SHORT COMMUNICATION

TRANSMISSION OF GRAPEVINE VIRUS A AND GRAPEVINE LEAFROLL-ASSOCIATED VIRUS 3 BY *HELIOCOCCUS BOHEMICUS*

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SUMMARY

Heliococcus bohemicus Sulc is very frequently found in vineyards in Northern Italy, where grapevine leafroll is widespread. *Grapevine leafroll-associated virus 1* (GLRaV-1) and *Grapevine leafroll-associated virus 3* (GLRaV-3) are also quite frequently observed, often in association with *Grapevine virus A* (GVA). The capacity of the mealybug to transmit these viruses to vine was therefore evaluated. Virus-free insects were fed on infected vines and then transferred to healthy test-plants. GLRaV-3 was transmitted to two out of 77 inoculated test-plants and GVA to one out of 38; GLRaV-1 was not transmitted. This is the first report of GVA transmission by *H. bohemicus* and the first report of the capacity of this mealybug to transmit GLRaV-3 to grapevine in Italy.

Key words: ampelovirus, mealybug, virus-vector, vitivirus, *Heliococcus bohemicus.*

The vitivirus *Grapevine virus A* (GVA) and the ampeloviruses *Grapevine leafroll associated virus 1* (GLRaV-1) and *Grapevine leafroll associated virus 3* (GLRaV-3) are phloem-limited viruses that are associated with two different grapevine diseases that are widespread in the most important viticultural areas, i.e. Kober stem grooving (GVA), belonging to the rugose wood complex, and leafroll (GLRaV-1 and -3). The same viruses are known to be transmitted by several species of mealybugs (Pseudococcidae), belonging to the genera *Planococcus* and *Pseudococcus* (Rosciglione and Castellano, 1985; Tanne *et al.*, 1989; Garau *et al.*, 1995; Cabaleiro and Segura, 1997; La Notte *et al.*, 1997; Petersen and Charles, 1997; Golino *et al.*, 1998), and by soft scale insects (Coccidae) belonging to the genera *Pulvinaria*, *Neopulvinaria* and *Parthenolecanium* (Belli *et al.*, 1994; Fortusini *et al.*, 1997).

Large populations of *Heliococcus bohemicus* Sulc, have been reported in Northern Italy (Marotta and Tranfaglia, 1990; Reggiani *et al.*, 2003) and frequently found in vineyards affected by leafroll. Moreover, the presence of this mealybug and its capacity to transmit GLRaV-1 and GLRaV-3 from infected to healthy grapevines were recently reported in France (Sforza and Greif, 2000). The objective of this study was to verify the capacity of *H. bohemicus* to transmit GVA, GLRaV-1 and GLRaV-3 from vine to vine.

Adult females of *H. bohemicus*, were collected during spring from grapevines in a vineyard located in the Oltrepò pavese, and then reared on healthy *Vitis vinifera* seedlings (grown in screen houses) until their use for inoculation assays. All insects were carefully moved with a small paintbrush to avoid damaging their stylets. Rooted cuttings of different *V. vinifera* cultivars (Moradella, Lambrusco viadanese and Schiava lombarda), infected with GLRaV-1, GLRaV-3 and GVA in single or mixed infections, were used as inoculum sources. Seedlings of *V. vinifera* cultivars Pinot noir and Riesling italico, and rooted cuttings of Barbera, Cabernet franc and Pinot noir, were used as recipients in the transmission tests; all these plants were pre-tested by ELISA to make sure they were free from the viruses being studied. For each plant, basal leaves were collected, ground in a mortar with liquid nitrogen, and mixed with an extraction buffer with the following composition: tris 0.26M, tris-HCl 0.24M, NaCl 0.8%, polyvinylpyrrolidone MW24000 2%, polyethyleneglycol MW6000 1%, NaN_3 0.02%, Tween 20 0.05% (pH 8.2). For the detection of GVA, microtiter plates were previously sensitised with protein A (Boscia *et al.*, 1992). Results were considered positive if the optical density at 405 nm was at least three times that given by the average healthy control.

Inoculations from infected to healthy vines were done in insect-proof cages in the greenhouse under controlled conditions (24°C, 70% RH). The insects were allowed to feed on infected grapevines for different acquisition access periods (AAP): 4 days, 1 week, 2 weeks, or 3 weeks. Live insects were then transferred to healthy grapevines and left to feed for inoculation periods (IP) of one or two weeks. In total, 77 test plants were inoculated: 39 in 2003, and 38 in 2004. Virus-free seedlings grown in the same conditions, but with insects reared on healthy grapevine seedlings feeding on them, were

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Table 1. Results of inoculations with GLRaV-3 and GVA in 2003.

Inoculum source	Test plant	GLRaV-3			GVA		
		Aug 03	Nov 03	Feb 04	Aug 03	Nov 03	Feb 04
GLRaV-3	Pinot noir (seedlings)	0/18*	0/18	0/18	0/18	0/18	0/18
GLRaV-3 + GVA	Pinot noir (seedlings)	1/19	1/19	1/19	0/19	0/19	0/19
GLRaV-3 + GVA	Cabernet franc (rootlings)	0/2	0/2	0/2	0/2	0/2	0/2
Healthy control	Pinot noir (seedlings)	0/1	0/1	0/1	0/1	0/1	0/1

* number of ELISA positive samples / number of samples tested

used as healthy controls. At the end of the transmission period, all grapevines were sprayed with an insecticide, maintained in the greenhouse and observed weekly for leafroll symptoms. Four months after the end of the transmission period, all test plants were checked by ELISA for the presence of GLRaV-1, GLRaV-3 and GVA. ELISA tests were repeated after three and six months.

In 2003, no virus transmission was obtained when plants singly infected with GLRaV-3 were used as inoculum, but there was one case of GLRaV-3 transmission (Pinot noir seedling 'LR18/03') from source plants infected also with GVA (Table 1). None of the 39 plants inoculated became infected with GVA. In 2004 (Table 2) one Pinot noir rootling ('LR123/04'), used as recipient plant, was found infected with GLRaV-3 in the first ELISA done four months after the transmission period. The same Pinot noir rootling was also found to be infected with GVA, seven months after the inoculation period. In this case, the inoculum source was infected with GLRaV-1, GLRaV-3 and GVA. No transmission of GLRaV-1 was observed.

Transmission of GLRaV-3 and GVA was confirmed by ELISA repeated in the following months. Virus concentration in infected plants increased over time (Table 3): the absorbance values registered for GLRaV-3 in LR18/03 were 0.76 in August 2003, 0.77 in November 2003 and 0.83 in February 2004 (negative control average: 0.09), whereas in LR123/04, the values registered for GLRaV-3 were 0.25 in September 2004, 0.96 in December 2004 and 1.65 in February 2005 (negative control average: 0.10). In the same test plant, the values registered for GVA were respectively 0.17, 0.82 and 1.36 (negative control average: 0.16).

Mild reddening and rolling appeared about four months after exposure to the insects, on the basal leaves of the plants that were shown to be infected. Symptoms became more evident during the following months. No symptoms appeared on the other recipient plants or on healthy controls.

This study shows, for the first time, that *Heliococcus bohemicus* can transmit GVA from infected to healthy vines. The same mealybug can also transmit an Italian isolate of GLRaV-3, confirming the results obtained in

Table 2. Results of inoculations with GVA, GLRaV-1 and GLRaV-3 in 2004.

Inoculum source	Test plant	GLRaV-1			GLRaV-3			GVA		
		Sep 04	Dec 04	Feb 05	Sep 04	Dec 04	Feb 05	Sep 04	Dec 04	Feb 05
GLRaV-1	Riesling italico (seedlings)	0/4*	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
GLRaV-1	Barbera (rootlings)	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
GVA	Riesling italico (seedlings)	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
GLRaV-1 + GVA	Riesling italico (seedlings)	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
GLRaV-1 + GVA	Pinot noir (seedlings)	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
GLRaV-1 + GVA	Barbera (rootlings)	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
GLRaV-1 + -3	Riesling italico (seedlings)	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
GLRaV-1 + -3 + GVA	Riesling italico (seedlings)	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9
GLRaV-1 + -3 + GVA	Pinot noir (seedlings)	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
GLRaV-1 + -3 + GVA	Pinot noir (rootlings)	0/3	0/3	0/3	1/3	1/3	1/3	0/3	1/3	1/3
GLRaV-1 + -3 + GVA	Barbera (rootlings)	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
GLRaV-1 + -3 + GVA	Cabernet franc (rootlings)	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Healthy control	Riesling italico (seedlings)	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

* number of ELISA positive samples/number of samples tested

Table 3. Mean ELISA absorbance values (405 nm) obtained from plants inoculated in 2003 and 2004 with *Heliococcus bohemicus*, and from negative and positive controls (LR18/03 and LR123/04 indicate the infected vines).

2003	GLRaV-3			GVA		
	Aug 03	Nov 03	Feb 04	Aug 03	Nov 03	Feb 04
LR18/03	0.76	0.77	0.83	0.12	0.12	0.12
Healthy values average	0.11	0.08	0.11	0.13	0.12	0.12
Negative control*	0.10	0.07	0.11	0.12	0.15	0.13
Positive control*	1.66	2.34	2.79	0.57	2.08	1.20

2004	GLRaV-1			GLRaV-3			GVA		
	Sept 04	Dec 04	Feb 05	Sept 04	Dec 04	Feb 05	Sept 04	Dec 04	Feb 05
LR123/04	0.09	0.11	0.09	0.25	0.96	1.65	0.17	0.82	1.36
Healthy values average	0.10	0.12	0.10	0.10	0.10	0.10	0.12	0.13	0.18
Negative control*	0.11	0.12	0.10	0.10	0.11	0.09	0.15	0.13	0.21
Positive control*	3.00	2.91	2.53	2.93	1.86	1.96	1.27	1.10	2.13

* from Agritest ® (Bari, Italy).

France by Sforza and Greif (2000).

In all cases of positive transmission, the inoculum contained GVA in association either with GLRaV-1 or GLRaV-3, or both. This might suggest a role of GVA as a helper in insect transmission of these viruses, as was observed by Fortusini *et al.* (1997), when the scale insect *Neopulvinaria innumerabilis* was able to transmit GLRaV-1 only in presence of GVA. The small size (the adult is about as big as a pinhead) and stylet fragility of *H. bohemicus* was a serious obstacle to their handling during the passages from plant to plant. Hence, some individuals could have lost the ability to feed and thus to transmit the viruses. This fact may explain the low percentage of virus transmission obtained in this work. Similar results were obtained by Sforza and Greif (2000) with the same insect species, although they were able to transmit GLRaV-1.

The frequent occurrence of leafroll in vineyards where large populations of *H. bohemicus* and other mealybug species, known to be vectors of GLRaV-3, were found, may be directly correlated with the capacity of mealybugs to transmit the virus.

The results of this work underline the need for controlling mealybug populations and intensifying diagnostic tests of rootstock material. Particularly important is the protection in insect-proof screenhouses of the mother plants used for propagating clonal material to keep them free from viral diseases.

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