Eur J Plant Pathol (2008) 121:185–188
DOI 10.1007/s10658-007-9244-3

Grapevine virus A transmission by larvae of *Parthenolecanium corni*

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Received: 20 March 2007 / Accepted: 25 October 2007 / Published online: 27 November 2007

Abstract *Grapevine virus A* (GVA, Vitivirus) was transmitted experimentally by first and second instars of the scale insect *Parthenolecanium corni* from grapevine to grapevine and to the herbaceous host *Nicotiana benthamiana*. This is the first report of GVA transmission by *P. corni*. *Grapevine leafroll-associated virus-1* (Ampelovirus) was always present in the donor grapevines and, in every case, GVA was transmitted simultaneously with this ampelovirus from grapevine to grapevine, suggesting possible interactions between the two viruses for transmission.

Keywords GLRaV-1 · GLRaV-3 · GVA · Homopteran · Virus vector

Introduction

Grapevine virus A (GVA, Vitivirus, *Flexiviridae*) is the agent of 'Kober stem grooving', a component of the 'Rugose wood' complex of grapevine, *Vitis vinifera*. Rugose wood occurs worldwide on grafted vines and induces lower vigour, wood alterations, lower amounts of bunches and, in some cases, decline of affected vines (Martelli and Boudon-Padieu 2006). In vineyards, GVA is often detected together with the *Grapevine leafroll-associated virus* species GLRaV-1 and/or -3 (Ampelovirus, *Closteroviridae*), with which it shares localisation in phloem tissues and transmissibility by grafting and mealybugs (*Pseudococcidae*) and soft scales (*Coccidae*). GVA is also mechanically transmissible and has also been transmitted experimentally from vine to vine and to herbaceous hosts (*Nicotiana benthamiana, N. clevelandii, Gomphrena globosa*) by six mealybug species: *Pseudococcus longispinus, Ps. affinis, Ps. comstocki, Planococcus ficus, Pl. citri*, and *Heliococcus bohemicus* (Rosciglione et al. 1983; Rosciglione and Castellano 1985; Agran et al. 1990; Engelbrecht and Kasdorf 1990; Pedroso et al. 1991; Garau et al. 1995; La Notte et al. 1997; Goszczynski and Jooste 2003; Nakano et al. 2003; Zorloni et al. 2004, 2006). The coccid *Neopulvinaria innumerabilis* was also capable of transmitting GVA, whereas another coccid, *Parthenolecanium corni*, was reported to be unable to do so (Fortusini et al. 1997).

Parthenolecanium corni, the European fruit lecanium, is a Palearctic and polyphagous species widespread in vineyards worldwide (Ben Dov 1993). In European vineyards where this species is monovoltine (Sforza 2000), adult females appear during spring and produce large amounts of eggs from which hatch first instar crawlers that colonize the leaves thereafter. When reaching high population levels,

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P. corni may cause direct damage, as well as indirect damage by depositing honeydew on leaves and fruits (Sforza 2000). Additionally, *P. corni* is recognized as a natural vector of GLRaV-1 (Fortusini et al. 1997; Sforza et al. 2003; Ciampolini and Guarnone 2003), that assigns to this species a new role in dispersing this virus. Therefore, the widespread distribution of both *P. corni* and GVA, and the frequent association with leafroll viruses in grapevine led us to assess the possibility of GVA transmission by *P. corni*.

Transmission by first instar nymphs to grapevines

A first experiment (A) was performed with first instar nymphs obtained in June 2004 from vineyard-sampled *P. corni* females. Freshly laid eggs were sampled and kept until hatching. About 100 newly hatched nymphs were deposited into plastic caps (9 mm diam) clipped onto the lower side of the leaves of pot-grown rooted cuttings of Pinot Noir (isolate P 70) infected with both GLRaV-1 and GVA. After an acquisition access period (AAP) of 12 days given to the nymphs, 1–3 leaf pieces with about 20–30 larvae in total were cut and then clipped on the leaves of 10 virus-free recipient cuttings (6–12 leaves), obtained from greenhouse-grown and insect-protected rooted cuttings cv. Pinot Noir (accessions P 14 and P 15). Each recipient plant was isolated under a 0.1 mm mesh microperfored plastic bag ('bread bags', Sealed Air SAS, Epernon, F). After an 8-day inoculation access period (IAP), recipient plants were sprayed with mevinphos (4 ml l^{-1} Phosdrin W10®) and kept at 20–23°C, under 16 h artificial light. Two plants without nymphs were maintained in the same conditions. Infection of recipient plants was checked by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). The isolates Chardonnay V 29 and Servant A 94 were used as positive controls for both GLRaV-1 and -3, and for GVA, respectively. Leaf fragments (1 g from several leaves in 5 ml buffer) were ground with a bullet blender (Homex 5®, BioReba). Polyclonal antibodies raised against GLRaV-1, GLRaV-3 or GVA previously produced in the laboratory were used in a biotine–streptavidine procedure (Zimmermann et al. 1990). Absorbance was recorded at 405 nm using a multiscan microplate reader (Thermo Labsystems). Optical density (OD) values above the mean of healthy controls (0.22 and 0.21, for GVA and GLRaV-1 respectively), + 3× their standard deviation were considered positive. Plants were checked by ELISA 13 months after IAP. This experiment showed that two vines out of 10 (20%) were tested positive for GVA (OD 0.51 and 0.84) and GLRaV-1 (OD 0.91 and 0.83) (Table 1). This demonstrates the ability of newly hatched nymphs of *P. corni* to transmit these two viruses, at least from a mixed infection after a 12-day AAP.

Transmission by second instar nymphs to grapevines

In experiment B, second-instar scales were collected from vineyards, located in Nothalten and Turckheim (eastern France). The plots were naturally infected with GLRaV-1 and -3, and with GVA (as determined previously by ELISA). GVA was always present in mixed infection with GLRaV-1 and/or -3, but GLRaV-1 or -3 singly infected plants could be identified. In October 2003, and between August and October 2004, leaf fragments taken from infected

Table 1 Rates of virus transmission by first or second instar nymphs of *Parthenolecanium corni*: Experiment A: from greenhouse-grown infected to healthy grapevine; Experiment B: from naturally-infected to healthy grapevine; Experiment C: from naturally-infected grapevine to *Nicotiana benthamiana*

Experiment	Virus in source plant	Test plant	Detection in test plants		
			GVA	GLRaV-1	GLRaV-3
A: 1st instar	GVA+GLRaV-1	Grapevine	2/10	2/10	–
B: 2nd instar	GVA+GLRaV-1	Grapevine	7/16	8/16	–
	GVA+GLRaV-3	Grapevine	0/6	–	0/6
	GVA+GLRaV-1+ GLRaV-3	Grapevine	3/4	4/4	0/4
C: 2nd instar	GVA+GLRaV-1	*N. benthamiana*	31/38	–	–

grapevines and bearing together about 50 second instar larvae of *P. corni* were clipped onto each pot-grown rooted cuttings of virus-free Pinot Noir. After a few days, the insects crawled off the leaf fragments and settled on recipient plants. The latter were 26 grapevine cuttings, whereas 18 other cuttings received nymphs from virus-free grapevines. Sixteen recipient grapevines were inoculated from source plants infected with both GLRaV-1 and GVA, 6 with both GVA and GLRaV-3, and 4 with GLRaV-1, -3 and GVA. Recipient plants, covered as described above, were sprayed with methidathion (Suprathion®) to eliminate the vectors. These plants were ELISA-checked, as described above, about 9–12 months after IAP. Ten grapevines out of 26 (38.5%, OD range 0.54–1.14) were GVA positive. From plants doubly infected by GVA and GLRaV-1, GLRaV-1 was transmitted simultaneously with GVA to seven plants (OD range 0.48–0.89) and was transmitted alone to one plant (OD 0.60). No transmission of GVA from the grapevine infected by both GVA and GLRaV-3 was observed, nor was any transmission of GLRaV-3 recorded. From plants infected with the three virus species, only GVA and GLRaV-1 were transmitted, either together (three plants out of four, OD ranges 0.55–0.98 and 0.85–1.30 respectively) or GLRaV-1 alone (one plant, OD 0.41). Healthy control plants were ELISA negative for GLRaV-1, -3 and GVA. This experiment showed that vineyard-sampled second instar *P. corni* larvae GVA are able to transmit GVA along with GLRaV-1 to grapevine.

Transmission by second instar nymphs to *N. benthamiana*

In October 2004, leaf fragments from vineyard-growing virus-infected grapevine with second instar nymphs were placed onto 38 recipient *N. benthamiana* seedlings (4–8 leaf stage): 36 seedlings with insects collected from donor vines co-infected with GLRaV-1 and GVA, and two others with insects from a vine mixed-infected by GLRaV-1, -3 and GVA. About 50 nymphs were allowed to settle down on each recipient plant; however they died within 3 weeks on this plant species, probably due to increasing leaf hairiness. Recipient plants were ELISA-checked 55 days after IAP. GVA was detected in 31 out of 38 plants (82%, OD range 0.68–3.59) (Table 1), demonstrating that second instar larvae are able to inoculate GVA to *N. benthamiana*.

To our knowledge, these results are the first reported evidence that *P. corni* acts as an efficient vector of GVA. Both larval stages of *P. corni* are capable of transmitting GVA from grapevine to grapevine and to the experimental host, *N. benthamiana*. *Parthenolecanium corni* nymphs thus inoculated GVA efficiently to *N. benthamiana*, which contrasts with negative results reported previously by Fortusini et al. (1997).

Parthenolecanium corni is commonly considered as a pest in European vineyards and is present worldwide (Sforza 2000; Ben Dov 1993). Our conclusions about the vector ability of larval stages reinforce the attention that should be paid to *P. corni* as a natural vector of GVA and GLRaV-1 in northern vineyard conditions. Even though adult females are sessile on stems, *P. corni* nymphs can account for plant-to-plant dispersal of virus, as shown for *Pl. citri* and GLRaV-3 (Cabaleiro and Segura 2006). Also, these small and flat larvae may be dispersed by wind and by ants, as observed for other scales (Barrass et al. 1994; Sforza 2000), as well as by the vinegrower's engines. *Parthenolecanium corni* has previously been shown to vector GLRaV-1 but not GLRaV-3 (Fortusini et al. 1997; Sforza et al. 2003), which is confirmed here, even in mixed infections.

In our experiments, *P. corni* transmitted GVA to grapevine always along with GLRaV-1, whereas GLRaV-1 was sometimes transmitted alone, suggesting that GVA would be assisted by GLRaV-1 during transmission. A similar hypothesis was raised by Engelbrecht and Kasdorf (1990), who observed that GVA was transmitted by mealybug vectors only from vines also infected with GLRaV-3. However, Fortusini et al. (1997) suggested that GLRaV-1 would benefit from GVA for transmissibility by *N. innumerabilis*. Moreover, La Notte et al. (1997) reported transmission of GVA alone by *Pl. ficus* from infected to healthy *N. clevelandii*, a non-host for GLRaV-1 and -3, which seems to rule out the need for a helper virus in this case. Further experiments are needed to ascertain whether reported associations between GVA and an ampelovirus are merely circumstancial or have

a biological significance, i.e. some form of 'dependent transmission' (Sylvester 1985), at least for certain specific virus–vector combinations and in grapevine, a plant where co-infections are very common.

Acknowledgements We are grateful to VINIFLHOR (Grant No.W 01 01 68 05 0001 10) and INRA Department 'Santé des Plantes et Environnement' for financial support. Many thanks to Dr R. Sforza (USDA, Montpellier) for very helpful comments, to Dr C. Greif (INRA Colmar) for useful advise, to P. Cornuet (INRA Colmar) for technical help and to J. Misbach (INRA Colmar) for greenhouse production of plants.

References

Agran, M. K., Di Terlizzi, B., Boscia, D., Minafra, A., Savino, V., Martelli, G. P., et al. (1990). Occurrence of *Grapevine virus A* (GVA) and other closteroviruses in Tunisian grapevines affected by leafroll disease. *Vitis, 29*, 43–48.

Barrass, I. C., Jerie, P., & Ward, S. A. (1994). Aerial dispersal of first- and second-instar longtailed mealybug, *Pseudococcus longispinus* (Targioni Tozzetti) (Pseudococcidae: Hemiptera). *Australian Journal of Experimental Agriculture, 34*, 1205–1208.

Ben Dov, Y. (1993). *A systematic catalogue of the soft scale insects of the world (Homoptera: Coccoidea: Coccidae) with data on geographical distribution, host plants, biology and economic importance. Flora & fauna handbook* (Vol. 9, 568 p.). Gainesville, FL: Sandhill Crane Press.

Cabaleiro, C., & Segura, A. (2006). Temporal analysis of *Grapevine leafroll associated virus-3* epidemics. *European Journal of Plant Pathology, 114*, 441–446.

Ciampolini, M., & Guarnone, A. (2003). Pullulazioni su vigneti di *Parthenolecanium corni. Informatore Agrario, 59*, 81–85.

Engelbrecht, D. J., & Kasdorf, G. G. F. (1990). Transmission of grapevine leafroll disease and associated closteroviruses by the vine mealybug, *Planococcus ficus. Phytophylactica, 22*, 341–346.

Fortusini, A. G., Scattini, G., Prati, S., Cinquanta, S., & Belli, G. (1997). Transmission of *Grapevine leafroll virus-1* (GLRaV-1) and *Grapevine virus A* (GVA) by scale insects. In Proceedings 12th Meeting of ICVG, Lisbon, Portugal, pp. 121–122.

Garau, R., Prota, V. A., Boscia, D., Fiori, M., & Prota, U. (1995). *Pseudococcus affinis* MASK., new vector of grapevine trichoviruses A and B. *Vitis, 34*, 67–68.

Goszczynski, D. E., & Jooste, A. E. C. (2003). Shiraz disease (SD) is transmitted by mealybug *Planococcus ficus* and associated with *Grapevine virus-A* (GVA). In Proceedings 14th Conference of ICVG, Locorotondo, Italy, pp. 219–220.

La Notte, P., Buzkan, N., Choueiri, E., Minafra, A., & Martelli, G. P. (1997). Acquisition and transmission of *Grapevine virus A* by the mealybug *Pseudococcus longispinus. Journal of Plant Pathology, 79*, 79–85.

Martelli, G. P., & Boudon-Padieu, E. (Eds.) (2006). Directory of infectious diseases of grapevines and viroses and virus-like diseases of grapevine: Bibliographic report 1998–2004. Options méditerranéennes. Série B, 55: Studies and Research. Bari, Italy: CIEHAM-IAMB, 279 p.

Nakano, M., Nakaune, R., & Komazaki, S. (2003). Mealybug transmission of grapevine viruses in Japan. In Proceedings 14th Meeting of ICVG, Locorotondo, Italy, p. 218.

Pedroso, E. I., Sequeira, O. A., Pinto, M. E. G., & Simões, V. (1991). Ensaios de transmissão de virus de videira por cochonillas. *Ciência e Técnica Vitivinicola, 10*, 39–46.

Rosciglione, B., Castellano, M. A., Martelli, G. P., Savino, V., & Cannizzaro, G. (1983). Mealybug transmission of *Grapevine virus A. Vitis, 22*, 331–347.

Rosciglione, B., & Castellano, M. A. (1985). Further evidence that mealybugs can transmit *Grapevine virus A* (GVA) to herbaceous hosts. *Phytopathologia Mediterranea, 24*, 186–188.

Sforza, R. (2000). Les cochenilles sur la vigne : Bio-éthologie, impact agronomique, lutte et prophylaxie. In J. Stockel (Ed.), Les Ravageurs de la Vigne (pp. 130–147). Bordeaux, France: Editions Féret.

Sforza, R., Boudon-Padieu, E., & Greif, C. (2003). New mealybug species vectoring *Grapevine leafroll associated viruses-1* and *-3* (GLRaV-1 and -3). *European Journal of Plant Pathology, 109*, 975–981.

Sylvester, E. S. (1985). Multiple acquisition of viruses and vector-dependent prokaryotes: Consequences on transmission. *Annual Review of Entomology, 30*, 71–88.

Zimmermann, D., Bass, P., Legin, R., & Walter, B. (1990). Characterization and serological detection of four closterovirus-like particles associated with leafroll disease on grapevine. *Journal of Phytopathology, 130*, 205–218.

Zorloni, A., Prati, S., Bianco, P. A., & Belli, G. (2004). Further data on the experimental transmission of *Grapevine leafroll-associated virus-1* and *-3* and of *Grapevine virus A* by mealybugs. *Journal of Plant Pathology, 86*, 339–340.

Zorloni, A., Prati, S., Bianco, P. A., & Belli, G. (2006). Transmission of *Grapevine virus A* and *Grapevine leafroll-associated virus-3* by *Heliococcus bohemicus. Journal of Plant Pathology, 88*, 325–328.