Eur J Plant Pathol (2008) 121:121–130
DOI 10.1007/s10658-007-9254-1

Spatial analysis of epidemics of *Grapevine leafroll associated virus-3*

Cristina Cabaleiro · Carolina Couceiro · Sonia Pereira · Miguel Cid · Martín Barrasa · Antonio Segura

Received: 7 July 2006 / Accepted: 8 November 2007 / Published online: 1 December 2007

Abstract Several vineyards in Rías Baixas and one in the Ribeira Sacra (Spain) were monitored and the spatial pattern of leafroll-diseased grapevine was analysed at several dates. Unidimensional aggregation analysis (ordinary runs), bidimensional analysis, and disease gradients analysis were used as methods of study of spatial aspects of epidemics of GLRaV. At very low insect populations the mealybug *Planococcus citri* transmitted the GLRaV-3 from infected plants to healthy ones planted between them in an experimental plot at Beluso; an initial focus of leafroll-infected grapevines was detected 2 years after planting at the area where the vectors had been located infesting the old plants. Within 8 years some new foci appeared and coalesced, and the incidence reached >80%. In three commercial plots where no vectors were observed, the spatial analysis of the diseased plants showed three different situations. In Meaño, the study of the evolution of the spatial patterns of diseased plants between 1992 and 2005 suggested slow vectorial field transmission of GLRaV-3. In Goián the analysis for only 2 years suggested random distribution; therefore the viruses were arriving with the planting material, but the runs analysis of some lines suggested incipient spread of GLRaV-3. In Portomarín the incidence of both GLRaV-2 and 3 was low and their distribution was random, without any evidence of field spread. These examples of the study of the spatial analysis of leafroll-infected plants may be helpful to determine whether or not spread of the viruses is occurring, and the best control measures to take.

Keywords Ampelovirus · GLRaV · Mealybugs · Runs analysis · Disease gradient

C. Cabaleiro (✉) · C. Couceiro
Departamento de Producción Vegetal,
Universidad de Santiago de Compostela,
Campus Universitario,
27002 Lugo, Spain
e-mail: pvcabsob@lugo.usc.es

S. Pereira · M. Cid · A. Segura
Departamento de Fisiología Vegetal,
Universidad de Santiago de Compostela,
Campus Sur,
15706 Santiago de Compostela, Spain

M. Barrasa
Departamento de Ingeniería Agroforestal,
Universidad de Santiago de Compostela,
Campus Universitario,
27002 Lugo, Spain

Introduction

Leafroll is one of the most widespread viral diseases of grapevine. Five *ampeloviruses*, one closterovirus and three viruses not assigned to a genus, all in the *Closteroviridae* family, have been associated with the grapevine leafroll disease (Gugerli 2003). The *Ampelovirus* GLRaV-3 is the

most common. Leafroll disease is an economically important graft-transmissible disease of grapevines and occurs in all grapevine-growing countries. Although grapevine leafroll can affect the growth, development, longevity and yield of the vines, its most serious effect is on lowering the sugar content and raising the acidity of must. The increase in spread has been mainly due to movement of infected plant material, especially asymptomatic rootstocks. For a long time it was believed that leafroll and related viruses were only transmitted through plant material and there were no reports on field spread of the disease until 1973 (Dimitrijevic 1973). The number of species of mealybugs (Homoptera: pseudococcidae) and scale insects (Homoptera: coccidae) cited as vectors of some grapevine leafroll ampeloviruses (*Grapevine leafroll-associated virus* 1, 3, 5, 9) and vitiviruses have increased considerably in recent years but no vectors are known for other leafroll viruses tentatively included in the genera (GLRaV-4, 6,7,8) (Gugerli 2003). In the past 25 years, the spread of GLRaV-3 has been reported in most of the grapevine-growing countries in the world, and in most cases the spread has been associated with vectors (Gugerli 2003). The transmission of *Ampelovirus* by mealybugs and scale insects is considered semi-persistent as for other members of the family *Closteroviridae* (Cabaleiro and Segura 1997a; Sether et al. 1998; Krüger et al. 2006). However, GLRaV-3 was detected recently in the salivary glands of *Planococcus citri* after an acquisition access time (AAT) of 7 days (Cid et al. 2007) and therefore further research is required to fully characterize the transmission of *Ampeloviruses*.

Knowledge about the spatial distribution of infected plants at a given date may help in understanding whether or not the infection was already present in nursery plants, whether the virus was vectored from neighbouring vineyards, whether there was field transmission within a plot, and which factors favour spread of the infection (Madden et al. 1987; Cabaleiro and Segura 1997b; Maixner and Reinert 1997). Study of the changes in the spatial distribution during a suitably long period allows validation of the hypotheses and predictions made in accordance with temporal epidemiological models (Madden 2006).

Plant epidemiologists agree about the importance of the study of the spatial components of plant disease epidemics (Madden et al. 2007) but most studies on virus diseases have been done for aphid, white fly or thrip-transmitted viruses (Jeger et al. 2004). For tree virus diseases, mainly viruses transmitted in a non- or semi-persistent manner by aphids (PPV, CTV), the study of the spatial aspects of epidemics has revealed a wide range of patterns (Gottwald et al. 1996; Dallot et al. 2003). The spatial distribution of viral diseases transmitted by members of the families Pseudococcidae and Coccidae is expected to follow specific patterns because they have few generations per year, they do not fly and they are associated with ants, which help in survival and dispersal (Sether et al. 1998). GLRaV-2 is the only grapevine leafroll virus included in the genus *Closterovirus*; no vectors have been identified and field spread has not been reported, but other members of the genus are transmitted by aphids in a semi-persistent way. If this is true for GLRaV-2, the pattern of its spread in the field would differ from *Ampelovirus* and be closer to that of *Citrus tristeza virus* (Gottwald et al. 1996). Aphids are not pests in vineyards but they occasionally establish short-lived colonies in spring; *Aphis illinoisensis* is the only species cited in America, and recently in Europe, it has been reported to cause some damage to grapes (Tsitsipis et al. 2005).

In the Rías Baixas (Galicia, Spain) *Planococcus citri* produces several generations that overlap and move mainly during the summer, and the life-cycle is strongly affected by temperature and rainfall (Cid et al. 2006). In vineyards cultivated for wine production in Galicia, mealybugs and scale insects are usually kept relatively well under control by natural enemies and insecticides used against other pests; despite this, when vectors are present the disease continues to spread year after year, at rates from 4 to 12% diseased trees per year (Cabaleiro and Segura 2006).

The field spread of GLRaV-3 has been monitored since 1991 in several vineyards in Galicia (northwest Spain) (Cabaleiro and Segura 1997b, 2006); some other vineyards have been added to the study more recently and in some plots GLRaV-2 has been included in the study because it has been recently shown to be present at least at the same levels as GLRaV-3. The aim of this work was to predict and confirm the predictions of virus spread using the analysis of the spatial distribution of leafroll-infected plants in several vineyards.

Materials and methods

Vineyards

Beluso (Bueu, Pontevedra)

This vineyard was described in previous papers (Cabaleiro and Segura 1997b, 2006). In 1996, 40 leafroll indicator plants (20 Pinot noir and 20 Cabernet sauvignon) grafted on 161.46C rootstocks were each planted between two plants already infected with leafroll virus (GLRaV-3) in a plot designated as 'C' (Cabaleiro and Segura 2006). The plants were tested by Double Antibody Sandwich and/or Direct Immuno Printing Enzyme-linked Immunosorbent Assay (DAS or DIP-ELISA) every year between 1996 and 2007. In harvest time (2003 and 2005), 10 leaves per plant were removed and the number of mealybugs feeding on them was recorded (Cabaleiro and Segura 2006).

In the same vineyard, a plot with 1648 plants (plot A), which had been monitored in 1994, was examined again in 2003 to detect any changes in the spatial distribution of plants showing symptoms of the disease (leafroll, yellowing, corky texture). At harvest time (2003), 10 leaves per plant were removed from the 42 plants used as control for temporal analysis (Cabaleiro and Segura 2006) and the number of mealybugs feeding on them was recorded.

Meaño (Pontevedra)

In 1989, 160 scions from four virus-free Albariño clones were grafted onto 196.17C or SO4 virus-free certified rootstocks planted the year before. In 1991 clear symptoms of leafroll were observed in the plants and in 1992 the presence of GLRaV-3 was confirmed by DAS-ELISA. The disease development was studied between 1992 and 1996 (Cabaleiro and Segura 1997b) and the plot was monitored again between 2003 and 2005. All plants in the plot were tested for GLRaV-3 once a year (August, two leaves per plant).

Goián (Pontevedra)

This is a commercial vineyard planted in 1991 with the Albariño cultivar grafted onto 110R rootstock, and with approximately 3000 vines spaced at 1.5×3 m. The vineyard was sampled along one diagonal in 2003 (50 plants) and tested for GLRaV-3 using DAS-ELISA. In summer 2004 all plants were assessed for symptoms of leafroll (yellowing, leafroll, corky texture of the leaf). Eight complete rows (335 plants) were tested by DAS-ELISA in August 2004 and by DIP-ELISA in August 2005 (for GLRaV-2 and GLRaV-3).

Portomarín (Lugo)

This vineyard was planted in 1998, with the Mencía cultivar grafted onto 101.14 rootstocks. There were 716 plants in 22 rows, spaced at 1.5×3 m and trained vertically up a trellis. All plants were examined for symptoms of leafroll in August 1999, 2005 and 2007, and random samples were tested by DAS-ELISA for the presence of GLRaV-1 and GLRaV-3 in 1999 and 2003; all plants were tested by DIP-ELISA for GLRaV-2 and GLRaV-3 in 2005 and 2007.

Analysis of the spatial distribution of diseased plants

Disease gradients, ordinary runs analysis and fixed grid analysis were performed with the data corresponding to leafroll symptoms or GLRaV tests. Most data were analyzed with the 'Patchy' spatial analysis package (Maixner 1993).

When possible, incidence data were subjected to regression analysis with row (or column) number as an independent variable (x), and the incidence of disease in that row (or column) as the dependent variable (y). The linearized forms of the exponential ($\ln(y) = a + bx$ with a: intercept and b: slope) and inverse Power Law models ($\ln(y) = a + b \cdot \ln(x)$) were evaluated. Significant deviation of the slope from 0 was checked by a t-Test and a test for linearity was performed by calculating the coefficient of correlation and performing an F-test. Regression lines with slopes significantly different from 0 were considered to indicate the existence of a disease gradient in the direction corresponding to the independent variable.

Unidimensional aggregation analysis (ordinary runs) was performed according to Madden et al. (1982) and a normal test was used to determine clustering; a row (or column) of plants was considered to have a non-random sequence of infected and healthy plants if the standardized Z was <-1.64.

Bidimensional analysis was performed by successively dividing an entire vineyard into identical

subplots of increasing size, calculating the mean number of infected vines in subplots of each size, and using these data to calculate Morisita's index of dispersion (Maixner 1993). The statistical significance of deviations of Morisita's index from unity was estimated. All calculations were performed using the PATCHY programme. The size of the units tested for clustering ranged from 2×2 (minimum allowed size) to a maximum of row-length/2×column-length/2 grapevines (Meaño); in Portomarín and especially in Goián the changing slope of the plots and the large alleys (3–4 m) did not make it advisable to use units with >4 crop lines (columns). The average sizes of aggregations (mean focus sizes) were estimated from the values of $I\delta$, which are maximal when the quadrat size equals focus size.

Virus testing

Samples in all cases consisted of mature leaves with petioles, collected in late summer after veraison. The antibodies were obtained from Bioreba AG (Basel, Switzerland). DAS-ELISA was performed following the supplier's indications. DIP-ELISA was performed as previously reported (Couceiro et al. 2006) with some modifications. Briefly, the samples were printed onto nitrocellulose membranes of 0.45 μm pore size (Sartorius, Goettingen, Germany). The membranes were blocked in non-fat dried milk at 1–2% in extraction buffer for grapevine (the same as for DAS-ELISA) for 1 h at room temperature or overnight at 4–6 °C. Membranes were then dipped directly in alkaline phosphatase conjugated specific antibody solution (in conjugated buffer as for ELISA) and incubated for 2 h at room temperature or overnight at 4–6 °C. (Alternatively, specific antibody (1.5 h) and alkaline phosphatase conjugated antirrabit antibody (30 min) may be used with same results). After incubation, the membranes were washed carefully three or four times, for at least 5 min each time (with stirring), in a saline buffer (0.085% NaCl, 0.05% Tween 20 in distilled water). The membranes were then covered with BCIP-NBT ready-to-use liquid substrate (SIGMA B-1911). A purple colour usually developed after 10–15 min of incubation, but occasionally it took up to 60 min or even more; therefore the reaction was only stopped (with tap water) when the positive control was clear and the membrane began to darken. A dissecting microscope was used to observe the purple colour because it only develops in the area corresponding to the phloem vessels in the case of leafroll-associated viruses. One membrane per virus is necessary only when all viruses are to be identified; in other cases the antibodies can be mixed and one membrane is enough to perform the test.

Results

Beluso

Most plants in the old plot A showed clear symptoms of leafroll; 41 of the 42 plants analysed along the diagonals were found to be infected with GLRaV-3 in 2003 and most plants randomly sampled were also positive for GLRaV-3. It was therefore not possible to repeat the spatial analyses of infected plants for comparison with the analysis carried out in 1994. The mealybugs were detected throughout the diagonals (76% plants with at least one insect) but insect numbers always remained very low (an average of 1.2 insects per plant). The spatial distribution of infected plants in each consecutive year in plot C planted in 1996 is shown in Fig. 1. The first infected plants appeared in 1998, but only in the area where mealybugs had been seen under the bark of the old infected vines before planting the new vines; this initial focus increased in size in the following years. In 1999 and 2000 two new foci appeared and increased until they coalesced. In 2003 and 2004 there were only three groups of two plants that remained free of GLRaV-3. In 2005 one more infected plant was detected and no more plants tested positive in 2006 and 2007. Sticky bands placed around trunks and branches of the vines did not catch significant numbers of crawlers or nymphs in spring/summer of 2003 in this plot. At harvest, mealybugs were found on 13 plants (Fig. 1), with an average of 1.5 leaves with insects per infested plant, and approximately 2.9 insects per leaf – mainly first and second stages nymphs (Cabaleiro and Segura 2006). In 2005 insects could not be found at harvest or under the bark during the winter. The virus moved both within rows (with infected plants in between two new plants) and across alleys.

Meaño

The spatial distribution of the diseased plants in 1992, 1996, 2003 and 2005 is shown in Fig. 2. There was an

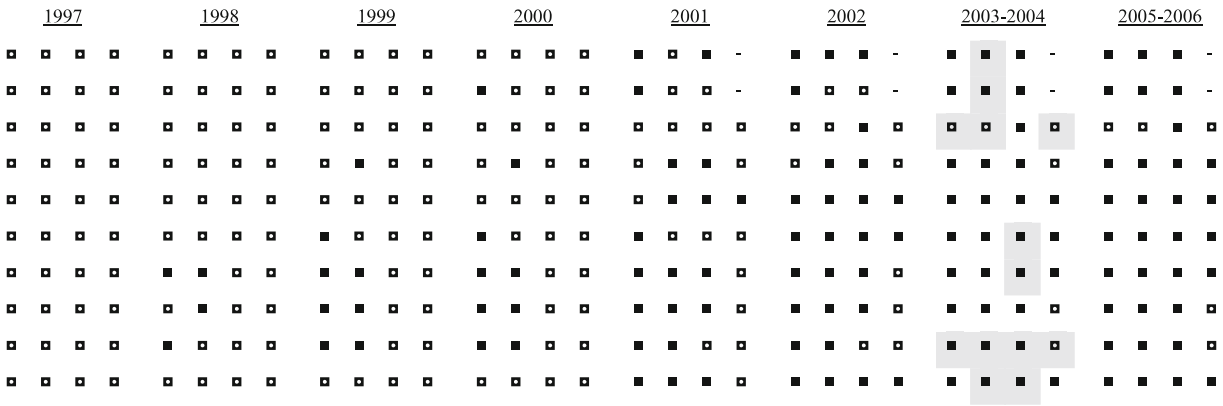

Fig. 1 Spatial distribution of leafroll infected plants between 1996 and 2005. *empty square* new plants leafroll free (planted between two old infected ones not shown in the figure); *filled square* new plants, GLRaV-3 infected. *Blank space*, after 2001, dead plants. *Shaded areas*, vines where mealybugs were observed at harvest in 2003

average of 1.4 newly infected plants per year between 1992 and 2005. The main focus, already present when the virus was first detected in the plot, increased slowly but some other foci appeared. Most newly infected plants were on the left side of the plot (predominant southwestly) and they were always close to an already-infected vine. The right side of the plot remained almost the same since 1992, with a few infected plants scattered within this area. There was a strong gradient towards the left side of the plot. The slopes and intercepts of the linearized forms of the fitted models increased every year because most of the newly infected plants were on the left side of the plot. In 2004 and 2005 the rate of spread of the virus was greater than average (three and four newly infected plants, respectively) but not all newly infected plants were close to already-infected plants and some occurred on the right side of the plot (Fig. 2). The results of the ordinary runs analysis for rows are summarised in Table 1; the percentage of rows with significant aggregations ($Z<-1.64$) increased from 25% during the first period (1992–1996) to 37.5% in 2003, and 50% in 2005. When all rows were considered together, there were no significant aggregations of infected plants in 1992, and the level of significance of aggregation increased in the following years, reaching a maximum in 2003 ($Z=-3.43$). The bidimensional analysis indicated that there were significant foci of infected plants from the beginning of the study (Fig. 3); the first significant foci ($I\delta>1$ with $P>95\%$) changed from 2×5 (1992–1996) to 2×4 (2003–2005) and the maximum $I\delta$ was at 3×5 in 1992 and1996 and 2×5 in 2003 and 2005.

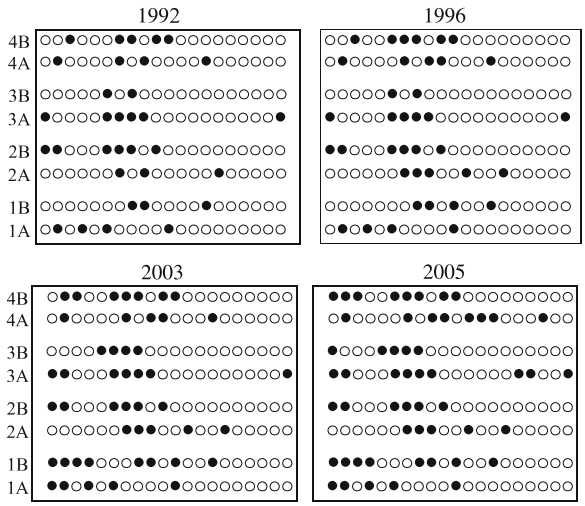

Fig. 2 Spatial distribution of diseased plants (*filled dots*) in the Meaño vineyard in 1992, 1996, 2003 and 2005

Portomarín

A few plants showing symptoms were observed in 1999 (6%) and 20 of those plants tested positive for GLRaV-3 by DAS-ELISA. In 2003, 54 plants showed symptoms of leafroll (7%) and 41 of those plants were GLRaV-2 or GLRaV-3 positive. In 2005 (all plants analysed) very few plants showed symptoms of leafroll (2%) at the beginning of September, but 4 and 9% of the plants were respectively GLRaV-3 and GLRaV-2 positive (as indicated by DIP-ELISA) and 10 plants showed mixed infection. In 2007 the percentage of leafroll-diseased plants was similar (5.2 and 8.3 for GLRaV-3 and GLRaV-2) and again no symptoms were present in many infected plants, especially those with GLRaV-2. No significant aggregations were observed, either

Table 1 Results of ordinary runs analysis for leafroll symptomatic or GLRaV positive plants within the rows or columns in the three vineyards

Plot	Year	Leafroll	%	Sr/Tr[b]	Sc/Tc[c]
Meaño	1992	GLRaV-3	21	2/8	–
	1996	GLRaV-3	24	2/8	–
	2003	GLRaV-3	29	3/8	–
	2005	GLRaV-3	34	4/8	–
Portomarín	1999	S[a]	6	0/42	0/22
	2003	S	8.5	1/42	0/22
	2005	GLRaV-2	9	0/42	0/22
	2005	GLRaV-3	4.3	1/42	0/22
	2005	S	2	0/42	0/42
	2007	GLRaV2	8.3	0/42	2/22
	2007	GLRaV-3	5.2	0/42	1/22
	2007	S	6	1/42	1/22
Goian	2004	GLRaV-1	3.6	–	0/8
	2004	GLRaV-3	13.8	–	1/8
	2004	S	22.8	–	0/8
	2005	GLRaV-2	28.5	–	3[d]/8
	2005	GLRaV-3	16.6	–	3[d]/8
	2005	S	11.9	–	0/8

% Percentage of symptomatic/virus infected plants

[a] *S*: leafroll symptoms observed in the field

[b] *Sr/Tr*: number of rows with significant aggregations ($P = 0.05$) relative to total number of rows tested ($Z < -1.64$)

[c] *Sc/Tc*: number of columns with significant aggregations ($P = 0.05$) relative to total number of columns tested ($Z < -1.64$)

[d] Not the same three rows

within grapevine lines (Table 1) or across the lines (columns and rows respectively), or quadrats, and there were no significant gradients of diseased plants towards any of the borders of the plot. Taking all the data in consideration, the spatial analysis of leafroll-infected plants indicates that diseased plants were randomly distributed.

Goián

The spatial distribution of plants with clear symptoms of leafroll at the end of summer was random in most areas. There was significant aggregation in 17.1% of the lines of vines (columns for the spatial analysis) (Table 1). But in several other columns with high

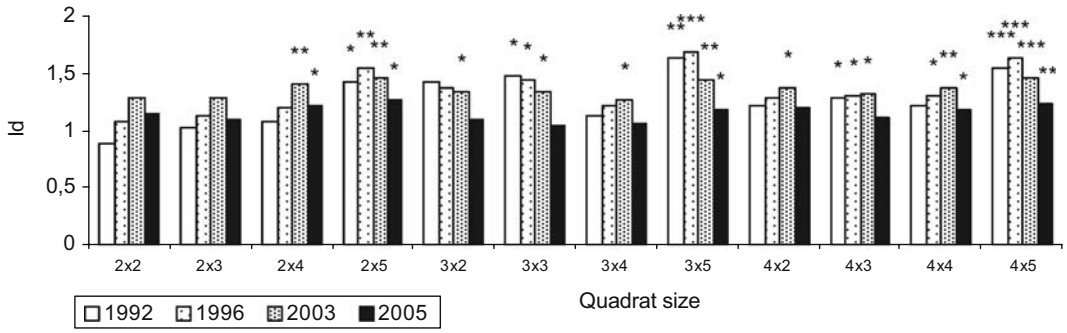

Fig. 3 Morisita's index of dispersion ($I\delta$) for a number of quadrat sizes (row × column) in Meaño for the years of study as in Fig. 2. Grapevine lines correspond to rows. *, ** and *** indicate significant $I\delta > 1$ with $P < 0.05$, $P < 0.01$ or $P < 0.001$ respectively

incidence (>60%), and several consecutive plants showing leafroll symptoms (4–5 and even one with 14), the runs analysis did not indicate significant aggregations. The number of GLRaV-3 positive plants increased 3% between 2004 and 2005. In addition, in 2005 there were more consecutive infected plants in the eight rows analyzed, with three rows showing significant aggregations for GLRaV-3. No aphids, mealybugs, scale insects or ants were observed in this plot. In 2005 a number of plants that did not show symptoms of leafroll in 2004 and 2005 tested positive for GLRaV-2. The symptoms observed in 2004 and 2005 corresponded, in >90% of cases, to GLRaV-3 positive plants in the 8 rows analysed using DIP-ELISA. There was a slight gradient of symptomatic plants towards the right side of the plot. Although most aggregations of plants showing symptoms were within columns, foci of mainly two crop lines were detected by the bidimensional analysis: the first significant focus was 7×2 plants and the maximum $I\delta$ (1.126) was reached for foci of 10×2 plants (Fig. 4).

Discussion

In vineyards with available virus inoculum and mealybugs present, GLRaV-3 spreads quite quickly from plant to plant as seen in the new plot (C) established in 1996; in new Zealand vineyards GLRaV-3 spread at a rate that doubled the number of infected vines each year (Jordan 1993) and in South Africa after 7 years 71% of the healthy plants became GLRaV-3 infected (Engelbrecht and Kasdorf 1990). The virus was not detected until 2 years after planting, possibly because of long latency periods, which would be consistent with the long time required to detect the virus in the plants after experimental transmission (Cabaleiro and Segura 1997a). The first infected plants appeared precisely in the area where the insects had been observed in 1996. In 2003 when mealybugs were found throughout the plot, the virus was already widely spread; the absence of new infected plants in 2006 and 2007 may be related to the good mealybug control since 2005. The movement of the insects was not appreciated in August 2005 when a greater number of insects were captured in other areas of the vineyard (Cid et al. 2006). The crawlers are responsible for the spread of mealybugs in vineyards because they move actively and are easily carried by the wind and spraying. However they do not feed while actively moving and so the adults and nymphs of any stage are probably responsible for spread of the virus, as at the end of the summer and during autumn, they move down to the trunks looking for the best place to take shelter during adverse weather. This period is when the concentration of virus on adult leaves is maximum (Monis and Bestwick 1996); pesticide spraying is stopped before harvest and therefore, even if there were not many vectors, they could feed without being disturbed and were probably effective enough to ensure transmission of the virus. If the virus accumulates inside the salivary glands of mealybugs (Cid et al. 2007), the probability of them being infective is higher; as they feed directly on the phloem in trunks and branches, the probability of transmission of the virus increases (Cohen et al. 2004). Virus-positive adult plants were the source of the virus at the beginning of the epidemic but around 2002 the newly infected plants

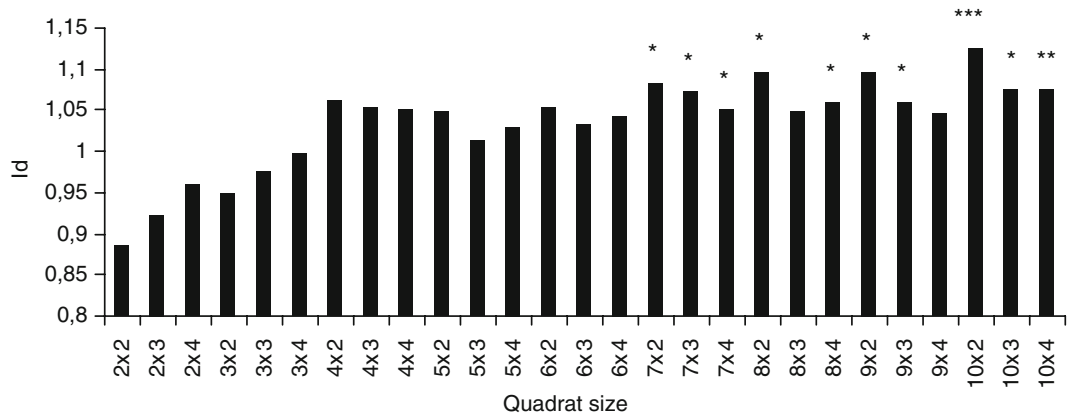

Fig. 4 Morisita's index of dispersion ($I\delta$) for a number of quadrat sizes (row × column) in Goian (2004). Grapevine lines correspond to columns. *, ** and *** indicate significant $I\delta>1$ with $P<0.05$, $P<0.01$ or $P<0.001$ respectively

appeared to act as sources of infection and there was an exponential increase in incidence of the disease between 2001 and 2003 (Cabaleiro and Segura 2006).

In the Meaño vineyard the pattern of spread appeared to be vectorial because clustering of infected plants increased year after year, indicating that a pathogen was predominately spreading from plant to plant, although the candidate insect vector was not found. Such slow (an average of one new infected plant per year) but constant plant to plant transmission is consistent with what would be expected if scale insects were the vectors because they have only one generation per year and only the first instars move. The scale insects which could be vectors of GLRaV-3 (Parthenolecanium corni, Pulvinaria vitis), are difficult to observe and therefore it is also possible that they act slowly and unobserved in a vineyard trained in a horizontal trellis 2m high. The area where the spread was most evident was the most humid, favourable for scale insects. The roots of individual plants that come into contact with each other could also graft together and share common vascular tissue, as has been described for several systemic pathogens (Epstein 1978) and suggested for several viruses such as *Apple mosaic virus* or *Plum Pox virus*, although always as a minor method of spread. However, it does not explain infection of some plants that are not close to infected ones. There have been other reports of spread of leafroll not related to the presence of known vectors (Dimitrievic 1973; Habili et al. 1995). The initial arrival of the virus could occur with vectors carrying external sources of the virus in their salivary glands. The fact that only GLRaV-3 was detected supports the idea of an external source of the virus and vectorial transmission.

In the vineyard in Portomarín the inoculum clearly originated exclusively from the plant material: there were few infected plants, no gradient towards any of the neighbouring vineyards, very slight and erratic symptoms of leafroll and many different viruses present (apart from those already mentioned, some GLRaV-1, GVA and GFkV). Spatial analysis of the data indicated a random and similar distribution of GLRaV-3 and GLRaV-2 infected plants and the viruses did not appear to be spreading. The small changes in the incidence of both viruses were due to a certain number of death plants (soil pathogens). No mealybugs or ants – or aphids – were observed in the plot, although some scale insects were observed in a vineyard about 200m away. The low intensity and year-dependent occurrence of leafroll symptoms may be due to environmental conditions, the characteristics of the cultivar or the vigour of the rootstock.

In Goian, in southern Galicia (close to the border with Portugal) a similar situation was observed, with a low incidence of leafroll symptoms, and the presence of at least GLRaV-1, -2 and -3, which probably originated from the plant material. But the field data suggested incipient field spread because the incidence and significant aggregations within columns increased between 2004 and 2005 (Table 1). The presence of significant runs of adjacent infected vines may represent initial foci where vectors have begun to transmit the viruses. The Goian vineyard was located in an area where citrus and fruit trees are grown and there are several ornamental plant nurseries close by, where mealybugs and scale insects may be present.

Apart from the relatively recent discovery of vectorial field transmission of leafroll viruses, the spread of leafroll disease, especially GLRaV-3, has been reported to occur in most grapevine-growing countries, although at different rates (Jordan 1993; Engelbrech and Kasdorf 1990; Martin et al. 2005; Pietersen 2006). Monitoring plants for symptoms of leafroll in new vineyards or performing rapid and inexpensive analysis by DIP-ELISA may help to detect very early stages of virus spread in the vineyards. The damage caused by leafroll viruses should not be underestimated, especially in some cultivars and regions (Cabaleiro et al. 1999). In grape-growing areas in Galicia the grapevine orchards are small, with an average plot size of <0.5 ha. When a new healthy vineyard is planted it may be surrounded by partially infected vineyards; when mealybugs are present (fortunately not very often under the current climate), virus transmission may be relatively fast and takes place before the insects are considered as pests. But since the spread of disease and the damage caused are not severe enough so as to require that the vines are uprooted, there is no mandatory removal order as for other tree virus-diseases (Dallot et al. 2003). In any case, if we are able to detect early spreading on the base of the spatial distribution of infected plants, it would be advisable to remove the infected plants as soon as possible, especially in young vineyards established with certified virus-free grapevines or in foundation blocks, and when insect vectors are present

in the zone. Pietersen et al. (2003) found that at first the virus kept spreading after roguing infected plants, but continuous roguing of the infected plants resulted in the virus being eradicated from the plots (Pietersen 2006).

Acknowledgements This work was partly funded by grants from the Xunta de Galicia (XUGA200074A93 and PGIDITO3P-XIC20001PN), the European Commission (ALVIRUS/SP1.P7), the FEDER and Ministerio de Ciencia y Tecnología (AGL2002-02438), and the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (RTA2005-00201-C03-03).

References

Bonfiglioli, T., Edwards, F., Hoskins, N., & Pantaleo, A. (2003). Graft incompatibility syndrome in New Zealand Merlot vines involves another possible variant of GLRaV-2. *The Australian and New Zealand Grapevine Grower and Winemaker*, *472*, 87–90.

Cabaleiro, C., & Segura, A. (1997a). Some characteristics of the transmission of grapevine leafroll associated virus 3 (GLRaV-3) by *Planococcus citri* Risso. *European Journal of Plant Pathology*, *103*, 373–378.

Cabaleiro, C., & Segura, A. (1997b). Field transmission of *Grapevine leafroll associated virus* 3 by *Planococcus citri* Risso. *Plant Disease*, *81*, 283–287.

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

Cabaleiro, C., Segura, A., & García-Berrios, J. (1999). Effect of grapevine leafroll associated virus 3 on the physiology and must of *Vitis vinifera* L. cv. Albariño following contamination in the field. *American Journal of Enology and Viticulture*, *50*(1), 40–44.

Cid, M., Pereira, S., Faoro, F., Cabaleiro, C., & Segura, A. (2007). Presence of GLRaV-3 on the primary salivary glands of *P. citri* suggest a circulative transmission mechanism. *European Journal of Plant Pathology*, *118*(1), 23–30.

Cid, M., Pereira, S., Gago, P., Couceiro, C., Cabaleiro, C., & Segura, A. (2006). *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) as GLRaV-3 vector. Extended abstracts 15th ICVG, South Africa, 3–7 April 2006, 252–253.

Cohen, D., van den Brink, R., & Habili, N. (2004). Detection of leafroll virus in newly infected grapevines. The Australian and New Zealand Grapevine Grower and Winemaker. Annual Technical Issue 2004, 56–59.

Couceiro, C., Cid, M., Pereira, S., Segura, A., & Cabaleiro, C. (2006). Use of direct immunoprinting (DIP)-ELISA for the detection of grapevine leafroll viruses. Extended abstracts 15th ICVG, South Africa, 3–7 April 2006, 180–181.

Dallot, S., Gottwald, T., Labonne, G., & Quiot, J. B. (2003). Spatial pattern analysis of Sharka disease (*Plum pox virus* strain M) in peach orchards of southern France. *Phytopathology*, *93*, 1543–1552.

Dimitrijevic, B. (1973). Some observations on natural spread of grapevine leafroll disease in Yugoslavia. *Rivista di Patologia Vegetale* (S.IV) *9*, 114–119.

Engelbrecht, D. J., & Kasdorf, G. G. F. (1990). Field spread of corky bark, fleck, leafroll and Shiraz decline diseases and associated viruses in South African grapevines. *Phytophylactica*, *22*(3), 347–354.

Epstein, A. H. (1978). Root graft transmission of tree pathogens. *Annual Review of Phytopathology*, *16*, 181–192.

Gottwald, T. R., Cambra, M., Moreno, P., Camarasa, E., & Piquer, J. (1996). Spatial and temporal analyses of *Citrus tristeza* in eastern Spain. *Phytopathology*, *80*, 45–55.

Gugerli, P. (2003). *Grapevine leafroll and related viruses*. Extended abstracts 14th Meeting ICVG, Locorotondo (Bari), Italia, 12–17 September, 2003, 25–31.

Habili, N., Fazeli, C. F., Ewart, A., Hamilton, R., Cirami, R., Saldarelli, P., et al. (1995). Natural spread and molecular analysis of grapevine leafroll-associated virus 3 in Australia. *Phytopathology*, *285*, 1418–1422.

Jeger, M. J., Holt, J., Bosch, F., & Madden, L. V. (2004). Epidemiology of insect-transmitted plant viruses: Modelling disease dynamics and control interventions. *Physiological Entomology*, *29*, 291–304.

Jordan, D. (1993). Leafroll spread in New Zealand vineyards. *Australian and New Zealand Wine Industry Journal*, *8*(4), 322–324.

Krüger, K., Saccaggi, D., & Douglas, N. (2006). *Grapevine leafroll associated virus* 3- vector interactions: Transmission by the mealybugs *Planococcus ficus* and *Pseudococcus longispinus* (Hemiptera: Pseudococcidae). Extended abstracts 15th ICVG, South Africa, 3–7 April 2006, 130–131.

Madden, L. V. (2006). Botanical epidemiology: some key advances and its continuing role in disease management. *European Journal of Plant Pathology*, *115*, 3–23.

Madden, L. V., Hughes, G., & van den Bosh, F. (2007). *The study of plant disease epidemics*. APS, St. Paul, Minesotta, USA

Madden, L. V., Louie, R., Abt, J. J., & Knoke, J. K. (1982). Evaluation of tests for randomness of infected plants. *Phytopathology*, *72*, 195–198.

Madden, L. V., Pirone, T. P., & Raccah, B. (1987). Analysis of spatial patterns of virus-diseased tobacco plants. *Phytopathology*, *77*(10), 1409–1417.

Maixner, M. (1993). PATCHY – Ein programm zur analyse rŠumlicher verteilungsmuster von Rebkrankheiten. *Nachrichtenbl Deut Pflanzenschutzd*, *45*, 157–164.

Maixner, M., & Reinert, W. (1997). Spatio-temporal analysis of the distribution of grapevine yellows in Germany. Extended abstracts 12th Meeting ICVG, Lisbon, Portugal, 29 September–2 October 1997, 75–76.

Martin, R. R., Eastwell, K. C., Wagner, A., Lamprecht, I., & Tzanetakis, I. E. (2005). Survey for viruses of grapevine in Oregon and Washington. *Plant Disease*, *89*, 763–766.

Monis, J., & Bestwick, R. K. (1996). Detection and localization of grapevine leafroll associated closteroviruses in greenhouse and tissue culture grown plants. *American Journal of Enology and Viticulture*, *47*(2), 199–205.

Pietersen, G. (2006). Spatio-temporal distribution dynamics of Grapevine leafroll disease in Western Cape vineyards. Extended abstracts 15th ICVG, South Africa, 3–7 April 2006, 126–127.

Pietersen, G., Kellerman, U., Oosthulzen, W. T., & Spreeth, N. (2003). Effect of roguing on natural spread of

grapevine leafroll disease in two vineyards in South Africa. 8th International Congress of Plant Pathology. Christchurch, New Zealand, 2–7 February 2003. pp 301.

Sether, D. M., Ullman, D. E., & Hu, J. S. (1998). Transmission of pineapple mealybug Wilt-associated virus by two species of mealybug (Dysmicoccus spp.). *Phytopathology*, *88*, 1224–1230.

Tsitsipis, J. A., Angelakis, E., Margaritopoulos, J. T., Tsamandaini, K., & Zarpas, K. D. (2005). First record of the grapevine aphid *Aphis illinoisensis* in the island of Kriti, Greece. OEPP/EPPO. *Bulletin*, *35*, 541–542.