Plum Pox disease in Poland: the past and current situation

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SPREAD OF *PLUM POX VIRUS* STRAIN M AND MONITORING OF APHID POPULATIONS IN STONE-FRUIT ORCHARDS IN GREECE

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SUMMARY - Two apricot orchards, established in Southern Greece with healthy planting materials in 1992 and 1993, were monitored since 1996 for *Plum pox virus* (PPV) spread. Trees were sampled in late April 1996 to 2000. Leaves were tested by DAS-ELISA. PPV strain identification was by DASI-ELISA with PPV-M or -D type specific monoclonal antibodies (MAbs). The orchards were established in different areas: orchard 1 in an area with high inoculum pressure (divided in two subplots 1a and 1b based on presence of a hedge planting) and orchard 2 was in an isolated site. In all infected trees, only PPV-M was identified. Differences in the rate of PPV spread were observed among plots and orchards. PPV incidence in plot 1a was 88.6%, in plot 1b 41% and in orchard 2, 40.8%. It would appear that the hedge might have effected aphid movements and virus spread. Five aphid vector species were identified, among which *Aphis gossypii*, was the most abundant. The statistical analysis applied here demonstrated that initial infections appeared in a random way and that no spatial disease gradient developed.

Key words: Greece, apricot, PPV, strain M, ELISA, epidemiology

RESUME *- Deux vergers d'abricotier mis en place dans le sud de la Grèce, en 1992 et 1993, en utilisant du matériel de multiplication sain, ont fait l'objet d'un suivi, à partir de 1996, pour la diffusion du Plum pox virus (PPV). Les échantillons ont été prélevés en fin Avril de 1996 à 2000. Les feuilles ont été testées en DAS-ELISA. L'identification des souches du PPV a été effectuée par la DASI-ELISA avec des anticorps monoclonaux (Mabs) spécifiques pour le PPV-M ou le PPV-D. Les deux vergers ont été installés dans différentes régions: le verger 1, dans une zone avec une pression de l'inoculum élevée et le verger 2, dans une zone isolée naturellement. En tout cas, seul le PPV-M a été identifié. Des différences du taux de diffusion du PPV ont été observées entre les parcelles et les vergers. L'incidence du PPV dans la parcelle 1a a atteint 88,6%, dans la parcelle 1b, 41% et dans le verger 2, 40,8%. La différence entre la parcelle 1a et 1b a été attribuée à l'effet de bordure sur le vol des pucerons et la diffusion des virus. Cinq espèces de pucerons vecteurs du PPV ont été identifiées et parmi ceux-ci, l'Aphis gossypii est le plus abondant. L'analyse statistique appliquée a montrée que les infections initiales ont fait leur apparition d'une manière aléatoire et qu'il n'existe pas un gradient spatial de la maladie.*

Mots-clés: *Grèce, abricotier, PPV, souche M, ELISA, épidémiologie*

INTRODUCTION

Sharka disease of stone fruits caused by *Plum pox virus* (PPV) was first described in Greece in 1967 and since then had spread throughout the country causing extensive damages. Apricot cultivation seems to suffer most, since the main commercial cultivars such as Tirynthos and Bebeco were highly susceptible. In most cases M-type isolates, which are generally considered as quite virulent and responsible for serious epidemics, were recovered (Varveri and Boutsika, 1998). Previous studies regarding virus spread in apricot orchards were done in Spain and France where D-type isolates are prevalent. In Spain 100% infection of trees was described to occur within 2-5 years (Llacer *et al.*, 1992), whereas in France 8-9 years were needed (Adamolle *et al*., 1994). Concrete epidemiological data, however, regarding the speed and mode of PPV-M spread by aphids in Greece or elsewhere are not available.

For this reason, a study on the development of the virus natural spread as well as a small scale aphid monitoring were undertaken in two young apricot orchards established in Argolida County, Peloponnese (Southern Greece).

MATERIALS AND METHODS

The apricot orchards under study were established with healthy materials of cv. Tirynthos originated from Italy and had the following site characteristics:

Orchard 1, had high PPV inoculum pressure and, comprised of two subplots: designated 1a established in 1992 with 158 trees next to an old infected apricot orchard, which was eradicated in 2000; and 1b, established in 1993 with 117 -trees and separated from 1a by a hedge and a house.

Orchard 2 was in an isolated area surrounded by hills and 30 km away from orchard 1. It was cultivated with olive trees. Orchard 2 contained 574 twenty-year-old trees, of which 225 were removed and replanted with new materials in 1992 and 1993. By 1996, 35% of the old trees showed symptoms.

Trees were sampled in late April 1996 to 2000. The canopy of symptomless trees was divided in three sectors and three leaves per sector collected and tested for PPV by DAS-ELISA. Virus strain identification was by DASI-ELISA with PPV-M or -D type specific monoclonal antibodies produced at the University of Bari and at IVIA, Spain (Boscia *et al*., 1997; Cambra *et al*., 1994).

In 1999 and 2000 aphid populations were monitored using sticky tree method from mid-May to August 1999 and mid-May to November 2000. The sticky tree method involving spraying of young shoots with a non toxic glue (Soveurode Aerosole, Rhone-Poulenc) and collecting the stuck aphids every week was used (Avinent *et al*., 1993). Three shoots on three different trees in each orchard were analysed. Aphids were kept in alcohol until identification.

The virus spread data were further analysed statistically using a distance method (Diggle *et al*., 1976) to determine whether infected trees were scattered randomly or not. The coordinates of the infected trees were determined based on a defined coordinate system. A distance-based Monte Carlo test was performed on the data of each sampling year to find whether infected trees were scattered randomly or not. The statistic used for the test was the mean of the distance between infected trees and their nearest infected neighbours (Diggle *et al*., 1976).

RESULTS AND DISCUSSION

PPV spread

The ELISA tests permitted detection of recent infections in symptomless trees (up to 7%) (Table 1) with only PPV-M which was also identified in all symptomatic trees. Table 1 demonstrate the horizontal spread of PPV since 1996 in both orchards. Within eight years after planting, 88.6% of apricot trees in plot 1a were infected, and in plot 1b, 41%. Plot 1a, established a year earlier than plot 1b, was eventually removed by the grower as being not economic. The differences in the disease development between the two adjacent plots can be attributed to the direct vicinity of plot 1a to the inoculum source found in the direction of the predominant wind (south-eastern), whereas plot 1b was protected both by the hedge and house to the west and by the citrus orchard to the south.

Table 1. PPV incidence (%) in two orchards surveyed in 1996, 1997, 1998, 1999 and 2000

Testing year	Orchard 1									Orchard 2				
	Plot 1a				Plot 1b									
	1996	1997	1998	1999	1996	1997	1998	1999	2000	1996	1997	1998	1999	2000
With symptoms	46.8	62	84.8	88	4.3	12.3	17	31	41	9.3	16	22	26	35.1
Without symptoms ELISA +	6.4	7	0.6	0.6	2.6	1.8	0.9	0.8	0	3.1	0.9	0.2	0.2	5.7
Total	53.2	69	85.4	88.6	6.9	14.1	17.9	31.8	41	12.4	16.9	22.2	26.2	40.8

The spread of PPV in the second orchard 2, located in an area surrounded by hills, was slower even though inoculum existed in the orchard (old infected trees). Within eight years 40.8% of the young apricot trees were infected. This area, which was isolated and not cultivated with fruit crops, was possibly less exposed to aphid flights, thus leading to slow virus spread.

Thus, it seems that the most important factor for PPV dissemination is the actual ecological conditions occurring in a very particular orchard or plot and influencing the number of viruliferous aphids. As a result, it is difficult to predict disease spread even in regions of "high risk" for virus infection. The Spanish data for PPV-D also showed spread under high inoculum pressure conditions (Gottwald *et al*., 1995) were comparable with our data for M strain.

Aphid population monitoring

A total of 654 aphids were caught during the monitoring and five known PPV vector species were identified in the apricot orchards (Table 2). *Aphis gossypii*, which is as a vector of low transmission efficiency (Llàcer *et al*., 1998) was the most abundant one, 13% and 18% in 1999 and 2000, respectively. On the other hand, only a few individuals of *Myzus persicae*, one of the most efficient vector, were caught. Few individuals of *Aphis craccivora* and *Aphis fabae* were also caught. *Hyalopterus pruni* represented 3.5% and 9% of the total aphids in 1999 and 2000, respectively. This species may also play a role in PPV transmission.

Table 2. Percentages of aphid species being PPV vectors caught in two apricot orchards in 1999 and 2000

Aphid species	1999	2000
Aphis gossypii	13.4	17.7
Hyalopterus pruni	3.5	9
Myzus persicae	2.6	0.2
A. craccivora	0.1	0.5
A. fabae	0	0.4

Statistical analysis

The statistical analysis done with the Monte Carlo test (Table 3) revealed that in plot 1a the pattern of PPV spread was clustered in 1996 (P<0.01%) and that uniformity was established after 1997. In plot 1b a complete spatial random pattern (P=0.014) was obtained in 1996, but loose clusters appeared over the next years (P=0.003 for 1997, P=0.002 for 1998, P<0.01% for 1999 and 2000) (Fig. 1). In orchard 2 a complete spatial randomness (P>0.01) was obtained during the first four years and a clustering at the fifth year (P<0.01%). Thus, the statistical analysis applied here demonstrated that initial infections appeared in a random way and that no spatial disease gradient developed. As the disease developed in all cases loose clusters of infected trees appeared around the initially infected ones showing the significance of internal inoculum sources. Similar results were obtained in Spain regarding apricot orchards infected with PPV-D and applying a different kind of statistical analysis based on quadrat count methods (Gottwald *et al*., 1995).

Table 3. Results of the Monte Carlo test in two PPV-M infected apricot orchards

Orchard/ Year	Observed average of nearest neighbouring distances (m)	Significant level (P) of Monte Carlo Test	Pattern
Plot 1a			
1996	5.29	<0.0001	clustering
1997	4.94	<0.0001	Clustering uniform
1998	4.78	<0.0001	uniform
1999	4.71	<0.0001	uniform
Plot 1b			
1996	8.43	0.014	CRS*
1997	9.43	0.003	CRS clustering
1998	8.40	0.002	CRS clustering
1999	7.05	<0.0001	non CRS clustering
2000	5.78	<0.0001	clustering
Orchard 2			
1996	12.70	0.013	CRS
1997	8.49	0.161	CRS
1998	7.74	0.052	CRS
1999	7.45	0.027	CRS
2000	6.09	<0.0001	clustering

* Complete spatial randomness

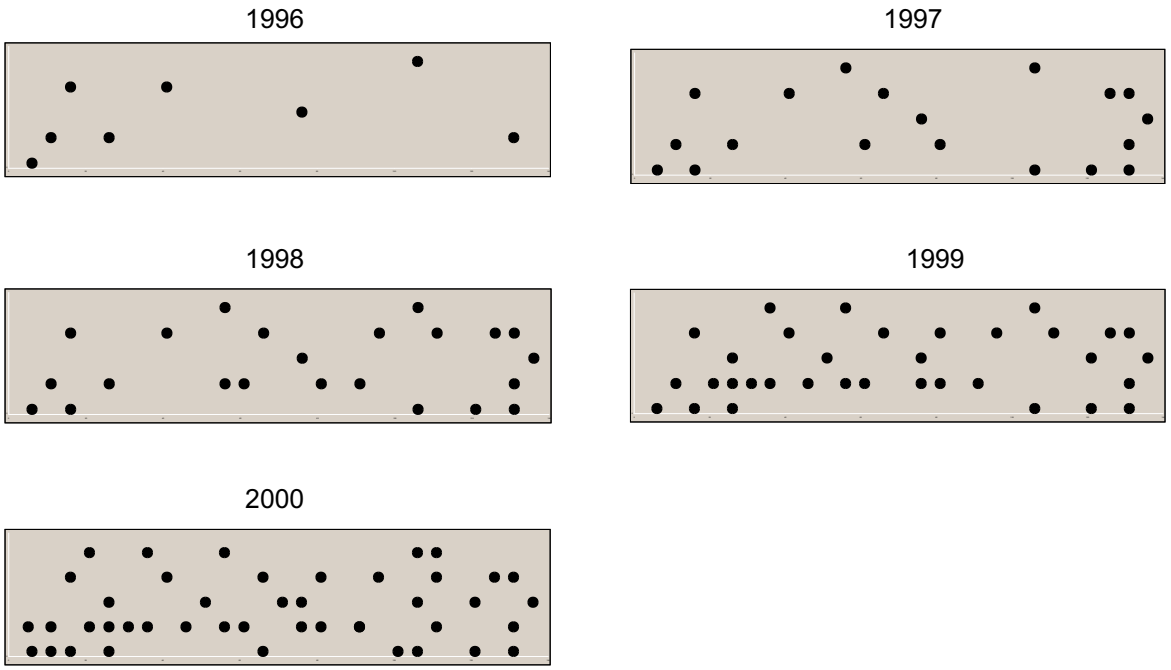

Fig. 1. Development of PPV spread in plot 1b of orchard 1 within five years.

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