the ELISA test have indicated the presence of symptomless sharka infection. Higher virus concentration has been recorded in plum flowers than in the leaves. The authors have recommended ELISA method as a reliable method for sharka detection in Bulgaria.

Polyclonal antisera against three isolates of PPV (apricot, peach, both Bulgarian and cherry - Moldova) produced by (42) are widely used now for routine PPV diagnoses in Bulgaria. The most suitable period for serological detection of PPV under Bulgarian climatic conditions, is from May to early August, but the virus is also reliable detected during the winter in forcibly developed dormant leaf and flower buds (Kamenova, unpublished).

**Molecular techniques.** The problem of low PPV concentration for reliable detection in infected trees has been overcome with application of cDNA and cRNA probes in the 1980’s (109) and later with the development of polymerase chain reaction technology when PPV was among the first viral targets amplified (111). In Bulgaria IC-RT-PCR method has been applied for PPV detection by (46). Recently, the author has successfully used IC-RT-PCR for PPV identification during the winter in forcibly developed leaf and flower buds from plum, peach and apricot (Kamenova, unpublished).

**Isolates and strains.** Numerous PPV isolates have been characterized the world over. To date, two main subgroups of PPV isolates, i.e. PPV-M and PPV-D have been determined, on the base of serological or molecular tools of the coat protein (CP) or CP gene. Both strains are widely distributed in Europe and show significant differences in their epidemiological properties. M strains are transmitted from infected to non-infected trees very efficiently by aphids and are considered as the “epidemic” form of PPV. Once established in a region, M strains can spread quickly and are very difficult to be eliminated. Because aphids are not very efficient in transmitting D strains from infected to the non-infected trees, these strains are referred as a “non-epidemic” form of PPV. Two additional minor subgroups include geographically limited apricot isolate from Egypt (EL Amar) and cherry infecting isolates (PPV-C).

One of the very first attempts for PPV strain differentiation and classification is the method developed by (85). Based on the reaction of the local host of PPV, Chenopodium foetidum, the authors have grouped the isolates studied in “yellow”, “intermediate” and “necrotic” strains. On the base of reaction of C. foetidum forty two PPV plum isolates from different Bulgarian regions (Kostinbrod, Trojan and Drjanovo) have been classified as yellow (3 isolates), intermediate (13) and necrotic (26) strains (39). Some differences among the grouped three strains in respect to their properties in vitro have been noted (39). In a study for biological characterization of number of isolates from different hosts (plum, apricot, peach), (94) has reported that some of the isolates have caused local lesions on C. quinoa and C. amaranticolor. Thirteen of the isolates (from plum, apricot and peach), (94, 95) has classified as “intermediate”, while two of the isolates (1 plum and 1 apricot) have not been distinguished for the lack of any symptoms on C. foetidum. Later on, (96) have studied the biological and immunoenzymic characteristics of several PPV isolates (5 from peach, 3 from plums, 1 from apricot and 1 from cherry) from south and north (Plovdiv and Silistra districts, respectively) regions of Bulgaria. Again on the reaction of C. foetidum the isolates (peach ones) have been determined as “intermediate”, while two of the isolates (1 plum and 1 apricot) have not been distinguished for the lack of any symptoms on C. foetidum. Later on, (96) have studied the biological and immunoenzymic characteristics of several PPV isolates (5 from peach, 3 from plums, 1 from apricot and 1 from cherry) from south and north (Plovdiv and Silistra districts, respectively) regions of Bulgaria. Again on the reaction of C. foetidum the isolates (peach ones) have been determined as “intermediate”. Based on the local lesions induced on the species C. amaranticolor and C. quinoa by two peach isolates, one plum and the cherry isolates the authors have assumed the presence of strains with higher immuno-
In comparative host range study of seven PPV isolates, including three Bulgarian isolates (1 apricot and 2 peach) (46) has found differences among the Bulgarian isolates that has allowed their classification into three biological groups. One of peach isolates (P45) being able to cause local chlorotic lesions on *C. quinoa* and local chlorotic lesion with necrotic center on *C. amaranticolor* has composed the first biological group. Since apricot isolate (PPV-A) has not induced symptoms on the above herbaceous species that isolate has been placed in the second biological group. On the base of very restricted host range (*N. benthamiana, N. clevelandii* and *N. occidentalis*), the second peach isolate (PPV-P48) has composed the third biological group. Electrophoresis mobility of the coat proteins of the above isolates and additionally of one more isolate from plum (PPV-P), together with the results from their reaction with monoclonal antibodies (MAbs) have defined the isolates studied to PPV-M and PPV-D group of strains. The molecular weights of 38 kDa, and the reaction with MAbAL, specific for PPV-M strains has allocated two of the isolates, PPV-A and PPV-P (apricot and plum isolates, respectively) to PPV-M group. On the base of molecular masses (33kDa) and their positive reaction with PPV-D specific MAb (4DG5), both peach isolates (PPV-P45 and PPV-P48) have been classified as PPV-D strains (46). Lack of correlation between the biological and serological classification of the studied Bulgarian isolates has been noted. Both peach isolates (PPV-P45 and PPV-P48) defined as PPV-D strains have shown different biological properties (first and third biological groups, respectively). The deletions (17 amino acids) found between the 14 and 31 residues in the coat proteins of peach isolates, together with DAL replacement of DAG amino-triplet, required for successful aphid transmission, have explained both, the lack of aphid transmissibility and the lower molecular weights of these peach isolates (43, 45). A comparison of amino acids sequence data of the mentioned above Bulgarian PPV isolates from different natural host (plum, apricot, peach), has revealed a high percentage of similarity (99.05%) between the two peach isolates - PPV-P45 and PPV-P48 and similarity of 97.58% between the apricot and plum isolates (PPV-A and PPV-P (43). The authors have shown that the two peach isolates (P45 and P48) have shared significant homology (97.49 and 96.55%, respectively) with the published sequences of D strain (78) and AT strain (97.77 and 97.13%, respectively) (59). The apricot (PPV-A) and plum (PPV-P) isolates, have had the highest identity (99.10% and 97.10%, respectively) with the Hungarian isolate SK68. As result of that comparison (43) have divinized the studied Bulgarian isolates into PPV subgroups A and B, proposed by (75).

In the last several years, typing of relatively high number of PPV isolates (total of 536 samples) from different host (plum, apricot, peach, cherry) by the use of MAbs (4DG5 - PPV-D specific, AL - PPV-M specific, EA24 - EL Amar specific and AC - PPV-C specific) from some main foci of PPV infection in Bulgaria has been carried out (44, 47, 48, 49)

Only PPV-M strain has been identified on peach and apricot cultivars. The most distributed strain on plum has been PPV-M (88%), followed by PPV-D (5.2%). Mixed infections of both strains (M+D) at a rate of 6% have been found, as well. All plum isolates coming from north and south of Bulgaria (Drjanovo and Plovdiv, respectively) and the isolates from Sofia region have been identified as M strain. PPV-M strain has been the predominant strain (74.8%) in Kyustendil region (the region of the first
Sharka discovery), too. PPV-D strain at a rate of 11.6%, however, and mixed M+D serotype infection (11.6%) in all tested trees of cv. Čačanski Seker and single trees of California Blue, Anna Spath, Lyumitsa Hranova and Stanley plums have been identified at that western part of the country (near the Serbia border) (48). So far, no Bulgarian isolates have been identified as PPV-EA and PPV-C but the number of tested samples has been limited (49).

Very recently, biological indexing by chip-bud inoculation of six Prunus genotypes and mechanical inoculation of ten herbaceous species with the studied by (96) several PPV isolates from peach, apricot, plum and cherry, and including two more peach isolates, together with sequence analyses of the 3'-terminal of their RNAs have been made (66). A limited correlation between the two biological tests of the isolates compared, concerning their virulence onto susceptible and non-susceptible Prunus genotypes and herbaceous hosts has been noted. Two of the isolates (one plum and the cherry isolates) have infected P. mahaleb. Due to the higher number of infected by cherry isolate herbaceous plants, that isolate has been allocated to the group of the isolates with higher virulence. Using serogroup-specific MAbs, PPV sub-group-specific RT-PCR and RFLP analyses the isolates have been confirmed to be PPV-M strain, showing 97-99% identity of their coat protein sequences with PS and SK68 isolates (PPV-M). On the base of protein sequence alignment, the isolates studied have formed 2 groups, except the apricot isolate who has appeared to be unique (66).

At present, PPV-M confirms its dominant and endemic presence in Bulgaria. Having in mind, the lack of any information concerning strain presence in some other main fruit growing regions of the country, mainly in the Central Balkan Mountain (Gabrovo, Lovech, Trojan districts) and Black sea cost (Varna, Pomorie, Burgas districts), the presence of natural mixtures of strains (M+D) found in one of the surveyed infection foci and the recent reports for natural recombinant isolates of PPV from some European countries (31, 32) imply the need of further strain differentiation and classification. On the other hand in the last several years, many small-sized stone fruit orchards (peach, apricot) have been established all over the country with propagating material frequently coming abroad. Investigations and sharka surveys in these new fruit growing areas are required, as well.

Existence of two serological subclusters of PPV-M strain, denoted as PPV-M1 and PPV-M2, corresponding to the isolates from two geographically defined areas (i.e., the Mediterranean and Central-Eastern Europe, respectively) has been reported (71). The isolate from Bulgaria, included by the above authors in the study, however, has been defined as PPV-D strain. The question is in which subcloster of PPV-M strains could be included the majority of PPV-M strains present in our country? In that aspect, further determination of the status of PPV-M strains in Bulgaria is highly required for better understanding and elucidation of sharka disease evolution, particularly in the country and at the Balkan peninsula and European levels, as well.

**Susceptibility of plum, peach, apricot cultivars**

**Plum.** Shortly after sharka discovery, (9) has reported that while severe symptoms on the fruits of Kyustendil ska sinja sliva and Italian kecha have been observed, including multiple and sunken rings and spots with injuries in the flesh, on the fruits of Monfort and Buhler Frühzwetsche, only single and very mild symptoms have been present. Today is well known that the different Prunus species and cultivars behave differently when infected with PPV. The differences in susceptibility between the cultivars available have encouraged the
stone fruit breeders to seek for resistance to PPV. Actually (10) is the first virologist who has carried out experiments (by grafting of buds from infected Kyustendiliska sinja sliva on the tested cultivar) for screening of plum cultivars resistant to PPV. On the base of the symptoms incidence on the fruits of the tested 24 cultivars the author has reported the plum cultivars Anna Spath, Bühler Frühzwetsche, Monfort, The Czar, Prune d’Agen, Violette Rein Claude, Big Shugar prune, Early read Mirabelle, Rivers Early, Belle of Louvain, Grosse Rein Claude, Yellow Afuska and Red Afuska (P. cerasifera) and Mirabelle Precoce (P. insititia) as resistant to sharka disease. Further on, in continuation of his experimental work with PPV resistance of plum cultivars (11, 12, 13, 14, 15) has reported the next cultivars with tolerance and resistance to sharka disease: Early Red English, Green Reine Claude, Mirabelle Monfort, Yellow Afaz, Big Rambeau, Enibakanka Red, Favorite, Gabrov ska, Karadjieka, Kirke, Monfort, Pribekja, Reine Claude Dorre, Sarjada, Shiptchen ska, Sinajkiza, Sinja Rakijniza, Earl u Blue, Belle delouvaine, Reine Claude Violette, Septemvrisija, Sofia, Sofia Wande, and Serdika.

The number of trees showing PPV symptoms, the aphid populations and the percentage of injured and shed leaves and fruits have been used by (99) as criterion for evaluation of susceptibility of 93 local and foreign plum cultivars to sharka infection. Kyustendiler Blaue Pflaume and Magareschjak have been assigned as the most sensitive, while Agen, Bühler Frühzwetsche and Stanley as the most tolerant cultivars. The last cultivar, Stanley has been recommended for growing in PPV infected areas and for top grafting of diseased susceptible trees (102, 103). As sources suitable for PPV resistance, Kirke, Tuleu grass and Wangenheim have been found (100). Later, (102) has recommended also the tests of plum cultivars for their reaction to PPV to be held not less than 8-10 years, because with the age the most plum cultivars become more susceptible to the disease. The high resistance of the cultivars, Damson, Winter Damson, Summer Damson and Nancy Mirabelle, (P. insititia) to sharka disease has allowed (103) to offer them for hybridization with cultivars of P. domestica.

Sensitivity of 88 plum cultivars, 3 interspecies hybrids, Myrobalana Alba root stocks and P. Simoni Carr. species have been studied by (110). The cultivars Vasilyovskaya 41, Yellow Afuska, Hard Yellow Afuska, Plovdivska Afuska, Krimska 141, Krimska ball-shaped, Nikitsa Golden, Blue Afuska, Urgan su, Myrobalana alba (P. cerasifera Ehrh.), Satsuma (P. salicina Lind.), Onthario (P. americana March.), the interspecies hybrid Partizanka (Burbank X Zheltaya pozdnyaaya), Serozabenek (P. domestica spp. domestica), Goccia doro und Konkurent and the species P. Simoni Carr have been found to be leaf and fruit resistant. The cultivars Bühler Frühzwetsche, Wilhelmina Schpet, Izobilie 242, Californian plum, Malvazinka, Man Ju Mary, Monfort plum and Stanley have been characterized as tolerant on the fruits, while Pineapple plum, Rencloda Aloise, Beautiful Luvenska as moderately sensitive to the disease.

The susceptibility of 47 Prunus domestica L. cultivars and hybrids in conditions of natural sharka infection has been assessed by (29). Fifty one percent of the genotypes have been evaluated as resistant to tolerant (Scolds, Cačanska Najbolja, Nevena, Opal, Kirke, Mirabelle de Nancy, Cačanska lepotica, Gabrov ska, Reine – Claude Chr amova), 17% as susceptible (Hermen, Strinava, Imperial Epinense, Pacific, Zhulta edra, Reine-Claude verte) and 32% as highly susceptible (Kyustendilska sinja, Tuleo dulce, de Marie Deniston, Tetevenska, Pop Hariton, Nikitsa Rana, Soperniza, Troyanska, Tragedy, Pazelt).
Several cultivars, Bühler Frühzwetsche, Kirke, Opal and Pacific have been found not infected with PPV under field condition of high infection level for a period of 12 years (21). Because of the absence or slight damages on the fruits of Čačanska lepotica, Čačanska Najbolja and Sentjabarskaya, the authors have defined these cultivars as PPV tolerant and recommended them for planting in highly infected regions with Stanley cultivar. The poor translocation of the disease in the trees of cv. Gabrovaska, together with very slight symptoms manifestation on the fruits has allowed (21) to classify Gabrovaska as cultivar with the highest field resistance to PPV. On that base Gabrovaska cultivar has been determined as very suitable for planting in pox-infected regions. For overcoming the great disadvantage of Gabrovaska as self-sterile cultivar the authors have recommended it planting together with some plum cultivar as Geley and Stanley.

The reported as completely resistant to PPV cultivar Zhulta butilkovidna (86) under Bulgarian environmental conditions has reacted with well pronounced symptoms on single leaves and fruits and on that base the cultivar has been defined as tolerant to sharka disease (84).

The reaction of Katinka and Elena and Ortenauer plum cultivars, reported in Germany as tolerant and poorly susceptible, respectively to PPV (7, 33) has been evaluated to PPV-M strain spread in Plovdiv region of Bulgaria (19). Severe leaf symptoms of PPV have been observed on all cultivars and the highest level of infection has been noted on the fruits of Kalinka (87.5%), followed by Ortenauer (66.6%) and Elena (57.1%).

In recent years more credibility has gained the opinion that interspecific hybrids should be used in the breeding of sharka tolerant cultivars (20, 34, 105, 107). Following that, (68) have investigated the reaction to PPV of 166 interspecific hybrids of the combinations: P. spinosa x P. domestica cv. Greengage, P. spinosa x P. domestica cv. Zhulta Albanska and P. spinosa x P. cerasifera on the base of symptoms presence and ELISA data. Field resistance has been found in the three mentioned above combinations since no sharka symptoms on the leaves have been observed and ELISA tests have detected PPV in only 10 plants. Thirteen perspective hybrids have been selected for further plant breeding experiments. Further on, 754 interspecific hybrids obtained from five hybrid families (four of them from the mother plant P. spinosa and in one myrobalan P. cerasifera) with male parents from P. cerasifera, P. spinosa and P. domestica have been studied by (69) for their growth performance, morphological and biological characters and response to PPV. Two of the hybrids, Hybrid 55/4 “Blackthorn” (P. spinosa) x “Green Gage” (P. domestica) and Hybrid 60/10 “Blackthorn” (P. spinosa) x Green Gage” (P. domestica) have been ased as tolerant to PPV. With filed resistance to PPV three hybrids: Hybrid 31/14 “Myrobalan” (P. cerasifera) x Blackthorn” (P. spinosa), Hybrid 10/4 “Blackthorn” (P. spinosa) x “Zhulta Ablanska Sliva” (P. domestica) and Hybrid 16/12 “Blackthorn” (P. spinosa) x “Zhulta Ablanska Sliva” (P. domestica) have been defined (69).

As a result from interspecific hybridization between P. domestica and the species P. cerasifera, P. ussurienis, P. armeniaca and P. dasycarpa, individual interspecific F1 progenies from Stanley x Modesto and Stanley x Early Divinity with tolerance to PPV have been obtained (30).

Difference in the reaction to PPV of three plum cultivars (Kyustendilskia sinja sliva, Stanley and Gabrovaska) grafted on two rootstock [Zhalta Dzanka (P. cerasifera) and Byala Uhrepka (P. insititia)] has been registered by (23). Slower virus spread and less infected trees have been found with plum cultivars grafted on Byala Uhrepka rootstocks. The most obvious ef-
fect of the rootstock used has been noted in the combination Gabrovska/Byala Uhrepka, where only 3 trees have been infected at the age of 15 years old and at the age of 19 years old the percentage of infected trees has become 4.8%. For comparison the rate of infected trees of Gabrovska grafted on Zhulata Dzanka at the age of 19 years old has been 38.9% (23).

Behavior of three forms of the cultivar type Kystendilska sinja, named Besztercei BT 2, Besztercei MM 116 and Besztercei MM 122 (introduced from Hungary) grown under conditions which have permitted natural infection with sharka virus has been evaluated on the base of the symptoms presence and DAS-ELISA results (36). Because of the low rate of discarded fruits (4.6%) of the average biological yield, the authors have recommended Besztercei MM 122 for cultivation in sharka infected regions.

Peach. Compared to the studies for susceptibility of plum cultivars to sharka disease, those conducted with peach and nectarine cultivars in Bulgaria are still scanty. Over 65 peach, nectarine cultivars and hybrids have been evaluated for their reaction to PPV infection for a period of 3 years and conditions of natural infection in South Bulgaria by (27). Only 4-7% of the cultivars, including Dupniska, Superior Pacific Star, Red Bird Cling, Early Glow have not shown PPV infection after their visual inspection for fruit symptoms and DAS-ELISA tests and 39% of the cultivars have shown mild symptoms on the fruits. 29-31% of the cultivars have been evaluated with moderate manifestation of fruit symptoms and 17-19% of the cultivars and hybrids with conspicuous leaf and fruit symptoms. Not a strict correlation between the ELISA virus concentration and the visual estimation has been observed (27).

Investigations for estimation of susceptibility of peach and nectarine cultivars, rootstocks and hybrids to PPV on the base of visual and DAS-ELISA data of naturally infected trees under field conditions have shown the presence of tolerant (Baladin, Centry, Chaya, Flamekist, Indipendence, New Jersey 59, Plovdiv 2, Plovdiv 3, Rubired, Slivenska kompotna, Spring lady and Summerset), poorly susceptible (Aheoi, Babygold 6, Babygold 7, Cardinal, Collins, Early Glow, Golden queen, Lamonwe, Maycrest, Piedmontgol, Sudanel, Tebana, Tundzha 1, J.H.Hale, Halford II, Shasta, the rootstocks GF 305, GF 677, Hansen 2 and Hansen 5), moderate susceptible Aurelia, Elberta, Fairtime, Early crest, Julcta edra, Petrichka, Summer beauty), susceptible (Andros, Babygold 9, Cresthaven, Fantazia, Maria Serena and Redtop) and strongly susceptible (Adriatica, Anderson, Glowhaven, Late le grand, Malo Konare, Maria Luisa, Marigold, Nectared 2, Redhaven, Red June, Sun crest and Fayette) ones (93).

Apricot. Among the apricot cultivars reported by (10) that have shown healthy fruits in spite of the mottling of their leaves are Mandoria Doice and Ungarishe beste Apricose. The next several varieties, White Large, Early Apricot, DE Versailles, Aleko, Pasha, Mush large, Golden, Yellow Apricot, Apricot from Leskovetz, La France the author has described as very susceptible due to the badly damaged by the disease fruits.

Virus control

Eradication. Among the various strategies to control virus diseases on perennial crops and especially those ones that are also vector-transmitted (aphids) is the removal of sources of infection by eradication of infected plants. In Bulgaria, eradication of sharka infected trees has started shortly after the discovery of the viral nature of the disease. In a poster entitled: “Fight with Sharka disease on plum”, issued by the Ministry of Agriculture and State Properties and the personal help of Prof. D. Atanassov being at that time a Minister of
Agriculture it can be read: “Sharka on the plums is an infectious and incurable disease. The only means against the disease is the eradication of the infected trees. Owners of plum trees! Yours own interest dictate the eradication of all sharka-infected trees. Do not put off the check and the eradication of the infected trees until tomorrow. Remember, yours still healthy trees are exposed to the danger to be infected each moment. Eradicate the single infected trees in your orchards, to not eradicate all trees later.” In such propaganda the authorities have persuaded the farmers to destroy the infected trees as the only way to protect plum growing in the country. That measure, however, has been applied with different success in the stone growing regions of Bulgaria. While in some Bulgarian districts (Elenska, Svistovska, Nicopolska, Gorno-Orjahovska), all sharka diseased trees have been eradicated in another ones the measure has been unacceptable and has met the strong resistance of the local farmers. For example in a large survey held on by the specialists from Plant Protection Institute in 1936 in one of the main plum growing region of Bulgaria (Pleven district) 105 649 (3%) out of 3 million surveyed trees have been found to be sharka infected. Only 56 652 trees, however, have been destroyed, while the other diseased trees have been only marked (3). Such a half-made clean up from sharka infected trees in many Bulgarian districts have resulted in further virus dissemination and increase of virus incidence. Later, around 1950s – 1960s a program for disease control by the removal of sharka infected trees has been undertaken again. The high intensity of infection has prevented the successful completing of this work and no results have been obtained (11). Also, the sporadically applied inspection and eradication measures in some fruit growing regions (North Bulgaria) of the country has lead to further sharka spread and dissemination, although at the beginning in that regions the level of infection has been low.

At present, owing to the existence of large number of infected trees on large areas of Bulgaria, the application of eradication control is not realistic is not more conducted.

Production of virus free plant materials. Traditionally, most of the nurseries producing standard propagation material use buds from established commercial plantations, which increase the risk of sharka dispersion in the new plantations. That’s why one of most important measures for sharka control is the production, planting and growth of sharka-free trees. A program for production of sharka-free planting material in Bulgaria has been initiated by the Central Institute of Fruit Growing (later Plant Protection Institute) in 1964 (107). In the frame of that program six mother plantations for production of virus free rootstocks and scions in different regions of the country have been established. The sanitary status of the propagated material has been established on the base of biological tests (herbaceous and woody indicator plants).

Nowadays, the success of the applied visual observations and biological indexing for virus presence during the mentioned period could be under question, having in mind that often infected trees do not show symptoms for up to 3 years after infection, the uneven virus distribution, the low virus concentration and lower sensitivity of indicator method (compared to the serological and molecular methods for virus detection).

According to the author (107) very often together with the tested materials not tested ones and proven to be virus-free have been sold by some irresponsible nursery administrators. Later on during 1990s-2000, following the economical difficulties Bulgaria has encountered, the nurseries control has not been enough, correct and for that reason ineffective. As a pre-accession country, however, and in compliance with the EU requirements, a low concerning the production of “virus-free” and “virus tested”
certified seed and planting material has been adopted in October 2000. A national system for production of certified fruit growing material has been established by the Ministry of Agriculture and Forestry with the help of FAMAD project (Fruit-Cultivation and Mountain Agriculture and Development Project) to the German Society for Technical Assistance (GTZ). Virus-free rootstocks, scions and trees have been imported in Bulgaria with the aim of their further propagation and dissemination in the fruit growing nurseries of several Bulgarian districts, as Plovdiv, Kyustendil, Sevlievo, and Sliven. In the Institute of Upland Stock Breeding and Agriculture, Trojan a mother plantation of virus-free plants according to the standard has been created with the possibility to produce over 30,000 healthy buds (21). The increased use of certified virus-free plant material could lead to decrease in disease incidence in the future years.

One of the most modern means of propagating virus-free material is by *in vitro* methods. *In vitro* propagation of own-rooted fruit cultivars has number of technological advantages over the traditional method used, expressed in avoiding labour intensive and specific grafting operations, accelerated production and a high propagation coefficient (53). A modern laboratory for *in vitro* propagation of fruit and small fruit species has been created at Fruit Growing Institute, Plovdiv in 1985. Recently, the technological parameters as introducing the explants into sterile culture, the nutrient media and the conditions of multiplication and rooting for the production of PPV-free planting material of the plum cultivars *Čačanska leptotiza*, *Altan’s gage* (both *P. domestica*), *Santa Rosa* and *Black Star* (*P. salicina*) have been reported by (53). *In vitro* rooted plants of the mentioned above plum cultivars and free of PPV infection have been successfully produced.

**Elimination of PPV through *in vitro* techniques.** In the cases when stone fruit cultivars are very susceptible and mass infected (such as *Kyustenilska sinya* sliva) in Bulgaria (Milusheva, unpublished data) to find healthy source plants for producing of certified planting material is extremely difficult and very often not possible. In such cases virus-free stocks can be obtained by virus elimination through heat therapy, chemotherapy and/or meristem tissue culture. Elimination of PPV up to 65% through in vitro heat therapy of plum plants has been reported (25). Elimination of PPV in plum up to 47% by tissue cultures of apical tips has been achieved (21).

The investigations conducted (77) with *in vitro* propagated plants of cv. *Stanley* have shown that the production of own-rooted planting material trees have had many advantages over the conventional graft propagation, thus providing an opportunity for rapid restoration of plum orchards with PPV free high-tech cultivars. Positive results in PPV elimination in two plum cultivars, *Kyustendilska sinya* and *Valjevka* using the method of clonal micropagation have been obtained (72). The authors have shown the significant importance of number of subcultures for tissue virus elimination and the good opportunity for PPV elimination. While 35.5% of the obtained sub clones of *Kyustendilska sinya* and 33.3% of Valjevka have been free to the fourth sub culturing that percentages have increased to 88% and 100% for *Kyustendilska sinya* and *Valjevka*, respectively after 8 passages.

**Use of resistant and tolerant cultivars.** The most efficient way for control of sharka disease in the infected regions is the establishment of new plantations with resistant, tolerant and slightly susceptible prune cultivars (5). Despite the fact that PPV resistant cultivars have been sought since the first discovery of sharka disease, no considerable progress in that aspect have been achieved due to the little resistant germplasm identified in *Prunus* species. Because of the excellent taste of the
fruits, *Kyustendiška sinja sliva* has been the major cultivar grown in Bulgaria before the World War II and after, which until 1965 has occupied about 90% of the plum orchards. The great losses caused by sharka disease on *Kyustendiška sinja sliva* and the desire to reduce the losses has urged the researchers to seek cultivars and forms less susceptible to PPV. Some plum cultivars as *Stanley*, *Yellow Afaska*, *Karlovskaya Afaska*, *Bühlers Frühlwetsch*, assessed as resistant on the fruits have been registered for planting in highly infected regions (101). Due to the small number of infected fruits (5-6% of the fruits have shown sharka symptoms), around the late 1960’s *Stanley* has occupied approximately 15-30 % of all plum cultivars grown in Bulgaria (103). After 1970’s and around 1985’s the areas planted with *Stanley* have increased to 70 - 90% and the cultivar has become the basic one, followed by *Strinava*, *Gabrovska* and *Čačanska Lepotica* (18, 34). At present, according to the report of Ministry of Agriculture and Forestry of Bulgaria from 2004 the most grown cultivars in the old orchards (more than 20 years old) are *Kyustendiška sinja sliva* and *Stanley*, while in the younger ones (1 to 12 years old) *Stanley*, *Čačanska Ranna*, *Čačanska Lepotica*, *Čačanska Najbolja*, *Gabrovska*, *Karlovskaya Afaska* and *Red Afaska* are the most cultivated plum cultivars.

**Genetic engineering.** As aphid transmitted, the control of PPV, particularly in areas where the disease is prevalent, is very difficult. An effective treatment to cure virus infected trees is currently not available. The lack of natural resistance genes and the very long periods need for conventional breeding procedures greatly favors the strategy of genetically engineered resistance. Coat protein protection technology for PPV control has been applied extensively during the past several years (57, 61, 79, 82, 89). In Bulgaria, hypocotyls slices from *Prunus insititia* San Giuliano rootstocks have been transformed with the coat protein (CP) gene of PPV (CP33), carrying *GUS* and *npt* II markers gene (52). Four transgenic clones have been identified out of 1832 hypocotyls slices. The transgenic nature of the putative transformants has been confirmed. It remains to be seen to what extent the obtained transgenic PPV resistance in *Prunus insititia* San Giuliano rootstocks is inheritable, stable and effective under the natural conditions and the local PPV strains.

**REFERENCES**
