# Closteroviruses and grapevine diseases: a review of the situation before the establishment of the network

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# Closteroviruses and grapevine diseases: a review of the situation before the establishment of the network

**M. Digiaro [1], R. Garau [2], V. Savino [3]**

*[1] Istituto Agronomico Mediterraneo, Valenzano-Bari; [2] Istituto di Patologia Vegetale, Università degli Studi, Sassari; Italy [3] Dipartimento di Protezione delle Piante, Università degli Studi and Centro di Studio del CNR sui Virus e le Virosi delle Colture Mediterranee, Bari Italy*

**SUMMARY** - A brief account is given of the knowledge on leafroll and rugose wood diseases that preceded the implementation of the network (1993). The paper reviews the biological, physico-chemical and epidemiological properties of closteroviruses and vitiviruses, the putative etiological agents of the two diseases, and the diagnostic tools available for their detection. An updated picture of the current knowledge on phloem-limited viruses of grapevine concludes the paper.

**Key words**: grapevine, rugose wood, leafroll, closteroviruses, vitiviruses

***RESUME -*** *On passe en revue brièvement les connaissances relatives aux maladies de l'enroulement foliaire et du bois strié avant la réalisation du réseau (1993). Les propriétés biologiques, physico-chimiques et épidémiologiques des closterovirus et des vitivirus, probables agents étiologiques des deux maladies et les outils de diagnostic disponibles pour leur détection sont ici décrits. Pour conclure, on présente les informations les plus récentes concernant les virus du phloème de la vigne.*

***Mots-clés****: vigne, bois strié, enroulement foliaire, closterovirus, vitivirus.*

## Introduction

The very first indication that closteroviruses could infect grapevines was provided by Mengden (1971) who observed accumulations of filamentous virus-like particles in thin-sectioned sieve tubes of German grapevines. Eight years after Mengden's paper, which was overlooked at the time of its publication, another report from Japan gave more convincing evidence that closteroviruses were indeed associated with diseased grapevines (Namba *et al.*, 1979). Ever since, the number of closterovirus records in *Vitis* increased steadily so that, in 1990 about 50 major contributions existed (Gugerli, 1991).

Although much information was gathered and the relationship between closteroviruses and grapevine diseases was being unravelled, a lot of ground remained to be covered for the ultimate understanding of the etiological role of these viruses and their epidemiology.

At the starting of the network, nine serologically distinct closteroviruses had been identified and partially characterised in diseased grapevines. Six such viruses had particles above 1500 nm in length ("long" closteroviruses) and three particles about 800 nm long ("short" closteroviruses, now included in the genus *Vitivirus*). All long closteroviruses, but one, were reported to occur in leafroll-infected vines and were named accordingly, i.e. grapevine leafroll-associated viruses I to V (GLRaV I-V). One long (grapevine corky bark associated virus - GCBaV) and three short closteroviruses (grapevine viruses A, B and C - GVA, GVB and GVC) were suspected to induce one or more syndromes of the rugose wood complex disease.

Encouraging perspectives in the study of these filamentous viruses were given by: (1) the progresses in electron microscopy that allowed the observation of virus particles directly in grapevine tissues (Namba *et al.*, 1979; Faoro *et al.*, 1981; Castellano *et al.*, 1983); (2) the transmission of some short closteroviruses to herbaceous hosts (Conti *et al.*, 1980; Boscia *et al.*, 1993), in particular using *in vitro* grown explants as inoculum (Monette *et al.*, 1990; Monette and James, 1991); (3) the successful purification of closteroviruses starting from grapevine infected phloem tissues (Gugerli *et al.*, 1984); (4) the improvement of the procedures of dsRNA extraction from grapevine (Mossop *et al.*, 1985; Rezaian and Krake, 1987; Rezaian *et al.*, 1991); (5) the advent of molecular biology in the characterisation and diagnosis of plant viruses (Matthews, 1991).

In this presentation, an attempt is made to summarise the state of the art, before the establishment of the network, on the two diseases thought to have a closterovirus aetiology, highliting the still unsolved questions.

## The diseases

### *Leafroll*

Leafroll is a long-known graft-transmissible disease which is fully symptomatic in *Vitis vinifera*, especially in the red-berried cultivars, and symptomless in American *Vitis* species and their hybrids, commonly used as rootstocks.

*Field symptoms*. Affected vines may be smaller than normal and express symptoms consisting of downward rolling of the leaves, accompanied by reddish-purple or yellow discoloration of the blades, according to whether the vines are red- or white-berried. Discoloured areas appear in the interveinal spaces of the lower leaves in early summer, becoming progressively stronger and extended so as to cover the whole foliar surface. The main veins may or may not retain the green colour in the advanced stages of the disease and there is a difference in the hue, intensity and distribution of the reddish pigmentation over the leaf surface. When the discoloration is particularly evident, necrotic areas may develop in the interveinal tissues. Ripening of the fruits is affected, bunches being smaller than normal and berries remaining greenish or whitish at harvest time

*Symptoms on indicators*. On the red-berried varieties commonly used as indicators (Cabernet franc, Cabernet sauvignon, Pinot noir, Mission and Barbera) the symptoms are pretty much the same as those shown by diseased vines in the field.

*Economic importance*. Losses are estimated to be around 20% in yield reduction (Goheen, 1988), with peaks up to 70%. The rooting ability is decreased as well as the sugar content of the berries. The damage persists for the whole duration of the vine's life.

### *Rugose wood*

Rugose wood is a graft-transmissible disease of *Vitis vinifera* and other *Vitis* species and hybrids (American rootstocks) first detected in Southern Italy and described under the name of "legno riccio" (Graniti and Ciccarone, 1961), but now recognised to have a world-wide distribution.

*Field symptoms*. The disease is specifically characterised by modifications of the woody cylinder. In general, affected vines may be undersized, less vigorous than normal and show delayed bud opening in spring, some decline and die within a few years from planting. Grafted vines often show a swelling above the bud union and a marked difference between the relative diameter of scion and rootstock. With certain cultivars, the bark of the scion above the graft-union is exceedingly thick and corky, has a spongy texture and a rough appearance. The woody cylinder shows typical pits and/or grooves which correspond to peg and ridge-like protrusions on the cambial face of the bark. These alterations may occur on

the scion, rootstock or both according to the cultivar/rootstock combination and, perhaps, individual susceptibility. In most cases no specific leaf symptoms are observed, but bunches may be smaller and fewer than normal. Certain cultivars may show leaf alterations similar to those induced by leafroll, i.e. rolling, yellowing or reddening of the leaf blades. These symptoms, when they occur, are more severe than those induced by ordinary forms of leafroll. Symptomless field infections may occur.

*Symptoms on indicators*. There is mounting evidence that rugose wood is a complex disease in whose frame four different disorders can be recognised and sorted by graft-transmission to three indicators: *Vitis rupestris*, Kober 5BB and LN 33 (Savino *et al.*, 1989):

(i) Rupestris stem pitting. In *V. rupestris* it induces a distinct basipetal pitting limited to a band extending downwards from the point of inoculation. LN 33 and Kober 5BB are symptomless.

(ii) Corky bark. Elicits grooving and pitting in all parts of the stem of *V. rupestris* and LN 33, but not in Kober 5BB. Furthermore, it induces proliferation of secondary phloem tissues of LN 33 giving rise to most typical internodal swellings with a cracked surface. Infected LN 33 indicators are severely stunted and show early rolling and reddening of the leaves. Sometimes, irregular yellow spots appear on the leaves of the spring flush. The canes ripen irregularly or not at all, and the vines may die within a year.

(iii) Kober stem grooving. Induces a marked grooving on the stem of Kober 5BB but is symptomless in *V. rupestris* and LN 33.

(iv) LN 33 stem grooving. It is characterised by extended grooves on the stem of LN 33 similar to those of corky bark, which, however, are not accompanied by internodal swellings and leaf discoloration. *V. rupestris* and Kober 5BB are symptomless.

*Economic importance*. Rugose wood is a disease of economic relevance. On the most sensitive graft-combinations it causes decline and death of the vines. Decline may cause up to 50% of yield reduction (Garau *et al.*, 1985), mainly when wood pitting affects both scion and rootstock (Savino *et al.*, 1985). Rupestris stem pitting is reported to affect adversely the growth of European grapes (Goheen, 1988) and corky bark can induce severe crop losses (up to 70%) and shortening of the longevity of the vines (Teliz *et al.*, 1980, Tanne *et al.*, 1991).

## Aetiology

The consensus was that all "long" closteroviruses, except one (GCBaV), were involved in the aetiology of leafroll, but whether the causal agents were also those with short particles (e.g. GVA) remained to be established. The high association of LR with the presence of long closteroviruses was repeatedly reported in field and in experimentally inoculated indicator vines (Gugerli *et al.*, 1984; Rosciglione and Gugerli, 1986; 1989; Zimmermann *et al.*, 1988;

Tanne *et al.,* 1989; Gugerli, 1991). The absence of GLRaV-1, GLRaV-2 and GLRaV-3 and of LR symptoms in LR affected vines after heat treatment provided additional evidence of the role played by these three viruses in the aetiology of LR disease (Gugerli *et al.,* 1984; Zimmermann *et al.,* 1990; Gugerli *et al.,* 1991). The role of GLRaV-3 in LR disease was further supported by the experimental transmission of the virus together with typical LR symptoms from grapevine to grapevine by *Planococcus ficus* (Rosciglione and Gugerli, 1986; Engelbrecht e Kasdorf, 1990) and *Pseudococcus longispinus* (Tanne *et al.,* 1989).

American *Vitis* rootstocks and hybrids are tolerant to LR disease (Goheen, 1970), and a low concentration of closterovirus particles in the phloem tissues can be observed (Boscia *et al.,* 1991).

Differently by the other long closterovirus, GCBaV seemed to be involved in corky bark (Namba *et al.*, 1991).

The available data accredited the possible role of "short closteroviruses" in the aetiology of RW disease.

Few years after its first report in RW infected plants (Conti *et al.,* 1980), GVA appeared to be distributed world-wide. Its exact role in the aetiology of grapevine diseases remained controversial since it was also repeatedly detected in LR diseased vines (Rosciglione *et al.*, 1983; Conti and Milne, 1985; Engelbrecht and Kasdorf; 1985 Engelbrecht and Human, 1989; Agran *et al.*, 1990; Monette *et al.*, 1990).

A substantial evidence of the etiological role of GVA in RW disease was given by heat treating LR and RW diseased vines in which GLRaV-1, GLRaV-3 and GVA had been detected. After the treatment GLRaV-1 and GLRaV-3 and the LR symptoms were eliminated, but not GVA and RW symptoms (Gugerli *et al.,* 1991).

## The viruses

### *Long closteroviruses*

Significant progresses in the knowledge of grapevine closteroviruses followed the development of purification procedures by using grapevine phloem tissues (Gugerli *et al.*, 1984). The morphological characteristics of the viruses were determined and specific antisera were produced. It was possible to assess the distribution of these viruses in grapevine and their serological differences (Gugerli *et al.,* 1984; Rosciglione and Gugerli, 1986). In a few years the closteroviruses type 1 and 2 in Switzerland (Gugerli *et al.,* 1984), type 3 in Italy (Rosciglione and Gugerli, 1986) and USA (Zee *et al.,* 1985; 1987), type 4 in California (Hu *et al.,* 1990) and type 5 in France (Walter and Zimmermann, 1991) were successfully identified and characterised.

The similarity in physico-chemical characteristics justified their grouping in the same genus: the length, between 1400 and 2200 nm, outward appearance, filamentous and flexuous, and the virus structure, as it appeared in the electron microscope, open and with cross banding. In particular, the length of virus particles was estimated to be 1800-2200 nm for GLRaV-1 and GLRaV-2 (Gugerli *et al.,* 1984), 1800-2100 in GLRaV-3 (Zee *et al.,* 1987; Hu *et al.,* 1990; Gugerli *et al.,* 1991; Walter and Zimmermann, 1991), 1400-1700 nm for GLRaV-5 (Walter and Zimmermann, 1991), whereas it was not determined for GLRaV-4, because of the low concentration in host tissues and the difficulty of purification (Hu *et al.,* 1990).

Grapevine closteroviruses were characterised by an unusual high molecular weight of the coat protein, ranging from 35 to 43 kDa instead of 22-27 kDa, typical of members of the same genus (Milne, 1988). An exception was GLRaV-2, which showed two different values of 26 kDa (Zimmermann *et al.,* 1990; Walter and Zimmermann, 1991) and 36 kDa (Gugerli *et al.,* 1990b; Boscia *et al.,* 1990). GLRaV-3 and the serological related "NY-1" isolate (Rosciglione and Gugerli, 1989; Zimmermann *et al.,* 1990), presented the highest Mol. wt. of coat protein (43 kDa) (Hu and Gonsalves, 1988; Hu *et al.,* 1990; Zimmermann *et al.,* 1990; Gugerli *et al.,* 1990b), followed by GLRaV-1, (38-39 kDa) (Zimmermann *et al.,* 1990; Gugerli *et al.,* 1990b; Walter and Zimmermann, 1991) and GLRaV-5, (36 kDa) (Walter and Zimmermann, 1991) .

The only information available on the characteristics of viral nucleic acids concerned GLRaV-3 whose heaviest dsRNA band was 20 kbp in size (Hu and Gonsalves, 1988; Hu *et al.,* 1990).

A new closterovirus, 1400-2000 nm long, was isolated in corky bark affected vines, for which the name "*grapevine corky bark-associated virus*" (GCBaV) was proposed (Namba *et al.,* 1991a). The Mol. wt. of its coat protein was 24 kDa and the dsRNA was 15,3 kbp in size (Namba *et al.,* 1991a).

### *Short closteroviruses*

These viruses showed a particle structure similar to that of long closteroviruses, but differed for the shorter length of their particles (725 - 825 nm) and for the ability to infect herbaceous plants, though with difficulty.

Detailed information was available for GVA (initially named "*grapevine stem pitting-associated virus*" - GSPaV) the first of short closteroviruses of grapevine (Conti *et al.,* 1980), but not for GVB and GVC (Namba *et al.,* 1991a; Monette and James, 1991). All these three viruses had been detected in RW affected vines.

The herbaceous host range of GVA included solanaceous species *(Nicotiana benthamiana, N. clevelandii, N. occidentalis, N. cavicola e N. megalosiphon*) in which the virus induced dwarfing, leaf malformation and vein clearing (Conti *et al.,* 1980; Rosciglione *et al.,* 1983;

Castrovilli and Gallitelli, 1985; Monette *et al.,* 1990). Based on *N. benthamiana* symptomatology, at least two different serologically identical GVA strains were distinguished (Monette and James, 1990). Serological variants were, however, reported in Switzerland (Gugerli *et al.,* 1991). GVA particles were 800 x 12 nm long (Conti *et al.,* 1980); the Mol. wt. of the coat protein ranged from 22 (Boccardo and D'Aquilio, 1981) to 27 kDa (Monette and Green, 1992), whereas that of RNA was 2,55 x $10^3$ kDa (Boccardo and D'Aquilio, 1981).

GVB was first detected in a corky bark (CB) affected vine cv. Semillon. It was similar to GVA in size and length, but not serologically (Namba *et al.,* 1991a).

GVC was detected in CB and LR affected Semillon vines. It was isolated in *N. benthamiana* plants by inoculating extracts from *in vitro* growing explants. *N. benthamiana* infected plants showed necrotic local lesions, systemic vein necrosis, wilt of apical leaves, before dying. Fainter symptoms were observed in *N. occidentalis*, consisting in chlorotic mottle among veins. GVC (725 x 10 nm) was not serologically related to any of the closteroviruses known (Monette and James, 1991).

## Epidemiology

### *Leafroll*

The natural spread of LR disease was first reported in a vineyard of cv. Gamay in Yugoslavia (Dimitrijevic, 1973), then in South Africa (Engelbrecht and Kasdorf, 1985) and in Mexico (Teliz *et al*., 1987).

Experimental evidence of the natural spread of LR was given by planting 100 healthy LN33 indicators in a LR diseased vineyard of cv. Tinta Barocca (Engelbrecht and Kasdorf, 1990). In this test the first symptoms on indicators appeared 2-3 years after planting, but they developed on 71% of the vines after 7 years. GLRaV-3 was detected in all symptomatic plants.

The role of pseudococcid mealybugs as vectors of the disease was suggested by the appearance of LR symptoms on healthy LN33 and *V. vinifera* plants in a greenhouse infested by *Pseudococcus longispinus*. Further experiments with LR and RW donor plants demonstrated the role of this vector in disease transmission (Rosciglione *et al*., 1983; Tanne *et al*., 1989). In other experimental tests, also *Planococcus ficus* was able to transmit LR symptoms and GLRaV-3 from grapevine to grapevine (Rosciglione and Gugerli, 1989), and GVA and GLRaV-3 but not GLRaV-1 and GLRaV-2. This latter virus was detected only in the vector (Engelbrecht and Kasdorf, 1990b).

### *Rugose wood*

The first indication of the natural spread of RW in the field was given by Teliz *et al.* (1980). The role of mealybugs in vectoring the disease was later demonstrated by Rosciglione *et al.* (1983) and by Rosciglione and Castellano (1985). In this latter test GVA was transmitted by LR and RW diseased vines of cv. Inzolia to *N. clevelandii* by *P. longispinus* and *Planococcus citri.* The same virus was also transmitted by *P. ficus* from LR diseased vines to *N. clevelandii* in South Africa (Elgelbrecht e Kasdorf, 1985). In Israel, the same vector induced CB symptoms on LN33 indicators 13 months after inoculation (Tanne *et al.*, 1989). The natural spread of GVA and the appearance of CB symptoms on LN33 was also reported in a vineyard of cv. Tinta Barocca infested by *P. ficus* (Elgelbrecht and Kasdorf, 1990b).

## Diagnosis

GVA was the first "closterovirus" to be characterised (Conti *et al.*, 1980; Boccardo and D'Aquilio, 1981) Notwithstanding the possibility of growing GVA in herbaceous hosts, the polyclonal antisera obtained could not be used for large scale analysis by ELISA tests, because of the low immunogenicity and the irregular distribution of the virus in grapevine tissues, at low concentration.

After the successful extraction and purification of closteroviruses from grapevine phloem tissues (Gugerli *et al.,* 1984), numerous polyclonal antisera were produced in many laboratories all over the world (Gugerli *et al.*, 1984; Rosciglione and Gugerli, 1986; Zee *et al.,* 1985; Hu *et al.*, 1990; Walter and Zimmermann, 1991; Namba *et al.*, 1991). The serology applied to electron microscopy (IEM) and ELISA permitted to distinguish at least six different long closteroviruses (Gugerli, 1991). Related to the symptoms they were associated with, five were named *grapevine leafroll-associated closteroviruses* from I to V (GLRaV I to V), and one was named grapevine corky bark associated virus (GCBaV).

Late summer and autumn were recognised as the best period for diagnosis of closteroviruses since their concentration in grapevine tissues increases with seasonal vegetative advancing (Teliz *et al.,* 1987). Cortical scrapings were the best tissue-sources for the diagnosis, chiefly when American *Vitis* species and their hybrids had to be tested. Closteroviruses (GLRaV-3, in particular), in fact, could hardly be detected by ELISA and ISEM in foliar extracts of American rootstocks, especially *V. rupestris* and its hybrids (Boscia *et al.,* 1991; Credi and Santucci, 1991).

Mixtures of different antibodies were successfully used to detect simultaneously groups of viruses in the same test (Zimmermann, 1990; Hu *et al.*, 1991). Furthermore, the sensitivity of ELISA was improved by amplifying virus detection by byotin and streptavidin

(Zimmermann, 1990; Hu *et al.*, 1991). These procedures allowed to detect closteroviruses earlier than with other DAS-ELISA procedures (Zimmermann, 1990).

The first monoclonal antibodies had meanwhile been obtained for GLRaV I (Gugerli, 1987), GLRaV III (Hu e Gonsalves, 1988; Gugerli *et al.*, 1990; Walter and Zimmermann, 1991) and GVA (Gugerli *et al.*, 1990).

Molecular diagnosis of grapevine closteroviruses was hindered by difficult in obtaining suitable nucleic acid preparations, free by host plant constituents (polyphenols, polysaccarids, tannins) interfering with molecular hybridisation and/or enzymatic reactions (Rezaian *et al*, 1987; Rohwani *et al.*, 1993).

The presence of different dsRNA bands (genomic and subgenomic) was repeatedly reported in phloem tissues of LR-infected grapevines (from 0,8 to 15 kbp) (Mossop *et al.*, 1985; Cameron and Walter, 1985; Rezaian and Krake, 1987; Hu and Gonsalves, 1988; Monette *et al.* 1989). Similarly, a dsRNA band of *c.* 15kbp and one or two of *c.* 5kbp were respectively detected in GCBaV and in "Rupestris stem pitting" infected grapes (Namba *et al*, 1991; Azzam *et al*, 1991; Walter and Cameron, 1991).

Unfortunately, the analysis of dsRNA patterns did not permit to identify the virus species for the high frequency in nature of mixed infections and the different influence of the host (species o varieties, age of the plant) on the electrophoretic profile (Monette *et al.*, 1989).

The possibility to obtain purified GVA-RNAs by *Nicotiana* plants permitted to construct radio-labelled cDNAs by random priming (Gallitelli *et al.*, 1985). This primer, that was not cloned, successfully hybridised GVA-infected grapevine extracts on nitro-cellulose membranes. This was the first application of molecular hybridisation in the diagnosis of grapevine viruses.

## Grapevine phloem-limited viruses: state of the art

A summarized updated picture on the current knowledge on grapevine phloem-limited viruses is shown in the following table.

***Tab. 1.*** Main properties of phloem-limited grapevine viruses

| **Viruses** | References | **Length of particles (nm)** | C.P. Mol. wt **(Kda)** | RNA **(Kb)** |
|---|---|---|---|---|
| **CLOSTEROVIRUSES** | | | | |
| GLRaV-1 *(gr. leafroll associated virus – 1)* | Gugerli *et al.,* 1984 | 1800-2200 | 38-39 | N.D. |
| GLRaV-2 | Gugerli *et al.,* 1984 | 1800-2000 | 24-26 | 15-19* |
| • GLRaV-2 | Zimmermann *et al.,* 1990<br>Zhu *et al.*, 1998<br>Abou-Ghanem *et al.,* 1998 | | | 15* |
| • GLRaV-2b | Gugerli and Ramel, 1993 | | | |
| • GCBaV | Namba *et al.,* 1991 | | | |
| GLRaV-3 | Gugerli *et al.,* 1984<br>Ling *et al.,* 1998 | 1800-2100 | 43-44 | 19-20* |
| • NY-1 | Zee *et al.,* 1987 | | | |
| GLRaV-4 | Hu *et al.,* 1990 | 1800 | 35-36 | N.D. |
| GLRaV-5 | Zimmermann *et al.,* 1990<br>Walter and Zimmermann, 1991 | 1400-1700 | 35 | N.D. |
| GLRaV-6 | Boscia *et al.,* 1995,<br>Gugerli *et al.*, 1997 | 1800 | 36 | N.D. |
| • GLRaV-2a | Gugerli and Ramel, 1993 | | | |
| GLRaV-7 | Choueiri *et al.,* 1996 | 1500-1700 | 37 | 15* |
| **VITIVIRUSES** | | | | |
| GVA *(gr. virus A)* | Conti *et al.,* 1980<br>Minafra *et al*., 1998 | 700-825 | 22,5 | 7.349 |
| GVB *(gr. virus B)* | Boscia *et al.,* 1993<br>Saldarelli *et al.*, 1997 | 800 | 22,5 | 7.598 |
| GVC *(gr. virus C)* | Monette and James, 1991 | 725 | 25,7 | N.D. |
| GVD *(gr. virus D)* | Bonavia *et al.,* 1996;<br>Abou-Ghanem *et al.*, 1997 | 825 | 20,5 | 7.6 |
| GBINV *(gr. berry inner necrosis virus)* | Yanase, 1985<br>Yoshikawa *et al.,* 1997 | 600-700 | 22 | 7.6 |
| **FOVEAVIRUSES** | | | | |
| GRSPaV *(gr. rupestris stem pitting associated virus)* | Zang *et al.*, 1998 | | | 8.725 |
| GRSPaV-1 | Meng *et al.,* 1998 | | | 8.726 |
| **FLECK-LIKE VIRUSES** | | | | |
| GFkV *(gr. fleck virus)* | Boulila *et al.*, 1990<br>Sabanadzovic *et al.*, 1997 | 30 | 28 | 7.4-8.8 |
| GAaV *(gr. ajinashika associated virus)* | Namba *et al.*, 1986 | 25 | 23 | 6.8 |
| GAMaV *(gr. asteroid mosaic associated virus)* | Boscia *et al.*, 1994 | 30 | N.D. | N.D. |
| GSaV *(gr. stunt associated virus)* | Namba *et al.*, 1986 | 25 | | |

* Kbp of dsRNA (x 1000)

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