Plant Biotechnology: Potential Impact for Improving Pest Management in European Agriculture

Stone Fruit – Virus-Resistant Case Study
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Leonard Gianessi
Sujatha Sankula
Nathan Reigner

NCFAP
National Center for Food & Agricultural Policy

The National Center for Food and Agricultural Policy
1616 P Street, NW Suite 100
Washington, DC 20036

Full Report: www.ncfap.org

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Introduction

The production of plums, peaches, nectarines, and apricot (stone fruit) in the European Union amounts to 6.1 billion kg/yr with a value of €3.7 billion. These stone fruits are produced on 440,000 hectares (see Table 1). Six countries (Austria, France, Germany, Greece, Italy, Portugal, and Spain) account for 99% of the E.U.'s production of stone fruit (see Table 1).

Plum Pox Virus

The disease referred to as Plum Pox Virus (PPV) was first reported in 1915 in Bulgarian plums. "Sharka" is the Slavic name for "Plum Pox" and is the most widely used common name for the disease around the world. The disease spread slowly north through Eastern Europe into Yugoslavia, Hungary, Russia, Romania, Albania and Czechoslovakia [6] [8]. Following World War II, PPV spread to Austria and Germany by 1956. By the mid-60s, the disease was reported in the Netherlands, Switzerland, Greece, England and Turkey. Efforts to eradicate the disease from England in 1965 appeared to be successful until it was reintroduced on imported rootstocks during the early 1970s.

Sharka spread throughout Europe during the 1970s into France, Italy, and Belgium [1]. The rapid spread and devastating effect of the disease began to have an impact on the peach, nectarine, apricot and plum industry of Europe. This prompted the European Plant Protection Organization (EPPO) to impose quarantine regulations and restrict the movement of stone fruit propagation material between European countries to slow down the spread of the disease. Despite this effort, Sharka continued to spread in Europe reaching the stone fruit orchards of Spain and Portugal by 1984.

In the 1980s, PPV began to spread to areas outside of Europe. It showed up in Egypt, Syria and Cyprus. In 1992, the disease was reported for the first time in the Western Hemisphere on peaches in Chile. PPV was discovered in Pennsylvania in the U.S. in 1999, and in Canada in 2000 [1]. The Pennsylvania Department of Agriculture quarantined the infected areas and 18 orchards were destroyed to prevent its spread. PPV has not been detected in the major U.S. stone fruit states (California, Georgia, Michigan).

Plum pox virus is a member of the genus potyvirus (potato virus Y). It is characterized by its flexuous, filamentous particles that contain a single-stranded RNA. The virus develops by replicating in the conducting tissue (phloem) of the host plant.

The long distance spread of PPV among and within European countries during the last 20 years is attributed mainly to the movement of diseased plant material. Buds taken from infected trees will carry the virus and transfer it when grafted to healthy trees.

Plum pox is spread over short distances by aphids. The virus can occur in high concentrations in leaf cells and when an aphid probes into an infected cell, some of the virus is sucked into the aphid's mouthparts. If the aphid then feeds on a healthy plant, the virus can detach and be squirted into the healthy plant cell [18]. Research has shown that there can be roughly 3.5 million aphids in a typical planted orchard acre and between 50,000 and 300,000 aphids can visit a single fruit tree in a one-year period [19].

Plum pox virus produces symptoms such as leaf discoloration, and rings or spots on fruit [18]. Symptoms include premature fruit drop, deformed fruit, and discoloration of the skin and flesh. Regardless of how severe leaf and fruit symptoms are, trees infected with plum pox will suffer from decreased production and reduced fruit quality with crop losses as high as 80 to 100% [6]. Much of the infected fruit drops prematurely, 20 to 30 days before normal maturity date [20]. The fruit that remains on the tree lacks flavor, and is low in sugar content. The virus so seriously affects the fruit of diseased trees that the fruit becomes unsuitable for direct consumption or industrial processing (dried, jams or brandied). Plum pox does not kill trees but it makes the fruit unmarketable and drastically reduces yields. The fruits drop before maturity and are unfit for use as they are bitter and unsweet. The yield of infected trees may be reduced by 20 to 30% [6].

Many trees fail to show symptoms for the first three years following the initial infection of the tree. PPV is economically important even on symptomless trees that become infected but fail to produce obvious symptoms. PPV infections reduce total quantity of even symptomless fruit.

The disease affected only plum and apricot for a long time. On peach, the virus first appeared in 1961 [9].

Plum pox has proven to be a very difficult disease to control in Europe. Control of plum pox disease is difficult and complicated for two reasons: first, the perennial nature of stone fruit trees provides a permanent source for both virus and insect vectors. Second, the internal multiplication of the virus is slow and infection is often confined to one or two limbs of a tree. Foliar symptoms are usually mild or transient as a result of which considerable spread can occur from new disease foci before infections are actually noticed. Thus, damage from the disease occurs long before control measures are undertaken.

Trees infected with PPV cannot be cured and infected trees must be removed to retard disease spread. In many European countries, the virus has quarantine status. In particular, plum pox virus appears on the EPPO A2 list of quarantine pests and in Annex II/A2 of E.U. Directive 77/93 with detailed requirements in Annex IV. Eradication programs and systematic surveys are carried out in commercial orchards and nurseries. If infection is found, diseased trees or orchards are to be destroyed.

The recommendation is made that growers wait three years before replanting the orchard to stone fruit, but this recommendation is not often followed by growers because of high land costs.

There is no treatment to cure virus-infected trees, and once a tree is infected it serves as a source for infection of other trees. Eradication has proven insufficient in most countries because newly planted healthy trees become infected in a short time.

In countries where the level of infection is low and infected trees are restricted to limited areas, eradication has made it possible to maintain a low level of infection and more rarely to eliminate the disease [7].

Since the virus can spread through root suckers, it is customary in Europe to either uproot an infected tree with in a week of noting the disease or cutting off the aerial portion and treating the stump with herbicides.

When virus-susceptible varieties are planted in orchards in regions where the disease is present, they become infected by plum pox within a few years. Studies have shown that removal of infected trees within 500m around an orchard does not prevent infection [8].

Control of aphid populations with insecticides may reduce their levels, but because it takes only one aphid a few seconds to transmit PPV from an infected tree to a healthy one, insecticide applications will not effectively control PPV infestations. Most European countries do not include insecticide sprays in sharka management.

In Bulgaria, losses in 1968 were estimated to be 30,000 tonnes. In some years, losses were as high as 60,000 tonnes [6]. In the last 3 decades, the average fruit yield in the Czech Republic dropped by 80%, and the total number of plum trees was reduced from 18 million to 4 million as a result of sharka [2].

PPV is widespread in Germany and Greece, and is present locally in Austria, Portugal, Spain, France and Italy [12].

Sharka disease of stone fruits was first described in Greece in 1967, and despite an eradication program, has spread all over the country, causing extensive damage [3][8]. The cultivated area of apricots in Greece used to be more than 7,000 hectares, but recently it has been reduced to 4,500 hectares because of the sharka problem [4]. Apricot production in Greece has declined from 113,000 MT to 33,000 MT.

Apricot production has been seriously threatened from some valleys in Northern Italy in the late 1970s (e.g. in Vintschgau) and threatened in some Austrian valleys (e.g. Wachau) [5]. The disease was detected in Austria for the first time in the 1960s and since then spread to all major fruit-tree growing regions [8]. By 1988 in Styria, the major plum growing area, 21,000 trees had to be eradicated due to PPV and another 3,000 in 1989. Since Austria joined the E.U. in 1995 and adopted the respective legislation on the phytosanitary standards for stone fruit planting material (Directive 92/33/EEC), the increased activities of fruit consultants advising on the danger of PPV, and the intensified inspections of nurseries by phytosanitary services have led to a general alert and increased awareness of the growers, and have therefore led to a more rapid identification and subsequent removal of PPV infected trees [37].

In France, PPV is mainly present in the southeast. At present, plum pox has a very limited distribution in France and infection levels are very low in regions where the disease is present (0.2% infection) [8]. All trees showing symptoms are eliminated and if infection levels are above 10%, the whole plot is destroyed. These methods have resulted in the destruction of about 27,000 trees in 1992 (of which 550 were found to be infected) and in 1993, 100 hectares (mainly peach) were eliminated [8]. From 1973 to 1990, it is estimated that 91,000 trees were destroyed in France [13].

Plum pox was first detected in Germany in 1955, but at that time infected trees were not immediately removed. Within a few years, the disease spread to the most important growing regions in the south and east of Germany, especially in the Rhine and main valleys and regions of Kaiserstuhl, Pfalz, and Rheinhessen [8]. In the eastern and southern regions of Germany, Sharka is a very large problem. In old orchards with sensitive varieties more than 90% of the trees may be infected [27].

PPV was first detected in Italy in 1973 in apricots, and has spread from north to south, mainly damaging apricots and plums [8]. In 1984, a survey of 261,000 plum trees in the Sarca Valley revealed that 13% (34,000) of the trees were infected [15]. Eradication programs became compulsory in 1992. If more than 30% of plants are infected, all plants must be destroyed [8]. Sharka was first discovered in Apulia (southern Italy) in 1988. 30,000 trees were inspected, and six plum plantings with an infection rate above 30% were completely pulled out. In plantings where the infection rate did not exceed 10%, only the infected trees were pulled [17]. In the Emilia-Romagna region between 1998 and 2002, 69,083 stone fruit trees were removed due to Sharka detection; €450,000 is being spent annually for their removal and replanting [35]. In Lombardia, approximately 25% of the stone fruit hectares are infected with PPV [34]. As a result, it is necessary to import fresh fruit into the region. In the Verona region, PPV led to the removal of trees from 5,000 hectares. The hectares were replanted and all of the replanted trees became quickly re-infected [34].

In Portugal, the disease has been maintained at a low level due to surveys and compulsory eradication of orchards takes place if more than 20% of trees are infected [8]. It was detected also in the Azores, where infected orchard material had been introduced from the mainland.

In Spain, the virus was introduced and spread on Japanese plum. The disease spread for several years without being noticed. Subsequently, PPV spread into all areas of early fruit production. In Valencia, early apricots can no longer be grown [29]. In Spain since 1988, 1.5 million trees have been removed at a cost of €17 million [31]. Eradication programs have not stopped the disease in Spain, and apricot production is seriously reduced in many areas [16]. The compensation averages about 1,000 pesetas per tree. The scale of compensation depends on the species, and age of the tree [14].

Transgenic Virus Resistant Stone Fruit

Many attempts to control PPV by classical breeding have been undertaken, but these have not resulted in a generally useful array of commercially important resistant varieties [10]. Only a few natural sources of resistance have been identified to date [11]. Traditional breeding of fruit trees takes a long time, spanning the careers of several breeders [1]. For example, for a relatively short generation fruit tree such as peach, the time required for developing an improved variety is twenty years [21]. As a result, the use of transgenic plants to control PPV has been chosen as a preferred research strategy [11].

Attempts to develop stone fruit trees resistant to plum pox virus use modern biotechnology methods have been successful in Austria and France [22] [23] [5]. Reported in 1992, the development of apricot plants resistant to PPV at the Institute of Applied Microbiology in Austria was the first reported case of successful transformation for plum pox resistance in stone fruits [22]. The durability of resistance in the transgenic apricots has been evaluated under greenhouse conditions in Austria [5] [38]. After more than ten years of preparation, the trees are currently kept in a contained system under approximately natural conditions, and insect-proof screenhouse [32].

A collaborative effort between researchers in France (INRA) and the U.S. (USDA-ARS) led to the development of genetically-modified plum plants resistant to PPV reported in 1997 [23]. Using *Agrobacterium*, they inserted viral coat protein genes into plum cells [25]. The plum pox resistance gene was provided by INRA [30]. The transgenic plum line (named as C-5) exhibited excellent resistance in three-year greenhouse tests when grafted with infected budwood or exposed to aphids that carried the virus. The transgenic plums remained virus-free for the entire three-year experiment. In contrast, 30% of the non-transgenic trees in the experiment showed symptoms of the virus infection after six months, 67% showed symptoms after twelve months, and 100% showed symptoms after three years [23]. Transgenic C-5 plum plants are currently being field-tested in Poland, Romania, and Spain to determine the long-term expression of resistance under varied climatic conditions, infection pressure and virus strains [24]. These European field tests have confirmed that C-5 is highly resistant to PPV.

Unlike other transgenic virus-resistant plants developed using coat-protein mediated mechanisms, C-5 plum plants do not contain any viral coat proteins. Researchers confirmed that the mechanism of PPV resistance in C-5 plums is due to Post-Transcriptional Gene Silencing (PTGS). The PTGS mechanism allows the plant cell to recognize and shut off invading viruses before they actually produce any proteins [24]. All copies of the PPV coat protein gene in C-5 were inherited as a single gene. Transgenic resistance is inheritable, and transmitted through seed and can be incorporated into other cultivars by standard plant breeding methods.

The trees are not being inoculated with PPV in the outdoors in the U.S. because of concerns about releasing the plum pox virus into the environment. The transgenic trees are being field-tested for agronomic traits in West Virginia [28]. A commercial interest is pursuing the registration of the virus-resistant plum for sale in the U.S.

Recent research at the Plant Pathology Research Institute in Italy has resulted in tobacco plants resistant to PPV as a result of being transformed with four distinct genome regions of an Italian PPV virus [36].

Planting of the genetically engineered stone fruit varieties has the potential to reduce yield losses and prevent the destruction of productive trees, which would no longer be subject to PPV infection.

There are no current estimates of the extent of economic losses due to PPV in Europe, although such estimates are being compiled at this time [33].

It is assumed that PPV-resistant stone fruit trees would initially be planted on 5% of the hectares of stone fruit in Europe and prevent current losses to PPV which have been estimated for this Study as follows: Austria/Germany (4%), Italy/Greece/Spain (3%), Portugal/France (1%). These estimates consist of current losses from infected less productive trees and losses due to trees being taken out of production on an annual basis.

Table 1a: Stone Fruit Area (000 ha)				
	Plums	***Peaches and Nectarines***	***Apricots***	***Total***
Austria	4.7	1.3	2.3	8.3
France	20.0	20.4	15.0	55.4
Germany	61.0	8.3	1.5	70.8
Greece	0.7	52.5	4.7	57.9
Italy	12.6	92.3	15.3	120.2
Portugal	2.1	8.0	0.7	10.8
Spain	20.0	70.0	24.5	114.5
Total E.U.	123.6	252.8	63.9	440.3
U.S.	51.6	76.1	7.9	135.6

Source: FAO

Table 1b: Stone Fruit Production (million kg)				
	Plums	***Peaches and Nectarines***	***Apricots***	***Total***
Austria	43.4	6.7	6.3	56.4
France	253.2	464.6	171.8	889.6
Germany	424.5	16.6	6.0	447.1
Greece	8.0	930.0	80.0	1018.0
Italy	181.7	1631.7	209.1	2022.5
Portugal	17.0	65.0	6.0	88.0
Spain	186.5	1247.4	119.2	1553.1
Total E.U.	1134.0	4362.0	598.4	6094.4
U.S.	591.2	1358.1	74.9	2024.2

Source: FAO

Table 1c: Stone Fruit Value (€ million)				
	Plums	***Peaches and Nectarines***	***Apricots***	***Total***
Austria	22.3	3.6	11.2	37.1
France	194.7	479.0	204.6	878.3
Germany	298.4	9.6	3.8	311.8
Greece	6.2	582.2	89.6	678.0
Italy	78.7	652.7	88.4	819.8
Portugal	13.1	56.6	6.0	75.7
Spain	79.6	728.5	50.0	858.1
Total E.U.	705.3	2512.2	453.6	3671.1
U.S.	173.2	624.0	26.5	823.7

Source: FAO
Dollars and Euros assumed equivalent.

Table 1d: Stone Fruit Area (000 a)				
	Plums	***Peaches and Nectarines***	***Apricots***	***Total***
Austria	11.6	3.2	5.7	20.5
France	49.4	50.4	37.1	136.9
Germany	150.7	20.5	3.7	174.9
Greece	1.7	129.7	11.6	143.0
Italy	31.1	228.0	37.8	296.9
Portugal	5.2	19.8	1.7	26.7
Spain	49.4	172.9	60.5	282.8
Total E.U.	305.3	624.4	157.8	1087.5
U.S.	127.5	188.0	19.5	335.0

Source: FAO

Table 1e: Stone Fruit Production (million lbs)				
	Plums	***Peaches and Nectarines***	***Apricots***	***Total***
Austria	95.6	14.8	13.9	124.3
France	557.8	1023.5	378.5	1959.8
Germany	935.2	36.6	13.2	985.0
Greece	17.6	2048.8	176.2	2242.6
Italy	400.3	3594.6	460.6	4455.5
Portugal	37.5	143.2	13.2	193.9
Spain	410.9	2748.0	262.6	3421.5
Total E.U.	2498.2	9609.5	1318.3	13426.0
U.S.	1302.4	2991.9	165.0	4459.3

Source: FAO

Table 1f: Stone Fruit Value (*$* million)				
	Plums	***Peaches and Nectarines***	***Apricots***	***Total***
Austria	22.3	3.6	11.2	37.1
France	194.7	479.0	204.6	878.3
Germany	298.4	9.6	3.8	311.8
Greece	6.2	582.2	89.6	678.0
Italy	78.7	652.7	88.4	819.8
Portugal	13.1	56.6	6.0	75.7
Spain	79.6	728.5	50.0	858.1
Total E.U.	705.3	2512.2	453.6	3671.1
U.S.	173.2	624.0	26.5	823.7

Source: FAO
Dollars and Euros assumed equivalent.

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