THE DETECTION OF PLUM POX AND OTHER VIRUSES IN WOODY PLANTS BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

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Abstract

The new technique of enzyme-linked immunosorbent assay (ELISA) was used successfully to detect plum pox virus in the fruits, flowers, leaves and roots of plum. Numerous samples from commercial nurseries and orchards and from experimental plants in the field and glasshouse were tested quickly and conveniently. The sensitivity of the technique and its suitability for handling many small samples of tissue were exploited in assessing differences in virus content within leaves and between different floral parts.

Arabis mosaic virus was detected by ELISA in extracts made direct from blackcurrant leaves and buds and from the leaves of raspberry, strawberry, elderberry and ivy. It was also possible to detect apple mosaic virus in apple flowers and in leaves collected in spring and early summer when leaf extracts failed to infect cucumber test plants that were inoculated mechanically.

The success of ELISA for detecting viruses in woody hosts is discussed in relation to other possible applications of the technique.

Introduction

The technique of enzyme-linked immunosorbent assay (ELISA) provides a quick and sensitive means of detecting plant viruses (Clark et al., 1976). It is particularly suitable for processing numerous samples and for detecting latent viruses and those that cause inconspicuous or ephemeral symptoms of limited diagnostic value. Many of the current procedures for detecting viruses of this type by indexing, serology or electron microscopy are slow, insensitive or otherwise inconvenient.

Such difficulties are avoided by using ELISA. Some of the many possible applications have been considered in our recent work on latent viruses of hop and in the work here described on plum pox and other viruses of woody plants.

Materials and methods

The assays were performed in polystyrene microtitre plates using the methods and antisera of Clark et al., (1976). Except where stated all plant extracts were prepared by pestle and mortar at a standard dilution of 1:50 (w/v) in phosphate buffered saline containing Tween 20 and 2% (w/v) polyvinyl pyrrolidone (MW 24.500 or 44.000). All extracts were loaded into the plates within 5 h.

The strength of the reaction was assessed from the intensity of yellowing due to the enzyme-produced end product. Quantitative data were obtained by measuring absorbance at 405 nm. Some reactions were simply assessed visually as positive or negative with a sensitivity of colour detection >0.10 OD.

Results

Plum pox virus (PPV)

In a comprehensive assessment of the ELISA technique for detecting plum pox virus (PPV), over 2 000 tests were done between March and August, 1976 using Prunus material from the field or glasshouse. PPV was detected readily in all field-grown material known to be infected, including leaves, dormant or developing buds and ripe or unripe fruits. PPV was also detected in the leaves of peach and apricot and in the roots, flowers and leaves of infected plum trees in the glasshouse.

Equivalent healthy tissues were included in all tests to assess possible interference or non-specific reactions. The latter became apparent in mid-June, but were avoided subsequently by including polyvinyl pyrrolidone in the extraction buffer as part of the standard procedure.

A purified preparation of PPV was included in tests done on three occasions in June and July so that the virus content of infected leaf samples could be estimated by reference to a standard dilution curve (see figure). All the leaf extracts gave ELISA values that greatly exceeded the limit of visual detection. Indeed, all but three of the 73 extracts would have given a detectable reaction even if diluted to 1 in 600 (w/v). Even greater dilution might be possible for appropriate tissues tested at the time of greatest virus content. This suggests that there are possibilities of testing bulk samples when simply assessing the presence or absence of virus in a group of trees, shoots or leaves.

The sensitivity of ELISA and its effectiveness with very small quantities of tissue make it ideally suited for assessing differences in virus content within and

between tissues. In tests on infected St. Julien rootstocks growing in the glasshouse, flower buds gave consistently lower ELISA values than vegetative buds on the same shoots. This was attributable to the low virus content of some floral parts. When the different parts of eight infected flowers were dissected and assayed separately, virus was detected less consistently and at lower concentrations in the petals and gynoecia than in the sepals and to a lesser extent the stamens (table 1).

The distribution of PPV within leaves was assessed in tests on pairs of discs taken by 7 mm cork borer from three different scion varieties with typical plum pox symptoms. Strong positive reactions were obtained with all the extracts from Victoria leaves, which had very diffuse symptoms. Virus was also detected consistently in the affected portions of Czar and Marjorie's Seedling but less frequently in normal-looking tissue of the same leaves. These results indicate the limitations of taking very small samples of leaf tissue in routine screening tests for PPV.

The technique of radial immunodiffusion has been used in West Germany for quick confirmatory tests on suspect material sent in by growers, advisors or plant health inspectors (Casper, 1975). In a comparison of the efficiency of the RID and ELISA techniques, both tests readily detected PPV in plum leaves with symptoms. ELISA also detected PPV in leaves containing little virus, whereas some of the RID tests on equivalent samples gave negative or doubtful results.

Arabis mosaic virus (AMV)

Arabis mosaic virus (AMV) has a wide natural host range including various fruit crops and other woody species. The virus is readily transmitted to C h e n o p o d i u m q u i n o a L. and other herbaceous indicator plants in which AMV can be identified serologically using expressed leaf sap for double diffusion tests in agar.

Using ELISA it is now possible to detect and identify AMV directly in at least some woody species without first having to inoculate herbaceous test plants. Early tests were done on extracts of dormant blackcurrant buds collected from healthy and graft-infected bushes (vars. Baldwin and Mendip Cross) in February, 1976. Polyvinyl pyrrolidone was not being used at that time in the extraction buffer and using PBS-Tween alone there was no detectable reaction with extracts made at dilutions of 1:10 (w/v). However, AMV was detected quite specifically in separate extracts made at 1:100 or 1:1 000 and in all extracts made with PBS-Tween containing 1% polyethylene glycol (table 2). These results are particularly notable

considering the presence in blackcurrant of protein-precipitating substances of the tannin type and the failure of all previous attempts to detect AMV in buds by double diffusion tests in agar.

In subsequent tests AMV was detected in blackcurrant, strawberry, raspberry (var. Glen Clova), elderberry (*Sambucus nigra* L.) and ivy (*Hedera helix* L.) leaves collected in mid-summer. Tests on comparable material from healthy plants gave no evidence of interfering substances or of non-specific effects. However, strong non-specific reactions prevented the detection of AMV in privet (*Ligustrum vulgare* L.), ash (*Fraxinus excelsior* L. and *F. americana* L.), *Spiraea douglassii* Hook and *S. bumalda* Burven in preliminary tests on leaf extracts made with PBS-Tween containing no other additives.

Apple mosaic virus (ApMV)

Apple mosaic virus (ApMV) is usually detected in apple by grafts to Lord Lambourne or other sensitive varieties that develop symptoms the year after inoculation. The virus has also been detected serologically by double diffusion tests in agar, but only in extracts made from the leaves of herbaceous hosts or from apple petals (Sequeira, 1967). This is a serious limitation because flowers are normally available for such a short period of the growing season and the only successful sap transmissions from apple to herbaceous hosts have been from petals.

The ELISA technique enables the successful detection of ApMV not only with extracts from petals but also in those made from Lord Lambourne leaves collected in spring and early summer. All inoculations from comparable material to cucumber were unsuccessful except those made with extracts of petals collected at full bloom. A small proportion of the inoculated plants then developed a few lesions before developing typical systemic symptoms.

It was impossible to detect ApMV by infectivity or by ELISA in later tests on leaves with conspicuous symptoms collected on 28th July after a prolonged period of unusually hot weather. Negative results were obtained with extracts from healthy and infected leaves which suggests that virus was below the detectable level rather than that there were interfering substances in the leaves.

Discussion

The ELISA technique has now been used successfully with diverse viruses including ApMV which is extremely labile and representatives of the nepovirus, carlavirus and potyvirus groups. The method is sensitive and versatile and numerous tests can be done quickly and economically. There are major advantages over existing methods of detection and in particular, the ability to assay viruses in woody hosts without using indicator plants in the field or glasshouse. This facilitates epidemiology studies and surveys of infection in commercial plantings and in stocks being grown for official certification schemes.

ELISA has been used already to monitor PPV in EMLA material of plum varieties and rootstocks from various sites in England. This has been done quickly and on a scale that would have been impracticable by inoculating C h e n o p o d i u m f o e t i d u m Schrad. or peach. Confirmatory tests have also been done on numerous samples from commercial nurseries and orchards and it may be possible to devise appropriate bulk sampling procedures for use during the winter months to check the health of imported planting material. Our successes with PPV and other viruses of woody plants and of hop suggest many additional applications of ELISA for studying viruses of other crops.

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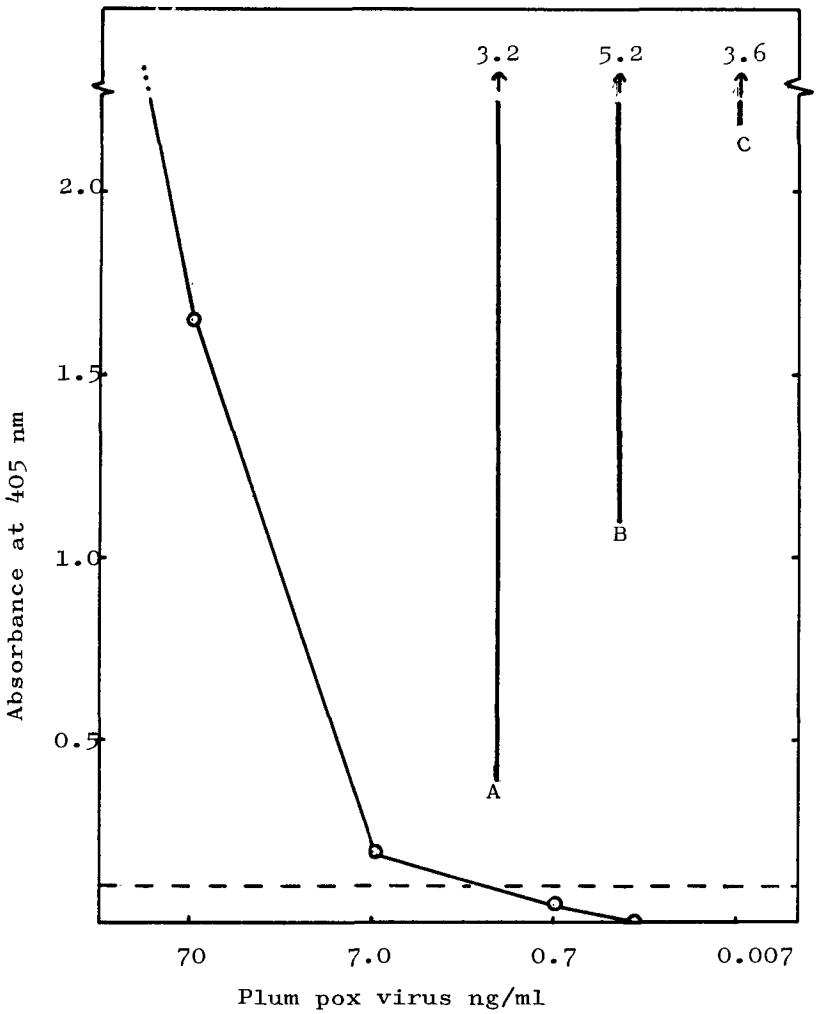

Figure 1 - ELISA absorbance values for purified plum pox virus (O——O) compared with the range of ELISA values for infected plum leaf extracts (vertical bars).
A 33 Czar and Marjorie's Seedling tested 9 June.
B 20 Czar and Marjorie's Seedling tested 19 July.
C 20 St. Julien and Brompton rootstocks tested 27 July.

Table 1. The distribution of plum pox virus in the different parts of infected St. Julien flowers

	Petals	Gynoecia	Stamens	Sepals
Proportion infected	4/8	2/8	8/8	8/8
Av. ELISA value	0.05	0.07	0.13	0.28
Sample wt (mg)	10	3	10	10
Sample dilution	100	300	100	100

Table 2. Mean ELISA values* for extracts made with and without polyethylene glycol from the buds of healthy and AMV-infected blackcurrant.

	Extraction ratios		
	1:10	1:100	1:1 000
PBS-Tween extracts			
Healthy	0.007	0.030	0.020
AMV	0.013	0.247	0.053
PBS-Tween + PEG			
Healthy	0.013	0.018	0.015
AMV	0.586	0.179	0.046

* The combined data for duplicate assays on samples from the varieties Baldwin and Mendip Cross which gave very similar results.