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Temporal analysis of *grapevine leafroll associated virus 3* epidemics

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Abstract

With the aim of investigating the dynamics of transmission of GLRaV-3, we report and analyse time-courses of infection incidence in various plots in one vineyard in Rías Baixas (Galicia, Spain). GLRaV-3 infection was close to 100% after 15 years monitoring the epidemic in several plots where *P. citri* was widely spread although with low density populations. In two plots, virus-free plants were planted close to infected ones and after 8–9 years more than 80% of the plants tested GLRaV-3 positive (average infection rates of 7.8–12.4% per year). The Gompertz model best fitted the epidemiological data.

Grapevine leafroll disease has been described in all grape-growing countries as one of the most important viral diseases of grapevine. Five *Ampelovirus*, one *Closterovirus* and three viruses not assigned to a genus, all in the *Closteroviridae* family, have been associated with grapevine leafroll disease (Gugerli, 2003). The *Ampelovirus* GLRaV-3 is the most common in Mediterranean countries. The spread of grapevine leafroll disease has been assumed to occur only through infected plant material, mainly of asymptomatic American grapevine rootstocks, but during the last 20 years several pseudococcids (*Planococcus citri*, *P. ficus*, *Pseudococcus longispinus*, *P. affinis*, *P. calceolaria*, *P. comstocki*, *P. maritimus*, *P. viburni*, *Heliococcus bohemicus, Phenacoccus aceris*) and coccids (*Parthenolecanium corni, Pulvinaria vitis*) were found to be vectors of several GLRaV (reviewed by Gugerli, 2003). The mechanisms underlying transmission of closteroviruses by mealybugs are still largely unknown. It is assumed that the transmission of GLRaV-3 is of the semi-persistent type, as it is for other viruses in the family *Closteroviridae* (Cabaleiro and Segura, 1997b).

In the field, the spread of GLRaV-3 associated with particular insect vectors has been reported in several countries, including South Africa (Engelbrecht and Kasdorf, 1985, 1990), New Zealand (Bonfiglioli et al., 2002); Spain (Cabaleiro and Segura, 1997a), Mexico (Teliz et al., 1989), Italy (Belli et al., 1993), Cyprus (Ioannou, 1993), France (Sforza et al., 2003), Israel (Tanne et al., 1989) and the USA (Golino et al., 1995). In some reports, however, no vector could be associated with the spread of the disease (Dimitrijevic, 1973; Teliz et al., 1989; Habili et al., 1995; Cabaleiro and Segura, 1997a).

However, little is known about the long-term time-course of the spread of this virus from vineyard to vineyard. We have been monitoring the field spread of GLRaV-3 by the mealybug *Planococcus citri* since 1991 in several vineyards in northwest Spain (Galicia) (Cabaleiro and Segura, 1997a) and we now have a good understanding of how epidemics develop under the conditions existing in the region.

The experiments were performed in a vineyard, in Beluso (municipality of Bueu), province of

Pontevedra in Galicia (NW Spain) with several plots A–F; the vineyard is 3 ha in size and the oldest plots (A and D) were planted in 1980. Mealybugs, identified as *Planococcus citri* have are present in all the plots (A–F) but their populations never reached pest level and therefore no specific treatments are programmed regularly; only in plot F occasionally a delayed dormant treatment with insecticide with oil is applied. The only insecticide spraying is done against the grape moth, usually one treatment, at the beginning of July. Fungicides against *Uncinula necator*, *Plasmopara viticola* and/or *Botrytis cinerea* are sprayed regularly (8–10 treatments) because the environmental conditions are highly favourable to fungal attacks. Any pesticide spray stops at the end of August or beginning of September (depending on the predictable harvest date).

Plot A is about 1.8 ha in area, with about 1600 plants in a rectangular pattern (3 × 4 m). Vines are trained on horizontal trellis to form a continuous canopy at a height of about 2 m. Forty plants along two diagonals were tested for GLRaV-3 by DAS-ELISA (see below) every year from 1991 to 2003. Plot B is included within plot A, in an area in which mealybugs are present on most plants but with very small populations. In 1995, twelve grapevines of cv Albariño, GLRaV-3-free, were interplanted close to some of the plants belonging to the diagonal monitored. Plot C is about 50 m from plot A. Forty leafroll indicator plants (Pinot Noir and Cabernet Sauvignon), GLRaV-3-free, were planted in the winter of 1996, each between two existing infected plants. Vines are trained on a vertical shoot trellis, which was reached by the new plants during the first vegetative period. Within this plot mealybugs had been seen only on a single cluster of plants in the year of planting. In 2003 sticky bands where placed in several places to check the mealybug movement; in harvest time (2003 and 2005), 10 leaves per plant were removed and the number of mealybugs feeding on them was recorded. The 40 new plants were tested once or twice a year by DAS-ELISA for the presence of GLRaV-3 and the symptoms of leafroll disease were recorded.

Plot D is close to plot A and is planted with Albariño plants of the same age; however, the plants are trained on a vertical trellis about 1.8 m high. Random samples of plants (n = 40) from this plot were tested by DAS-ELISA for GLRaV-3 in 1993 and 2004; in addition, in 1994 all plants were examined for leafroll symptoms. Plot E is similar to plot D but younger (15 years old), with Albariño plants trained on a vertical trellis. All plants in eight rows (total 160 plants) were tested by DAS-ELISA for the presence of GLRaV-3 in 2003. Plot F is close to plot E, and is planted with 20-year-old plants of 'Tinta Femia'; this is the local name for a red traditional cultivar of the region ('Caiño Tinto'). This is the plot in which mealybugs were first seen in Beluso and it is the plot where the mealybug infestation is more easily observed. Random samples (minimum 10 per year) were tested by DAS-ELISA for the presence of GLRaV-3 in 1990, 1994 and 2003.

Disease progress curves (DPCs) were constructed for each of plots A, B, and C, on the basis of the incidence of ELISA-determined GLRaV-3 infection in that plot in each year of study (plot A, 1991–2003, n = 40 plants; plot B, 1995–2005, n = 12; plot C, 1996–2005, n = 40. Overall rates of disease spread were estimated as the slope (% of plants per year) from linear regressions considering all years of monitoring. For DPC modelling, we fitted the monomolecular, logistic and Gompertz models (Campbell and Madden, 1990), in each case with assessment of goodness of fit on the basis of coefficient of determination (R^2) and mean square error (MSE).

All the analyses for the presence of virus were by double antibody sandwich (DAS)-ELISA. Samples consisted of mature leaves with petioles, collected in late summer, or wood shavings collected in winter. The antibodies were from Bioreba AG (Basel, Switzerland) and the tests were performed following the indications of the supplier. Plants were tested for GLRaV-1 and 3 but only GLRaV-3 was found.

In plot A, the percentage of infected plants increased from 35% in 1991 to 97.5% (39/40 plants) in 1998 (Figure 1); in winter 2003 one of four wood shaving samples taken from different branches of the single remaining vine tested positive. This incidence of 95–100% appears to be broadly constant throughout the vineyard: in plot D, 44.6% of plants were infected in 1994, reaching 96.9% in 2003, in plot E, 99.4% of plants were infected in 2003, and in plot F all plants tested in 1990, 1994 and 2003 were infected. The oldest plants in the Beluso vineyard are 25 years old, and most of them are infected; but younger plants

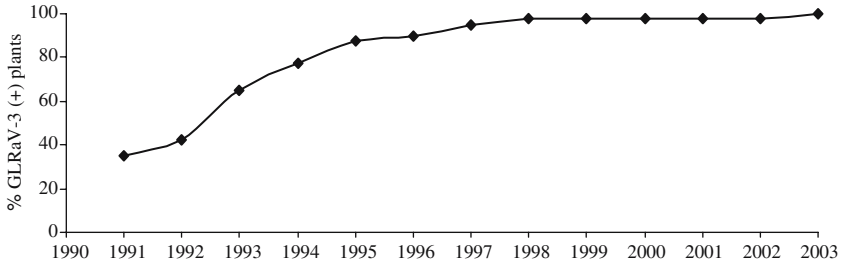

Figure 1. Disease progress curve for Plot A (n = 40 plants). GLRaV-3 infection was detected by DAS-ELISA.

(15–20 years) are likewise almost 100% infected. In plot B, 82% of the newly planted healthy plants became infected over the study period, some of them in the first year after planting (Figure 2). Some mealybugs were occasionally seen on these vines, at least in the first years of monitoring, but the level of infestation was always very low.

In plot C, the first infected plants (4/40 plants) were detected in 1998, two years after planting, within the main focus of mealybug infestation. Subsequently, the number of infected plants increased every year, with a particularly dramatic increase between 2000 (incidence 24%) and 2001 (incidence 58%). In 2001 most of the plants close to the initial mealybug infestation focus were infected, and mealybugs could be observed in other foci; by 2003, the infected plants were distributed all over the plot, with an incidence of 84% (Figure 3); in 2005 it reached 86.8%. Throughout the study period mealybug population density remained very low even at the initial focus; in 2003, the examination of plants at harvest revealed nymphs feeding throughout the plot in 13 of the 40 plants, with insects averaging of 1.5 leaves per plant and an average of 2.9 in 10-leaf samples; however, in 2005 no insects were detected in the detached leaves.

The Gompertz model fitted all DPCs fairly well, with R^2 ranging from 0.79 for plot B (MSE = 0.272), to 0.97 for plot C (MSE = 0.068). In plot A, the Gompertz model showed $R^2 > 0.90$ (MSE = 0.506), for the period 1991–2003 (Figure 1) and 0.99 for the period 1991–1998, when 97.5% of virus incidence was reached. The monomolecular model gave good fit to the DPCs for plot B ($R^2 > 0.88$, MSE = 0.059) and plot A ($R^2 > 0.90$, MSE = 0.209). In plot A this result is probably because no data were obtained for the early years of the epidemic, thus lacking the typical initial delayed phase (Campell and Madden, 1990). In the case of plot B the monomolecular model fitted the data well because the inoculum and vectors were evenly distributed so that infection was from already-infected surrounding plants, and was not dependent on transmission via initially uninfected plants (Kranz, 2002). The monomolecular model was not expected to fit because it is generally more appropiate for describing annual data in monocyclic pathogens (Madden and Campell, 1990; Kranz, 2002), and not for polyetic epidemics.

The rate of disease spread differed from plot to plot. The rate of infection of healthy plants surrounded by mostly infected plants, in vineyards

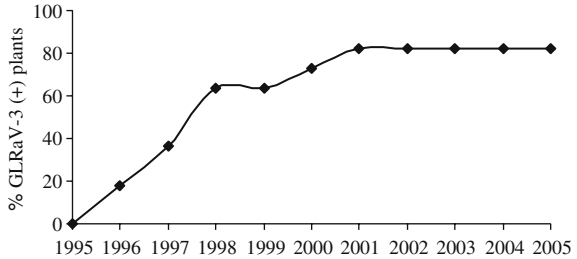

Figure 2. Disease progress curve for Plot B (n = 12 plants). GLRaV-3 infection was detected by DAS-ELISA.

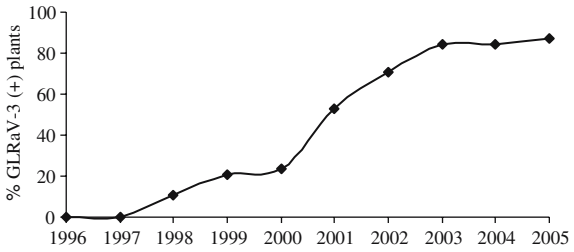

Figure 3. Disease progress curve for Plot C (n = 40 plants). GLRaV-3 infection was detected by DAS-ELISA.

with the mealybug vector present, was 7.8% (B) and 12.4% (C) of plants per year. In both plots the epidemic slowed down or stopped (B) at about 80% incidence: in plot B, 5 years have now passed without infection of the remaining virus-free plants. In plot C, after 2 years without new infections, in 2005 one more plant tested postive for GLRaV-3 and showed leafroll symptoms and we expect that the remaining uninfected plants in these plots will eventually become infected. The fastest rate of spread was registered in plot C, but was initially higher in plot B (up to 24% between 1997 and 1998), probably because the horizontal training system favoured the contact of the new plants with the many neighbouring infected plants (up to 8) with vectors likewise present in most plants; the probability of an infectious vector reaching a healthy plant was thus higher. If the whole period is considered, the virus spread faster in plot C, presumably because the vertical training system favoured the movement of mealybugs from plant to plant without having to move far from the trunk base where they overwintered; the initial delay in transmission was perhaps attributable to the fact that mealybugs were present in 1996 apparently only in one focus though later they spread throughout the plot. The year of fastest spread was between 2000 and 2001 (25%).

In plot A, the incidence of infection increased at a rate of about 4.3% per year, with a particularly marked increase in 1992/3 (Figure 1); since there was no change between 1998 and 2002 (when only one plant remained healthy) the rate of spread during the period 1991–98 was about 9.3%. The percentage of infected plants at planting in 1980 is not known, but we would expect any diseased plants to initially have been randomly distributed. Mealybugs were first seen in the plot in summer 1994, 3 years after the first sampling in 1991, and with the disease having already entered its exponential phase (Figure 1); this suggests that mealybugs may have been spreading through the plot for several years before detection. Mealybug population densities in this vineyard were always very low, and the insects usually feed on leaves or shoots and under the bark, rarely on grapes even at harvest time. Taking 1988 as a likely date for the start of mealybug infestation, and back-extrapolating with the different epidemiological models, we can estimate the incidence of GLRaV-3 at planting to have been between 6% (Gompertz model) and 20% (logistic model); with the monomolecular model the predicted values are out of range because this model predicts that the start of infection was only a couple of years before 1991, which is biologically implausible. That incidences of GLRaV-3 at planting appear rather low for the cultivar Albariño 25 years ago, when commercial viticulture was starting in the Rías Baixas and very few old clones without any control were used as sources of grafts for all new vineyards. In 1991 we tested most of the own-rooted 100-year-old Albariño plants, and about 30% were GLRaV-3-positive (Segura et al., 1993). In a survey throughout the Rías Baixas region in 1993 about 40% of plants were found to be infected and these were found in 80% of the vineyards sampled (Segura et al., 1993). If on the contrary we consider that 20–30% is a more likely incidence of GLRaV-3 at planting, this would suggest that vectors did not start transmitting the virus until 1990, only four years before they were detected throughout the plot.

The leafroll virus spreads quite fast in the field. In a crop like grapevine with a very long productive lifespan infection rates of between 4 and 12% are high, especially if we take into account that mealybug population densities are maintained at very low levels in this vineyard so that vector control measures are unlikely to be effective. When healthy plants are planted in or close to old vineyards with vectors present, they can be expected to be totally infected by the time they reach full production.

When we first sampled the vineyard in 1990, the grower had already detected delayed ripening of the grapes, low sugar content and high acidity of the musk? In fact, the Albariño cultivar in this vineyard is usually harvested about 15–20 days later than is normal in Rías Baixas DOC (15–30 September, M. Tubio, personal communication). Besides reducing sugar content and increasing total acidity (Cabaleiro and Segura, 1996; Cabaleiro et al., 1999), a late harvest increases the risk of rain, making botrytis a great concern, and also hampers the marketing of the grapes in years of abundant harvest in the region.

It is clear that the transmission of GLRaV-3 by mealybugs in Beluso happens even with very small mealybug populations and apparently few insects feeding on the plants, but Albariño is a vigorous cultivar and its canopy can easily hide enough

insects. The mealybugs move actively in August just when GLRaV-3 is best detected in leaves and probably most efficiently acquired by the vector from adult leaves and petioles. The spread of the disease is totally dependent on vector spread and behaviour, which in turn depends heavily on the weather conditions in a particular year, the vine training systems used and products and frequency of spraying (Lucas, 2002). Ants, which are the best indicator of mealybug or scale insect activity, could also be responsible for the spread of GLRaV-3, as happens with *Diysmicoccus* spp and mealybug wilt of pineapple (Sether et al., 1998). The number of insects (coccids and pseudococcids) known to be leafroll vectors increases every year, and the expected increase in average temperatures in Europe due to climate change favours most of them. Reduced pesticide application is a main objective of IPM programmes, and such reductions may have effects on the populations of vectors that up to now were controlled indirectly by insecticides (Lucas, 2002). Careful monitoring for the presence of coccids and pseudococcids (and ants as indicators) in nurseries and vineyards is of critical importance for detection of initial foci and prevention of their spread, especially in vineyards with old infected material which could be a source of virus inoculum for new healthy plants.

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