Vitis **30**, 37—43 (1991)

Detection of a closteroviruslike particle from a corky bark-affected grapevine cultivar

by

P. L. MONETTE and D. JAMES

Agriculture Canada, Saanichton Plant Quarantine Station, Sidney, British Columbia, Canada

Détection de particules virales de type clostérovirus à partir d'une vigne atteinte de la maladie de l'écorce liégeuse

Résumé : Des particules virales de type clostérovirus ont été transmises à des pousses de *Nicotiana benthamiana* à partir de cultures *in vitro* d'une vigne atteinte de la maladie de l'écorce liégeuse. Des lésions nécrotiques sont apparues sur les feuilles inoculées 9 à 12 d après l'inoculation. Des symptômes systémiques sont apparus 14—16 d après l'inoculation. L'infection était léthale pour *N. benthamiana*. Les particules virales avaient une largeur d'environ 10 nm, une longueur d'environ 725 nm et contenaient des striaisons avec un avancement de 3.4 nm. Des tests sérologiques (ISEM) ont révélé que les particules n'étaient pas décorées par les anticorps préparés envers les virus GVA, NY-1, CA-4, GLRV-1, GLRV-3, ASGV, PVT ou CLSV.

Key words: virosis, corky bark, closterovirus, test plant, tissue culture, serology, ISEM.

Introduction

Grapevine corky bark (CB) disease was first observed in California (HEWITT 1954; HEWITT *et al.* 1962). It has been reported to occur in Mexico, Brazil, France, Spain, Switzerland, Italy, Yugoslavia, Bulgaria, South Africa and Japan, and it is probably more widespread than has been reported (BOVEY *et al.* 1980). On most *Vitis vinifera* cultivars CB does not show symptoms. It produces only a reduction of vigor. In California, CB induces a growth retardation at leaf burst and dieback of a few shoots on Palomino, Petite Sirah, Mondeuse, Cabernet franc and Gamay. CB produces cane and leaf symptoms on these cultivars. Some canes on a diseased vine are soft and rubbery and tend to bend downwards. They may have longitudinal cracks at their base. Leaves are often smaller than normal and, on red cultivars, turn red and roll downwards during summer. In autumn the leaves do not abscise normally, but persist 3—4 weeks longer than those of healthy plants (BEUKMAN and GOHEEN 1970; BOVEY *et al.* 1980).

CB is detected using the indicator grapevine cultivar LN 33 (Couderc 1613 × Thompson Seedless), on which it produces severe symptoms. The growth of the vines is badly stunted. The leaves are smaller than normal, becoming reddish as the season advances, and tend to droop. The bark on the canes is thick, spongy and soft. It often splits longitudinally. The cracks then heal along the margins, producing rough longitudinal fissures as the canes grow older. The wood under these areas is deeply pitted and grooved. The canes may remain immature or mature unevenly, and are limber or rubbery. Symptoms develop first at the bases of the canes and become more extensive as the season progresses (HEWITT *et al.* 1962; BEUKMAN and GOHEEN 1970; BOVEY *et al.* 1980).

The disease is transmissible by budding or grafting (HEWITT *et al.* 1962; CASTILLO *et al.* 1975). No natural vector has been identified, but CB has been experimentally transmitted using the leafhopper *Scaphoideus littoralis* BALL. (Homoptera Jassidae) (MOUTOUS and HEVIN 1986). CB is assumed to be viral in etiology, but the causative agent has not been determined. This communication reports on the detection of a closteroviruslike particle (CVLP) from a vine of *V. vinifera* L. Semillon affected with CB disease.

Materials and methods

Source of virus and transmission to herbaceous hosts

The source of the virus reported in this study was a vine of *V. vinifera* L. Semillon. This vine was CB-affected, grapevine leafroll- (GLR-)affected and rupestris stem pitting-free, based on indexing results obtained with the woody indicators LN 33, *V. vinifera* Cabernet franc and *V. rupestris* St. George. *In vitro* shoot tip cultures of the CB-affected Semillon vine were established and maintained using media and procedures described elsewhere (MONETTE *et al.* 1989). Cultures at the shoot proliferation stage were homogenized in 5 volumes (w:v) of 0.01 M potassium phosphate buffer containing 2.5 % nicotine, final pH 9.5 (CADMAN *et al.* 1960) and the extract was rub-inoculated into corundum-dusted leaves of three seedlings each of *N. benthamiana*, *N. occidentalis*, *Chenopodium quinoa* sspp. *milleanum* and *quinoa*, *C. amaranticolor* and *Gomphrena globosa*. This inoculation procedure had previously been used for the mechanical transmission of grapevine virus A (GVA) from a leafroll-affected grapevine to a herbaceous host (MONETTE *et al.* 1990). All plants were inoculated at the 6-leaf stage. Control plants were inoculated with buffer only and all plants were maintained in the same greenhouse.

Electron microscopy

Grids backed with Formvar-carbon films were coated for 3 h with a 1:1000 dilution of rabbit anti-GVA serum (MONETTE and JAMES 1990) in 0.06 M potassium phosphate buffer, pH 7.0 (6-PB). Although this pre-treatment of the grids was not necessary for detection of the CVLPs, it generally increased the number of particles observed, due to an apparently non-specific interaction between the viruslike particles and some component of the serum. The grids were rinsed with a stream of 6-PB (about 1 ml). Symptomatic leaves from systemically infected *N. benthamiana* seedlings were ground in

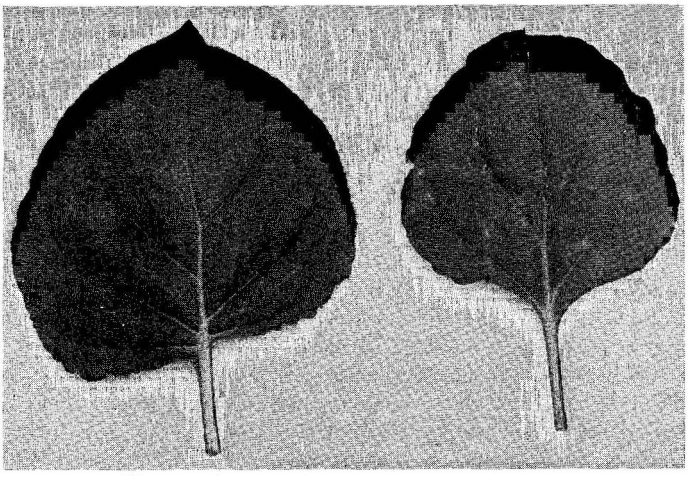

Fig. 1: Leaf from a buffer-inoculated *N. benthamiana* plant (left). Leaf from a *N. benthamiana* plant inoculated with extract from *in vitro* shoot tip cultures of a CB-affected Semillon grapevine, showing local necrotic lesions (right).

Une feuille de *N. benthamiana* inoculé avec une solution tampon (à gauche). Une feuille de *N. benthamiana* inoculé avec un extrait d'une vigne Semillon atteinte de la maladie de l'écorce liégeuse (à droite).

10 volumes (w:v) of 6-PB and particle trapping was allowed to take place for 1 h. The grids were then rinsed with a stream of distilled water, stained with 2 % uranyl acetate and examined with a JEOL JEM-100C electron microscope. The instrument was calibrated using a JBS number 401A grating replica (J. B. EM Services Inc., Québec), and 85 closteroviruslike particles were measured. For normal length calculations, all particles belonging to size classes (20 nm increments) between 600 and 860 nm were included. This corresponded to 55 % of all particles measured. Immunosorbent electron microscopy (ISEM), similar to that described elsewhere (VAN REGENMORTEL 1982), was used to test the serological relationship between the virus under study and previously reported CVLPs. Antiserum coating of grids, particle trapping and decoration were conducted as previously described (MONETTE *et al.* 1990).

Results and discussion

N. benthamiana seedlings inoculated with extract from *in vitro* shoot tip cultures of a CB-affected Semillon grapevine developed local necrotic lesions (Fig. 1) at 9—12 d post-inoculation. Systemic symptoms, consisting of auxiliary vein necrosis and collapse of the upper leaves, were observed at 14—16 d post-inoculation. Leaves and stems all

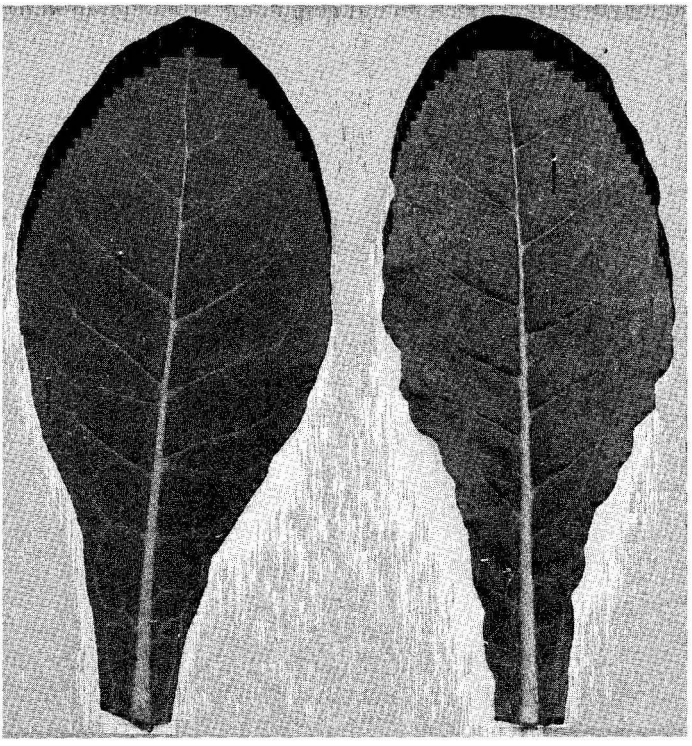

Fig. 2: Leaf from a buffer-inoculated *N. occidentalis* plant (left). Leaf from a *N. occidentalis* plant inoculated with extract from *in vitro* shoot tip cultures of a CB-affected Semillon grapevine, showing systemic interveinal chlorotic flecking (right).

Une feuille de *N. occidentalis* inoculé avec une solution tampon (à gauche). Une feuille de *N. occidentalis* inoculé avec un extrait d'une vigne Semillon atteinte de la maladie de l'écorce liégeuse (à droite).

progressively died. All *N. benthamiana* plants inoculated with the Semillon extract were dead at 34 d post-inoculation while buffer-inoculated controls showed no disease symptoms. Of the other herbaceous plants inoculated with extract from Semillon, only *N. occidentalis* developed disease symptoms. These consisted of an interveinal chlorotic flecking (Fig. 2), which appeared within 16 d after inoculation. This virus was not lethal to *N. occidentalis*, suggesting that this plant could serve as a propagation host in the greenhouse.

The virus detected in symptomatic leaves from systemically infected *N. benthamiana* seedlings was a flexuous rod (Fig. 3) with striations 3.4 nm in pitch. The particles were approximately 10 nm wide with a normal length of about 725 nm (Fig. 4). The particle length distribution showed two peaks, one at 400—420 nm and another at 720—740 nm. Except for their length, the smaller particles were indistinguishable by electron microscopy from the larger ones. As closteroviruslike particles are prone to breakage, the smaller particles may be fragments of the larger CVLPs. On the basis of particle morphology, the virus might belong to the closterovirus subgroup II or III (FRANCKI *et al.* 1985). The only closterovirus of similar size reported to date to occur in grapevines was grapevine virus A (GVA; CONTI *et al.* 1980; MILNE *et al.* 1984). The virus under study was serologically distinct from GVA as it was not decorated in ISEM tests with rabbit anti-GVA serum. The virus was also readily distinguishable from GVA on

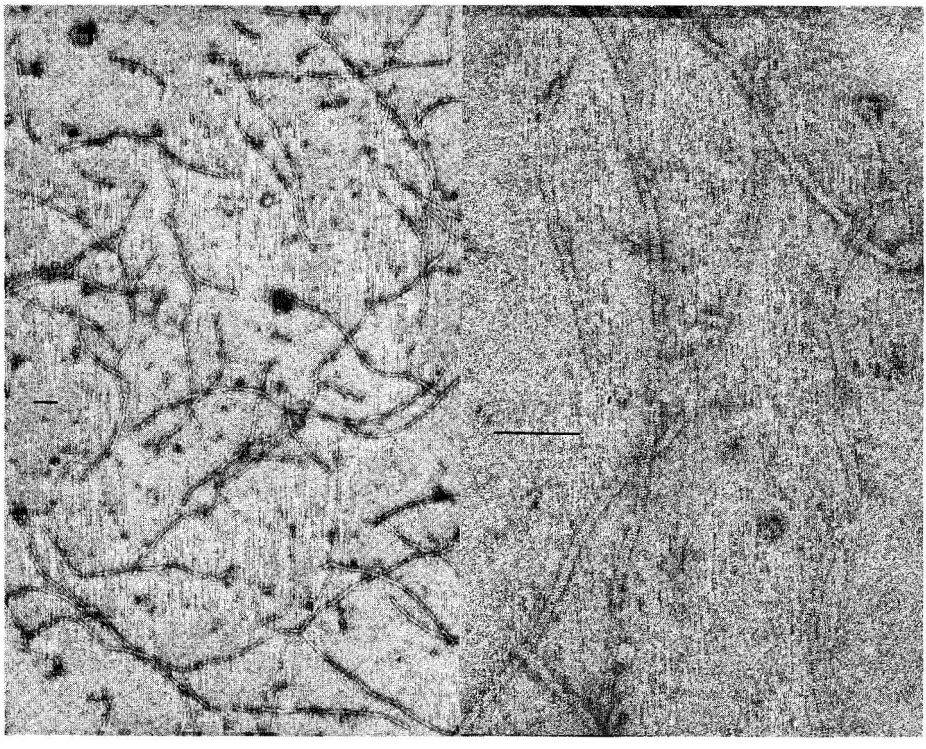

Fig. 3: Closteroviruslike particles, at two magnifications, from a CB-affected Semillon vine. Uranyl acetate stain. Bars = 100 nm.

Particules virales de type clostérovirus, à deux magnifications, obtenues à partir d'une vigne Semillon atteinte de la maladie de l'écorce liégeuse. Coloration à l'acétate d'uranyle. Les barres représentent 100 nm.

the basis of symptoms produced on *N. benthamiana* (MONETTE *et al.* 1990; MONETTE and JAMES 1990). Antisera prepared against three other closteroviruses, apple stem grooving virus (ASGV), potato virus T (PVT) and apple chlorotic leafspot virus (CLSV, Mink strain), also failed to decorate the virus under study.

As the Semillon grapevine from which the virus was isolated produced leafroll symptoms on graft-inoculated Cabernet franc, it was of interest to determine whether this virus was serologically related to other closteroviruslike particles previously reported to be associated with grapevine leafroll disease (ZEE *et al.* 1987; ZIMMERMANN *et al.* 1988; HU *et al.* 1990). In ISEM tests, the virus from Semillon was not decorated

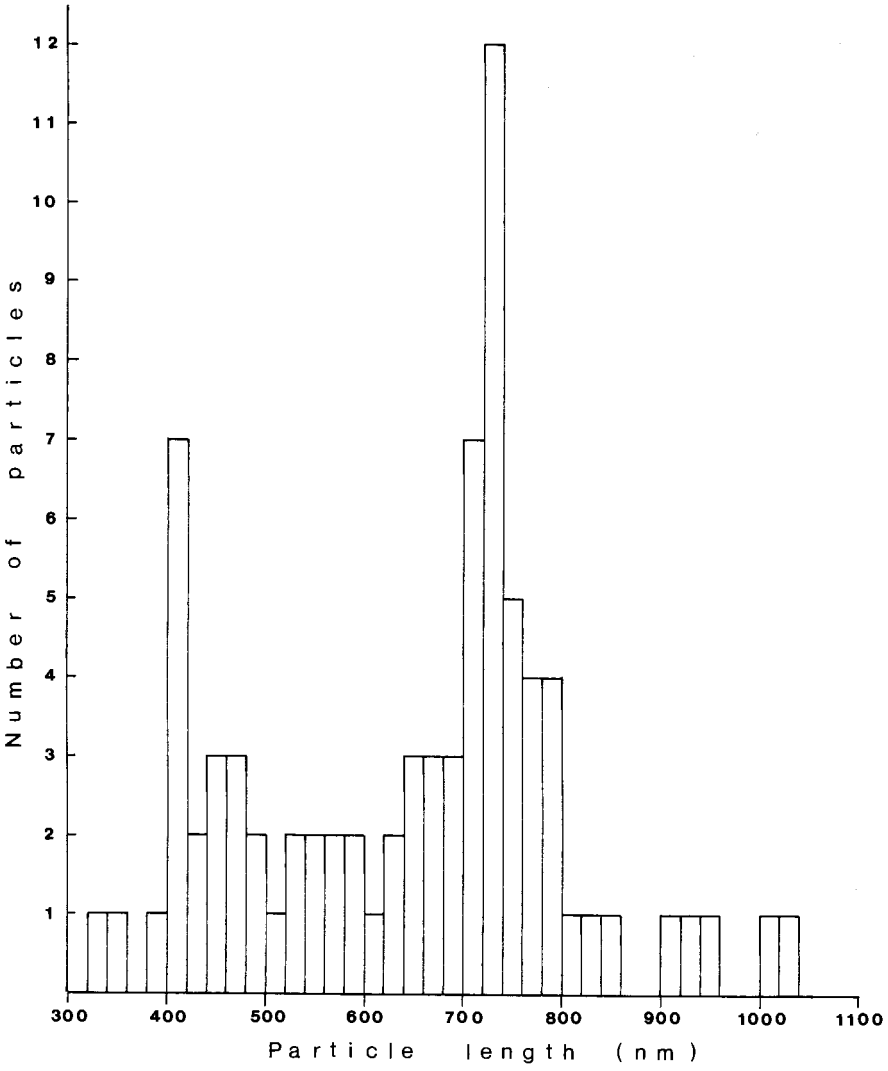

Fig. 4: Length distribution of closteroviruslike particles in symptomatic leaves of *N. benthamiana* inoculated with extract from CB-affected Semillon grapevine.

Longueur de particules virales obtenues à partir d'une vigne Semillon atteinte de la maladie de l'écorce liégeuse.

with antisera prepared against the grapevine closteroviruses NY-1, CA-4, GLRV-1 or GLRV-3. These results indicate that the CVLPs under study were not fragments from these GLR-associated CVLPs. It is conceivable that the Semillon virus might be responsible for both the CB symptoms on LN 33 and the GLR symptoms on Cabernet franc, but this has not been established. Further transmission trials using additional CB-affected cultivars and a serological survey using antibodies currently being prepared against the purified Semillon virus should help to determine whether this virus is in fact CB-associated. Further characterization of the purified virus should also be valuable in determining whether a provisional grouping within the subgroup II or subgroup III closteroviruses would be justified.

Summary

Closteroviruslike particles were mechanically transmitted from *in vitro* shoot tip cultures of a grapevine affected with corky bark disease to seedlings of *Nicotiana benthamiana*. Local necrotic lesions appeared 9—12 d and systemic symptoms 14—16 d post-inoculation. This virus had a normal length of 725 nm, striations with a pitch of 3.4 nm and was 10 nm wide. In ISEM tests, the virus was not decorated with antibodies prepared against GVA, NY-1, CA-4, GLRV-1, GLRV-3, ASGV, PVT or CLSV.

Acknowledgements

Antisera to leafroll-associated CVLPs were generously provided by D. GONSALVES (NY-1 and CA-4) and D. ZIMMERMANN (GLRV-1 and GLRV-3). Antisera to apple stem grooving virus and apple chlorotic leafspot virus were from the East Malling Research Station and antiserum to potato virus T was kindly supplied by L. F. SALAZAR. The expert technical assistance of S. E. GODKIN for electron microscopy and photography is gratefully acknowledged. Information on the virus disease status of the grapevine used in this study was kindly provided by Station personnel.

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Received 26. 6. 1990

Correspondence to:

P. L. MONETTE
Agriculture Canada
Saanichton Plant Quarantine Station
8801 East Saanich Road
Sidney, British Columbia
Canada, V8L 1H3