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Research Note

Detection of capillovirus-like particles in a grapevine affected with rugose wood

P. L. Monette and S.E. Godkin

K e y w o r d s : ISEM, stem grooving, stem pitting.

Introduction: All of the filamentous viruses which have been isolated to date from diseased grapevines have very flexuous closterovirus-like particles (CVLPs). The grapevine leafroll-associated virus types I-IV (GLRaV I-IV; Hu *et al.* 1990; Zimmermann *et al.* 1990) and grapevine corky bark-associated virus (CBaV); (Namba *et al.* 1991) are morphologically similar to the closteroviruses. The type member of the *Closterovirus* genus is citrus tristeza virus (Bar-Joseph and Murant 1982). Short CVLPs (≤ 800 nm long) have also been isolated from diseased grapevines. These are grapevine virus A (GVA), from stem pitting- or leafroll-affected vines (Conti *et al.* 1980; Monette *et al.* 1990), grapevine virus B (GVB) from corky bark-affected vines (Boscia *et al.* 1993), and grapevine virus C (GVC), also from corky bark-affected vines (Monette and James 1991 a; Monette and Godkin 1993). GVA and GVB have been tentatively reassigned to the newly established *Trichovirus* genus (Martelli *et al.* 1994). The objective of this communication is to report on the detection of filamentous virus particles morphologically similar to capilloviruses in a vine of *Vitis vinifera* cv. Sauvignon blanc affected with a rugose wood disease. This appears to be the first report of filamentous virus particles distinct from CVLPs in a diseased grapevine.

Materials and methods: G r a p e v i n e s : A vine of *V. vinifera* Sauvignon blanc was used in this study. It was tested by ELISA (Clark and Adams 1977) with antisera to tomato black ring virus (TBRV), grapevine fanleaf virus (GFLV), Arabis mosaic virus (ArMV) and tomato ringspot virus (TRSV). The Sauvignon blanc vine was also subjected to indexing tests with the herbaceous indicator plants *Chenopodium quinoa* and *C. amaranticolor*, and with the woody indicators *V. vinifera* Pinot noir, *V. rupestris* cv. St. George, and the hybrids Kober 5 BB (*V. berlandieri* x *V. riparia*) and LN 33 (Couderc 1613 x Thompson Seedless).

G r a p e v i n e *i n v i t r o* s h o o t t i p c u l t u r e : *In vitro* shoot tip cultures of the Sauvignon blanc vine were used for the electron microscopy studies. Such cultures have been successfully used for the detection of viral antigens and of disease-associated nucleic acids, for the transmission of filamentous viruses to herbaceous plants and as starting material for virus purification (Monette 1985; Monette *et al.* 1990; Monette and James 1991 b; Monette and Godkin 1993). Media and procedures for the initiation and maintenance of grapevine shoot tips *in vitro* have been reported elsewhere (Monette 1988).

E l e c t r o n m i c r o s c o p y : Extracts for this procedure were prepared by grinding stem tissue (including the necrotic base of the stem) from *in vitro* cultures of the Sauvignon blanc vine with about 10 volumes (w:v) of 0.03 M potassium phosphate buffer, pH 7.0, containing 0.005 M $MgCl_2$. Immunosorbent electron microscopy (ISEM) was conducted as described previously (Monette and Godkin 1993). Grids were stained with 2 % uranyl acetate and examined with a Hitachi Model H-7100 electron microscope. Rabbit antisera prepared against apple stem grooving virus (ASGV) were from Bioreba (Chapel Hill, NC) and the American Type Culture Collection (ATCC, Rockville, MD).

Antisera prepared against apple chlorotic leafspot virus (ACLSV), apple stem pitting virus (ASPV), potato virus T (PVT), lilac chlorotic leaf spot virus (LCLV) and GVB were from G. Mink, H. Yanase, L.F. Salazar, S. Phillips and D. Boscia, respectively.

Antisera against GVA and GVC were prepared at the the Centre for Plant Health.

Results and discussion: I n d e x i n g r e s u l t s : The results of the ELISA tests indicated that the Sauvignon blanc vine was free of TBRV, GFLV, ArMV and TRSV. No disease symptom developed on *C. quinoa* or *C. amaranticolor.* No grapevine leafroll (GLR) disease symptom developed on *V. vinifera* Pinot noir, indicateding that the Sauvignon blanc vine was GLR-free. Kober 5 BB exhibited no disease symptom, indicating that the Sauvignon blanc vine was free of Kober stem grooving (KSG). Wood pitting symptoms, consistent with *rupestris* stem pitting (RSP) disease, developed on the stem of the woody indicator vine *V. rupestris* St. George. Very severe grooving (Fig. 1) developed on the wood of the indicator vine LN 33, but this symptom was not accompanied with the leaf reddening usually associated with corky bark (CB) disease on this hybrid.

The rugose wood complex is comprised of the following diseases: KSG, RSP, LN 33 stem grooving (LNSG) and CB. These diseases can be distinguished from one another based on the symptoms they produce on various rootstocks (Garau *et al.* 1994). The Sauvignon blanc vine used in this study may thus have been affected with both RSP and LNSG. This interpretation is very tentative, however, as the causal agents for these diseases have yet to be definitively identified. For the moment, we prefer to consider the Sauvignon blanc vine as affected with a "rugose wood" disease.

E l e c t r o n m i c r o s c o p y r e s u l t s : *In vitro* shoot tip cultures of the Sauvignon blanc vine developed stem necrosis about 10 weeks after initiation. Filamentous virus particles (Fig. 2) 600-700 nm long were observed in extracts from symptomatic stem segments. They were morphologically similar to capilloviruses, such as ASGV. The particles lacked the high degree of flexuousness and the open structure typical of closteroviruses. Three "short" (≤ 800 nm long) CVLPs have to date been isolated from diseased grapevines. These are GVA (Conti *et al.* 1980),

Agriculture Canada, Centre for Plant Health, 8801 East Saanich Road, Sidney, BC V8L 1H3, Canada. Fax: (604)363-6661.

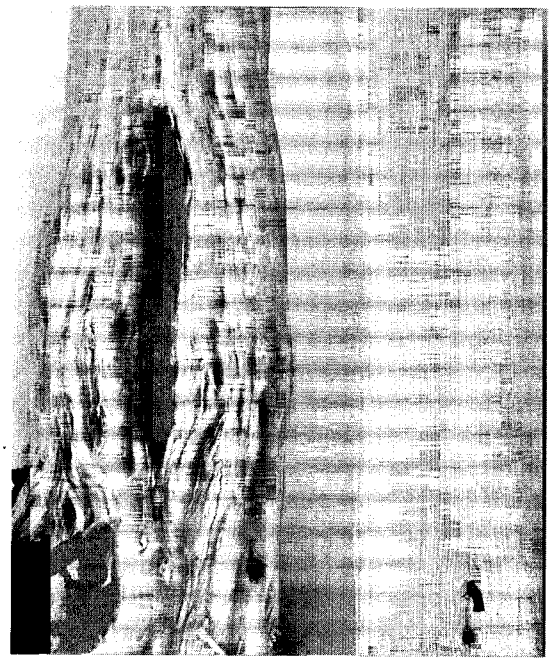

Fig. 1: Healthy LN 33 rootstock (right) and LN 33 showing severe stem grooving (left) after graft-inoculation with a Sauvignon blanc vine affected with a rugose wood disease.

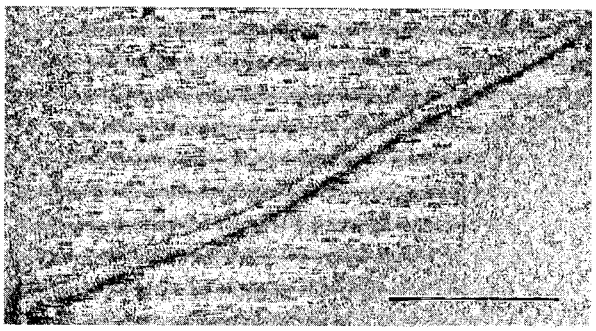

Fig. 2: Capillovirus-like particle detected in a Sauvignon blanc vine affected with a rugose wood disease. Bar = 200 nm.

GVB (BOSCIA *et al.* 1993) and GVC (MONETTE and JAMES 1991 a). Antisera prepared against each of these viruses failed to decorate the filamentous virus particles detected in the Sauvignon blanc vine. The capillovirus-like particles detected in this vine were also serologically distinct from ASPV, ACLSV, PVT and LCLV, based on ISEM tests. The anti-ASGV serum from Bioreba failed to decorate the particles, but the anti-ASGV serum from ATCC provided faint and irregular decoration. The serological relationship between the particles from the Sauvignon blanc vine and ASGV needs further study.

Only a few viruses have been detected to date in grapevines affected with rugose wood diseases. GVA is thought to be involved in the production of rugose wood symptoms, specially in KSG-affected grapevines (CHEVALIER *et al.* 1993; GARAU *et al.* 1994), and a longer CVLP has been detected in vines affected with RSP (PRUDENCIO 1985). This appears to be the first report of capillovirus-like particles in diseased grapevines. The purification and characterization of this virus should help elucidate its possible relationship to the *Capillovirus* genus and whether this virus can be associated with one or more of the rugose wood syndromes. This report should be of interest to plant quarantine and regulatory authorities as well as to virologists working with woody plants.

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