

INRA — Station de Pathologie Végétale, Colmar, France  
IBMC — Laboratoire d'Immunochimie, Strasbourg, France

## Grapevine fanleaf virus detection in various grapevine organs using polyclonal and monoclonal antibodies

by

BRIGITTE HUSS, B. WALTER, L. ETIENNE and M. H. V. VAN REGENMORTEL

### Détection du GFV dans différents organes de vigne à l'aide d'anticorps polyclonaux et monoclonaux

**Résumé :** Il est possible de détecter le GFV (agent de la maladie du court-noué) non seulement dans les feuilles et les radicelles de vignes, mais aussi dans des copeaux de bois. Les extraits de plante peuvent être préparés dans des tampons ne contenant pas de nicotine. Des comparaisons faites avec différents protocoles ELISA montrent que l'utilisation d'anticorps monoclonaux est avantageuse pour le dépistage de routine du GFV.

**Key words :** fanleaf, virus, analysis, serology, leaf, shoot, root.

### Introduction

Nepoviruses are among the most widespread and damaging viruses of grapevine and their detection is essential for a good sanitary condition of the vineyards. ELISA techniques have been shown to allow a rapid and reliable detection of these viruses in grapevine tissues (WALTER *et al.* 1979, 1984) and are now used for large-scale sanitary selection programs in many countries around the world. One of the remaining difficulties concerns the sampling of grapevine tissues as shown by the fact that detection is unreliable during some periods of the year (BOVEY *et al.* 1980; LEHOCZKY *et al.* 1983). The classical extraction medium used in the serological detection of nepoviruses in grapevine tissues, especially for leaves, contains nicotine (VUITTENEZ and KUSZALA 1972; BOVEY *et al.* 1980; WALTER *et al.* 1984). Rootlets or wood shavings are also a good source of virus (STELLMACH and BERRES 1985; WALTER *et al.* 1985). It would be advantageous to replace nicotine because of its toxicity and to develop a simple extraction medium suitable for large scale diagnostic tests.

In this paper, we show that ELISA can be used during the whole year for detecting grapevine fanleaf virus (GFV) in different grapevine organs and that several extraction media are suitable for this purpose. We also describe the use of monoclonal antibodies prepared against GFV (HUSS, MULLER, SOMMERMEYER, WALTER, VAN REGENMORTEL, in preparation) for routine diagnostic tests.

### Materials and methods

Grapevines from different varieties, naturally or experimentally infected with GFV, were kept in the glasshouse or in the fields. Healthy grapevines were plants subjected to thermotherapy. Different GFV isolates were maintained on *Chenopodium quinoa*.

Virus was purified by butanol clarification, polyethylene glycol (PEG) precipitation, ultracentrifugation and sucrose gradient fractionation.

Antibodies were obtained from immunised rabbits and chickens, as described previously (WALTER *et al.* 1984). The preparation of mouse GFV-monoclonal antibodies (MCA) is described elsewhere (HUSS, MULLER, SOMMERMEYER, WALTER, VAN REGENMORTEL, in preparation). All the results presented here were obtained using ascitic fluids of the monoclonal antibody (3 × 1). Rabbit IgG's were extracted by the rivanol method of HARDIE and VAN REGENMORTEL (1977) and egg immunoglobulins by PEG precipitation, as described by POLSON *et al.* (1980). Rabbit IgG's and monoclonal antibodies were conjugated with alkaline phosphatase (Boehringer 567 752) using glutaraldehyde (AVRAMIAS 1969). Goat anti-mouse (GAM) conjugate was obtained from Sigma (A 5153). Details of the successive steps of the different ELISA procedures are presented in Table 1. Optical density (OD) readings were performed with a Titertek Multiskan photometer, zero being adjusted on an empty plate.

The plant extracts were obtained from grapevine leaves, wood shavings or rootlets or from *C. quinoa* leaves. They were prepared by two techniques. By the first, the tissues were ground in the presence of various extraction media, the compositions of

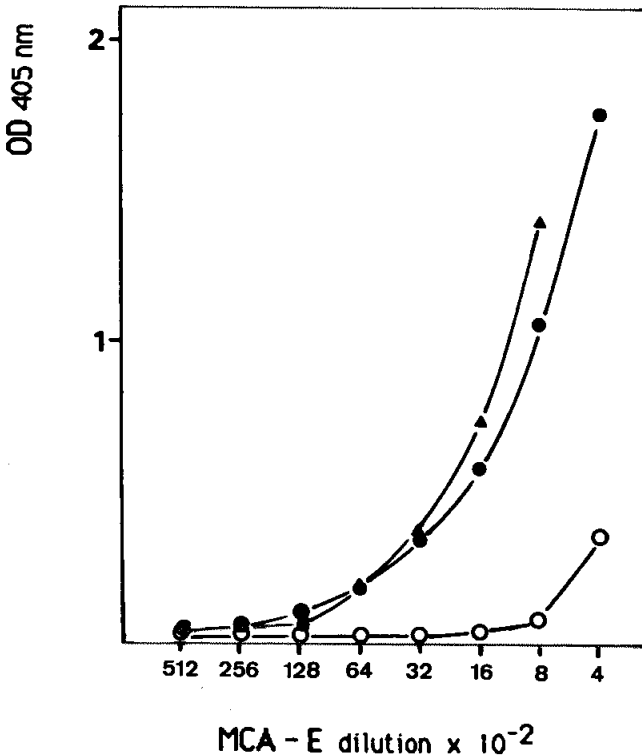


Fig. 1: Detection of GFV by ELISA using a MCA phosphatase conjugate at various dilutions. — Coating with 4 µg/ml chicken antibody for 4 h at 35 °C. Antigen: (▲) purified virus at 640 ng/ml; crude sap from (●) infected and (○) healthy *C. quinoa*. Substrate hydrolysis time was 1 h.

Détection du GFV à l'aide d'un anticorps monoclonal conjugué à la phosphatase.

Table 1

Comparison between different ELISA methods for the detection of GFV in leaf sap · Figures are mean OD<sub>405nm</sub> readings, after 1 h substrate incubation

Comparaison de différentes méthodes ELISA pour la détection du GFV dans des extraits de feuilles · Les chiffres indiquent une moyenne des valeurs de la DO<sub>405nm</sub> après 1 h d'incubation du substrat

| ELISA method                                | <i>Chenopodium quinoa</i> |          | Grapevine |                 |      |
|---|---------------------------|----------|-----------|-----------------|------|
|   | Healthy                   | Infected | Healthy   | Infected<br>S 1 | S 2  |
| 1. Ab <sup>R</sup> , Ag, Ab <sup>R</sup> -E | 0.12                      | 1.64     | 0.16      | 0.46            | 1.25 |
| 2. Ab <sup>R</sup> , Ag, MCA-E              | 0.22                      | 0.61     | 0.15      | 0.20            | 0.43 |
| 3. Ab <sup>C</sup> , Ag, MCA-E              | 0.13                      | 0.86     | 0.19      | 0.18            | 0.38 |
| 4. Ab <sup>C</sup> , Ag, MCA, GAM-E         | 0.13                      | 1.55     | 0.16      | 0.54            | 1.48 |
| 5. MCA, Ag, MCA-E                           | 0.0                       | >2.0     | 0.14      | 0.17            | 0.58 |

Abbreviations:

Ab<sup>R</sup> = rabbit antibody; Ab<sup>R</sup>-E = rabbit antibody enzyme conjugate; Ag = antigen; MCA = monoclonal antibody; Ab<sup>C</sup> = chicken antibody; GAM-E = goat anti-mouse enzyme conjugate; MCA-E = monoclonal antibody enzyme conjugate.

Plant extracts:

*C. quinoa*: 1 g/5 ml PBS 0.01 M pH 7.4, 1 % Tween, 1 % bovine serum albumin; grapevine (S 1 = sample no. 1; S 2 = sample no. 2): 1 g/5 ml nicotine 2.5 % in water.

Conditions of each ELISA method were as follows:

Method 1: Ab<sup>R</sup> at 0.125 µg/ml, incubated 4 h; Ab<sup>R</sup>-E, 1/8000, 4 h

Method 2: Ab<sup>R</sup>, 0.125 µg/ml, 4 h; MCA-E, 1/1000, 4 h

Method 3: Ab<sup>C</sup>, 4 µg/ml, 4 h; MCA-E, 1/1000, 4 h

Method 4: Ab<sup>C</sup>, 4 µg/ml, 4 h; MCA, 10<sup>-6</sup>, 3 h; GAM-E, 1/1000, 2 h

Method 5: MCA, 1/2500, 4 h; MCA-E, 1/1000, 4 h

Table 2

Grapevine extracts allowing GFV detection in leaves, wood and rootlets · Samples were considered positive when OD readings were at least twice the OD readings of healthy controls

Détection du GFV dans des extraits de feuilles, de bois et de racelles de vigne · Les échantillons sont considérés comme positifs quand les valeurs de la DO représentent au moins deux fois les valeurs de la DO des témoins sains

| Grapevine tissues | Extraction buffer                          |  |
|-------------------|--|--|
|                   | Nicotine <sup>1)</sup> (extract dil. 1/60) | PBS <sup>2)</sup> (extract dil. 1/600) |
| Leaves            | +  | —                                      |
| Wood              | —  | +                                      |
| Rootlets          | +  | +                                      |

<sup>1)</sup> Nicotine 2.5 % in water.

<sup>2)</sup> PBS buffer 0.01 M pH 7.4, 4 % insoluble polyvinylpyrrolidone (Polyclar AT), 1 % Triton X 100, 0.2 % mercaptoethanol.

ELISA method no. 1 as described in Table 1.

which are detailed under 'Results'. After a low-speed centrifugation, the supernatant was used as virus source. The second technique is a modification of that described by BLAICH and WIND (1982): 0.5 g leaves were ground in a mortar in the presence of 2.5 ml extraction medium (2.5 % NaHCO<sub>3</sub>, 0.5 % ascorbic acid in water). The pellet obtained after 10 min centrifugation at 20,000 *g* was dissolved in 2 ml 2 % PEG (MW 6,000) in water; the supernatant obtained after 1 min centrifugation at 20,000 *g* was used as virus extract.

The wood shavings were obtained by scratching grapevine canes after discarding the bark, as described previously (WALTER *et al.* 1985). The shavings were left for soaking during 4 h at 4 °C, without any grinding, in various extraction media. The juice, after sedimentation of the shavings by low speed centrifugation, was used for ELISA. In all tests, antigens were incubated in the plates overnight at 4 °C.

## Results

### 1. Use of MCA for detecting GFV in plant sap

The use of phosphatase conjugated MCA (3 × 1) for detecting the virus was investigated. When chicken antibodies were used at 4 µg/ml for coating the plates, the MCA conjugate must be diluted at least 1/1,000, in order to avoid high background reading with healthy plant sap (Fig. 1). In the case of purified virus or virus-infected *C. quinoa* sap, detection was satisfactory with dilutions of the MCA conjugate till 1/5,000. Instead of using chicken antibody for coating the plates, MCA could also be used for this purpose. In the case of purified virus or virus-infected *C. quinoa* sap, MCA 3 × 1 was diluted to 2 µg/ml for coating the plates and the MCA conjugate was diluted to 1/1,000.

Table 3

Detection of GFV in grapevine wood and rootlets using different extraction media · Figures are OD readings after 1 h substrate incubation

Détection du GFV dans du bois et dans des radicelles de vigne à l'aide de différents milieux d'extraction · Les chiffres indiquent les valeurs de la DO après 1 h d'incubation du substrat

| Extraction media | Wood shavings |         | Rootlets |         |
|------------------|---------------|---------|----------|---------|
|                  | Infected      | Healthy | Infected | Healthy |
| a) Carbonate     | 0.18          | 0.18    | 0.18     | 0.19    |
| b) PBS-T-BSA     | 1.97          | 0.20    | > 2      | 0.21    |
| c) PBS-T-PVP     | > 2           | 0.21    | > 2      | 0.20    |
| d) PBS-Polyclar  | > 2           | 0.20    | > 2      | 0.17    |
| e) Tris-PVP-PEG  | > 2           | 0.21    | > 2      | 0.19    |

a: NaHCO<sub>3</sub> 2.5 %, 0.5 % ascorbic acid, pH 7.3.

b: Phosphate buffer saline 0.01 M pH 7.4, 1 % Tween 20, 1 % BSA.

c: PBS 0.01 M pH 7.4, 0.05 % Tween 20, 2 % Polyvinylpyrrolidone.

d: PBS 0.01 M pH 7.4, 4 % insoluble PVP, 1 % Triton X 100, 0.2 % mercaptoethanol.

e: Tris-HCl 0.5 M pH 8.2, 0.05 % Tween 20, 2 % PVP, 1 % Polyethyleneglycol, 0.8 % NaCl, 0.02 % NaN<sub>3</sub>.

1 g tissue/5 ml extraction medium.

ELISA method no. 4 (Table 1).

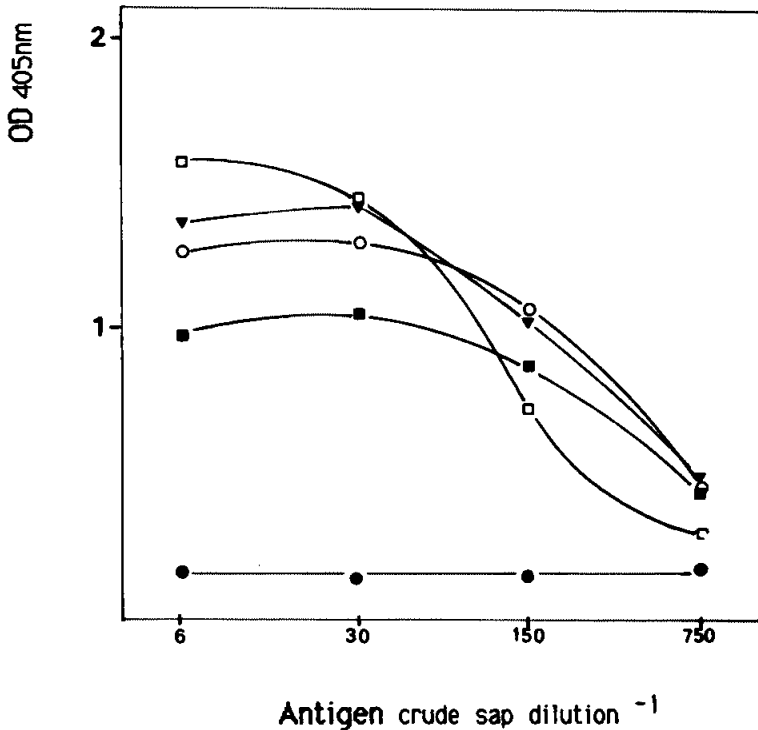


Fig. 2: Detection of GFV in grapevine rootlets. — Rootlets were ground in various media: (□) Tris-HCl-PVP-PEG; (▼) PBS-Tween-PVP; (○) PBS-Tween-BSA; (■) PBS-Polyclar; (●) carbonate. ELISA method no. 4. Substrate hydrolysis for 30 min.

Détection du GFV dans des radicelles de vigne.

For detecting GFV in infected grapevine leaves, the MCA had to be used for coating at a concentration of 10  $\mu\text{g/ml}$  (data not shown).

Different ELISA methods have been compared for their sensitivity in detecting GFV in *C. quinoa* and grapevine extracts (Table 1). In each case, methods 2 and 3, using a MCA conjugate were the least sensitive. With *C. quinoa* extracts, method 5 (using the same MCA as coating and conjugate) was the most sensitive, as shown in Table 1. Methods 4 and 1 were the most sensitive for detecting GFV in grapevine extracts.

## 2. GFV detection in various grapevine organs

The detection in leaves, wood, or rootlets of infected grapevines was highly dependent on the nature of the buffer used to prepare the extract (Table 2). These experiments confirmed that nicotine is essential for leaf extracts and that rootlets and wood shavings are good virus sources.

Different media were compared for their efficiency in the preparation of antigen extracts:

### 1. Wood and rootlets (Table 3 and Fig. 2)

The carbonate-ascorbic acid medium was not suitable, whereas the four other buffers allowed the detection of GFV.

Table 4

GFV detection in grapevine leaves with various extraction methods using monoclonal and polyclonal antibodies · Figures are OD readings, after 1 h substrate incubation

Détection du GFV dans des feuilles de vigne à l'aide de réactifs mono- et poly-clonaux · Les chiffres indiquent les valeurs de la DO après 1 h d'incubation du substrat

| Extraction method            | Monoclonal antibodies |         | Polyclonal antibodies |         |
|------------------------------|-----------------------|---------|-----------------------|---------|
|                              | Infected              | Healthy | Infected              | Healthy |
| a) Nicotine                  | 0.69                  | 0.16    | 0.47                  | 0.14    |
| b) Tris-PVP-PEG              | 1.69                  | 0.16    | 1.00                  | 0.13    |
| c) Carbonate followed by PEG | 1.07                  | 0.19    | 0.68                  | 0.18    |

a: 1 g leaves in 5 ml 2.5 % nicotine in water.

b: 1 g leaves in 5 ml Tris-HCl 0.5 M pH 8.2, 2 % polyvinylpyrrolidone, 1 % polyethyleneglycol, 0.8 % NaCl, 0.02 % NaN<sub>3</sub>.

c: 1 g leaves ground in 5 ml 2.5 % NaHCO<sub>3</sub>, 0.5 % ascorbic acid, pH 7.3; centrifugation for 10 min at 20,000 g; pellet resuspended in 2 ml polyethyleneglycol 6,000 at 2 % in water.

ELISA methods no. 4 for monoclonal antibody and no. 1 for polyclonal antiserum (Table 1).

## 2. Leaves

The results obtained using both rabbit antiserum and monoclonal antibodies are presented in Table 4. Positive samples could be identified when, after grinding the leaves in carbonate buffer, the low speed centrifugation pellet was resuspended in PEG (line c). When the carbonate grinding buffer was replaced by the classical PBS-Tween-PVP buffer (CLARK and ADAMS 1977), no virus could be detected (data not shown).

The detection was also possible by simply grinding the leaves in Tris-HCl-PVP-PEG buffer (line b). This method is very interesting because it avoids using nicotine and is less time consuming than the carbonate + PEG method.

## 3. Berries

The detection of GFV was possible in mature berries from different varieties, by grinding one berry in 1 ml Tris-HCl pH 8.2 buffer containing PEG 1 %, PVP 2 %, NaCl 0.8 %, Tween 20 0.05 % (data not shown).

## 3. Essential characteristics of the nicotine-free grinding buffer

### 1. PEG concentration (Fig. 3)

The presence of PEG in the grinding buffer did not improve the ELISA test. There was even a decrease in the sensitivity of virus detection at higher PEG concentration (5 %).

### 2. PVP concentration and NaCl (Fig. 4)

When PVP was not added in the Tris-HCl grinding buffer, virus was not detected. With 2 % or 5 % PVP, the detection was much better than with nicotine. The addition of NaCl at 0.8 % enhanced significantly the sensitivity of the test, particularly when the PVP concentration was 2 %.

### 3. pH

No significant differences were noticed when the pH of the extraction buffers varied between 7.4 and 8.2 (data not shown).

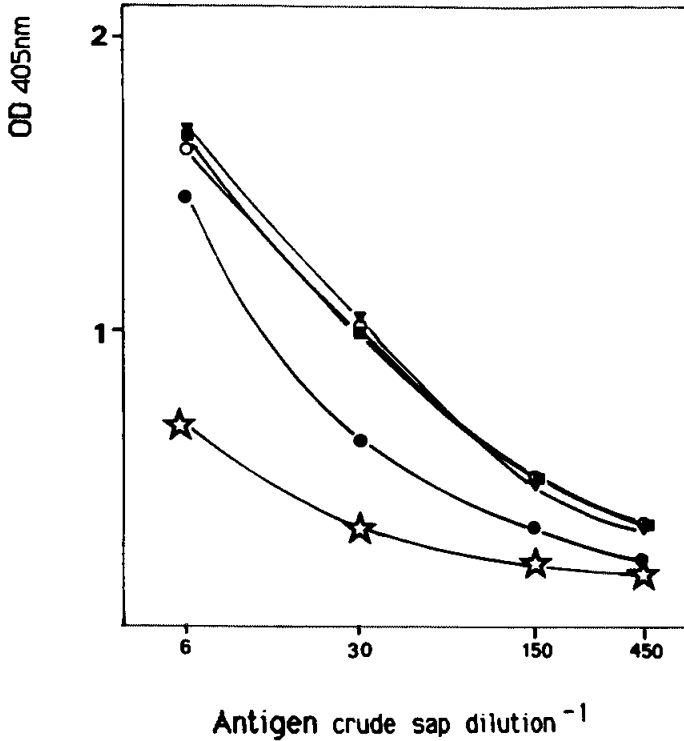


Fig. 3: Detection of GFV in grapevine leaves at various PEG concentrations in the extraction buffer. — Leaves were ground in nicotine (☆) or Tris-HCl buffer containing PEG at various concentrations: (○) 0 %; (■) 1 %; (▼) 2 %; (●) 5 %. ELISA method no. 4. Substrate hydrolysis time was 1 h.

Détection du GFV dans des feuilles de vigne avec des concentrations variables de PEG.

#### 4. Molarity and nature of the buffer (Table 5)

When the molarity of the buffer was low (0.01 M) the detection of GFV was not possible in grapevine leaves, although it was still possible in *C. quinoa* leaves. The nature of the buffer (Tris-HCl or phosphate) seemed to have little effect on the sensitivity of the test.

Our experiments showed that a buffer (PBS or Tris-HCl) with a molarity above 0.1 M and a pH around 8, containing PVP, is suitable for GFV detection in all grapevine organs.

#### 4. Simultaneous incubation of MCA and GAM-E

In order to shorten the ELISA test, we compared a slight modification of method 4 (in Table 1) which consists in incubating MCA and the anti-mouse-globulin conjugate simultaneously instead of sequentially (Table 6). Coating chicken immunoglobulins were incubated at 4 µg/ml for 4 h at 35 °C. The *C. quinoa* and grapevine extracts, prepared as described before, were incubated for the night at 4 °C. When incubated successively, the MCA at 10<sup>-6</sup> dilution was incubated for 3 h, and the GAM conjugate for 2 h, both at 35 °C. Alternatively, the MCA-GAM conjugate mixture in PBS-Tween

Table 5

Effect of the molarity of extraction buffer on GFV detection · Figures are OD readings after 1 h substrate incubation

Effet de la molarité du tampon d'extraction sur la détection du GFV · Les chiffres indiquent les valeurs de la DO après 1 h d'incubation du substrat

| Extraction buffer | Grapevine |         | <i>C. quinoa</i> | Buffer  |
|-------------------|-----------|---------|------------------|---------|
|                   | Infected  | Healthy | Infected         | Control |
| a1) PBS 0.5 M     | 1.33      | 0.17    | 1.90             | 0.18    |
| a2) PBS 0.1 M     | 1.42      | 0.15    | 1.82             | 0.17    |
| a3) PBS 0.01 M    | 0.16      | 0.16    | 1.82             | 0.20    |
| b1) Tris 0.5 M    | 1.41      | 0.17    | >2.0             | 0.20    |
| b2) Tris 0.1 M    | 1.55      | 0.16    | 1.92             | 0.18    |
| b3) Tris 0.01 M   | 0.16      | 0.18    | >2.0             | 0.18    |

a1, a2, a3:  $\text{KH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$ , 0.8 % NaCl, 0.02 % KCl, 2 % polyvinylpyrrolidone, 0.05 % Tween 20, 0.02 %  $\text{NaN}_3$ .

b1, b2, b3: Tris-HCl, 0.8 % NaCl, 2 % PVP, 0.05 % Tween 20.

ELISA method no. 4 (Table 1).

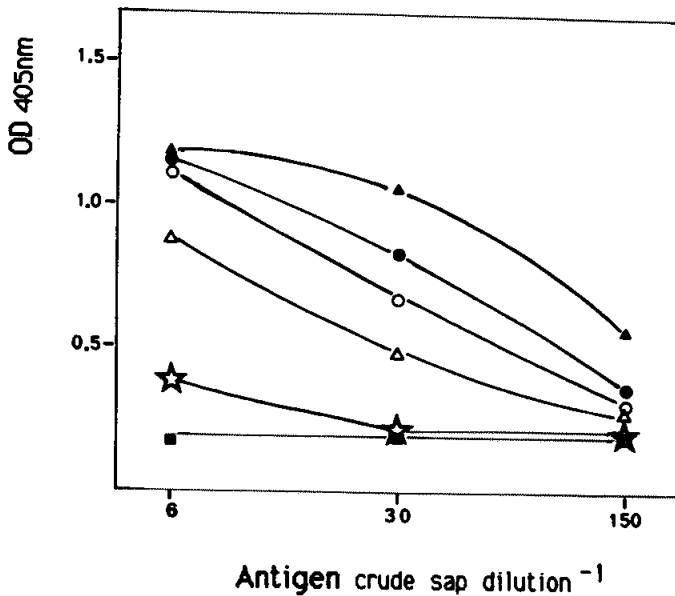


Fig. 4: Detection of GFV in grapevine leaves using various PVP concentrations in the extraction buffer. — Extraction media were (☆) nicotine or Tris-HCl buffer with: (■) 0 % PVP; (△) 2 % PVP; (○) 5 % PVP; (▲) 2 % PVP, 0.8 % NaCl; (●) 5 % PVP, 0.8 % NaCl. ELISA method no. 4. Substrate hydrolysis for 1 h.

Détection du GFV dans des feuilles de vigne avec des concentrations variables de PVP.



Table 6

Comparison between simultaneous and sequential incubations of MCA and anti-mouse globulin conjugate for GFV detection · Figures are OD readings after 1 h or 3 h substrate incubation

Détection du GFV en incubant l'anticorps monoclonal et le conjugué anti-souris simultanément ou successivement · Les chiffres indiquent les valeurs de la DO après 1 h ou 3 h d'incubation du substrat

| Samples          | Simultaneous incubation |         |       |         | Successive incubations |         |
|------------------|-------------------------|---------|-------|---------|------------------------|---------|
|                  | 4 °C                    |         | 16 °C |         | 1 h                    | (3 h)   |
|                  | 1 h                     | (3 h)   | 1 h   | (3 h)   |                        |         |
| <i>Grapevine</i> |                         |         |       |         |                        |         |
| no. 1            | 0.92                    | (> 2.0) | 0.77  | (1.93)  | 1.33                   | (> 2.0) |
| no. 2            | 0.45                    | (0.86)  | 0.51  | (1.19)  | 0.88                   | (> 2.0) |
| Healthy          | 0.15                    | (0.17)  | 0.14  | (0.16)  | 0.16                   | (0.24)  |
| <i>C. quinoa</i> |                         |         |       |         |                        |         |
| Infected         | 1.21                    | (> 2.0) | 1.10  | (> 2.0) | 1.45                   | (> 2.0) |
| Healthy          | 0.16                    | (0.21)  | 0.15  | (0.18)  | 0.16                   | (0.20)  |

*C. quinoa* extracts: 1 g leaves/5 ml PBS 0.01 M, 1 % Tween 20, 1 % BSA.

Grapevine extracts: No. 1: 1 g leaves/5 ml Tris-HCl 0.5 M, 0.8 % NaCl, 2 % PVP, 0.05 % Tween 20.

No. 2: 1 g leaves/5 ml PBS 0.5 M, 2 % PVP, 0.05 % Tween 20.

ELISA method no. 4 (Table 1).

buffer was first incubated for one night at 4 °C or 16 °C (Table 6) and then placed in the ELISA plate for 3 h at 35 °C.

In the conditions tested here, the method using sequential incubations was always much more sensitive than the method using simultaneous incubation. If necessary, the sensitivity of the method using simultaneous incubation could be improved by increasing the length of the substrate incubation time, or by increasing the concentrations of reagents.

### Discussion

Monoclonal antibodies have been found very useful for discriminating between closely related virus strains and for studying antigen structure (VAN REGENMORTEL 1984; ALTSCHUH *et al.* 1985). The use of GFV specific MCA for these purposes will be discussed elsewhere (HUSS, MULLER, SOMMERMEYER, WALTER, VAN REGENMORTEL, in preparation).

There are few reports concerning the use of MCA for large-scale virus detection. The fact that the MCA used in this work recognized all the GFV isolates tested until now indicates that they are likely to be useful for routine detection of the virus in grapevine extracts. The best ELISA format was to trap the virus with chicken anti-virus globulins and to reveal it with MCA and a goat anti-mouse phosphatase conjugate.

The use of serological techniques for virus detection in tissues of phenol-rich plants, such as grapevine, was previously possible only by adding nicotine or caffeine in the extraction media. We demonstrated that GFV could be detected even in grapevine leaves in PVP containing buffers, without nicotine.

The possibility of detecting GFV in various grapevine organs is also of considerable importance. Since the virus was readily detected in wood shavings, it was possible to detect virus in grapevine plants during the whole year. Another advantage is the possibility of a quick and reliable control of commercial grapevine wood (for instance, when crossing borders) without the need of forcing cuttings to obtain leaves. Experiments are under way in order to determine if virus detection is still reliable after grapevine wood has been stored for long periods of time.

### Summary

Monoclonal antibodies prepared with grapevine fanleaf virus (GFV) are useful for detecting the virus in plant extracts. In this paper we describe comparisons between different ELISA techniques using rabbit and chicken immunoglobulins as well as monoclonal antibodies (MCA). The technique using chicken immunoglobulins for coating the plates followed by MCA and goat anti-mouse phosphatase conjugate was the best one for detecting GFV in plant sap. In this technique, ascitic fluids containing MCA could be diluted up to  $10^{-6}$ .

Our experiments clearly demonstrate that the detection of GFV is possible in grapevine not only from leaves or rootlets, but also from wood shavings, without grinding them. We replaced the classical nicotine containing extraction medium by a harmless phosphate or Tris-HCl buffer. To detect GFV with these media it is essential that the buffer should contain polyvinylpyrrolidone and that its molarity should not be less than 0.1 M.

### References

- ALTSCHUH, D.; AL MOUDALLAL, Z.; BRIAND, J. P.; VAN REGENMORTEL, M. H. V.; 1985: Immunochemical studies of tobacco mosaic virus. VI. Attempts to localize viral epitopes with monoclonal antibodies. *Molec. Immunol.* **22**, 329—337.
- AVRAMEAS, S.; 1969: Coupling of enzymes to proteins with glutaraldehyde. Use of the conjugates for the detection of antigens and antibodies. *Immunochemistry* **6**, 43—52.
- BLAICH, R.; WIND, R.; 1982: Anreicherungsverfahren für den serologischen Nachweis von Virus in Rebblättern. *Vitis* **21**, 40—42.
- BOVEY, R.; BRUGGER, J. J.; GUGERLI, P.; 1980: Detection of fanleaf virus in grapevine tissue extracts by enzyme-linked immunosorbent assay (ELISA) and immune electron microscopy (IEM). *Proc. 7th Meeting ICVG, Niagara Falls*, 259—275.
- CLARK, M. F.; ADAMS, A. N.; 1977: Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.* **34**, 475—483.
- HARDIE, G.; VAN REGENMORTEL, M. H. V.; 1977: Isolation of specific antibody under conditions of low ionic strength. *J. Immunol. Methods* **15**, 305—314.
- LEHOCZKY, J.; KÖLBER, M.; FARKAS, G.; 1983: Effect of spring and summer high temperature on detection of grapevine fanleaf virus and yellow mosaic virus with ELISA. *Kertgazdasag* **15**, 37—45.
- POLSON, A.; VON WECHMAR, M. B.; VAN REGENMORTEL, M. H. V.; 1980: Isolation of viral IgY antibodies from yolks of immunized hens. *Immunol. Commun.* **9**, 475—493.
- STELLMACH, G.; BERRES, R.; 1985: Investigations on mixed infections of nepoviruses in *Vitis* spp. and *Chenopodium quinoa* WILLD. by means of ELISA. *Phytopathol. Mediter.* **24**, 125—128.
- VAN REGENMORTEL, M. H. V.; 1984: Monoclonal antibodies in plant virology. *Microbiol. Sci.* **1**, 74—78.
- VUITTENEZ, A.; KUSZALA, J.; 1972: Expérimentation d'améliorations récentes apportées aux techniques de détection des virus chez la vigne, par des procédés physiques et sérologiques. *Ann. Phytopathol. (N° H-S)*, 155—163.
- WALTER, B.; ETIENNE, L.; CLOQUEMIN, G.; 1985: Détection des virus du court-noué dans des bois de vigne. *Progr. Agric. Vitic.* **102**, 393—396.

- — ; KUSZALA, J.; VUITTENEZ, A.; 1979: Diagnostic sérologique par les tests PALLAS et ELISA. Application aux virus de la rhizomanie de la betterave et du court-noué de la vigne. *Ann. Phytopathol.* **11**, 568—569.
- — ; VUITTENEZ, A.; KUSZALA, J.; STOCKY, G.; BURCKARD, J.; VAN REGENMORTEL, M. H. V.; 1984: Détection sérologique des virus du court-noué de la vigne par le test ELISA. *Agronomie* **4**, 527—534.

*Eingegangen am 17. 3. 1986*

BRIGITTE HUSS  
B. WALTER  
L. ETIENNE  
INRA  
28 rue de Herrlisheim  
68021 Colmar  
France

M. H. V. VAN REGENMORTEL  
IBMC  
15 rue Descartes  
67084 Strasbourg  
France