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Studies on "corky rugose wood" of grapevine and on the diagnosis of grapevine virus B

by

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Summary: Vines affected by corky rugose wood (CRW), a field syndrome characterized by pronounced cork production by the scion of several grapevine varieties just above the graft union, contain a number of filamentous and isometric phloem-limited viruses, such as grapevine leafroll-associated virus 1, 2, and 3 (GLRaV-1, GLRaV-2, GLRaV-3), grapevine virus A and B (GVA and GVB), and grapevine fleck virus (GFkV). However, the same viruses, with the exception of GVB, are widely represented also in vines with rugose wood without excessive corkyness. Although GVB was found in all vines indexing positive in LN 33 for corky bark disease, its occurrence in CRW-affected vines was not consistent enough to suggest that it may have a determining role in the induction of this syndrome. Monoclonal antibodies to GVB raised previously were characterized and their possible use for reliable detection of GVB in field-grown vines investigated in detail. A triple antibody sandwich ELISA protocol that under our experimental conditions afforded consistent and repeatable results, was based on the use of crude cortical scraping extracts from mature canes collected in autumn, antibodies from a polyclonal antiserum for plate coating (trapping) and a monoclonal antibody for antigen detection.

Key words: grapevine, rugose wood, corky rugose wood, phloem-limited viruses, closterovirus, trichovirus, diagnosis, serology, ELISA, monoclonal antibodies.

Introduction

Rugose wood of the grapevine is a disease complex in which four different disorders participate, i.e., corky bark (Hewitt *et al.* 1962), Rupestris stem pitting (Goheen 1988), Kober stem grooving (Savino *et al.* 1989) and LN33 stem grooving (Garau *et al.* 1989). Individual disorders can be distinguished on the basis of the differential reactions of *Vitis* indicators but not in the field, due to the absence of specific and consistent characterizing symptoms.

In general, rugose wood-affected vines are less vigorous than normal, show a more or less pronounced swelling at the bud union and a marked difference between the diameter of scion and rootstock. The woody cylinder of the scion, rootstock, or both, is typically marked (when symptoms are expressed) by pits and/or grooves that represent the major characterizing feature of the disease complex in nature, and can be seen upon removal of the cortex (reviewed by Martelli 1993). In most cases, the outward aspect of the trunk at the bud union is rather normal, except for the swelling, and does not necessarily betray the presence of the disorderly condition of the wood underneath (Fig. 1 A). However, there are cases in which the scion of diseased vines shows an atypical production of corky tissues just above the graft union, which gives rise to a condition called "corky rugose wood" (CRW) (Fig. 1 B). Because of the exceedingly thick and corky bark, vines affected by CRW are readily identified in the field, also at a distance.

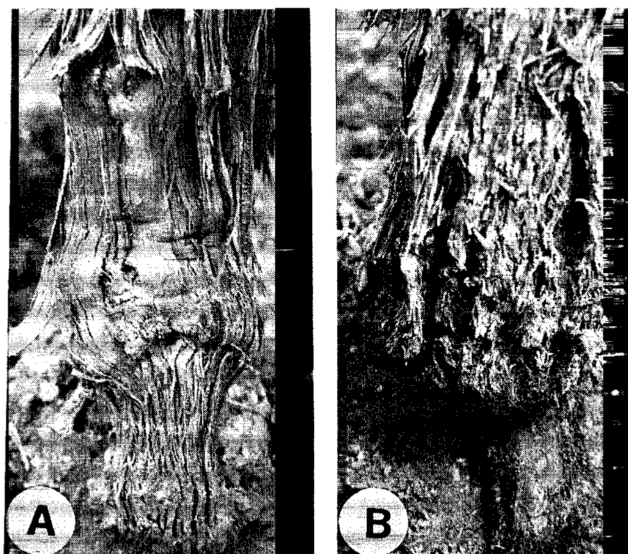

Fig. 1: Normal (A) and corky (B) cortex in two rugose wood-affected vines, both of which show a remarkable swelling at the graft union.

In Italy, CRW was observed in a wide number of varieties (Anonymous 1979) but is especially frequent in cv. Italia. Its presence in the vineyards is erratic, so that rugose wood affected-vines with normal and exceedingly corky bark often coexist in the same planting. This implies that the corky condition may not be due to varietal differences. However, whether the nature of CRW differs from the more

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frequent "classical" rugose wood with normal bark is unknown. In Israel, spectacular cases of CRW in cv. Thompson Seedless were reported to be closely associated with corky bark disease (CB) (TANNE *et al.* 1989), which contrasted with previous Italian findings that had failed to detect such an association in Italia vines (SAVINO *et al.* 1985 and unpublished information).

The recurrent observation of widespread CRW cases in young table grape stands in Apulia (Southern Italy), reproposed the unsolved issue of its nature, prompting specific investigations, the outcome of which is reported in the present paper. The results are also reported of trials for optimizing the detection of grapevine virus B, the putative agent of corky bark disease (BOSCIA *et al.* 1993; GREIF *et al.* 1995), thus possibly implicated in the aetiology of CRW.

Materials and methods

Field surveys and collection of samples: Surveys were conducted in the grape-growing district of the Ionian coastal plains of Apulia. Five vineyards aged 7 to 14 years were selected, two of cv. Italia and one each of cvs Primus, Michele Palieri and Regular seedless. In these vineyards a total of 231 vines with CRW were identified (112 of Italia, 50 each of Michele Palieri and Primus, 19 of Regular seedless). Petioles (ca. 20 g) were collected from basal leaves of each of these vines in September-October and mature canes in December 1993. Controls consisted of similar samples collected from 10 vines of each variety with normal bark. Petioles were stored in a freezer at -70 °C and canes in a cold room at 4 °C until used. Observations of the presence of wood pitting were done at the time of sample collection and were repeated in June 94 by lifting a strip of cortex across the graft union.

Mechanical transmission to herbaceous hosts: All tests were carried out in a climatized glasshouse at 24-26 °C, using *Nicotiana occidentalis*, *N. cavicola*, *N. benthamiana* and *Chenopodium quinoa* as test plants by rubbing the inoculum on their celite-dusted leaves. Transmissions were attempted from two types of material:

(a) Leaf petioles. The inoculum was prepared by grinding petioles in a chilled mortar in the presence of 5 volumes of phosphate buffer 0.01 M containing 3 % nicotine and 0.01 M cysteine HCl.

(b) *In vitro* cultures. These were prepared essentially as described by MONETTE *et al.* (1989). The explants were grown in a climatized artificially lighted cabinet (16 h photoperiod) at 25 °C, in a modified MS proliferation medium containing 0.6 ppm benzylaminopurine and 0.01 ppm naphthaleneacetic acid. The inoculum consisted of explant tissues ground in a chilled mortar in the presence of the same buffer as above.

Graft-transmission to LN33 indicators: Material from each of the selected vines was used as inoculum for graft transmission tests to LN 33, the indicator specific for corky bark disease. For each donor vine a total of twelve LN 33 rooted cuttings were chip-budded, whip-grafted, and green-grafted in groups of four. Graft-inoculated plants were grown in a greenhouse for three months before transferring to a shadehouse, then outdoors. A corky bark-infected vine was used as donor for positive controls.

Serology: All selected vines were tested with ELISA for the presence of the following phloem-limited viruses: grapevine leafroll-associated virus 1 (GLRaV-1), grapevine leafroll-associated virus 3 (GLRaV-3), grapevine corky bark-associated virus (NAMBA *et al.* 1991), which was recently found to be the same as grapevine leafroll-associated virus 2 (GLRaV-2) (BOSCIA *et al.* 1995 b), grapevine virus A (GVA), grapevine virus B (GVB), and grapevine fleck virus (GFkV). The reagents were monoclonal antibodies (MAbs) to GVA (BOSCIA *et al.* 1992), GVB (BOSCIA *et al.* 1994 a) and GFkV (BOSCIA *et al.* 1995 a) and polyclonal antisera to all the above viruses produced locally at different times.

Tests were done for identifying the most suitable sources of antigen for efficient and consistent ELISA responses. To this aim, crude extracts of cortical scrapings from mature canes and micropurified extracts from petioles and cortical scrapings (GUGERLI *et al.* 1984) were compared.

The characterization of eight recently produced MAbs to GBV isolate SS-D (BOSCIA *et al.* 1994 a) was completed by determining: (i) MAb isotype using "isotyping strips" (Sigma Immuno Type Kit ISO-1); (ii) the specificity of individual MAbs to identify known isolates of GVB (BOSCIA *et al.* 1994 a); (iii) ability to detect GVB in extracts of different *Vitis* species and hybrids. For these tests six grapevine accessions known to be infected by GVB were used, four of which belonged to *Vitis vinifera* (MN18M1, MN18M2, BA14, SS6I2), and one each to *Vitis rupestris* (E3) and the hybrid *Vitis berlandieri* x *Vitis riparia* 157.11 (MT47). Controls consisted of healthy LN 33 and *V. rupestris*, as well as healthy and GVB-infected *N. benthamiana* plants.

Specific tests were also made to optimize ELISA conditions for the detection of GVB in different plant organs and types of tissue extracts in view of a possible large-scale use of the MAbs. Two sets of trials were made to evaluate: (i) GVB antigen concentration in different plant organs, i.e. cortical scrapings from mature canes collected in autumn, green canes collected in late spring, and green shoots collected in early spring, young leaves, mature leaves, petioles collected in spring and used immediatetely, petioles collected in autumn and used after storage in the cold; (ii) the suitability of micropurified extracts, obtained with three different procedures (Tab. 1), versus non-concentrated extracts of cortical scrapings.

Standard DAS-ELISA procedure (CLARK and ADAMS 1977) was used for the detection of GLRaV-1, GLRaV-2 and GLRaV-3, whereas TAS-ELISA (i.e. antigen trapping with polyclonal IgGs and revealing with a monoclonal antibody followed by an enzyme-labelled antimouse antibody) was adopted for GVA, GVB and GFkV. ELISA plates were pre-coated with Protein A (0.5 µg/ml) as described (BOSCIA *et al.* 1992) for GVA detection.

Immunoelectron microscopy (IEM) (MILNE 1993) was used for all grapevine samples that gave erratic or doubtful ELISA responses. IEM was also used for confirming the identification of viruses isolated by mechanical transmis-

sion and for checking the particle decorating ability of MAbs. Western blots were done as described by Hu *et al.* (1990).

	Micropurification according to GUGERLI *et al.*, 1984	Modified micropurification 1	Modified micropurification 2
EXTRACTION	0.5M Tris-HCl pH 8.2 0.01M Mg SO4 4% PVPP 0.5% bentonite 0.2% 2-mercaptoetoh 5% TRITON X-100	0.01M K2HPO4 0.01M cysteine-HCl 3% nicotine 2% PVPP	PBS-Tween buffer plus 20g PVPP
CLARIFICATION	Cheesecloth filtration low-speed centri-fugation at 6,000 g for 20 min	Addition of 2% bentonite and low -speed centri-fugation at 6,000 g for 20 min.	Low-speed centri-fugation at 6,000 g for 20 min
CONCENTRATION	High-speed centri-fugation at 80,000 g for 2h	High-speed centri-fugation at 180,000 g for 45 min	High-speed centri-fugation at 180,000 g for 45 min
RESUSPENSION	0.1M Tris-HCl pH 7.6 plus 0.01M MgSO4	0.1M Tris-HCl pH 7.6 plus 0.01M MgSO4	PBST buffer

Table 1

Micropurification procedures for the extraction of GVB from grapevine tissues

Results

Field observations: Regardless of the cultivar, vines with very severe CRW symptoms were stunted, which was the second most obvious trait, besides excessive corkyness, differentiating CRW-affected plants from those with a normal bark. The incidence of the disorder in the five surveyed vineyards was 2 % in Regular seedless, the least affected, 5.8 % in Michele Palieri, 8.2 % in Primus, and 8.3 % in Italia. Diseased vines were distributed at random in the field never giving rise to distinct foci. Less than 30 % of the vines with a normal bark showed wood pitting. However, all vines with CRW exhibited a variously intense pitting and/or grooving of the stem, thus confirming that the abnormal corky condition of the trunk is strictly connected with xylem disturbances typical of the rugose wood complex.

Mechanical transmission: Transmissions were made from petioles of all 271 samples collected (231 from CRW-affected vines and 40 from control vines with a smoother trunk) but no virus could be isolated. However, a virus was recovered from eight of sixty *in vitro*-grown explants from CRW-affected vines, 20 of which proved to be GVB-positive in ELISA. This virus induced chlorotic-necrotic local lesions in *N. occidentalis* 10-11 d after inoculation, followed by systemic vein clearing and mild deformation of the leaves. As ascertained by ELISA and IEM, seven of the virus isolates proved to be GVB strains serologically indistinguishable from one another, whereas one of the isolates was not recognized, nor its particles decorated, by antisera to GVA, GVB and grapevine virus C (GVC) (Monette and James 1991). This virus, which is still under study, had filamentous particles with the same outward appearance and length (ca. 800 nm) of those of GVA and GVB but a different dsRNA profile (not shown). Despite repeated attempts, no virus was isolated from explants of 10 different control vines.

Graft transmission: In these experiments, which encompassed the making of 3,275 grafts, an average of ca. 55 % take was obtained. Thus, all 271 accessions were successfully grafted to a varying number of LN 33 indicators with one or more of the techniques used (chip-budding, whip-, and green-grafting). Typical corky bark symptoms, i.e. swelling and cracking of the basal internodes of green shoots of LN 33 (Martelli 1993), began to show about three months after grafting (mid-June 1994) both in the indicators grafted with material from positive controls (a vine known to be infected by corky bark disease) and in some of the vines under testing. The last reading made in August 1995 disclosed that 37 out of the 231 (16 %) grapevine accessions had CRW but none of the 40 vines with normal bark (controls) gave clear-cut corky bark reactions.

Characterization and performance in ELISA of monoclonal antibodies to GVB: As shown in Tab. 2, the 8 stabilized hybridoma lines obtained in previous studies (Boscia *et al.* 1994 a), secreted MAbs of three different isotypes (IgG2a, IgG2b, and IgM). Two of these MAbs (MAb1 and MAb2), both belonging to isotype IgG2a, did not decorate virus particles but were able to recognize dissociated virus coat protein subunits in Western blot assays (Fig. 2) and gave consistent ELISA responses, but only with extracts from cortical scrapings of *V. vinifera* canes (Tab. 3). This behaviour is consistent with the likelihood that both MAbs were originated by cryptotopes that became available upon disassembling of the viral coat protein.

MAb3 to MAb8 decorated virus particles (Tab. 2) but did not react with dissociated coat protein preparations in Western blots (Fig. 2) suggesting their origin from conformational determinants or neotopes (van Regenmortel 1966) present, and therefore recognizable, only on intact virus

Table 2

Properties of eight monoclonal antibodies to GVB

Monoclonal antibody	Hybridoma line	Isotype	Decoration in IEM	Reaction in Western blot
MAB1 (MAbGB1E2G)	GB1E2G	IgG2a	-	+
MAB2 (MAbGB5G11B)	GB5G11B	IgG2a	-	+
MAB3 (MAbGB3H10C)	GB3H10C	IgG2a	+	-
MAB4 (MAbGB6G11D)	GB6G11D	IgG2b	+	-
MAB5 (MAbGB2H9D)	GB2H9D	IgG2b	+	-
MAB6 (MAbGB6E5E)	GB6E5E	IgG2b	+	-
MAB7 (MAbGB7C6E)	GB7C6E	IgM	+	-
MAB8 (MAbGB9C2E)	GB9C2E	IgM	+	-

+ = positive reaction; - = negative reaction.

particles. These MAbs did not react with extracts from infected vines, regardless of the species, but gave strong responses with leaf extracts from GVB-SS-D- infected *Nicotiana benthamiana* plants (Tab. 3), possibly as a consequence of the low viral antigen concentration in grapevine tissues at the time of testing.

Specificity of individual MAbs: The supernatants of individual hybridoma cell cultures were used as a second (revealing) antibody in TAS-ELISA tests made with extracts of *N. benthamiana* leaves infected with six GVB isolates (GVB-SS-A, GVB-SS-B, GVB-SS-D, GVB-Se,

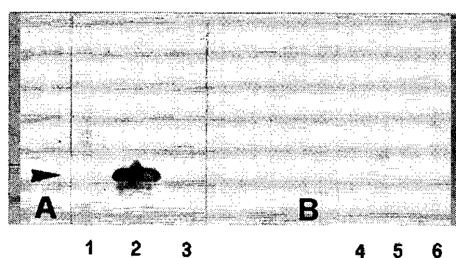

Fig. 2: Western blots of dissociated virus coat protein (CP) preparations exposed to GVB MAb2 and MAb5. MAb2 has clearly recognized the CP preparation of GVB (panel A, lane 2, arrow head) but not that of GVA (panel A, lane 3 and panel B, lane 4), whereas MAb5 did not react with CP preparations of either GVB (panel B, lane 5) or GVA (panel B, lane 4). Pre-stained markers in lanes 1 and 6.

Table 3

GVB recognition by MAbs in extracts from different *Vitis* species and in a *Nicotiana* control

Source	MAb1	MAb2	MAb3	MAb4	MAb5	MAB6	MAb7	MAb8
MN18M1 (*V. vinifera*)	+	+	-	-	-	-	-	-
MN18M2 (*V. vinfera*)	+	+	-	-	-	-	-	-
BA14 (*V. vinifera*)	+	+	-	-	-	-	-	-
SS6I2 (*V. vinifera*)	+	+	-	-	-	-	-	-
E3 (*V. rupestris*)	-	-	-	-	-	-	-	-
MT47 (hybrid)	-	-	-	-	-	-	-	-
LN 33 (control)	-	-	-	-	-	-	-	-
V. rupestris (control)	-	-	-	-	-	-	-	-
N. benthamiana (healthy)	-	-	-	-	-	-	-	-
N. benthamiana (infected)	+	+	+	+	+	+	+	+

+ = positive reaction; - = negative reaction.

GV-LRNOV, and GVB-BA) previously characterized (BOSCIA *et al.* 1994 b), and with GVA isolate PA3, GVC and GLRaV-2. Healthy *N. benthamiana* was used as control. These tests were done twice and their results, expressed as mean ratio of the absorbance values of infected samples versus those of the controls, are reported in Tab. 4. This table does not contain the absorbance values of the infected/healthy ratio determined for GVA, GVC and GLRaV-2 because the values did not differ from those of the control. Given a discriminating threshold for infected samples equal to twice the value of the healthy control made equal to 1, it is clear that MAb4 did not recognize GVB-BA (I/H ratio = 1.5) but it reacted with all other GVB isolates. All other

Table 4

Specificity of GVB MAbs for different GVB isolates

Virus isolates	MAb1	MAb2	MAb3	MAb4	MAb5	MAb6	MAb7	MAb8
GVB-SS-A	6.4	6.6	4.6	7.0	3.2	5.2	3.0	4.0
GVB-SS-B	>10	8.8	>10	9.0	>10	>10	>10	>10
GVB-SS-D	>10	>10	>10	3.5	>10	>10	>10	5.6
GVB-Se	>10	8.6	>10	>10	>10	9.6	>10	4.4
GV-LRNOV	>10	>10	>10	>10	>10	>10	>10	>10
GVB-BA	>10	>10	8.3	1.5	>10	8.8	9.2	2.5

Figures are the mean ratio of absorbance values of infected samples/healthy control. Values below 2 identify negative reactions.

MAbs reacted with all GVB isolates but only MAb1, MAb2, and MAb6 gave a I/H ratio consistenly higher than 5.

Based on the combined results of performance and specificity tests, MAb1 (MAbGB1E2G) was selected for use in the experiments that followed.

Identification of the most suitable source of antigen for ELISA detection of GVB: As shown in Fig. 3, antigen distribution in grapevine tissues varied greatly with the plant's organ and the season. The highest ELISA response was obtained with extracts from mature canes collected in autumn, followed at some distance by cortical scrapings from lignified canes collected in late spring and by petioles from autumn leaves. The use of extracts from green tissues, regardless of whether they were shoots or leaves, always resulted in responses that could be discriminated from the controls but not as clearly as with mature aged tissues.

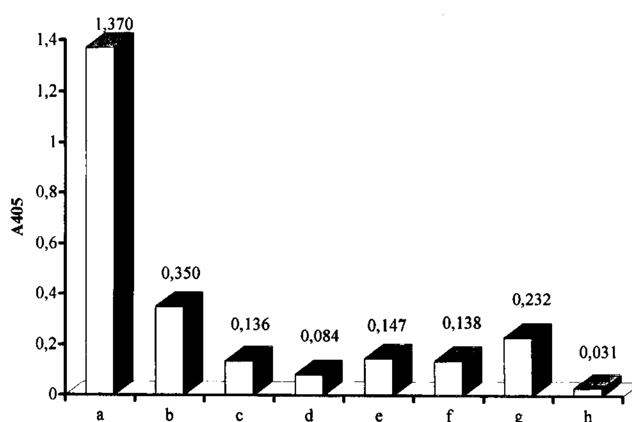

Fig. 3: Comparative concentration of GVB antigen as assessed by ELISA in extracts from different vine organs: a, cortical scrapings from mature canes collected in autumn; b, cortical scrapings from lignified canes collected in late spring; c, cortical scrapings from green shoots; d, aged leaves; e, young leaves; f, petioles collected in spring and tested immediately; g, petioles collected in autumn and used after storage in a freezer; h, healthy control (mean value of readings of extracts from tissues of different organs).

Influence of extraction methods on ELISA detection of phloem-limited grapevine viruses: In a first set of experiments in which extracts from cortical cane scrapings and leaf petioles micropurifed according to GUGERLI *et al.* (1984) were compared with non concentrated extracts from cortical cane scrapings, it was observed that for leafroll-associated closteroviruses (GLRaV-1, GLRaV-2, and GLRaV-3) concentrated extracts produced consistently higher absorbance values. The reverse was true for trichoviruses (GVA and GVB) and GFkV but the difference between the two extraction methods was much larger in the case of GVB (Fig. 4).

The extraction methods for ELISA detection of GVB were therefore investigated in more detail. The results (Fig. 5) proved that the four extraction methods tested were suitable for the detection of the virus in cortical scrapings from mature grapevine canes, as they yielded distinctly higher readings than the controls. However, it was confirmed that, contrary to observations with closteroviruses, non concentrated crude extracts gave the best responses (Fig. 5).

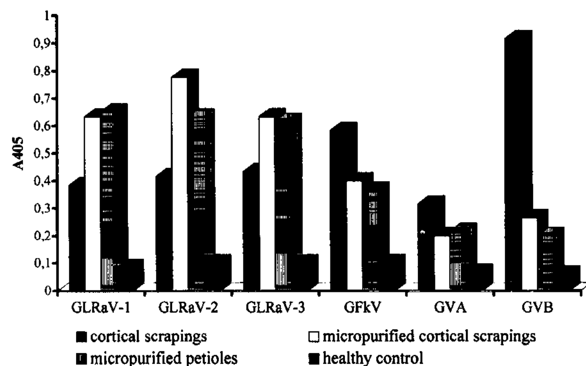

Fig. 4: Influence of extraction methods (micropurification versus crude extracts from cortical scrapings) on ELISA detection of six phloem-limited grapevine viruses.

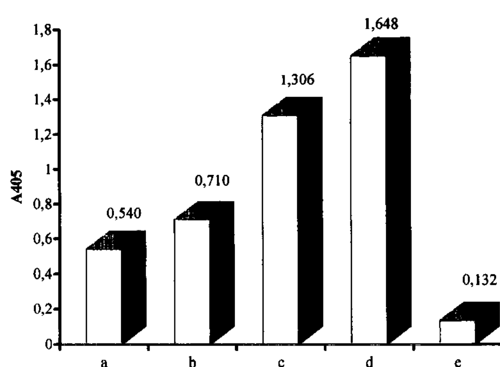

Fig. 5: ELISA response of extracts from GVB-infected vines obtained by the methods reported in Tab. 1: a, micropurification according to GUGERLI *et al.* (1984); b, modified micropurification 1; c, modified micropurification 2; d, non concentrated cortical scraping extracts; e, healthy control (mean value of readings of micropurified and non concentrated extracts from healthy vines).

There are several possible reasons accounting for these results: (i) much antigen is absorbed by bentonite and lost during low-speed centrifugation, as suggested by the fairly good performance of the procedure where the addition of bentonite was omitted (modified micropurification 2); (ii) GVB particles are fragile and fragment readily, so that they are lost with the supernatant discarded after high-speed centrifugation, a likelihood corroborated by previous findings (BOSCIA *et al.* 1993; LUPO *et al.* 1994); (iii) a substantial amount of antigen occurs in the cells as non assembled coat protein subunits, the form recognized by MAb1, which would be readily lost during sample manipulation.

Whatever the explantion, the point remains that non concentrated cortical scrapings extracts from canes collected in atumn seem to constitute the best material for ELISA detection of GVB.

Viruses associated with CRW: Phloem-limited viruses were found in almost all CRW-affected vines of Italia, Michele Palieri, and Primus, but in a lower proportion of CRW-affected Regular seedless, so that the overall infection rate was 95 %. The most common virus was GLRaV-3 (81 % infection) followed by fleck (70 %), GVA (56 %), GLRaV-2 (36 %), GLRaV-1 (35 %), and GVB (16 %) (Fig. 6). Mixed infections of three or more viruses prevailed by far (62%) over infections by one (8 %) or two (25 %) viruses (Fig. 7).

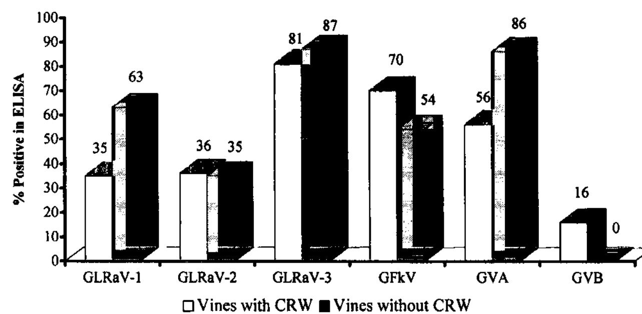

Fig. 6: Presence of different phloem-limited viruses, expressed as percentage values, in vines with and without CRW.

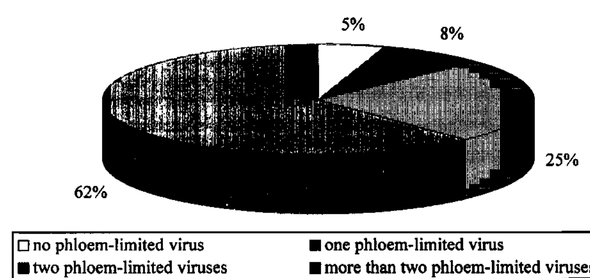

Fig. 7: Incidence of infections by phloem-limited viruses singly or in mixture in CRW-affeced vines.

The same viruses as above were also widely represented in vines with a normal bark, and exhibited a comparable distribution pattern. A notable exception was the apparent lack for GVB, which was not detected in any of these plants (Fig. 6). By converse, all CRW-affected varieties, but Regular seedless, contained GVB, though with an infection rate varying greatly with the cultivar, i.e. 52 % in Michele Palieri, 10 % in Primus, and 2 % in Italia, so as to give an average occurrence of 16 %. Interestingly, GVB was found in all 37 vines indexing positive for corky bark in graft-transmission tests to LN 33, whereas GLRaV-2 (the former grapevine corky bark-associated virus) was detected only in eight of these vines.

Discussion

A noteworthy result of the present investigation is that filamentous viruses other than those already known (MARTELLI 1993; BOSCIA *et al.* 1995 b) do occur in grapevines, as exemplified by the still uncharacterized trichovirus-like virus recovered from a CRW-affected vine.

It was confirmed that isolation of GVB by mechanical transmission directly from field-grown vines, although possible (BOSCIA *et al.* 1993), is not easy. As reported for other phloem-limited grapevine viruses (MONETTE and GODKIN 1993; CASTELLANO *et al.* 1995), transmission of GVB is more likely to succeed when *in vitro*-grown explants are used as inoculum source.

The eight MAbs to GVB characterized in this study, beside being virus-specific as previously reported (BOSCIA *et al.* 1994 b), recognized with various levels of confidence six different isolates of this virus. However, only two of them (MAbGB1E2G and MAbGB5G11B), both probably originating from cryptotopes, were useful for ELISA detection of GVB in grapevines.

Type of antigen source, timing of sample collection, and procedure for antigen preparation were all investigated in detail, and proved of paramount importance for reliable GVB identification. In the end, a triple antibody sandwich ELISA protocol was devised, that under our experimental conditions afforded consistent and repeatable results. It was based on the use of crude extracts from cortical scrapings of mature canes collected in autumn as antigen source, antibodies from a polyclonal antiserum for plate coating (trapping), and of MAb1 (MAbGB1E2G) as antigen revealing antibody.

This system allowed the largest survey carried out so far in Italy for GVB detection in field-grown vines. The results confirmed that: (i) data from field surveys are difficult to interpret, because the overwhelming coexistence of all sorts of phloem-limited viruses in vines affected by rugose wood and leafroll makes the sorting out of the putative causal agents of these complex diseases highly problematic; (ii) an extremely close relationship exists between GVB and corky bark disease; (iii) the same does not apply to GLRaV-2, this being in line with the notion that this virus may be involved in the induction of graft incompatibility and leafroll rather than in rugose wood syndromes (GREIF *et al.* 1995); (iv) in Southern Italy corky bark is not the most represented among the diseases of the rugose wood complex, although the present survey disclosed a 16 % incidence, which far exceeds the 0.5-2 % rate previously recorded (SAVINO *et al.* 1985 and unpublished information); (v) CRW has a complex nature and inducing factors that still escape our understanding. GVB may be one of these factors, as suggested by its absence in vines with rugose wood but no abnormal cork production. However, GVB occurrence in CRW-affected vines is not consistent enough to support conclusively the idea that it has a determining role in the development of the corky condition of grapevine trunks.

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