SHORT COMMUNICATION

MONITORING *AMERICAN PLUM LINE PATTERN VIRUS* IN PLUM BY ELISA AND DOT-BLOT HYBRIDISATION THROUGHOUT THE YEAR

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SUMMARY

American plum line pattern virus (APLPV) was monitored monthly throughout a year in a Japanese plum tree by using ELISA and dot-blot hybridisation. Leaf samples were tested from March to November and dormant buds were tested from December to February. Flowers, cortex and fruits, when available, were also tested. For leaf samples, detection by ELISA and hybridisation was reliable from March to May. For dormant bud samples, detection was reliable from December to February. Detection levels decreased from June to August, and were unsatisfactory from September to November. During this latter period, molecular hybridisation was more sensitive than ELISA. Leaves were better sources of APLPV than flowers and cortical tissues in spring, whereas mature fruits were better sources than leaves in summer.

Key words: APLPV, detection, ELISA, molecular hybridisation.

American plum line pattern virus (APLPV), an ilarvirus characterized by Paulsen and Fulton (1968), was first reported from North America where it infects Japanese plum, peach and flowering cherry inducing chlorotic lines, bands, or oak leaf patterns in the leaves (Fulton, 1982). APLPV is not serologically related to any of the other ilarviruses of stone fruits and can be detected by ELISA (Fulton, 1982). The full genomic sequence of an American isolate of the virus was determined by Scott and Zimmerman (2001), who detected the virus by molecular hybridisation and RT-PCR. More recently, Alayasa *et al*. (2003) and Herranz *et al*. (2003) published additional information on detection and sequencing of other isolates.

Although APLPV is included in the EPPO Quarantine List A1 of plant pathogens (Smith *et al*., 1992), it was recently recorded from Albania, Italy, Tunisia (Myrta *et al*., 2002), and Palestine (Alayasa *et al*., 2003). The presence in the Mediterranean of such a potential threat to the regional stone fruit industry and the need to develop effective detection protocols, prompted an investigation aimed at establishing the influence of the season, of the plant tissue to be sampled, and the position of the sample in the plant's canopy on the reliability of laboratory detection assays.

APLPV was monitored monthly by ELISA and dot-blot hybridisation in a naturally infected field-grown Japanese plum tree in Apulia (Southern Italy) that showed striking chlorotic rings and lines in spring, which turned yellow-cream in summer. With ELISA and molecular hybridisation tests, this symptomatic tree was shown to be free from *Apple chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV), *Prune dwarf virus* (PDV), *Prunus necrotic ring spot virus* (PNRSV), and *Plum pox virus* (PPV). ELISA for *Tomato ring spot virus* (ToRSV), *Tomato black ring virus* (TBRV), *Cherry leaf roll virus* (CLRV), *Arabis mosaic virus* (ArMV), *Raspberry ringspot virus* (RpRV) and *Strawberry latent ringspot virus* (SLRSV) were also negative.

Samples were collected from leaves in March to November and from dormant buds in December to February. Three leaves were sampled from each of the main four branches of the tree (in apical, central and basal position of one-year-old shoots) and divided in half along the mid vein. Extracts from the two halves were used either in ELISA or molecular hybridisation. Two adjacent buds were used separately for ELISA and molecular hybridisation. During the growing season, samples were collected also from flowers, cortical tissue from one-year-old shoots, and fruits. Tests were made on thirteen flower and 5 cortex samples in March, on 10 unripe fruit samples in June, and on 73 mature fruit samples in July.

All samples were tested by DAS-ELISA (Clark and Adams, 1977) with a commercial kit (Agdia, Elkhart, IN, USA) and by dot-blot hybridisation (Alayasa *et al*., 2003). Samples that gave ELISA readings after 1 h at least three times higher than the healthy control were retained as positive. Leaves and flowers from screen-house-grown Japanese plums were used as negative con-

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trols. Positive controls were fresh leaves or lyophilised leaf tissues from infected Japanese plums.

As shown in Table 1, the rate of serological and molecular detection of APLPV in leaves was excellent from March to May, when all samples were positive, regardless of the technique used. With ELISA, however, difficulties began in June, when only 9 of 12 samples (75%) gave a positive reaction, and lasted till November, when the last leaf samples were tested. The detection level was adversely affected by summer temperatures that were higher than 30°C for 12 days in June, for 16 days in July and for 9 days in August. During the hot season, dot-blot hybridization was more sensitive than ELISA; in July it gave positive responses for 9 of 12 leaves tested (75%) *versus* 2 out of 12 (18.3%), and in August it gave positive responses for 9 out of 12 leaves (75%) *versus* 3 out of 12 (25%). These findings are in line with those obtained with other ilarviruses (e.g. PNRSV in peach), that serology gives erratic results in summer (Uyemoto *et al.*, 1989), whereas detection is more efficient during active vegetative growth (Scott *et al.*, 1992).

APLPV was detected at a very low rate by both techniques in September-November, when only one sample out of 36 (2.8%) and 4 out of 36 (11.1%) were positive in ELISA and dot-blot hybridization, respectively, or not at all. These results resemble those reported for *Hop stunt viroid* (HSVd) in apricot, where a significant drop in viroid concentration was observed from September to November (Amari *et al.*, 2001). The effectiveness of serological and molecular detection in dormant buds was highest (100%) from December to February (Table 1) in agreement with results reported by Fulton (1982).

Different tissues (leaves, flowers and cortical scrapings from young shoots) were tested simultaneously in March. All leaves (12) and cortical samples from young shoots (5) were positive in ELISA and dot-blot hybridisation, but the concentration of the virus was higher in the leaves, their average O.D. values being about three times higher than those for cortical scrapings and five times higher than those for healthy controls (data not shown). All 13 flower samples, collected in different shoot positions (apical, central and basal) were positive in dot-blot hybridisation but negative in ELISA, which contrasts with the report that floral tissues are best for PNRSV detection (Scott *et al.*, 1989). Fruits were collected at random and tested in June and in July. Ten unripe June fruits were all negative in ELISA but positive in dot-blot hybridisation, thus indicating that in this month, leaves are apparently better antigen sources than fruits (Table 1). By contrast, in July, 55 of 73 mature fruits (75.3%) were ELISA positive as compared with 2 of 12 leaves (18.3%), thus reversing the June situation. This is similar to findings with PNRSV that virus concentrations were 125 times higher in mature fruits than in leaves (Sanchez-Navarro *et al.*, 1998).

The location of the leaves in the shoot seemed to have a bearing on virus detection. Basal mature leaves were slightly better source during the whole year with

Table 1. Detection of APLPV by ELISA and molecular hybridisation during a whole year [a].

Month		ELISA					Molecular hybridisation				
		B	C	A	Total		B	C	A	Total	
		(i/t)	(i/t)	(i/t)	N.	%	(i/t)	(i/t)	(i/t)	N.	%
January*		4/4	4/4	4/4	12/12	100	4/4	4/4	4/4	12/12	100
February*		4/4	4/4	4/4	12/12	100	4/4	4/4	4/4	12/12	100
March **		4/4	4/4	4/4	12/12	100	4/4	4/4	4/4	12/12	100
April**		4/4	4/4	4/4	12/12	100	4/4	4/4	3/4	11/12	92
May**		4/4	4/4	4/4	12/12	100	4/4	4/4	4/4	12/12	100
June**		3/4	3/4	3/4	9/12	75	4/4	4/4	4/4	12/12	100
July**		1/4	1/4	0/4	2/12	18	3/4	3/4	3/4	9/12	75
August**		2/4	1/4	0/4	3/12	25	3/4	3/4	3/4	9/12	75
September**		1/4	0/4	0/4	1/12	8	0/4	0/4	0/4	0/12	0
October**		0/4	0/4	0/4	0/12	0	0/4	0/4	0/4	0/12	0
November**		0/4	0/4	0/4	0/12	0	3/4	0/4	1/4	4/12	33
December*		4/4	4/4	4/4	12/12	100	4/4	4/4	4/4	12/12	100
TOTAL	N.	31/48	29/48	27/48	87/144	60	37/48	34/48	34/48	105/144	73
	%	64	60	56			77	71	71		

[a] Leaves (**) or dormant buds (*) from the basal (B), central (C), and apical (A) portion of a vegetative shoot; i/t: number of infected samples number of tested samples.

31/48 (64.4%) positives in ELISA and 37/48 (77.1%) in dot-blot hybridisation. Central leaves gave positive reactions in 29/48 (60.4%) and 34/48 (70.8%) of the cases, and apical leaves in 27/48 (56.3%) and 34/48 (70.8%) of the cases in ELISA and molecular hybridisation, respectively (Table 1). These data resemble those for PDV and PNRSV, which were detected equally well serologically in young and mature plum leaves, but not for ApMV, for which young leaves were a better source of antigen (Torrance and Dolby, 1984).

In conclusion, dot-blot hybridisation was more effective than ELISA in hot months and when flowers or unripe fruits were used for the assays. On a whole year basis, the responses to molecular tests were positive with 105 of 144 samples (73%), whereas ELISA responses were positive in 60.4% of the cases (87 of 144 samples). Considering that our results originate from testing of a single tree, confirmation from other hosts and environmental conditions is desirable. However, since ELISA and molecular hybridisation do not seem in any case to constitute fool-proof methods for an efficient identification of APLPV the whole year round, the use of more sensitive detection techniques, such as single-step or nested PCR should be investigated.

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