Mealybug transmission and natural spread of grapevine closterovirus in Cyprus

Ioannou N., Hadjinicolis A., Hadjinicoli A.

in

Martelli G.P. (ed.), Digiaro M. (ed.).
Proceedings of the Mediterranean network on grapevine closteroviruses 1992-1997 and the viroses and virus-like diseases of the grapevine a bibliographic report, 1985-1997

Bari : CIHEAM
Options Méditerranéennes : Série B. Etudes et Recherches; n. 29

1999
pages 93-101

Mealybug transmission and natural spread of grapevine closterovirus in Cyprus

N. Ioannou, A. Hadjinicolis, A. Hadjinicoli

Agricultural Research Institute, Nicosia, Cyprus

SUMMARY - Research activities carried out in Cyprus within the framework of MNGC concerned primarily the epidemiology of grapevine leafroll - associated closterovirus type lll (GLRaV III), which is widespread in Cyprus. Since 1991 the incidence of this virus has been monitored by ELISA in four variety collections grown in the open field at various sites in the southern coast of the island. The results prove beyond any doubt that the virus is spreading naturally by means other than infected propagating material. The rate of spread varies widely, depending on the site, grapevine cultivar and year. The highest rate of spread was noted at Zigi Experimental Station, where virus incidence increased from 19.7% in 1991 to 67.4 % in 1995, and the lowest at Kornos, where virus incidence remained around 5% in all years. Among different years, the biggest increase in virus incidence occurred from 1992 to 1993 and the smallest from 1993 to 1994. Virus incidence was much higher in *V. vinifera* varieties than in American rootstocks. The most important primary source of inoculum appeared to be the greenhouse used for the production of rootlings for transplanting in the field. Plants grown in the greenhouse were heavily infested by mealybugs (Pseudococcidae), namely *Planococcus ficus* and *P. citri.* Transmission tests in the laboratory showed that GLRaV III was readily transmitted to healthy grapevine plants by both *P. ficus* and *P. citri.* The former vector appeared to be much more efficient than the latter. As in the field studies, the levels of virus transmission recorded in American rootstock varieties were much lower than in vinifera varieties. This varietal difference in the degree of virus transmission by mealybugs is being pursued further by laboratory and field studies.

Some work was also carried out with corky bark in order to assess the possibility of its transmission to healthy vines by *P. ficus* and *P. citri.* Work is also carried out with Rupestris stem pitting which appears to be naturally spreading under field conditions. The possible role of mealybugs in this spread is being investigated but results so far are inconclusive.

Key words: Grapevine, Cyprus, leafroll, epidemiology, mealybugs

RESUME *- Les activités de recherche menées à Chypre dans le cadre du MNGC ont essentiellement porté sur l'épidémiologie du closterovirus type III associé à l'enroulement foliaire de la vigne (GLRaV III), qui est largement répandu à Chypre. Depuis 1991, l'incidence de ce virus a fait l'objet d'un monitorage par ELISA dans quatre collections variétales élevées au plein champ dans différents endroits de la côte du sud de l'île. Les résultats montrent indubitablement que le virus est disséminé naturellement par des voies autres que le matériel de multiplication infecté. Le taux de diffusion varie considérablement selon les endroits, les cultivars et l'année. Le taux de diffusion le plus élevé a été enregistré à la Station Expérimentale de Zigi, où l'incidence du virus a augmenté de 19,7% en 1991 à 67,4% en 1995, alors que le plus faible a été estimé à Kornos, où l'incidence du virus a demeuré égale à environ 5% pendant les mêmes années. Au fil des années, l'augmentation la plus sensible de l'incidence du virus s'est produite entre 1992 et 1993 et la moins sensible entre 1993 et 1994. L'incidence du virus a été de loin plus élevée chez les variétés de Vitis vinifera que chez les porte-greffes américains. La principale source d'inoculum a été la serre utilisée pour la production de plants racinés destinés à être transplantés au champ. Les plants élevés en serre étaient infestés dans une grande mesure par les cochenilles pseudococcidés, à savoir le Planococcus ficus et le Planococcus citri. Les tests de transmission au laboratoire ont révélé que le GLRaV III était facilement transmis à des plants de vigne sains par P. ficus et par P. citri. Le premier vecteur s'est avéré beaucoup plus efficace que le deuxième. Comme démontré par les enquêtes au champ, le taux de transmission du virus sur les porte-greffes américains était plus faible que chez les variétés de V. vinifera. La différence variétale en termes de degré de transmission du virus par les cochenilles pseudococcidés a été mise en évidence par les études au laboratoire et au champ.*

Par ailleurs, on s'est aussi intéressé à l'écorce liégeuse afin d'examiner la possibilité de transmission à des vignes saines par Planococcus ficus et Planococcus citri. Des travaux ont aussi été réalisés sur le rupestris stem pitting qui semble se répandre naturellement dans des conditions de champ. Le rôle possible des cochenilles pseudococcidés dans cette diffusion fait l'objet de certaines études qui, cependant, jusqu'à présent, n'ont pas encore produit de résultats concluants.

Mots-clés*: vigne, Chypre, enroulement foliaire, épidémiologie, cochenille pseudococcidé*

Introduction

During the 1st MNGC Meeting, which was held in Bari, Italy from 9 to 12 December 1992, it was decided that the participation of Cyprus in the Network should be concentrated primarily on the epidemiology of GLRaV III, a study which had already been initiated by the Agricultural Research Institute. In agreement with this decision, most of the work carried out in Cyprus in the context of MNGC since 1992 concerns GLRaV III whereas work on other closterovirus related diseases, like Rupestris stem pitting and corky bark, has been rather limited.

The particular research activities in connection with each of the above three diseases are outlined in Tab. 1. Results obtained so far from these studies are summarised in this report.

Table 1. Research activities of the Cyprus Agricultural Research Institute within the context of MNGC

1. ***Epidemiology of GLRaV III***	• Monitoring natural spread in different vine cultivars and sites • Experimental transmission tests with *Planococcus ficus* • Experimental transmission tests with *Planococcus citri* • Mealybug *vs* graft-inoculation of *V. vinifera* and rootstock cultivars (lab and field tests) • ELISA detection in rootstock cultivars: direct *vs* vinifera grafting
2. ***Corky bark***	• Experimental transmission tests with *P. ficus* and *P. citri* • Detection with ELISA and biological indexing
3. ***Rupestris stem pitting***	• Monitoring natural spread in the field • Mealybug transmission tests in the field

Grapevine leafroll-associated virus (GLRaV III)

Leafroll is the most widespread virus disease of grapevines in Cyprus and it has been detected in virtually all introduced and traditional varieties, at an average incidence of about 80% and 45%, respectively. Although its aetiology is still not fully established, a number of different closteroviruses have been associated with the disease in recent years. In Cyprus, four different types of GLRaV, designated I, II, III and IV, have been identified. Of these, the most prevalent appears to be GLRaV III, which was detected in over 30% of the grapevine specimens tested.

The widespread distribution of leafroll disease and GLRaV III led us to investigate the possibility of natural spread of this virus by means other than infected propagating material, namely by an efficient air-born vector. These investigations comprised both field studies on

the epidemiology of the disease, in particular its rate and pattern of spread, and transmission tests under controlled conditions in the laboratory-greenhouse (Tab. 1).

Epidemiological studies in the field. The first indication that this virus was spreading naturally in Cyprus by means other than propagating material were obtained in the late 1980's, when clean stocks introduced from the University of California, Davis or produced locally became infected after having grown for a few years in a greenhouse in Nicosia or in variety collections in the open field.

In order to further substantiate the natural spread of GLRaV III, since 1991 we have been monitoring virus incidence in three variety collections situated at Kornos, Zigi and Akhelia. Each year all vines in these collections are tested at least once with ELISA to determine the rate and pattern of GLRaV III spread. The results so far could be summarised as follows (Tab. 2 and 3):

a) At Kornos the virus was detected in only five out of 53 cultivars. The average virus incidence was 4.9% and remained unchanged since 1991, indicating lack of natural spread. This collection was planted in 1985-87, using cuttings introduced directly from the University of California, Davis.

b) By contrast, virus incidence determined at Zigi was much higher and increased from about 20% in 1991 to 30% in 1992, 48% in 1993, 57% in 1994 and 67% in 1995, suggesting the involvement of an efficient natural vector. Out of about 60 varieties grown in this collection only 17 (mostly American rootstock varieties) remained apparently free of GLRaV III. The apparent lack of infection in rootstock varieties like *V. rupestris* St. George and other American species and their hybrids is being further investigated. It must be noted that the Zigi plantation was established in 1989 using propagating material from Kornos (at least for the 53 out of 60 varieties).

c) High levels of infection and a definite year-to-year increase of GLRaV III incidence was also determined in a collection of 6 indicator varieties maintained at Akhelia (Old Station). Thus, infection increased from 44% in 1991 to 51%, 60%, 62% and 68% in 1992, 93, 94, and 95, respectively. Despite these high levels of infection, *V. rupestris* St. George remained again almost free of infection, as indicated by ELISA tests on petioles and phloem tissue from mature canes. The levels of infection determined for each variety during 1991-95 are summarised in Tab. 4.

d) In 1993 a new indicator variety collection was established at the New ARI Station at Akhelia using propagating material free of GLRaV III. The cuttings were rooted and grown in an insect-proof screenhouse to prevent infection of the transplants in the nursery, which appears to be a major source of primary inoculum. No infections were

detected in this plantation during 1994 and 1995, indicating the importance of using healthy propagating material in the establishment of a new vineyard.

Table 2. Incidence of type III of grapevine leafroll-associated closterovirus (GLRaV III) in four grapevine plots during 1991 -95.

Site	% GLRaV III infection determined by ELISA				
	1991	**1992**	**1993**	**1994**	**1995**
Kornos	4.9	4.9	4.9	4.9	4.9
Zigi	19.7	29.7	48.4	56.6	67.4
Akhelia (old station)	43.6	51.0	60.3	61.5	67.8
Akhelia (new station)	-	-	-	0	0

Table 3. Number of grapevine cultivars found infected by GLRaV III at different sites

Site	Number of varieties	
	Total	**Infected**
Kornos	53	5
Zigi	60	43
Akhelia (old station)	6	6
Akhelia (new station)	6	0

Table 4. Natural spread of GLRaV III in six vine cultivars at Akhelia Experimental Station during 1991-95 (%infected plants)

Cultivar	1991	1992	1993	1994	1995
Cabernet Franc	33	47	63	66	73
Mission	70	73	83	83	83
Propupac	56	63	73	73	80
LN-33	40	57	73	73	83
Baco	60	63	67	67	80
V. rupestris	3	3	3	7	7

The pattern of year-to-year spread within the Zigi plot is illustrated in Fig. 1. This pattern frequently appears to involve neighbouring vines, implicating the involvement of a slow-moving vector such as mealybugs. So far, however, no detailed epidemiological studies have been conducted which would relate the severity of mealybug infestation to the rate of virus spread in the field.

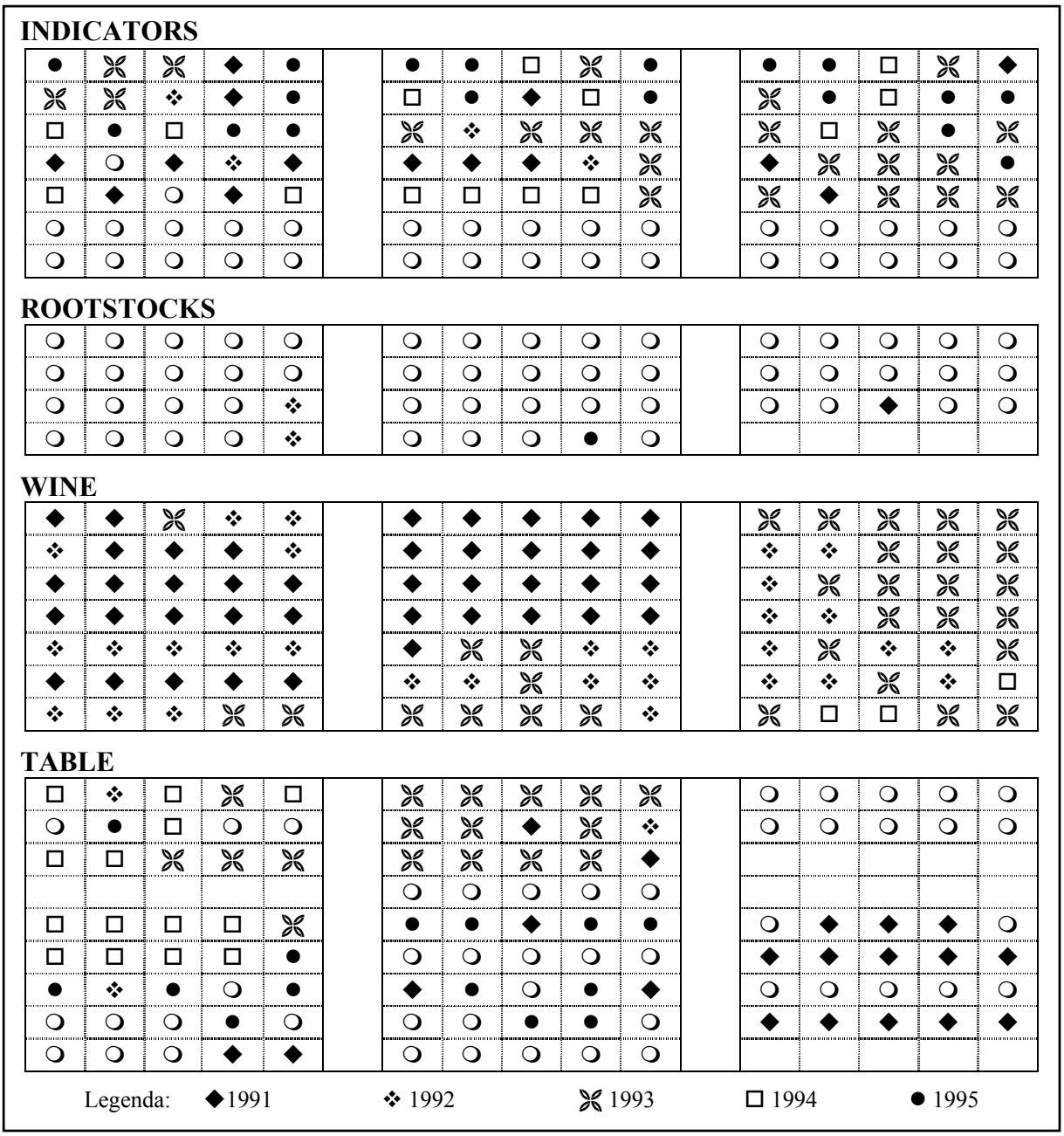

Fig. 1. Zigi collection: natural spread of GLRaV III in 1991-95

Mealybug transmission tests in the laboratory. In potted plants grown in the greenhouse, the spread of GLRaV III was associated with heavy infestation by the mealybug *Planococcus ficus* (Signoret). The ability of this mealybug to transmit GLRaV III to V. *vinifera cv.* Cabernet Franc and the hybrid LN-33 was verified with transmission tests carried out under controlled conditions in the laboratory-greenhouse (Tab. 5). These tests indicated that 25 insects per plant resulted in 35-75% transmission and 75 insects gave 100% transmission in both vine varieties. The tests indicate also that transmission to rootstock varieties V. *rupestris* St. George and 110 R was effected with difficulty by *P. ficus.* These results are in agreement with epidemiological data obtained from field studies and further implicate the mealybug *P. ficus* as a natural vector of GLRaV III.

Table 5. Experimental transmission of GLRaV III by *Planococcus ficus* in four grapevine cultivars.

N° insects per plant	**% plants infected (ELISA detection**			
	Cabernet Franc	**LN-33**	***V. rupestris* St George**	**110 R**
0	0	0	0	0
25	35	75	0	20
75	100	100	10	30

Tests with other mealybug species implicate also *P. citri* (Risso) as a potential vector of GLRaV III; even though its transmission efficiency appears to be lower than that of *P. ficus* (Tab. 6).

Table 6. Experimental transmission of GLRaV III by *Planococcus ficus* and *P. citri* in Cabernet Franc.

N° insects per plant	**% plants infected (ELISA detection)**	
	P. ficus	*P. citri*
0	0	0
25	35	7
75	100	45

Further tests with American rootstock varieties. During 1995 a series of tests were conducted to investigate further the apparent lack or very low levels of infection observed in American rootstock varieties, both under natural field conditions and in mealybug

transmission tests under controlled laboratory conditions. Results obtained towards the end of the year showed that American rootstock varieties were in some cases infected by GLRaV III but such infections were very difficult to detect by routine ELISA tests. Currently efforts are being made to improve the sensitivity of ELISA using various procedural modifications and also various grafting combinations with European varieties in which GLRaV III can be readily detected by ELISA.

Rupestris stem pitting and corky bark

Epidemiological studies and laboratory transmission tests were also conducted with two other closterovirus-related diseases, namely rupestris stem pitting and corky bark. The former is widely distributed in Cyprus and in several plots where its incidence is being monitored it appears to spread naturally by means other than infected propagating material. For example, in a plot at Akhelia (old Station) the incidence of rupestris stem pitting increased from 46% to 56% during 1993-1995. In order to investigate possible involvement of mealybugs various transmission tests are carried out under field conditions by enclosing infected vines with healthy potted plants in an insect-proof cage in which mealybug populations are artificially increased.

Corky bark is of limited distribution in Cyprus, even though in some other countries, like Mexico and Israel, it is very common and appears to spread naturally. Results from mealybug transmission tests carried out in the laboratory with this disease are still inconclusive.

Conclusions

Results presented in this paper indicate that GLRaV III and possibly other grapevine closteroviruses are spreading naturally in Cyprus by means other than infected propagating material. The role of mealybugs in the transmission of GLRaV III has been established where for other closterovirus-related diseases such as rupestris stem pitting and corky bark is still under investigation.

The rate of leafroll spread in some areas is alarmingly high whereas in other areas natural spread is hardly detectable. This points out the importance of microclimate in the development of mealybug infestation and virus spread (Tab. 7). Relative humidity appears to be a decisive factor and in this respect considerable virus spread is expected to occur in the irrigated table grapes of Cyprus grown along the south and south-western coastal plain of the island rather than in the rainfed wine grapes grown in mountainous areas. In this study severe mealybug infestation associated with natural spread of GLRaV III was observed in a greenhouse/nursery possibly due to favourable climatic factors, especially humidity. In as much as mealybugs are implicated as natural vectors of grapevine viruses, it is essential to

study their ecology and population dynamics and to relate the results of such studies to the regional variability observed in virus spread.

Another decisive factor in the natural spread of GLRaV III appears to be the sanitary status of planting material (primary inoculum). The use of clean propagating material, produced under conditions that prevent its infection in the nursery, appear to be of paramount importance for the control of virus spread in the vineyard. Finally the excessive use of insecticides against other insect pests, notably the grape berry moth, is known to encourage mealybug infestation and thus the natural spread of GLRaV III.

The difference in virus spread between European varieties and American rootstock varieties, such as V. *rupestris* St. George, is an observation of particular interest and should be pursued further. In its simplest form this difference may be due to the difficult detection of GLRaV III by ELISA in American rootstock varieties. In such a case the methodology must be improved to enable more reliable virus detection. However, it is also possible that such varieties possess a mechanism of resistance to the mealybug vector which should be thoroughly studied. Further work is also needed with regard to the epidemiology of rupestris stem pitting and corky bark.

Table 7. Epidemiological factors that appear to favour the development of mealybug infestation and the natural spread of GLRaV III within vineyards in Cyprus

- Site of the vineyard/microclimate: favoured in coastal, humid-areas.
- Cultural practices: favoured in irrigated vineyards.
- Pest management practices: favoured by excessive use of insecticides.
- Preparation of plants in the nursery: conditions most favourable in indoor nurseries.
- Sanitary status of planting material: freedom from GLRaV III very important.