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Rapid detection and serotyping of prunus necrotic ringspot virus in perennial crops by enzyme-linked immunosorbent assay

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SUMMARY

Strains of prunus necrotic ringspot virus (NRSV) in hop and plum cultivars were rapidly and conveniently detected and serotyped by enzyme-linked immunosorbent assay. Antisera produced against the G isolate of NRSV from cherry and the Paradise isolate of apple mosaic virus (ApMV) from apple were used concurrently to ensure detection of all serotypes and to differentiate between them. Both NRSV and ApMV serotypes occurred in plum either as single or mixed infections. By contrast, the NRSV serotype was not found in hop, which contained either ApMV or a serotype intermediate between NRSV and ApMV.

INTRODUCTION

Studies on the aetiology and epidemiology of virus diseases are facilitated by rapid and convenient methods of identifying the viruses involved, and of discriminating between strains. Prunus necrotic ringspot virus (NRSV) and apple mosaic virus (ApMV) have been described separately, although they are distantly related serotypes (De Sequeira, 1967; Fulton, 1968), and intermediate strains occur in hop (Bock, 1967) and rose (Casper, 1973). It is usually difficult to categorise the strains encountered as they can cause very similar symptoms and some strains are particularly difficult to transmit mechanically from woody hosts to herbaceous indicator plants. Grafting to woody indicators may be used to test some hosts but this is laborious and slow to give results (Fulton, 1970, 1972). Differentiation is possible by gel diffusion or other conventional serological techniques but it is usually necessary to transmit the virus to an herbaceous host and produce concentrated extracts to obtain satisfactory results (Bock, 1967, Fulton, 1968).

This paper describes a quick, convenient and sensitive method of detecting and discriminating between ApMV, NRSV and intermediate strains or mixtures of these viruses in plum and hop plants by the recently described technique of enzyme-linked immunosorbent assay (ELISA) (Clark & Adams, 1977). Many samples were assessed without preliminary treatment or resorting to woody or herbaceous indicators. The technique has great potential for use in large scale surveys and epidemiological investigations involving the many important crops affected by ApMV and/or NRSV.

METHODS

ELISA tests were done in polystyrene microtitre plates using the methods of Clark & Adams (1977). All plant extracts were prepared by grinding tissue in a mortar and pestle at 1 : 50 (w/v)

with phosphate-buffered saline containing 0·5 ml/l Tween 20, 20 g/l (w/v) polyvinyl-pyrrolidone (mol.wt 44 000) and 2 g/l ovalbumin. Reactions were assessed quantitatively by measuring absorbance at 405 nm.

Serotyping strains by ELISA involved concurrent tests with antisera produced against an American isolate of NRSV from cherry (NRSV-G) and against the Paradise (Californian) isolate of ApMV (ApMV-P) (Fulton, 1968). Unless otherwise stated all tests were made with extracts of leaves from plum or hop.

For sap inoculation, tissue was ground at 1 : 4 (w/v) in 0·025 M sodium phosphate buffer (pH 8·0) containing 10 g/l polyethylene glycol (mol.wt 6000). Extracts were then inoculated manually to Carborundum-dusted cotyledons of cucumbers cv. Ohio and the plants kept in a shaded greenhouse. Such tests done early in the growing season usually detect NRSV in *Prunus* leaf material (Seneviratne & Posnette, 1970) and all known strains in hop (*Humulus lupulus* L.) (Thresh, Adams, Barbara & Clark, 1977).

RESULTS

Behaviour of homologous and heterologous antigens in ELISA

A virus isolate from cherry (ch-106) with properties similar to NRSV-G and an isolate from apple (ApMV-S) resembling ApMV-P were tested using antisera to NRSV-G and ApMV-P in all possible permutations both as coating and conjugate. The homologous permutations of virus

Table 1. *The reactions of two NRSV serotypes in ELISA tests using homologous and heterologous permutations of virus and antiserum*

Coating antiserum	Sample	Conjugate antiserum	A_{405}
NRSV-G	ch-106	NRSV-G	2·00
NRSV-G	ApMV-S	NRSV-G	0·04
NRSV-G	ch-106	ApMV-P	0·02
NRSV-G	ApMV-S	ApMV-P	0·07
ApMV-P	ApMV-S	ApMV-P	0·62
ApMV-P	ch-106	ApMV-P	0·00
ApMV-P	ApMV-S	NRSV-G	0·02
ApMV-P	ch-106	NRSV-G	0·21

with coating and conjugate antisera gave strong positive reactions (Table 1). ApMV-P coating anti-serum trapped sufficient ch-106 to be just detectable by NRSV-G conjugate but no other heterologous permutation gave a significant reaction.

Infection in plum

In tests with leaf extracts from infected plum cultivars, those that reacted strongly with NRSV-G antiserum but not with ApMV-P antiserum could usually be distinguished from those that reacted strongly with ApMV-P antiserum but insignificantly with NRSV-G antiserum (Fig. 1a). However, a few extracts reacted with both antisera suggesting the presence either of intermediate strains or of mixtures of NRSV and ApMV. To test these possibilities sap inoculations were made to cucumber; using the extraction buffer described in Methods strains of the NRSV type usually infect cucumber but those of the ApMV type do not. In all cases testing the infected cucumbers by ELISA revealed only NRSV strains, suggesting that these plum sources were doubly-infected. Previous work has shown that strains of NRSV and ApMV can co-exist in plum without evidence of interference or antagonism (Seneviratne & Posnette, 1970).

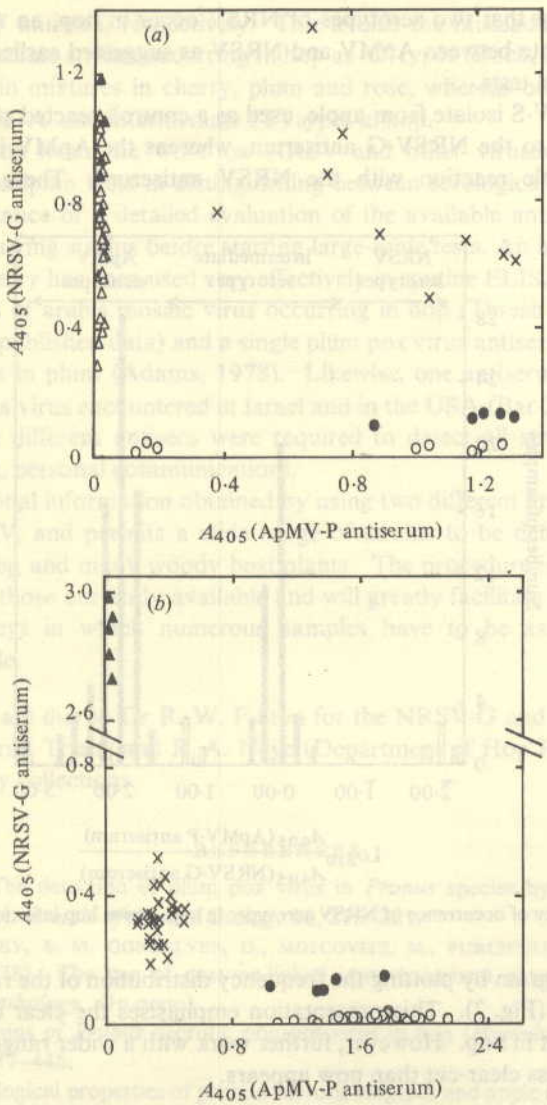

Fig. 1. ELISA reactions of NRSV infections using two antisera, NRSV-G and ApMV-P (a) in plum (b) in hop. ▲ = NRSV ch-106 (control) ● = ApMV-S (control). △, ○, × = infections reacting as NRSV, ApMV and mixed or intermediate serotypes respectively.

Infection in hop

When hop plants of several cultivars were tested using the two antisera the results were different from those with *Prunus* spp. Some samples reacted strongly with ApMV-P antiserum but not with NRSV-G antiserum, while others reacted moderately with both antisera (Fig. 1b). The latter isolates always reacted less strongly to ApMV-P antiserum than did the former and less strongly to NRSV-G antiserum than did ch-106 maintained in cucumber and included as a control.

Twelve of the isolates reacting to both antisera were sap-inoculated to cucumber cotyledons and then passaged three times in cucumber using single lesions taken soon after they had appeared, to minimise the possibility of maintaining any mixed infections. When tested by ELISA, all twelve isolates reacted to both antisera as in the original tests direct from hop.

These results confirm that two serotypes of NRSV occur in hop, an ApMV type and a type serologically intermediate between ApMV and NRSV as suggested earlier by Bock (1967) using conventional serological tests.

In all tests the ApMV-S isolate from apple, used as a control, reacted strongly to the ApMV-P antiserum and weakly to the NRSV-G antiserum, whereas the ApMV isolates from plum and hop gave no detectable reaction with the NRSV antiserum. These results are displayed

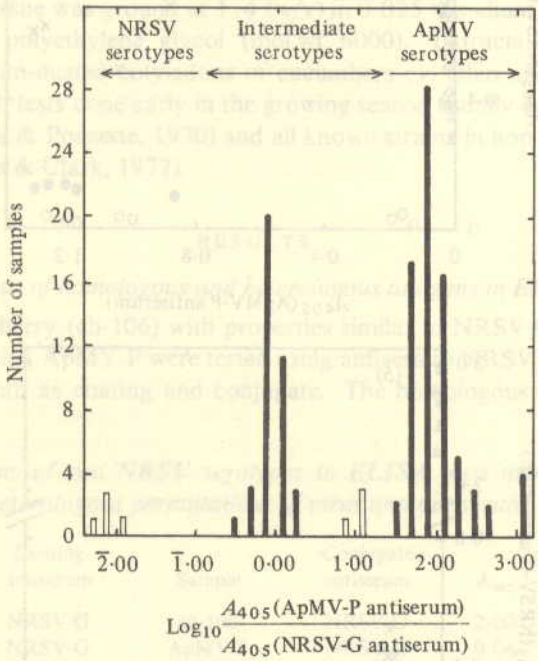

Fig. 2. Frequency of occurrence of NRSV serotypes in hop. ▬ hop infections ▭ controls.

conveniently as a histogram by plotting the frequency distribution of the ratios of the reactions to the two antisera used (Fig. 2). This presentation emphasises the clear distinction between the two serotypes prevalent in hop. However, further work with a wider range of isolates may reveal that the distinction is less clear-cut than now appears.

DISCUSSION

These results confirm and extend previous findings on the effectiveness and convenience of ELISA for detecting strains of ApMV in apple (Clark, Adams, Thresh & Casper, 1976) and hop (Thresh *et al.*, 1977). By using the NRSV-G antiserum as well as the ApMV antiserum used previously, it was possible to distinguish between weak reactions due to low concentrations of ApMV strains and those due to less closely related serotypes.

The distant serological relationships between NRSV and ApMV isolates and the occurrence of intermediate strains suggest that they should be regarded as serotypes of NRSV rather than as separate viruses. This view is supported by our confirmation of previous reports of the widespread occurrence in hop of intermediate strains that react well both with ApMV and with NRSV antisera. Bock (1967) referred to all isolates from rosaceous fruit plants and hop as NRSV but distinguished 'A' and 'C' strains according to their reactions with the two antisera. We support this usage which has advantages in categorising the wide range of strains encountered. However, we prefer to restrict the terms 'A' and 'C' strains to those reacting strongly only with

the ApMV and NRSV antisera, respectively. This avoids the misleading impression given by referring to the intermediate strains occurring in hop as 'C' types (Bock, 1967). True 'A' and 'C' types occur singly or in mixtures in cherry, plum and rose, whereas only 'A' types have been reported from apple and 'A' and intermediate ('I') types in hop.

There are indications from the work on NRSV and other viruses that ELISA is more discriminating than precipitin tests in distinguishing between serologically related strains. This emphasises the importance of a detailed evaluation of the available antisera against the whole range of naturally occurring strains before starting large-scale tests. An antiserum raised against an isolate from strawberry has been used very effectively in routine ELISA tests to detect but not to serotype the strains of arabis mosaic virus occurring in hop (Thresh *et al.*, 1977) and other hosts (M. F. Clark, unpublished data) and a single plum pox virus antiserum apparently detected all isolates of the virus in plum (Adams, 1978). Likewise, one antiserum sufficed to detect all strains of citrus tristeza virus encountered in Israel and in the USA (Bar-Joseph *et al.*, 1978). By contrast at least three different antisera were required to detect all strains of Andean potato latent virus (R. Koenig, personal communication).

The valuable additional information obtained by using two different antisera is well illustrated by our work on NRSV, and permits a wide range of strains to be detected and serotyped in extracts direct from hop and many woody host plants. The procedure is far quicker and much more convenient than those currently available and will greatly facilitate epidemiological studies and large scale surveys in which numerous samples have to be assessed as quickly and conveniently as possible.

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