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Investigation of Plum Pox Virus in Bulgaria for the past 70 years

V. T. DZHUVINOV, V. B. BOZHKOVA, S. A. MILUSHEVA and P. S. GERCHEVA
Fruit Growing Institute, BG-4004 Plovdiv, Bulgaria

Abstract

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Plum Pox Virus (Sharka) disease was described in Bulgaria 70 years ago by Dr. Dimitar Atanasov. The investigations on sharka in Bulgaria were carried out in the following ways during the last 70 years: Diagnostics, identification and characterization of the pathogen; Epidemiological evaluations and economic importance of PPV and disease control.

Reduction of the yield and worsened flavour and commercial qualities were found even in cultivars tolerant to sharka.

The most recent investigations on PPV have been aimed at establishing the correlation between the biological, serological and molecular properties of the Bulgarian PPV isolates

The results of the above mentioned items are presented in this paper.

Key words: plum pox virus, diagnostic, identification, characterization, epidemiological evaluations, control

Introduction

The first symptoms of "sharka"-"pox" were observed in plums by fruit growers in the South-West region of Bulgaria after World War I.

Dr. Dimitar Atanasov (1933) was the first scientist who described the viral nature of this new disease and named it "sharka"in the paper: "Sharka po slivite. Edna nova virusna bolest - Plum pox. A new virus disease" published in the Annuaire de L'Universite de Sofia, Faculte D'Agronomie et de Sylviculture, vol. XI, 1932 - 1933.

For more than 70 years, sharka has been a major problem for plum and prune cultivars, for the plum industry in the Balkans and in many countries in Europe. At present Plum pox virus (PPV) is a

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e-mail address: vcd@valan.net

problem for many stone fruit cultivars in the world.

During the last 70 years, the investigations on sharka in Bulgaria were carried out in the following ways.

· Diagnostics, identification and characterization of the pathogen;

·Epidemiological evaluations and economic importance of PPV; and

·Disease control.

Diagnostics, Identification and Characterization of the Pathogen

Dr. Atanasov started the evaluation of this new disease in 1926. He determined that its symptoms were found generally in plum trees of Kyustendilska (Figures 1 and 2) and on single trees of Dolanska, Bardaklia, Afazka and Green Gage cultivars.

In August 1931 he grafted buds from infected trees of the Kyustendilska plum on seedlings of Afazka (*Prunus cerasifera*).

In May 1932 he observed symptoms on the leaves. By this experiment he demonstrated the viral nature of this disease and the possibility of its transmissionby grafting.

For the first time in 1934 Atanasov described symptoms of sharka and published photographs of those symptoms on leaves and fruits of sweet cherry and peach and on leaves of apricot (Figures 3, 4, 5, 6 and 7).

Another famous Bulgarian scientist Dr. Alexander Christov began studies on sharka in 1937. He established that Prunus virus 7 was the sharka disease agent and it could be found in living cells of the infected plants.

Christov (1944) also used artificial indexing by grafting buds from infected plums and myrobalans, taking into consideration that the sharka virus did not attack sweet cherry, *Prunus mahaleb*, sour cherry and peach. He found out that *Prunus triloba* was a natural host of the sharka virus (Christov, 1947).

At this first stage of the investigations on sharka, the major methods of diagnostics were visual observations and biological testing by grafting on *Prunus* species.

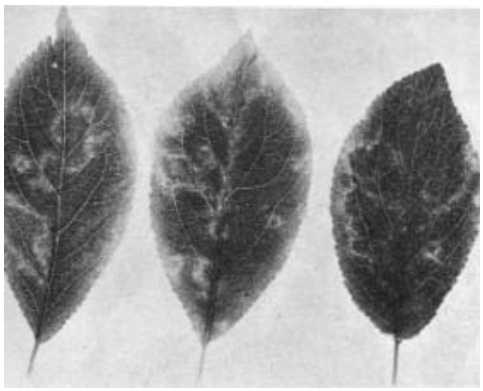

Fig. 1. Sharka on leaves of Kyustendilska

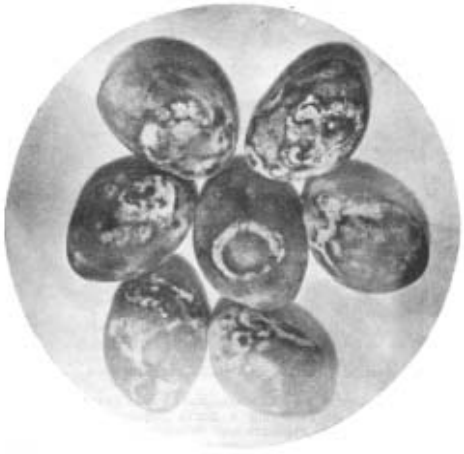

Fig. 2. Sharka on fruits of Kyustendilska

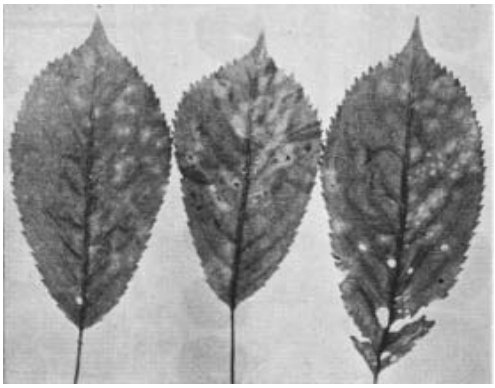

Fig. 3. Sharka on leaves of Ranna lomska

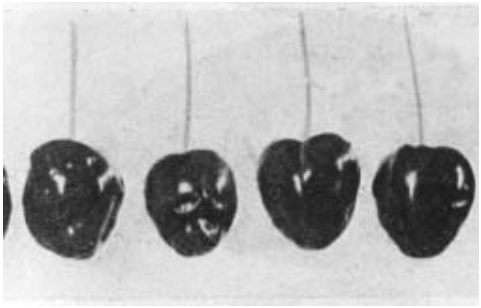

Fig. 4. Sharka on fruits of Ranna cherna

In the middle of the 1960^{s} the peach cultivar Elberta was used as the woody indicator and *Chenopodium foetidum* and *Nicotiana clevelandii* as the herbaceous ones for diagnostics of the sharka virus.

During this period Dr. Dimitar Trifonov (1965), after conducting investigations, added *Prunus salicina* to the range of the natural host plants.

Later Dr. I. Kamenova (1987), while studying herbaceous species for reliable detection, discovered that *Nicotiana benthamiana* was a very suitable indicator with systemic reaction for identification and investigation of the sharka virus.

In the 1970^{s} the use of a new method - serological analyses based on precipitation

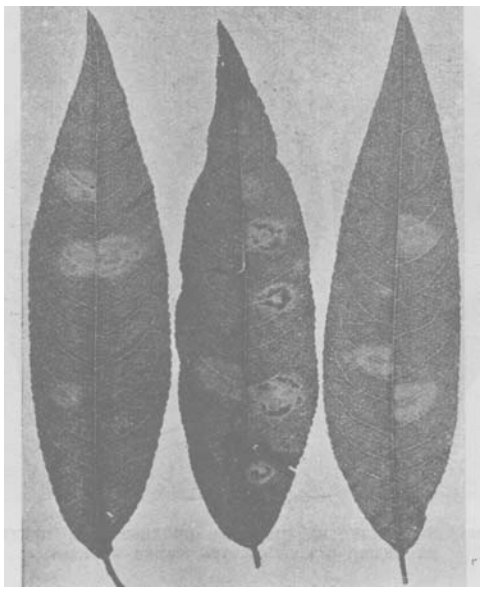

Fig. 5 Sharka on leaves of Byala snezhna

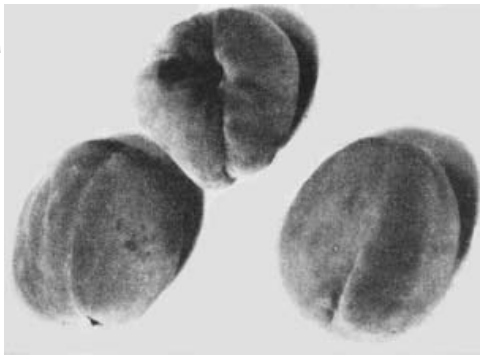

Fig. 6 Sharka on fruits of peach

–began to be applied in the detection of the virus. Another important step in its progress was made during the 1980^{s} after the introduction of the ELISA test.

At the end of the same period and the beginning of the 1990^{s} it was proved by ELISA that peach (Gabova et all. 1989) and sweet cherry were host plants of sharka virus too. PPV was identified also in sour cherry (Topchiiska, 1992).

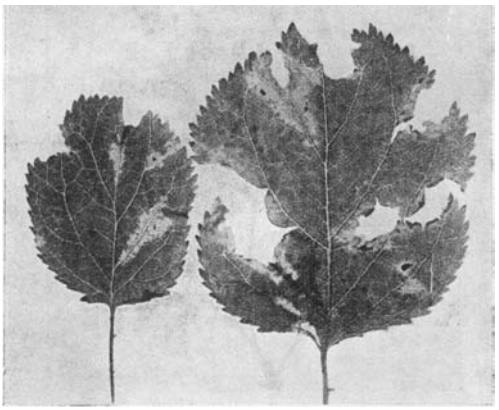

Fig. 7 Sharka on leaves of apricot

Strain characterization of Bulgarian isolates of PPV started during that period. Initially by biological indexing on *C. Foetidum,* it was found (Kamenova, 1987; Topchiiska, 1994) that the isolates were related to a chlorotic-necrotic /intermediate/ strain according to the classification of Sutic et al. (1971). Later, investigations on the spread of Bulgarian strains were conducted by using monoclonal antibodies. At that stage, the results showed that the most distributed strain in plum cultivars was PPV-M (88.3 %), followed by PPV-D (5.2 %). Mixed infection of both strains - 6.2 % was also observed (Kamenova and Borisova, 2002; Kamenova et al., 2002a; Kamenova et al., 2002b). Until now only PPV-M strain has been recorded in apricot and peach. During the last few years, a molecular technique has been applied as well (Kamenova, 2002).

The most recent investigations on PPV have been aimed at establishing the correlation between the biological, serological and molecular properties of the Bulga-rian PPV isolates (Kamenova, 2002; Topchiiska et al., 2002).

Epidemiological evaluations and Eco-nomic Importance of PPV

Atanasov (1934) suggested that the sharka virus could be transmitted by the aphid *Brachycaudus helichrysi* and *Lecanium corni* scale.

According to Christov (1944, 1947) that virus was transmitted by suckers from infected plum trees and by the aphids *Phorodon humili* and *Brachycaudus cardui* but not by seeds. The possibility of transmission of PPV by seeds was also investigated by other Bulgarian authors but the results were negative (Vitanov and Marinova, 1990; Milusheva and Gercheva, 2003).

Trifonov (1965) detected PPV in the pollen and reported that sharka virus could be transmitted by pollination from infected to healthy plum trees.

The role of weeds in PPV epidemiology was also investigated. For this purpose species forming weed associations in plum and apricot orchards were studied. Until now PPV has been detected in *Veronica hederifolia, Lactuca serriola, Rumex crispus, Capsella bursa-pastoris* and *Lithospermum arvense* (Milusheva and Rankova, 2002).

Initially, Dr. Atanasov (1933) found infected plum trees in the South-Western part of Bulgaria and later in the North-Western and in the Central part of North Bulgaria.

Now, 70 years after the first investigation, the shares of infected trees by PPV in some *Prunus* species in the country are as follows: plum - 62.2 %, apricot - 24.3 % and peach - 19.5 % (Kamenova et al., 2002b).

Investigations were carried out on the effect of the virus on the vegetative and reproductive behavior of susceptible and tolerant plum cultivars infected with sharka. The changes of the biochemical content and the biometric parameters were also studied (Christov, 1944; Trifonov, 1972; Iliev, 1989; Kovachevski et al., 1977; Marinova et al., 1994; Dragoiski et al., 1995).

In 1944 Christov reported that fruits of infected plum and apricot trees were characterized by premature ripening, low quality and dropping about 10-15 days earlier than the healthy ones. Velkov et al. (1951) reported that premature dropping of fruits started about two to four weeks before normal picking time. Trifonov (1972) established that due to premature dropping of the fruits, losses in the case of very susceptible cultivars could reach from 80-95 % up to 100 %, depending on the cultivar.

Reduction of the yield and worsened flavour and commercial qualities were found even in cultivars tolerant to sharka.

During a four-year period, a decrease in the yield of infected trees of Strinava and Stanley cultivars by 60% and 12%, respectively, in comparison with healthy plants, was reported by Vitanov and Marinova (1990).

Disease Control

On the basis of his studies, Atanasov (1933, 1934) recommended eradication of all the infected trees,prohibition of distribution of the budwoods and planting material from infected regions; quarantine control in fruit nurseries, and chemical control against aphids and scales as vectors of sharka.

In the subsequent stages of studying the disease, evaluations on the susceptibility of plum and apricot cultivars grown in Bulgaria, and breeding for tolerant and resistant new cultivars began at the end of the 1930, after artificial inoculation, Christov (1943, 1945)classified the susceptibility of 25 plum and 37 apricot cultivars.

Similar investigations on plum germplasm susceptibility have continued to the present day by using field resistant criteria and the ELISA test (Gabova and Iliev, 1995). Evaluations on susceptibility of peach cultivars, rootstocks and hybrids were done by Topchiiska (1994). The results of those investigations were used when choosing donors in the breeding programmes for stone fruit species.

Breeding for resistance, using methods and results:

· Individual selection of seedlings – Serdica 2, Sofia, Septemvriiska, Sofiisko chudo (Christov, 1967);

· Clonal breeding, clones of very susceptible Kjustendilska sinya tolerant to PPV (Yoncheva, 1983);

· Interspecific hybridization – Dryanovska, Gulyaeva, Strinava, Gabrovska, Nevena, Balvanska slava, Plovdivska, Pulpudeva, and selected hybrids (Vitanov, 1977; Iliev,1990; Iliev and Marinova, 1990; Zhivondov,1994; Zhivondov and Djouvinov, 2002; Dragoiski et al., 1995)

· Intraspecific hybridization – selected hybrids (Minev and Dragoiski, 1995; Zhivondov and Djouvinov, 2002);

· Induced mutagenesis – treat-ments with g-rays (P. Iliev, unpublished data).

The results from the investigation on susceptibility of cultivars to sharka virus has been taken into consideration for recommendations for the National list of cultivars for use by regions. Chronologically the following plum cultivars have

been recommended for commercial growing:

· **Practically resistant cultivars** – Monfort, Buehler (Christov, 1943, 1944), Anna Spath, Green Gage, Belle de Louvain, Zhalta afazka, Agen etc. (Christov, 1956) and Serdica 2, Stanley, Mirabelle de Nancy etc. (Christov, 1976).

· **Tolerant to PPV cultivars** in the contaminated regions – President, Gabrovska, Stanley, Ontario, etc. (Trifonov, 1965).

· **Resistant to tolerant** – Scoldus, Cacanska najbolja, Nevena, Opal, Kirke, Zhalta butilkovidna, Renklod Hramovih, Cacanska lepotica, Tuleu Timpuriu (Iliev, 1990; Gabova and Iliev, 1995).

Christov (1947) and Trifonov (1969) recommended thermotherapy and propagation of healthy plant material in the regions free of this virus, for the restriction and prevention of sharka disease. At the end of the 1980^{s} investigations on PPV elimination by *in vitro* thermo- and chemotherapy began.. By *in vitro* thermotherapy 64 % of the PPV-free regenerants from plum trees were obtained. The results from *in vitro* chemotherapy showed that 25-75 % of PPV-free regenerants were obtained at different virucide-nutrient media combinations (Gabova, 1989à; Gabova, 1989b; Gabova 1995). Studies were conducted on the possibility of using *in vitro* subcloning for the elimination of the PPV virus. The results obtained offered a good opportunity for producing sources of PPV-free trees and establishing mother stock orchards using the method of clonal micropropagation (Nacheva et al., 2002).

Djouvinov and Vitanova (2002) recommended the introduction of the Council Directive 92/34 of EU and the scene of EPPO for the production of certified fruit planting material as the main measures against the distribution of sharka virus in Bulgaria.

The Major Problems

The investigations of Bulgarian scientists on sharka virus were closely related to the following critical issues:

· Sharka virus has a lot of host plants, vectors and strains and it can also be found in combination with other stone fruit viruses on the host plants;

· Possibility of sharka virus transmission by seeds or from weeds to different stone fruit plants;

· Stone fruits cultivars that are resistant or tolerant to all PPV strains;

· Problems of GMO.

Conclusion

In order to solve these very complex problems there is a need for more international projects on stone fruit breeding programmes and for the establishment of teams involving different specialists such as breeders, geneticists, virologists, biotechnologists, etc.

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