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Production and evaluation of monoclonal antibodies for the detection of beet mild yellowing luteovirus and related strains

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Abstract

A sugar-beet-infecting isolate of beet mild yellowing luteovirus (BMYV), and a *Brassica*-infecting isolate of beet western yellows luteovirus (BWYV) were used to produce monoclonal antibodies for epidemiological studies with BMYV and related field strains. Thirty-four monoclonal antibodies were tested for their reaction with 9 luteoviruses in triple- antibody-sandwich enzyme-linked immunosorbent assay. One (MAFF 24) is now routinely used in the UK for detecting BMYV and BWYV in plants and aphids, although it does not discriminate between them. Heterologous reactions were detected between some of the monoclonal antibodies and potato leafroll virus (PLRV), bean leafroll virus (BLRV) and barley yellow dwarf virus (BYDV-RPV). 38% of antibodies raised to BWYV reacted with PLRV compared with 4% of those raised to BMYV. Monoclonal antibodies were produced which distinguished a sugar-beet-infecting isolate of BMYV with differing host range and serological properties from the commonly-occurring field strain.

Introduction

Virus yellows is the most important disease of sugar beet (*Beta vulgaris* L.) in the UK, affecting on average 5–10% of the national root crop each year, and up to 60% in epidemic years. Plants infected early in their growth can lose more than 50% of their potential sugar yield.

The most common cause of yellowing in the UK is beet mild yellowing luteovirus (BMYV). Polyclonal antibodies to BMYV have been used routinely for diagnosis of this virus, and in epidemiological studies [Govier, 1985; Smith and Hinckes, 1987]. However, they do not provide sufficient sensitivity to detect BMYV in the aphid vector, and they cannot be used to distinguish between virus strains such as BMYV and the non-beet-infecting strains of beet western yellows luteovirus (BWYV) which commonly infect *Brassica* crops, including oilseed rape (*B. napus* ssp. *oleifera*), in the UK [Smith and Hinckes, 1985], France and Germany [Häni, 1988]. Monoclonal antibodies have been shown to offer considerable potential for discriminating between luteoviruses and their strains, and for improving sensitivity of detection [D'Arcy *et al.*, 1989].

This paper describes work in which a sugar-beet-infecting isolate of BMYV, and a *Brassica*-infecting isolate of BWYV were used to produce panels of monoclonal antibodies. The aims of the studies were to produce monoclonal antibodies for detecting BMYV in plants and aphids in epidemiological and resistance studies, and for discriminating between BMYV and BWYV, and between field strains of BMYV with differing properties.

Materials and methods

Virus strains

The standard BMYV isolate (BMYV-BB-Std) originated from sugar beet in the UK, and was maintained in glasshouse-grown sugar beet at Broom's Barn

Experimental Station. Each year leaves from infected sugar-beet plants are collected from 10–20 fields. Aphids are fed on those leaves which are shown by ELISA to contain BMYV, and then transferred to sugar-beet seedlings to transmit. Four weeks later these inoculated sugar-beet plants are further checked by ELISA; aphids are then used to transfer BMYV from them, and from a plant infected with the stock 'culture' from the previous year, into a single sugar-beet plant. This stock 'culture' (BMYV-BB-Std), is presumed to be representative of the naturally-occurring field strains. Similarly, the standard BWYV isolate (BWYV-BB-Std) is comprised of a number of isolates collected from infected oilseed rape crops, and was maintained for these studies in glasshouse-grown oilseed rape. A field strain of BMYV which, unlike the standard strain is non-infective to the common weed species *Capsella bursa-pastoris* L. Medic. (BMYV-BB-NC), was maintained in glasshouse-grown sugar beet, as were five isolates of BMYV which originated from sugar-beet fields in different parts of the UK, (Allscott, Kidderminster, King's Lynn, Newark 1 and Newark 2). Isolates of BWYV originated from oilseed rape (Chatteris-OSR and Newark-OSR), calabrese (Boston-Cb and Great Holland-Cb) and Brussels sprouts (Spalding-B) and were maintained in oilseed rape.

A field isolate of potato leafroll virus (PLRV) was supplied by Mr A Philips, ADAS, Wolverhampton. Bean leaf roll virus (BLRV) in bean was supplied by Dr A Murant of the Scottish Crop Research Institute, Invergowrie, Dundee, Scotland. Isolates of barley yellow dwarf virus (BYDV-PAV, BYDV-RPV and BYDV-MAV) in oats were supplied by Professor R T Plumb, Rothamsted Experimental Station, Harpenden.

Monoclonal antibodies

In addition to the panels of monoclonal antibodies produced for these studies, a monoclonal antibody to BWYV (510H) was obtained from Dr R R Martin, Agriculture Canada Research Station, Vancouver, Canada. This antibody reacts with *Brassica*-infecting strains of BWYV, and sugar-beet-infecting BMYV. A second monoclonal antibody, prepared against barley yellow dwarf virus (BYDV-PAV-IL-1) was obtained from Dr C J D'Arcy, University of Illinois, Urbana, USA; this antibody reacts with BMYV from sugar beet, but not with *Brassica* isolates of BWYV [D'Arcy *et al.*, 1989].

Purification : virus sources

Seedlings of *Montia perfoliata* (Donn.) How. were inoculated with BMYV-BB-Std using viruliferous *Myzus persicae* Sulz. The plants were grown at 18–20 °C with a 16 h photoperiod under 400 W high-pressure sodium lights (Simplex SON/T), and harvested six weeks after inoculation. Enzyme-linked immunosorbent assay (ELISA) results showed that highest concentrations of virus occurred in leaves with red symptoms, and hence only these leaves plus their stems were harvested for virus extraction. *C. bursa-pastoris* seedlings were inoculated with BWYV-BB-Std; five weeks later all parts of the plants were shown to contain high concentrations of virus, and therefore the whole plants, excluding the roots, were harvested for virus extraction.

Purification method

The procedure was based on those described by Takanami and Kubo [1979], and Govier [1985]. Virus concentration was estimated by determining absorbance in a Pye Unicam SP6–500 uv spectrophotometer, and assuming

$$A\frac{0.1\%,}{10\text{mm}, 260\text{nm}} = 8.6$$

[Takanami and Kubo, 1979].

Polyclonal antiserum production

A Dutch/Lop rabbit was immunised using 25 μg of purified BMYV, diluted in phosphate buffered saline (PBS) and emulsified in Freund's adjuvant, administered at weeks, 1, 4, 9, 13 and 19. Immunoglobulin was partially purified by ammonium sulphate precipitation; solutions in PBS were adjusted to approximately 1 mg/ml, and stored at −20 °C.

Hybridoma production and screening

LOU/OLA rats were injected intramuscularly with 17 to 83 μg of pure virus in 250 μg of sterile PBS, emulsified with Freund's adjuvant; total immunisation periods varied between 30 and 80 days.

The methods for cell fusion and maintenance of hybrids were essentially as described by Galfré and Milstein [1981]. The basic medium used was Dulbecco's Modified Eagles Medium (DMEM) (Gibco 41965-039), containing 2 mM sodium pyruvate (Gibco 11360-039), 2 mM L-glutamine (Gibco 25030-024), 100 units/ml penicillin/streptomycin (Gibco 15070-022) and 2 mM Hepes buffer (Gibco 15630-049). Foetal calf serum (FCS) was heat-inactivated at 56 °C

Table 1. Triple-antibody-sandwich ELISA reactions of 26 BMYV and 8 BWYV monoclonal antibodies to nine virus strains

Number of monoclonal antibodies	Immunogen	BMYV-BB-Std	BMYV-BB-NC	BWYV-BB-Std	PLRV-potato	BLRV-bean	BYDV-PAV oats	BYDV-RPV oats	BYDV-MAV oats
10	BMYV-BB-Std	+	+	+	–	–	–	–	–
2	"	+	–	+	–	–	–	–	–
6	"	–	+	+	–	–	–	–	–
6	"	+	+	+	–	–	–	+	–
1	"	+	–	+	–	–	–	+	–
1	"	+	+	+	+	+	–	+	–
4	BWYV-BB-Std	+	+	+	–	–	–	–	–
2	"	+	+	+	+	–	–	–	–
1	"	–	+	+	+	–	–	–	–
1	"	–	–	+	–	–	–	–	–

Absorbance values (A_{405}nm) obtained after 1 h incubation.
+ = A_{405} values greater than the sum of the mean and three standard deviations of this mean for the corresponding virus-free samples.

for 30 min before being added to the DMEM in various percentages as appropriate.

Spleen cells were fused with the rat myeloma line Y3 Ag 1.2.3 [Galfré *et al.*, 1979] in a ratio of approximately 4:1 using 50% (w/v) polyethylene glycol (PEG) 1500 in 75 mM Hepes buffer (Boehringer 783641). Hybridomas were selected by plating-out cells in 20% (v/v) FCS-DMEM containing azaserine/hypoxanthine (HAZA) (Sigma A9666), and hybridoma enhancing supplement (HES) (Sigma 8142) in NUNC 96 well tissue culture plates. Hybridomas were transferred to 20% (v/v) FCS-DMEM-H (Hypoxanthine) (Gibco 11364-015) on day 7, and to 20% FCS-DMEM alone on day 8.

For BMYV fusions, tissue culture supernatants were screened by TAS-ELISA against BMYV in sugar-beet leaf sap and healthy sugar beet. For BWYV fusions tissue culture supernatants were screened against BWYV in oilseed rape, and healthy oilseed rape. Selected cell lines were cloned by dilution plating in 96 well tissue culture plates containing 20% FCS-DMEM-HES.

Triple-antibody-sandwich ELISA (TAS-ELISA)
The method was based on that described by D'Arcy *et al.*, 1989. Microtitre plates (NUNC Immunoplate II F96 Maxisorb, Denmark) were coated with 100 µl per well of polyclonal BMYV immunoglobulin at 2 µg/ml in 0.05 M sodium carbonate buffer pH 9.6, and incubated for 1 h at 37 °C. The wells were blocked using 200 µl per well of phosphate buffered saline (PBS) containing 0.1% non-fat dried milk, and incubated at room temperature for 1 h. Antigens were extracted in PBS containing 0.05% Tween 20 and 0.1% dried milk (PBS + T + milk), added to the wells in 100 µl aliquots and left overnight at 4 °C. Tissue culture supernatants were diluted ten-fold in PBS + T + milk, and monoclonal antibodies from ascites were used at 1 µg/ml; 100 µl aliquots were added to selected wells and incubated at 37 °C for 2 h. Alkaline phosphatase enzyme conjugated to anti-rat or anti-mouse antibody (Sigma Chemical Co., A9529, A1902) was added to each well at 1 µg/ml and incubated for 2 h at 37 °C. Plates were washed four times for 3 min with PBS + T between each step. Substrate (p-nitrophenyl phosphate, Sigma 104–105) was added at 0.5 mg/ml in 10% (v/v) diethanolamine pH 9.6 in 100 µl aliquots, and absorbance values at 405 nm recorded after 1 h, using a Titertek Multiskan plate reader (Flow Laboratories) or a Molecular Devices E Max Colorimeter.

Results

Twenty of the 26 monoclonal antibodies raised to BMYV-BB-Std reacted with their homologous antigen; all reacted with BWYV-BB-Std. One antibody reacted with an epitope common to PLRV, BLRV and BYDV-RPV, and seven other antibodies also reacted with BYDV-RPV. Three did not react with the non-*Capsella* infecting strain, BMYV-BB-NC.

The eight antibodies raised to BWYV-BB-Std all reacted with their homologous antigen, and six reacted with BMYV-BB-Std. Three also reacted with PLRV, but none reacted with BYDV-RPV. One did not react with BMYV-BB-NC (Table 1).

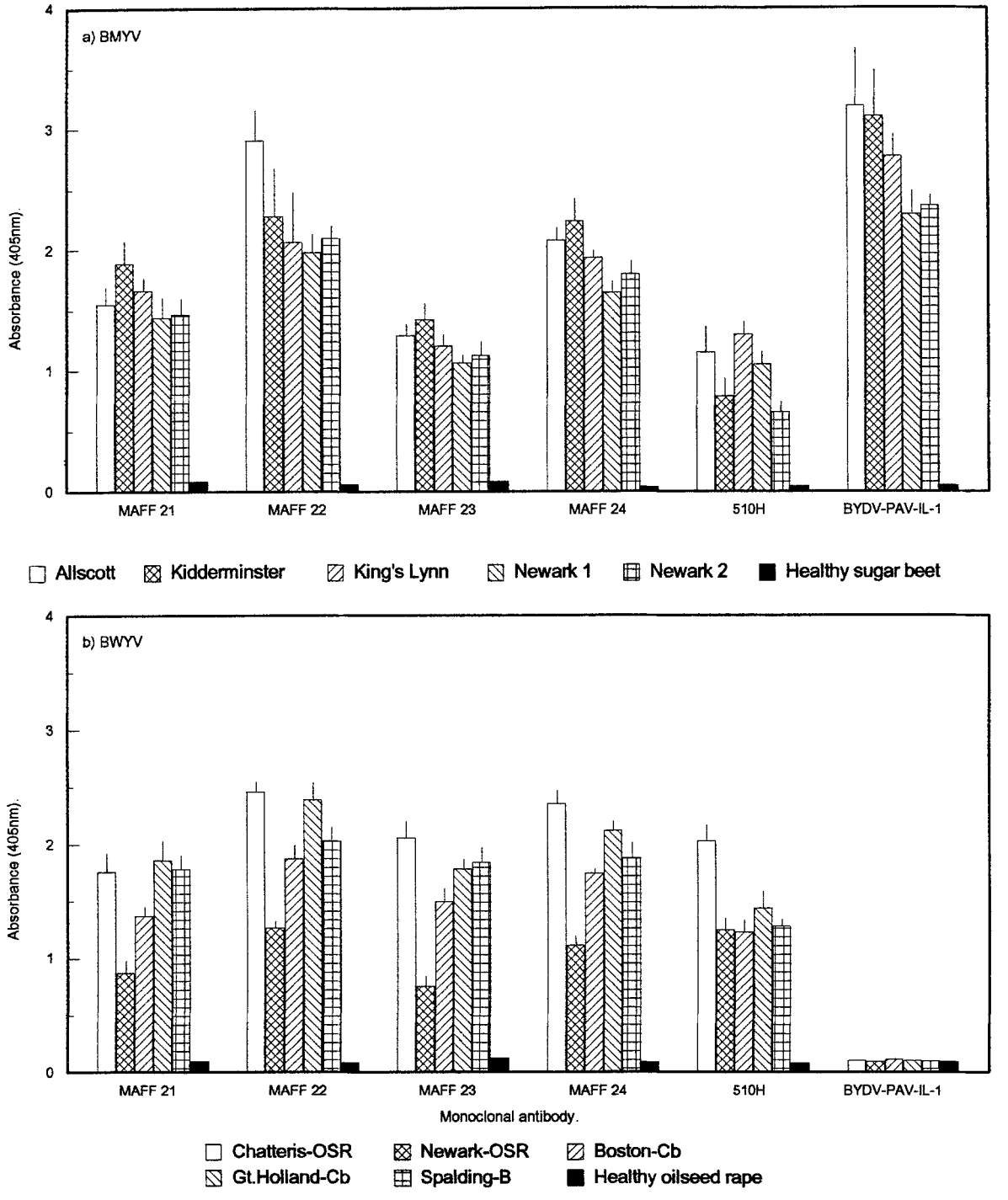

Fig. 1. Absorbance values obtained for five field isolates of BMYV from sugar beet (a) and five BWYV from brassicas (b) screened against six monoclonal antibodies.Each A_{405} value is the mean of 10 replicates. I = Standard error.

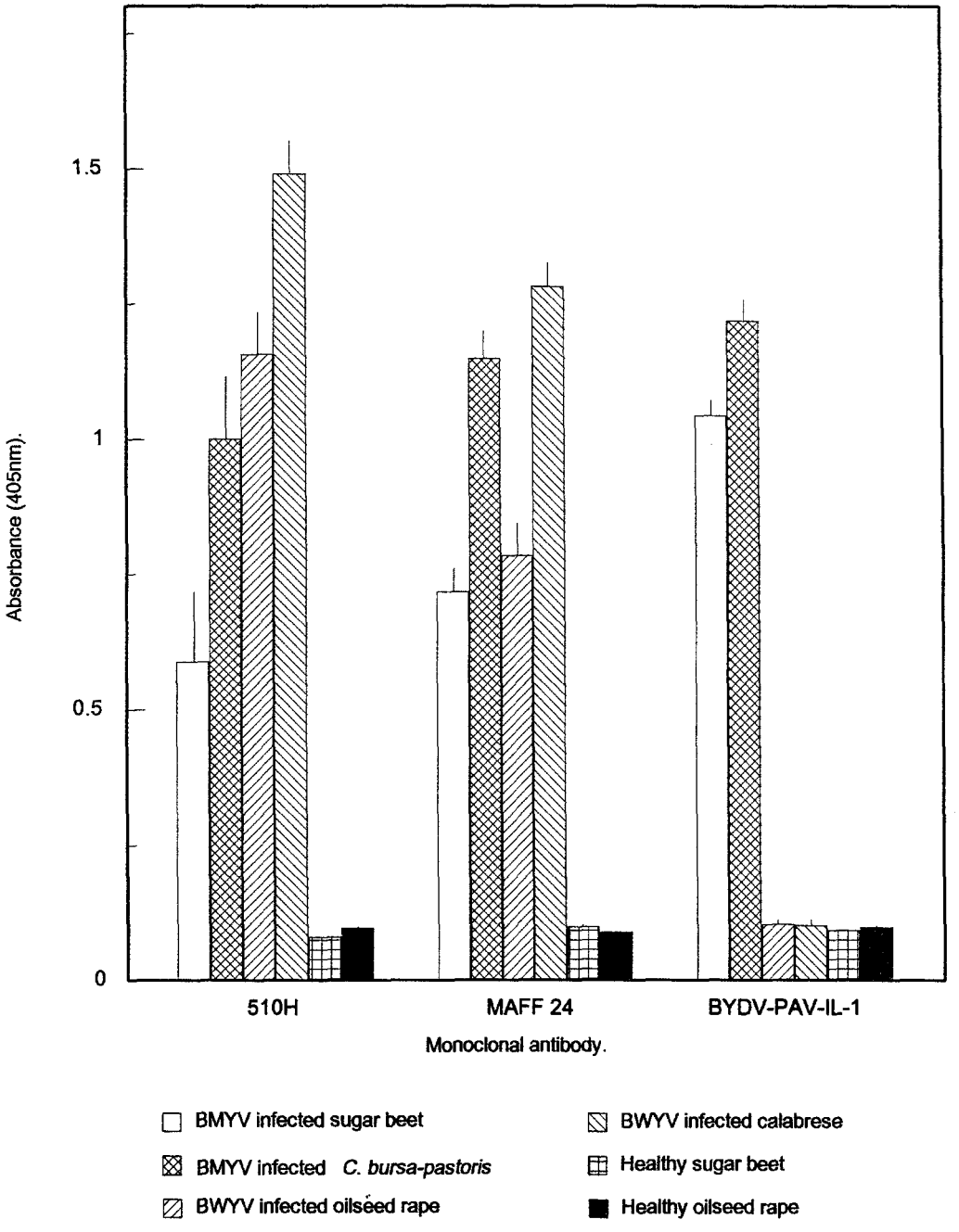

Fig. 2. Comparison of activity of monoclonal antibodies MAFF 24, 510H and BYDV-PAV-IL-1 when screened against two isolates of BMYV and two of BWYV. Each A_{405} value is the mean of 10 replicates. I = Standard error.

Monoclonal antibodies designated MAFF 21, 22, 23 and 24, all of which were isotype 1gG2a, were selected for further studies. All gave positive absorbance values in ELISA when screened against five field isolates of BMYV from sugar beet, as did antibodies 510H and BYDV-PAV-IL-1 (Fig. 1a). These four antibodies and 510H also all reacted positively with five field isolates of BWYV originating from *Brassica* crops. In contrast BYDV-PAV-IL-1 gave negative absorbance readings with these non-beet-infecting isolates (Fig. 1b).

Monoclonal antibody MAFF 24 was selected as a good general purpose antibody to BMYV, which did not react with PLRV, BLRV, or BYDV-RPV but which had shown activity comparable with 510H and BYDV-PAV-IL-1 (Fig. 2).

Studies with field isolates of BMYV from sugar beet have identified a distinct strain which, unlike BMYV-BB-Std, does not infect the common weed host, *C. bursa-pastoris*. This virus strain reacted with MAFF 24 and 510H, but not with BYDV-PAV-IL-1 [Stevens *et al.*, 1994a and b]. A monoclonal antibody isotype 1gM and designated MAFF 31, reacted strongly with these non-Capsella-infecting isolates, but weakly with the standard isolates.

Discussion

BMYV and BWYV are considered to be strains of the same virus [Casper, 1988], although they have fundamental differences in host range. The nucleotide sequence of the genomic RNA of a French isolate of BWYV from lettuce was found to be similar, but not identical to that of a German isolate of BMYV from sugar beet [Veidt *et al.*, 1988]. Therefore there should be potential for discriminating between the two viruses.

A monoclonal antibody (MAFF 24) was produced which showed considerable increase in sensitivity compared with polyclonal antibodies in detecting BMYV and BWYV. This antibody is now used routinely in the UK in epidemiological studies, including aphid infectivity. However, none of the antibodies produced reacted specifically with BMYV or BWYV.

Studies of the viral coat protein sequences of luteoviruses have shown that the nucleotide and amino acid sequences of BYDV-RPV isolates have greater similarity to those of BWYV and PLRV than to those of BYDV-MAV and BYDV-PAV isolates [Vincent *et al.*, 1990]. The serological results observed in these studies confirmed these similarities. However, a higher proportion (38%) of cell lines produced to BWYV-BB-Std reacted with PLRV, compared with 4% of those produced to BMYV-BB-Std, suggesting a closer relationship between BWYV and PLRV than between the latter and BMYV. Raising a panel of antibodies to PLRV may increase the chances of finding one which reacts with BWYV but not with BMYV, and which could be used for the identification of BWYV.

Considerable variations in host ranges and serological reaction have been shown to exist between members of the luteovirus group [Russell, 1965; Duffus, 1973; Duffus, 1981; D'Arcy *et al.*, 1989; Ellis and Wieczorek, 1992]. Little is known about the field strains of BMYV in the UK, although studies have identified a strain (BMYV-BB-NC), [Stevens *et al.*, 1994a and 1994b] which infects sugar beet but not *C. bursa-pastoris* or *M. perfoliata*, and which does not react with BYDV-PAV-IL-1. BMYV-BB-NC was detected in 11% of 102 infected sugar-beet plants tested between 1989 and 1993. BYDV-PAV-IL-1 is used to identify BMYV in winged aphids migrating into sugar-beet crops in studies which aim to improve the advice given to growers on the application of aphicidal sprays to control virus yellows [Smith *et al.*, 1991]. Since BYDV-PAV-IL-1 does not detect BMYV-BB-NC, these studies are likely to be underestimating the numbers of virus-carrying aphids, emphasising the need for methods for identifying specific strains. Several monoclonal antibodies reacted with BMYV-BB-NC but not with the standard strain of BMYV, offering the possibility of discriminating between these strains, both in plants and in aphids.

In conclusion, monoclonal antibodies provide valuable tools for identifying luteovirus strains, although they may ultimately need to be used in conjunction with more sensitive molecular methods to discriminate between strains with differing host ranges.

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