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Comparison of rapid detection assays for grapevine leafroll disease associated closteroviruses

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Vergleich verschiedener Testverfahren zum schnellen Nachweis Grapevine-leafroll-assoziierter Closteroviren

Zusammenfassung: Drei schnelle Nachweisverfahren für Grapevine-leafroll-assoziierte Closteroviren (ELISA, dsRNA-Analyse und ISEM) wurden im Hinblick auf ihre Empfindlichkeit, Spezifität und Einfachheit der Anwendung verglichen. Jede Methode hat ihre Vor- und Nachteile für den Einsatz in Routinetests. ELISA ist empfindlich und einfach durchzuführen, für den Nachweis verschiedener GLRaV-Typen sind jedoch verschiedene Antiseren erforderlich. Da gute Testergebnisse auch bei Vermischen der Antiseren erzielt werden, sind Tests mit einzelnen Antiseren nur notwendig, wenn der in einer Probe vorhandene GLRaV-Typ bestimmt werden soll. Durch dsRNA-Analyse war zwar der Nachweis aller getesteten GLRaV-Typen möglich, die Empfindlichkeit dieses Verfahrens ist jedoch relativ niedrig und der Test arbeitsaufwendig. Er ist daher für die Prüfung umfangreichen Probenmaterials ungeeignet. ISEM ist sowohl empfindlich als auch schnell, jedoch ist wie bei ELISA ein spezielles Antiserum für jeden GLRaV-Typ erforderlich. Weiterhin muß ein Elektronenmikroskop verfügbar sein. Wir empfehlen ELISA mit mehreren kombinierten Antiseren für Testprogramme im großen Maßstab. Proben, bei denen ELISA keine klaren Ergebnisse liefert, sollten mit ISEM und/oder dsRNA-Analyse überprüft werden. Zur eindeutigen Bestimmung des Infektionszustandes einer Einzelprobe sollten wenige Gramm zur partiellen Reinigung des Virus verarbeitet und dieses Präparat sodann für ELISA und elektronenmikroskopische Untersuchung (negative Kontrastierung) herangezogen werden. Ebenso sollte eine dsRNA-Analyse durchgeführt werden.

Key words: virosis, leaf roll, closterovirus, detection, ELISA, dsRNA analysis, ISEM.

Introduction

Grapevine leafroll (GLR) disease is one of the most important diseases of grapes worldwide (GOHEEN 1988). Various types of virus particles have been associated with the GLR disease, including isometric virus-like (CASTELLANO *et al.* 1983), potyvirus-like (TANNE *et al.* 1977, 1989), and closterovirus-like particles (e.g. NAMBA *et al.* 1979; CONTI *et al.* 1980). In recent years, GLR associated closteroviruses (GLRaV, about 1,800—2,200 nm long) have been consistently associated with the GLR disease (GUGERLI *et al.* 1984; ZEE *et al.* 1987; ZIMMERMANN *et al.* 1988; BOSCIA *et al.* 1990; HU *et al.* 1990 b). A number of these closteroviruses have been purified from GLR-affected grapevines. Some of them have been partially characterized for their coat protein molecular weight, double stranded RNA (dsRNA) patterns, and particle morphology (GUGERLI *et al.* 1984; BOSCIA *et al.* 1990; HU *et al.* 1990 b). Serologically distinct types of GLRaV exist, and are referred to as I, II, III, and IV (ROSCIGLIONE *et al.* 1986; HU *et al.* 1990 b).

Since the GLR disease is primarily spread through infected propagation materials, the establishment of vineyards with virus-free vines is the primary control measure (GOHEEN 1988). The current GLR indexing bioassay with grapevine indicators is reliable, but it is time consuming (minimum of 18 months), expensive, and unsuitable for detection of GLR in large numbers of samples (GOHEEN 1988). Thus, other rapid virus detection assays, such as enzyme-linked immunosorbent assay (ELISA), dsRNA anal-

ysis, and immunosorbent electron microscopy (ISEM), were evaluated for their potential to complement or eventually replace the bioassay. Although ELISA is an ideal assay for detection of many plant viruses, the existence of serologically distinct types of GLRaV necessitates the use of several different antisera (Hu *et al.* 1990 b). In this study, several approaches were taken to improve ELISA to detect GLRaV and to compare ELISA with dsRNA analysis and ISEM. Advantages and disadvantages of each assay are discussed and a recommendation given for detection of GLRaV.

Materials and methods

The isolates of GLRaV used in this study were those from GLR-diseased grapevines from New York (designated NY-1, cv. Pinot noir), Arkansas (AK-1, cv. Chardonnay), China (China-1, cv. Bei-Mei), California (cvs Italia, Marsanne, Teroldego, Thompson Seedless (T.S.) Enns, T.S. Midget, and T.S. 1A), and Italy (SS9, cv. LN 33). They have been classified into type I (AK-1), II (Marsanne, Teroldego, SS9), III (NY-1, AK-1, Italia), and IV (T.S. Enns, T.S. Midget, and T.S. 1A) (Boscia *et al.* 1990; Hu *et al.* 1990 b). Pure isolates of GLRaV type I were not available.

Standard double antibody sandwich (DAS) direct ELISA was carried out as described previously (Clark and Adams 1977). Antibodies against GLRaV type II, III, and IV were produced at Geneva, New York (Zee *et al.* 1987; Boscia *et al.* 1990; Hu *et al.* 1990 b) and used in the ELISA procedure. ELISA plates were coated with immunoglobulins at 2 μg/ml for all three kinds of antibodies at 30 °C for 5 h. Virus preparations were made by grinding grape petiole tissue in ELISA extraction buffer at 1:10 and centrifuging for a few seconds in an Eppendorf microcentrifuge at top speed. Supernatants of the preparations were incubated in ELISA plates at 4 °C overnight. Dilution of antibody-phosphatase conjugate for type II AB was 1:1000, for type III was 1:2000, for type IV was 1:750. The conjugates were incubated at 30 °C for 5 h. Finally, p-nitrophenyl phosphate substrate was added into the plates. In simple indirect ELISA, plates were coated with crude tissue extracts in pH 9.6 carbonate buffer at 4 °C overnight (Lommel *et al.* 1982). The conjugate and substrate steps were the same as those in DAS direct ELISA. In biotin-avidin ELISA, labeling of antibody with biotin was as previously described (Hu and Rochow 1988). Plates were coated with immunoglobulins at 30 °C for 5 h; virus preparations were added and incubated at 4 °C overnight. Then, biotin-labeled anti-GLRaV antibody was added to wells (1:2000) and incubated at 37 °C for 3 h. Avidin-phosphatase conjugate (Sigma, St. Louis, MO, USA) was used at 1:2000 to react with biotin for 3 h at 37 °C. Finally, the ELISA plates were incubated with p-nitrophenyl phosphate substrate at room temperature. Absorbance at 405 nm was measured with a Dynatech MR 580 reader (Dynatech Laboratories Inc., Alexandria, VA, USA) about 45 min after the addition of substrate. Controls, which consisted of virus extraction buffer, healthy and known infected samples, were included in all ELISA tests. Absorbance readings were recorded as the mean value of two wells. Maximum recorded absorbance was 1.5. A reaction was considered positive only if the absorbance was at least 0.100. This threshold was at least 4 times higher than the twice-background range of healthy controls.

DsRNA isolation was accomplished with a standard phenol procedure (Hu *et al.* 1990 b) or a non-phenol procedure using sodium perchlorate (Rezaian and Krake 1987). Silver staining, previously used for protein gels (Hu *et al.* 1990 b), was compared with ethidium bromide staining for its sensitivity in dsRNA gel analysis (Hu *et al.* 1990 b). ISEM and negative staining were conducted as described previously (Hu *et al.* 1990 b).

Results and discussion

DAS direct ELISA has been used widely for detection of GLRaV. However, in DAS direct ELISA, the anti-NY-1 polyclonal antiserum reacted with type III isolates, but not with isolates in other types (ZEE *et al.* 1987). There were two possibilities which might explain this specific, narrow reaction. First, the conjugation of immunoglobulins with alkaline phosphatase decreases the binding ability of antibodies (avidity), which is a result of conformation change of binding sites of the immunoglobulins, so that the conjugate only reacts with homologous antigens, but not with heterologous antigens (KOENIG and TORRANCE 1986). Second, the isolates belong to different types which are serologically distinct (ROCHOW and CARMICHAEL 1979). Although previous works (ROSCIGLIONE and GUGERLI 1986; BOSCIA *et al.* 1990; HU *et al.* 1990 b) on GLRaV indicated that the second possibility is one reason for the narrow specificity, the first possibility had not been tested. Thus, we tested it by conjugating anti-NY-1 immunoglobulins with biotin. Since biotin is a very small molecule, the conjugation does not change the immunoglobulin structure (STAHLI *et al.* 1983). Immobilized virus on ELISA plates was reacted with immunoglobulin-biotin complex, which was then detected with avidin-

Table 1

Reaction of anti-NY-1 polyclonal antiserum to different GLRaV types in DAS direct and biotin/avidin ELISA assays

Reaktion von Anti-NY-1-polyklonalem Antiserum mit verschiedenen GLRaV-Typen in direkten DAS- und Biotin/Avidin-ELISA-Tests

Grape variety (= Isolates)	GLRaV type	Absorbance at 405 in ELISA	
		DAS-direct[1])	Biotin/avidin[2])
Marsanne	II	−0.002	0.048
Teroldego	II	−0.003	0.053
Bei-Mei	II	−0.005	0.057
SS9	II	−0.008	0.062
Chardonnay (AK-1)	I + III	1.010	0.879
Pinot noir (NY-1)	III	1.030	0.913
Italia	III	1.230	0.891
T.S. Enns[3])	IV	−0.009	0.028
T.S. Midget	IV	−0.005	0.045
T.S. 1A	IV	−0.009	0.053
Healthy Pinot noir		−0.004	0.048
Buffer		−0.009	0.072

[1]) In double antibody sandwich (DAS) direct ELISA tests, ELISA plates were coated with 2 µg/ml anti-NY-1 IgG at 30 °C for 5 h. Virus preparations were made by grinding 0.1 g petiole tissue in 1 ml ELISA extraction buffer; and incubated in ELISA plates at 4 °C overnight. The alkaline phosphatase-anti-NY-1 IgG conjugate was diluted at 1 : 2000, and incubated at 30 °C for 5 h. Absorbance readings were recorded as the mean value of two wells, and obtained 10 min following the addition of substrate.

[2]) In biotin/avidin ELISA, coating and antigen steps were the same as in DAS ELISA. Anti-NY-1 IgG was conjugated with biotin, which was used to react immobilized virus. Avidin-alkaline phosphatase conjugate was then used to detect the IgG-biotin complex. Samples from these two steps were incubated at 37 °C for 3 h. Absorbance readings were recorded as the mean value of two wells, and were obtained 15 min following the addition of substrate.

[3]) All three isolates of GLRaV type IV were from different selections of Thompson Seedless (T.S.).

enzyme conjugate. Results from this study showed that the anti-NY-1 antibodies still only reacted with type III isolates (Table 1). Therefore, the narrow specificity is likely due to serologically distinct GLRaV isolates.

Efforts were made to use simple indirect ELISA for detection of GLRaV. This type of ELISA is generally much less antigen specific than DAS-ELISA, partially because of the denaturation of the antigens (MARTIN and D'ARCY 1990). But in our simple indirect ELISA, polyclonal antibodies (ZEE *et al.* 1987) and monoclonal antibodies (HU *et al.* 1990 a) did not effectively react with homologous or heterologous viruses from crude tissue preparations (data not shown).

The narrow specificity thus necessitated the use of homologous antibodies to the different types of GLRaV. However, for general diagnosis for any type of GLRaV DAS direct ELISA could be simplified by using a blend of antibodies. We tested the possibility by using antibodies to different types of GLRaV which had different titers in ELISA tests. Our anti-NY-1 polyclonal and monoclonal antibodies to the NY-1 isolate (type III) gave excellent reaction in ELISA (ZEE *et al.* 1987; HU *et al.* 1990 a), while the polyclonal antibodies to type II gave good but weaker reaction than the anti-NY-1 antibodies (BOSCIA *et al.* 1990). Our anti-type IV antibodies gave much weaker reactions in ELISA, but could nevertheless detect the virus in crude tissue (HU *et al.* 1990 b). Results with greenhouse samples showed that this approach was promising (Table 2). All three serological types were detected with a blend of the antibodies. In this way, the cost and labor of screening samples can be reduced by 65 %.

DsRNA analysis has been used for detection of several plant viruses (MORRIS *et al.* 1983; VALVERDE *et al.* 1990). Previously, we characterized dsRNA patterns of types II and III of GLRaV (BOSCIA *et al.* 1990; HU *et al.* 1990 b). In this study, dsRNA pattern of

Table 2

Detection of GLRaV in ELISA with individual or blended antibodies to different GLRaV types

Nachweis von GLRaV durch ELISA mit einzelnen oder kombinierten Antikörpertypen gegen verschiedene GLRaV-Typen

Grape variety (= Isolates)	GLRaV types	Absorbance at 405 in ELISA[1])			
		II	III	IV	Blend
Marsanne	II	0.482	0.000	0.015	0.457
Teroldego	II	0.997	0.032	0.013	0.940
Bei-Mei	II	0.793	0.015	0.008	0.645
SS9	II	0.804	0.023	0.009	0.742
Pinot noir (NY-1)	III	0.024	1.500	0.009	1.500
T.S. Enns[2])	IV	0.047	0.025	0.325	0.428
T.S. Midget	IV	0.054	0.023	0.405	0.610
T.S. 1A	IV	0.016	0.022	0.525	0.524
Healthy Pinot noir		0.027	0.019	0.000	0.051
Buffer		0.018	0.024	0.005	0.019

[1]) ELISA plates were coated with antibody (2 µg/ml) specific to GLRaV type II, III, IV, or a blend of three. Crude tissue extracts were diluted 1 : 20. Conjugates were used individually at the following dilutions; anti-II 1 : 1000, anti-III 1 : 2000, anti-IV 1 : 700. When conjugates were blended the following dilutions were used; anti-II 1 : 2000, anti-III 1 : 4000, anti-IV 1 : 700. Absorbance readings were means of two wells, 45 min after substrate was added. Maximum recorded absorbance was 1.500.

[2]) All three isolates of GLRaV type IV were from different selections of Thompson Seedless (T.S.).

type IV was compared with type III. The high molecular weight dsRNA band was similar to that of type III (ca. 10 × 10^6 Mr), whereas the lower molecular weight bands varied (Fig. 1). In 1985, MOSSOP *et al.* reported the presence of a high molecular weight dsRNA in GLR-affected grapevines. More recently, MONETTE *et al.* (1989) have isolated dsRNA, ranging from 0.24 × 10^6 to 15 × 10^6 Da, from GLR-affected grapevines.

However, dsRNA analysis for GLRaV is not as sensitive as ELISA (at least 20 g tissue/sample are required compared to 0.1 g of tissue for ELISA) and it is more time consuming (at least 2 d, <8 samples/d/person). Thus, dsRNA analysis is unsuitable for large scale indexing, though it could be useful for sampling imported materials since they are usually limited in number. Furthermore, it is more difficult to isolate sufficient dsRNA for analysis from isolates of type II and IV, than from isolates of type III. Preliminary examinations of seasonal variability in dsRNA concentrations in leafroll infected grapes showed differences. This was also observed by DODDS *et al.* (1987) in a study with citrus tristeza virus. A non-phenol dsRNA extraction procedure was tried with sodium perchlorate (REZAIAN and KRAKE 1987). DsRNA bands were detected; but the sensitivity of this procedure was not better than that of the phenol procedure (data not shown). Since 8.5 % of insoluble PVP is used in this procedure, it is not simpler than that of the phenol procedure. Silver staining was compared with ethidium bromide staining, the sensitivity was similar for both staining methods (data not shown).

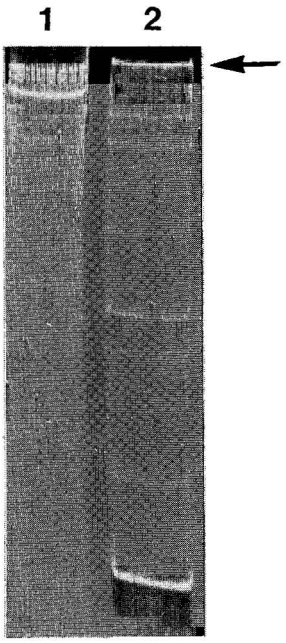

Fig. 1: Comparison of dsRNA patterns of GLRaV type IV with type III. About 10 g leaf petiole tissue was used for dsRNA extraction. The dsRNAs purified from grapevine leafroll diseased grapevines were analyzed in 6 % polyacrylamide gel electrophoresis. Lane 1: dsRNAs from the CA-4 isolate (type IV). Lane 2: dsRNAs from the NY-1 isolate (type III). Arrow indicates the high molecular weight dsRNA band.

Abb. 1: Vergleich der dsRNA-Muster von GLRaV Typ IV und Typ III. Etwa 10 g Blattstielgewebe erkrankter Reben wurden für die Extraktion der dsRNA verwandt. Die Analyse erfolgte durch Gelelektrophorese (6 % Polyacrylamidgel). Bahn 1: dsRNAs des CA-4-Isolats (Typ IV). Bahn 2: dsRNAs des NY-1-Isolats (Typ III). Der Pfeil kennzeichnet die hochmolekulare dsRNA-Bande.

ISEM is another very rapid and sensitive assay for detecting plant viruses (Milne and Lesemann 1984). For example, results can be obtained in 30 min. Furthermore, decoration of virions with antibodies (Fig. 2) can provide definitive results with some GLRaV isolates that give weak ELISA reactions due to low virus content or low antibody titer. ISEM was reliable in singly detecting isolates of type II, III, and IV. When AS to type II, III, and IV were used as a blend in ISEM, we were able to detect isolates in the three types (Table 3). Thus, the time for ISEM analysis of GLRaV types can be reduced considerably, if the objective is to simply detect GLRaV particles. However, ISEM requires an electron microscope and antisera to different GLRaV types.

In addition to their use for detection of GLRaV infections, the three assays described in this paper can be used to study the properties of GLRaV. Previous work by Teliz *et al.* (1988) showed that ELISA reactions were stronger with mature leaves than with young leaves. It was possible that the young leaves have less virus; or perhaps there are greater amounts of materials that inhibit ELISA reactions in young leaves. Using ISEM assay, we observed that the young leaves contained much less virus (data not shown), indicating that the lower ELISA reactions were due to low virus titers rather than other factors. Results from ISEM also indicated that stem phloem and petiole tissues had more virus than leaf tissue.

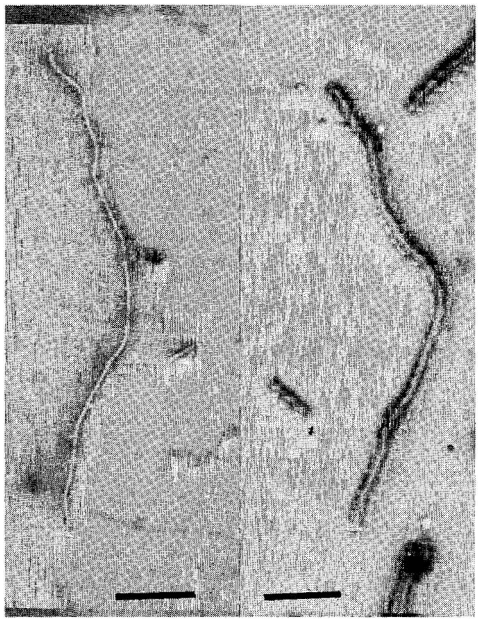

Fig. 2: Electron microscopy of GLRaV virus particles. A negatively stained virion (left) and an anti-GLRaV type IV antibody decorated virion of GLRaV type IV (the CA-4 isolate). Bar = 450 nm (left), or 380 nm (right).

Abb. 2: Elektronenmikroskopische Abbildungen der GLRaV-Partikel. Links ein negativ kontrastiertes Virion und rechts ein mit Anti-GLRaV-Typ IV-Antikörpern dekoriertes Virion des GLRaV-Typs IV (CA-4 Isolat). Der Maßstab entspricht 450 nm (links) bzw. 380 nm (rechts).

Each of the 3 assays (ELISA, dsRNA analysis and ISEM) have advantages and disadvantages in detection of GLRaV (Table 4), but they can be used to complement each other. ELISA is a very sensitive assay capable for processing large numbers of samples with minimal facilities, but at the present time, different antisera are needed for detec-

Table 3

ISEM detection of GLRaV using individual or blended antibodies to different GLRaV types

Nachweis von GLRaV durch ISEM mit einzelnen oder kombinierten Antikörpertypen gegen verschiedene GLRaV-Typen

Grape variety (= Isolates)	GLRaV types	Number of Virions/10 squares[1])	
		Individual	Blend
Marsanne	II	21	16
SS9	II	19	16
Pinot noir (NY-1)	III	109	95
T.S. Enns[2])	IV	39	39
T.S. Midget	IV	64	17

[1]) Antisera to GLRaV type II, III, and IV were used individually (Individual) or together (Blend) for detection of GLRaV from crude extracts. Dilutions of the antisera in both cases were the same. Formvar-filmed 400 mesh grids were coated with antisera diluted 1 : 1000 for 10 min. Virions were trapped onto the grids for 20 min, decorated with the antisera diluted 1 : 25 for 10 min, and stained with 2 % uranyl acetate for 1 min. The grids (10 squares/grid) were examined with a JEM-100SX electron microscope.

[2]) Both isolates of GLRaV type IV were from different selections of Thompson Seedless (T.S.).

tion of different GLRaV types. However, it is not necessary to use the antisera separately unless it is important to determine the type of GLRaV present. DsRNA analysis has the advantage of being a nonspecific assay, but it is not suitable for large scale testing and is not as sensitive as ELISA and ISEM. ISEM is a rapid and sensitive assay, but as mentioned it requires an electron microscope and different antisera. Considering the advantages and disadvantages, our current recommendation for detection of GLRaV is that ELISA should be done with multiple antisera to test many samples. Samples for which ELISA results are inconclusive should be retested with ISEM and/or dsRNA. A few grams of important samples should be processed to partially concentrate the virus prior to ELISA (Zee *et al.* 1987). This would maximize detection in tissue with low virus concentrations. The samples should also be examined with negative staining in electron microscopy to see if closterovirus-like particles are present. Finally, a dsRNA analysis should be conducted.

Since ELISA is the most widely-used assay for virus detection, we are characteriz-

Table 4

Advantages and disadvantages of the rapid detection assays for GLRaV

Vor- und Nachteile der Schnellverfahren zum Nachweis von GLRaV

Assays	Advantages	Disadvantages
ELISA	easy sensitive	need different antisera
dsRNA	nonspecific	not sensitive time consuming need skill
ISEM	very rapid sensitive	need different antisera need electron microscope need skill

ing different GLRaV types and producing antisera to them. We are also evaluating nucleic acid probes in molecular hybridization tests, in an effort to develop an assay capable of detecting a broad range of GLRaV isolates. The rapid detection assays are also being compared with the grapevine indicators for indexing GLR disease.

Summary

Three rapid detection assays (ELISA, dsRNA analysis and ISEM) were compared for their sensitivity, specificity, and simplicity in the detection of grapevine leafroll associated closteroviruses (GLRaV). Each was found to have advantages and disadvantages for routine testing. ELISA is sensitive and easy to use, but different antisera are needed to detect different GLRaV types. Because mixing or blending of antisera can produce good results in a single ELISA test, each antiserum does not need to be used separately unless it is important to determine the type of GLRaV present. DsRNA analysis can detect all the types of GLRaV tested but has a relatively low sensitivity and is labor intensive, which makes it unsuitable for testing large numbers of samples. Furthermore, dsRNA does not give unequivocal diagnosis of GLRaV infections. ISEM is sensitive and rapid. However, like ELISA, this technique requires an antiserum to each GLRaV type tested and an electron microscopy. Our recommendation is that ELISA should be used with multiple antisera for large scale testing programs. Samples for which ELISA results are inconclusive should be retested with ISEM and/or dsRNA. When the disease status of an individual sample must be determined conclusively, a few grams of tissue should be processed to concentrate the virus and then subjected to ELISA and examination by electron microscopy with negative staining. A dsRNA analysis should be carried out as well.

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Literature cited

Boscia, D.; Hu, J. S.; Gonsalves, D.; Golino, D.; 1990: Characterization of grapevine leafroll associated closterovirus (GLRaV) serotype II and comparison with GLRaV serotype III. [Abst.]. Phytopathology **80**, 117.

Castellano, M.; Martelli, G. P.; Savino, V.; 1983: Virus-like particles and ultrastructural modifications in the phloem of leafroll-affected grapevines. Vitis **22**, 23—39.

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.

Dodds, J. A.; Jarupat, T.; Lee, J. G.; Roistacher, C. N.; 1987: Effects of strain, host, time of harvest, and virus concentration on double-stranded RNA analysis of citrus tristeza virus. Phytopathology **77**, 42—47.

Goheen, A. C.; 1988: Leafroll. In: Pearson, R. C.; Goheen, A. (Eds.): Compendium of Grape Diseases, 52. APS Press, St. Paul.

Gugerli, P.; Brugger, J. J.; Bovey, R.; 1984: L'enroulement de la vigne: Mise en évidence de particules virales et développement d'une méthode immuno-enzymatique pour le diagnostic rapide. Rev. Suisse Viticult. Arboricult. Horticult. **16**, 299—304.

Hu, J. S.; Boscia, D.; Gonsalves, D.; 1990 a: Use of monoclonal antibodies to characterize grapevine leafroll associated closterovirues. Phytopathology **80**, 920—925.

— —; Gonsalves, D.; Teliz, D.; 1990 b: Characterization of closterovirus-like particles associated with grapevine leafroll disease. J. Phytopathol. **128**, 1—14.

— — ; ROCHOW, W. F.; 1988: Anti-idiotypic antibodies against an anti-barley yellow dwarf virus monoclonal antibody. Phytopathology **78**, 1302—1307.

KOENIG, R.; TORRANCE, L.; 1986: Antigenic analysis of potato virus X by means of monoclonal antibodies. J. Gen. Virol. **67**, 2145—2151.

LOMMEL, S. A.; MCCAIN, A. H.; MORRIS, T. J.; 1982: Evaluation of indirect enzyme-linked immunosorbent assay for the detection of plant viruses. Phytopathology **72**, 1018—1022.

MARTIN, R. R.; D'ARCY, J. D.; 1990: Relationships among luteoviruses based on nucleic acid hybridization and serological studies. Intervirology **31**, 23—30.

MILNE, R. G.; LESEMANN, D. E.; 1984: Immunosorbent electron microscopy in plant virus studies. Meth. Virol. **8**, 85—101.

MONETTE, P. L.; JAMES, D.; GODKIN, S. E.; 1989: Comparison of RNA extracts from *in vitro* shoot tip cultures of leafroll-affected and leafroll-free grapevine cultivars. Vitis **28**, 229—235.

MORRIS, T. J.; DODDS, J. A.; HILLMAN, B.; JORDAN, R.; LOMMEL, S. A.; STANLEY, J. T.; 1983: Viral specific dsRNA: diagnostic value for plant virus disease identification. Plant. Molec. Biol. Reptr. **1**, 27—30.

MOSSOP, D. W.; ELLIOT, D. R.; RICHARDS, K. D.; 1985: Association of closterovirus-like particles and high molecular weight double stranded RNA with grapevines affected by leafroll disease. N. Z. J. Agricult. Res. **28**, 419—425.

NAMBA, S.; YAMASHITA, S.; DOI, Y.; YORA, K.; YANO, R.; 1979: Grapevine leafroll virus, a possible member of closteroviruses. Ann. Phytopathol. Soc. Japan **45**, 497—502.

REZAIAN, M. A.; KRAKE, L. R.; 1987: Nucleic acid extraction and virus detection in grapevine. J. Virol. Meth. **17**, 277—285.

ROCHOW, W. F.; CARMICHAEL, L. E.; 1979: Specificity among barley yellow dwarf viruses in enzyme immunosorbent assays. Virology **95**, 415—420.

ROSCIGLIONE, B.; GUGERLI, P.; 1986: Maladies de l'enroulement et du bois strié de la vigne: analyse microscopique et sérologique. Rev. Suisse Viticult. Arboricult. Horticult. **18**, 207—211.

STAHLI, C.; MIGGIANO, V.; STOCJER, J.; STAEHELIN, T.; HARING, R.; TAKACA, B.; 1983: Distinction of epitopes by monoclonal antibodies. Meth. Enzymol. **92**, 242—253.

TANNE, E.; NAVEH, L.; SELA, I.; 1989: Serological and molecular evidence for the complexity of the leafroll disease of grapevine. Plant Pathol. **38**, 183—189.

— — ; SELA, I.; KLEIN, M.; HARPAZ, I.; 1977: Purification and characterization of a virus associated with the grapevine leafroll disease. Phytopathology **67**, 442—447.

TELIZ, D.; TANNE, E.; GONSALVES, D.; ZEE, F.; 1988: Field serological detection of viral antigens associated with grapevine leafroll disease. Plant Dis. **71**, 704—709.

VALVERDE, R. A.; NAMETH, S. T.; JORDAN, R. L.; 1990: Analysis of double-stranded RNA for plant virus diagnosis. Plant Dis. **74**, 255—258.

ZEE, F.; GONSALVES, D.; GOHEEN, A.; KIM, K. S.; POOL, R.; LEE, R. F.; 1987: Cytology of leafroll-diseased grapevines and the purification and serology of associated closteroviruslike particles. Phytopathology **77**, 1427—1434.

ZIMMERMANN, D.; WALTER, B.; LEGALL, O.; 1988: Purification de particules virales associées à l'enroulement de la vigne et mise au point d'un protocole ELISA permettant leur détection. Agronomie **8**, 731—741.

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