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Comparison of virus infection patterns in Austrian vineyards with simulated ones and some conclusions about transmission

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Spatial patterns of virus infection (with GLRaV-1 and -3, GFkV and ArMV) of randomly chosen vineyards in Austrian winegrowing regions were compared with those that we got as results of simulating the movement of vectors of determined infectivity, longevity and mobility in an artificial vineyard. The reason for this analysis was the fact that for the chosen viroses either the vector is not known or the known vectors are too seldomly found in Austrian vineyards to explain the observed frequency of the viroses. The aim of the study was to determine several features of transmission which reduce the number of species that come into consideration as local vectors for the analysed viruses. ArMV and GLRaV-1 infected vines lump together and therefore are not randomly distributed. This indicates a vector with high infectivity and longevity and low mobility. ArMV infection occurs preferably along the vine rows but not GLRaV-1 infection. Both observations are unexpected because ArMV is transmitted by a soil nematode that does not move especially along vine rows, whereas the known vectors of GLRaV-1, mealybugs and soft scales, do. GLRaV-3 vines do not cluster within the vineyard and so we must expect a vector with low infectivity - especially if its longevity is high - and high mobility. Since the known vector spectrum of GLRaV-1 and -3 overlap and all known vectors have a low mobility this result is surprising, too. The distribution of GFkV within a vineyard is at random but not so its distribution within the winegrowing regions. The most likely explanation for this observation is that there exists a very mobile vector with low infectivity. In any case spreading by human activity seems to be of minor importance.
Keywords: ArMV, GLRaV-1, GLRaV-3, GFkV, virus infection patterns, virus transmission

Vergleich der räumlichen Verteilung von Virusinfektionen in simulierten Weingärten und realen österreichischen Weingärten und einige Schlussfolgerungen hinsichtlich ihrer Übertragung. *Die räumliche Verteilung der Virusinfektion (GLRaV-1 und -3, GFkV und ArMV) in zufällig ausgewählten Weingärten österreichischer Weinbauregionen wurde mit Mustern verglichen, die das Ergebnis einer Simulation der Bewegung von Vektoren mit bestimmten Eigenschaften wie Mobilität, Lebensdauer und Infektiosität in einem artifiziellen Weingarten waren. Die Ursache für diese Untersuchung war, dass für die ausgewählten Virosen entweder der Vektor unbekannt ist, oder aber er ist in den österreichischen Weingärten zu selten, um die beobachtete Häufigkeit zu erklären. Ziel dieser Studie war es, die besonderen Charakteristika der Pathogenübertragung herauszufinden und damit die Anzahl der lokal als Vektoren in Frage kommenden Spezies möglichst zu reduzieren. Durch ArMV- oder GLRaV-1 infizierte Rebstöcke zeigen eine herdförmige, nicht zufällige Verteilung. Dies weist auf einen Vektor hin, der sehr infektiös ist, eine hohe Lebenserwartung aufweist, aber wenig mobil ist. Die ArMV-Infektion erfolgt bevorzugt entlang der Rebzeile, nicht aber die von GLRaV-1. Beide Beobachtungen kommen unerwartet, da ArMV durch einen Bodennematoden übertragen wird, der sich nicht bevorzugt entlang der Rebzeile bewegt, während sich die bekannten Vektoren von GLRaV-1, Cocciden und Pseudococciden, entlang der Zeilen verbreiten. GLRaV-3 infizierte Rebstöcke zeigen keine herdförmige Verbreitung, woraus folgt, dass man einen Vektor geringer Infektiosität annehmen muss – besonders wenn dessen Lebenser-*

wartung relativ hoch sein sollte – und hoher Mobilität. Da das Spektrum der bekannten GLRaV-1 und GLRaV-3 Vektoren überlappt und alle bekannten Vektoren eine geringe Mobilität aufweisen, überrascht dieses Ergebnis. Die Verteilung von GFkV-infizierten Reben innerhalb der Weingärten ist zufällig, aber nicht dessen Verteilung in den Weinbauregionen. Die wahrscheinlichste Erklärung für diese Beobachtungen bietet die Annahme, dass ein sehr mobiler Vektor mit geringer Infektiosität existiert. Die Verbreitung durch den Menschen scheint von geringer Bedeutung zu sein.
Schlagwörter: ArMV, GLRaV-1, GLRaV-3, GFkV, Virusinfektionsmuster, Virusübertragungt

La répartition et la fréquence des viroses de la vigne dans les régions viticoles de l'Autriche. *La répartition de 14 différents viroses de la vigne dans les régions viticoles autrichiennes a été étudiée et cartographiée. GLRaV-1, -2, -3 et -6, GFkV, GFLV, ArMV, TBRV et SLRSV ont été détectés. GLRaV-1 est le virus le plus fréquent, près de 24 % des vignes sont atteintes. À l'échelle locale, l'abondance peut cependant monter jusqu'à 44 % ; elle est particulièrement élevée le long du Danube, au Burgenland central et en Styrie de l'ouest. Le deuxième virus associé de l'enroulement de la vigne, par ordre de fréquence, est GLRaV-3, près de 5 % des vignes examinées étaient infectées. Malgré le spectre des vecteurs se chevauchant avec GLRaV-1, les diagrammes de répartition des deux virus comportent des différences marquées, surtout au sud de l'Autriche. GLRaV-6 est rare en Autriche (0,4 %) et n'a pas du tout été détecté au sud du pays. Seul un nombre minime d'échantillons de vignes a été examiné en vue de détecter GLRaV-2. Les népovirus GFLV et ArMV, eux aussi, ne sont pas fréquents en Autriche, leur répartition étant assez inhomogène, ils peuvent quand-même avoir une grande importance économique à l'échelle locale. Par exemple, ArMV a été détecté en Styrie de l'ouest dans 59 % des échantillons. Seules quelques vignes individuelles ont été infectées par TBRV et SLRSV. GFkV est le deuxième virus par ordre de fréquence ; sa présence a pu être révélée dans 13 % des vignes examinées. Dans la région examinée, le virus GFkV est celui qui est répandu de la manière la plus homogène.*
Mots clés: GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-6, GFkV, GFLV, ArMV

Hewitt et al. (1958) were successful in demonstrating the transmission of a grapevine disease by an animal vector for the first time. They showed that the grapevine fanleaf disease is transmitted by the nematode *Xiphinema index*. This discovery opened a new research field in plant pathology. Soon more vectors of nepoviruses were found, the genera *Xiphinema*, *Longidorus*, *Trichodorus* and *Paratrichodorus* were identified as transmitters of plant viruses in Europe and other countries.

By contrast, it was believed untill the eighties of the last century that leafroll and related viruses were only transmitted through plant material. This changed when the role of mealybugs (Pseudococcidae) in the leafroll transmission was discovered. Afterwards an increasing number of observations on the natural spread of leafroll viruses were published, mainly from Southern European regions and non-European countries. From some of these studies we may assume that beside Coccids and Pseudococcids other, yet unknown, vectors exist.

Obviously these reports have a significant influence on the practice of producing virus-free rootstocks, because it is now evident, that regular control of vector and virus infestation of nurseries is necessary. There is no steady state of soundness. Transmission of viruses from infected vineyards to virus-free plants in their neighbourhood may occur at any time.

Most epidemiological studies and field spread analyses take place in selected, pathogen-rich vineyards, where the spatiotemporal pattern of infection has been studied over some years. Here we took a different approach. We analyzed spatial patterns of virus infection in a great number of randomly chosen vineyards in all Austrian winegrowing regions and compared them with those patterns we got by simulating the movement of vectors with known infectivity, mobility and longevity in an artificial vineyard. In doing so, we wanted to discover the fundamental features of the grapevine virus transmitters active in Austria. This knowledge may be helpful in the search for hitherto unknown virus vectors.

Method

Sampling technique

Frequency and distribution of grape viroses in all Austrian winegrowing regions were analyzed during several years using a global positioning system (Personal Navigator, Garmin, GPS 12). Wherever local conditions made it possible, five vine samples per geographical raster unit were taken, where the raster unit length typically was one arc minute north-south (ca. 1.85 km) x east-west (1.24 km). Within the raster unit a vineyard was chosen randomly. As sampling position

within the vineyard the same grape row and vine number was always selected by the person sampling.
At the sampling location tendrils of five vines forming a cross were taken for serological detection of viruses, a central one, the neighbouring ones (before and behind) in the same row and the vines in the adjacent rows. All samples were tested for six grapevine viruses using DAS-ELISA (antisera purchased from Bioreba, Reinach, CH): Grapevine fanleaf virus (GFLV), Arabis mosaic virus (ArMV), Grapevine fleck virus (GFkV) and Grapevine leafroll associated viruses (GLRaV-1, -3, -6).

Clustering of viroses

Every cross of five vines (further on called sampling unit) may contain from zero (k = 0) up to five (k = 5) virus-positive vines of one virus species. The number of virus-positive vines per sampling unit k defines the class of infection. The frequency distribution of the classes within a winegrowing region allows conclusions concerning the distribution of the pathogen, e. g. whether it is randomly distributed or shows a tendency to cluster. If we assume that the pathogen distribution is a consequence of transmitter activity, we may even gain information about some characters of it, like infectivity, longevity or mobility of this organism. There are several different ways to get the desired information:

a) Comparison of the cluster distributions with a random distribution. Every vine may either be non-infected or infected, so if we select repeatedly five vines out of a vineyard by random, a binomial distribution will follow:

1) $P_k = [n!/(k!(n-k)!]\ p^k\ (1-p)^{n-k}$,

n = 5 (n is the number of vines per sampling unit), k = 0,…,5; p is the relative frequency of virus-positive vines and must be estimated from the data.
Of course, our five vines are not picked out randomly, they are nearby, and hence since they are not randomly distributed within the vineyards, some divergence from the binomial distribution will be observed. The divergence will be high, if the virus-positive vines within a vinegrowing region are lumping together, e. g., because they are transmitted by a slow, not very mobile transmitter with high infectivity. The divergence D between observed distribution O and expected one (binomial one) e (values scaled so that the sum for all k is one) was measured, using eq. 2:

2) $D = [\sum_k (O_k - e_k N)^2]/N^2$,

where N is the number of sampling units, analyzed within a winegrowing region. eN = E. Besides D the chi-square statistics was used to characterize the divergence.

b) If distribution patterns are characterized by scattered clusters and thus diverge from binomial distribution, a common approach in ecology is to fit the observed distribution to the so-called negative binomial distribution Q_k (Timischl, 1990; Poole, 1974):

3) $Q_k = (1+F)^{-g}$, if k = 0 and

4) $Q_k = F^k\ (1+F)^{-g-k}\ [g\ (g+1)\ \ldots\ (g+k-1)]/k!$, if k = 1, 2, ...

The reciprocal of g is a measure of aggregation, e. g. of the lumping of the virus-positive vines.
To fit observed data to the distribution, two approximation procedures were used, the one of Newton and one that bases upon the concept of function space.

c) Neither of these two approaches allows a further description of the characters of the virus transmitter. Thus we performed a simulation study written in Object Pascal (Borland International, Scott's Valley CA, USA), using Borland Developer Delphi 7.

The simulated vineyard contains 250.000 vines (500 x 500), each of them may exist in two states, either non-infected or infected. We avoid boundary effects, giving the vineyard the topology of a torus. At the beginning all vines are non-infected. At a random point a transmitter starts a random course. It has two characters:

1) Infectivity, which may vary between 1 % and 100 %. 100 % means that all vines met on the random course are infected and thus switch from non-infected to infected if they are not already infected. If infectivity is lower, the probability of infection decreases in a linear manner with the value. We assume that transmission occurs persistently, that is to say that infection probability remains the same during the vector's tour and that it is infectious from the beginning.
2) Longevity (or mobility) defines how many vines are visited during lifetime. We assume that longevity is normally distributed with a standard deviation that is the squareroot of the mean. The mean lifetime may vary from 1 to 100. So up to 100 vines and with a lesser probability even more, may be visited by one vector. A more unrealistic assumption – taking into account that some vectors may be flying animals – in this model is that on the random course vines are never left out. Transmitters move through the vineyard until a certain degree of infection is reached. The

degree of infection is defined as the percentage of infected vines. As a result of the activity of the vectors a characteristic infection pattern occurs (Fig.1).
In order to get the frequency distribution of the infection classes, within the simulation we used a sampling unit similar to the one utilized in practice: five neighbouring vines arranged in a cross. The position of the central vine was chosen by random and the number of infected vines within the sampling unit was determined. This step was repeated 500 times. Afterwards the whole procedure was repeated 100 times to get a statistical conclusion. Figure 2 gives some examples of frequency distributions that correspond to Figure 1.
In order to create simulated distributions that can be fitted to the observed ones the degree of infection (percentage of infected vines) was estimated from the observed data. Afterwards we varied longevity and infectivity increasing both alternatively by one per step and combined all values so that 10.000 different distributions occurred. The quality of fit to the observed distribution was measured using eq. 5:

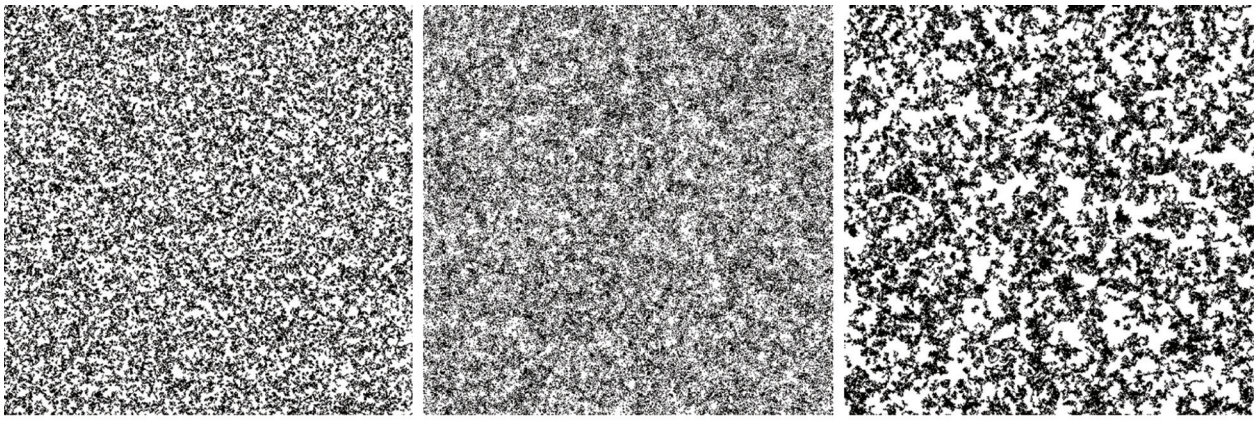
5) $D = \sum_k Abs(o_k - e_k)$,

Abs is the absolute value, o the observed and e the expected distribution (both were scaled so that the sum of all ok, or ek, respectively, is one).
A more detailed description of the model is given in Tiefenbrunner et al., 2010.

Results

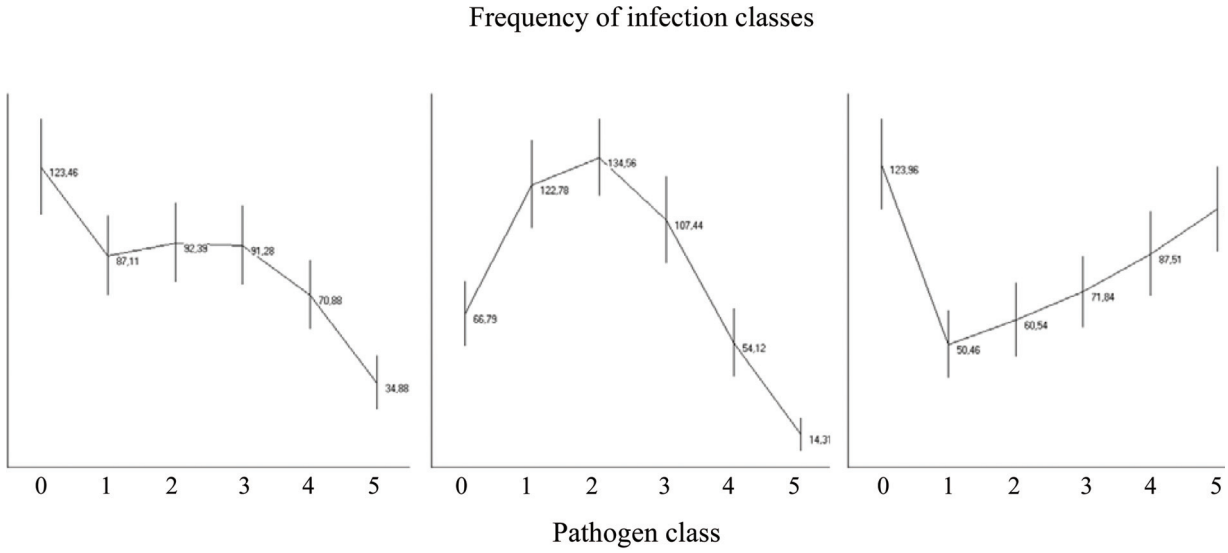
Initial situation

To gain detailed information about frequency and distribution of grape damaging viroses, 5081 vines of all winegrowing regions of Austria were analyzed. Most common is the leafroll disease, the leafroll virus GLRaV-1 is dominating, on average 24 % of all vines are infected (Fig. 3). The estimated frequency of infected vines varies considerably with the region. In Western Styria, Mittelburgenland, Traisental and the Wachau the frequency is about 40 % or even more, whereas only 4 % of the grapevines are infected in the eastern Weinviertel. Low frequencies of this virus type also occur in the western Weinviertel and south-east Styria (about 16 % to 17 %).
The leafroll virus GLRaV-3 is of minor importance, infecting roughly 5 % of the vines. Local fluctuations are not as high, in Southern Burgenland and in the Kamptal the highest frequencies were observed (around 8 to 9%), the lowest in Eastern Styria (0 %).
The Grapevine fleck virus (GFkV) is the second-most frequent virus in the Austrian vineyards, infecting on average 13 % of the vines. Much higher frequencies can be observed in Southern Burgenland (29 %) and



Frequency: 40%;	Frequency: 40%;	Frequency: 40%;
Longevity: 10;	Longevity: 100;	Longevity: 100;
Infectivity: 100%	Infectivity: 10%	Infectivity: 100%

Fig. 1: Examples of infection patterns that arise in the simulation as a consequence of vector activity with distinct transmitting characters: Frequency: frequency of infected plants; Longevity: average number of visited vines per transmitter; Infectivity: Probability to infect a visited vine in percent



Frequency: 40%; Longevity: 10; Infectivity: 100%

Frequency: 40%; Longevity: 100; Infectivity: 10%

Frequency: 40%; Longevity: 100; Infectivity: 100%

Fig. 2: Frequency distribution of infection classes that correspond to the examples of Figure 1. The line connects the average absolute frequencies of the infection classes; the vertical bars give the 95 % range of values for each infection class.

in the bordering area (Upper South-Eastern Styria 27 %). The lowest frequency occurs in the east of the Weinviertel (2 %).

Arabis mosaic virus (ArMV) is of great relevance in the southern parts of Austria, Styria and Southern Burgenland, but has virtually no significance in the rest of the country. In Western Styria ArMV is the most frequent virus, infecting 59 % of all grapevines (random sampling). Analyses concerning the abundancy of the ArMV-vector *Xiphinema diversicaudatum* showed somehow surprisingly that it is not frequent in the vineyards, although in Styria this nematode is numerous in the surrounding orchards and meadows (Gangl et al., 2002). In the more northern winegrowing regions *X. diversicaudatum* is very scarce with the exception of the riparian woods of these regions (Tiefenbrunner and Tiefenbrunner, 2004).

GLRaV-6 and GFLV are of minor importance in Austria.

Virus infection patterns

For the analysis of virus infection patterns data from all the winegrowing regions where the virus was detected in at least 15 vines and where the number of vines that were analysed was not less than 200, were used (this corresponds to 40 sampling units). The shaded cells in the table of Figure 3 show which regions were used for which virus. The data of Styria were always analyzed as a whole whereas in the other regions the utilization of subregions was preferred due to the distinction of the regional parts. In the subregions 3 (Mittelburgenland) and 9 (Thermenregion) an alternative sample unit was applied and therefore they were not used for infection pattern analysis. GLRaV-6 and GFLV were only rarely detected and thus could not be analysed. In GFkV and GLRaV-1 sample units of 11 subregions were available, 7 for GLRaV-3 and only one for ArMV.

At first we were interested in finding out which general factors (e. g. virus type, region, dimension of infection) influenced the infection patterns. Thus we performed a multivariate comparison (Principal Component Analysis - PCA) of the regional frequency distributions of the infection classes (Fig. 4).

The main result is that the frequency distributions cluster depending on the virus type, but not on the region. Furthermore the result is not simply univariate

Vine growing regions		Samples (total)	Frequency in % GLRaV I	GLRaV III	GLRaV VI	GFkV	GFLV	ArMV
Burgenland	1	705	20,6	5,1	0,1	13,0	1,1	0,3
	2	575	14,1	4,3	0,2	17,4	1,4	1,4
	3	270	41,5	4,1	0,4	12,2	0,4	1,5
	4	140	30,7	9,3	0,0	28,6	0,7	12,1
Lower Austria	5	363	30,9	5,2	0,0	11,0	0,3	0,6
	6	400	25,8	4,8	1,3	11,3	0,3	0,3
	7	314	25,2	8,3	1,0	18,5	1,3	3,8
	8	245	30,6	7,3	0,8	13,9	0,0	0,8
	9	163	22,7	4,3	0,6	17,8	0,0	0,6
	10	216	39,4	5,6	0,9	13,9	0,0	1,9
	11	200	39,0	5,0	0,0	11,0	0,0	2,5
	12	355	3,7	3,7	0,0	2,0	0,3	0,8
	13	300	25,0	4,7	1,0	7,7	0,0	0,0
	14	455	16,3	5,1	0,2	10,8	1,3	0,2
Styria	15	75	16,0	1,3	0,0	26,7	0,0	12,0
	16	95	16,8	3,2	0,0	22,1	0,0	3,2
	17	120	21,7	6,7	0,0	19,2	0,0	7,5
	18	90	44,4	0,0	0,0	7,8	0,0	58,9
		5081	23,7	5,1	0,4	13,2	0,6	2,7

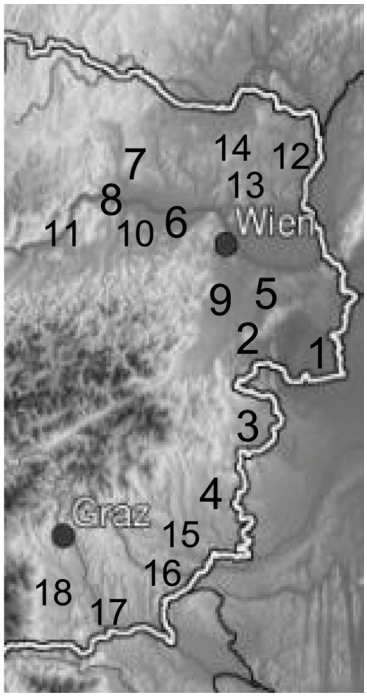


Fig. 3: Frequency of grape damaging viruses in Austrian winegrowing regions: 1) Neusiedlersee, 2) Neusiedlersee-Hügelland, 3) Mittelburgenland, 4) Southern Burgenland, 5) Carnuntum, 6) Wagram, 7) Kamptal, 8) Kremstal, 9) Thermenregion, 10) Traisental, 11) Wachau, 12) Eastern Weinviertel, 13) Southern Weinviertel, 14) Western Weinviertel, 15) Upper South-Eastern Styria, 16) Lower South-Eastern Styria, 17) Southern Styria, 18) Western Styria. The shaded cells in the table indicate which of the regions were used for virus infection pattern analysis. The data of Styria were utilized as a whole (black framing).

which would be the case if the similarity between the distributions were a function of the frequency of virus-postive vines alone. The similarity of distributions is especially high concerning GLRaV-3 and GFkV, where there are no outliers. GLRaV-1 clusters less and there are a lot of outliers. GLRaV-1 – Western Weinviertel and GLRaV-1 – Neusiedlersee tend to the GFkV cluster. These two and GLRaV-1 – Neusiedlersee-Hügelland, that is also an outlier, have relatively low infection frequencies and therefore this factor may be of importance concerning GLRaV-1. However, the other two outliers, GLRaV-1 – Kremstal and GLRaV-1 – Wachau do not have low infection frequencies.
It is the premise for all further analyses that the frequency distributions of infected vines within a sampling unit show a virus type dependent characteristic.

Comparison with a random distribution

Using equation 1 and 2 we compared the deviance of a binomial distribution from the region-typical frequency distribution of the infection classes (virus-positive vines per sampling unit k = 0,…,5), where p was estimated from the local relative frequency of infected grapes. The less the region-typical distribution fits, the higher the value of D and thus the degree of infected vine clustering.
Each point in Fig. 5 meets D for a special subregion and virus type. Figure 5 shows that the virus types differ concerning the randomness of the infection patterns.In order to enhance this conclusion, ANOVA was performed. ANOVA's P = 0.0 and the additionally accomplished Multiple Range Test (95 % LSD) indicates a significant difference between GFkV and GLRaV-1, GLRaV-1 and GLRaV-3, but not between GFkV and GLRaV-3. There is only a single value for ArMV. In a Levene's Test applied P = 0.17, thus there is not a statistically significant difference amongst the standard deviations at the 95.0 % confidence level, so the criterion of variance homogeneity, a premise for ANOVA, is fulfilled.
Hence we can argue that infected vines tend to lump together if they are infected with ArMV or GLRaV-1, but not – or to a significant minor degree – if infected with GFkV or GLRaV-3. Of course we can only affirm

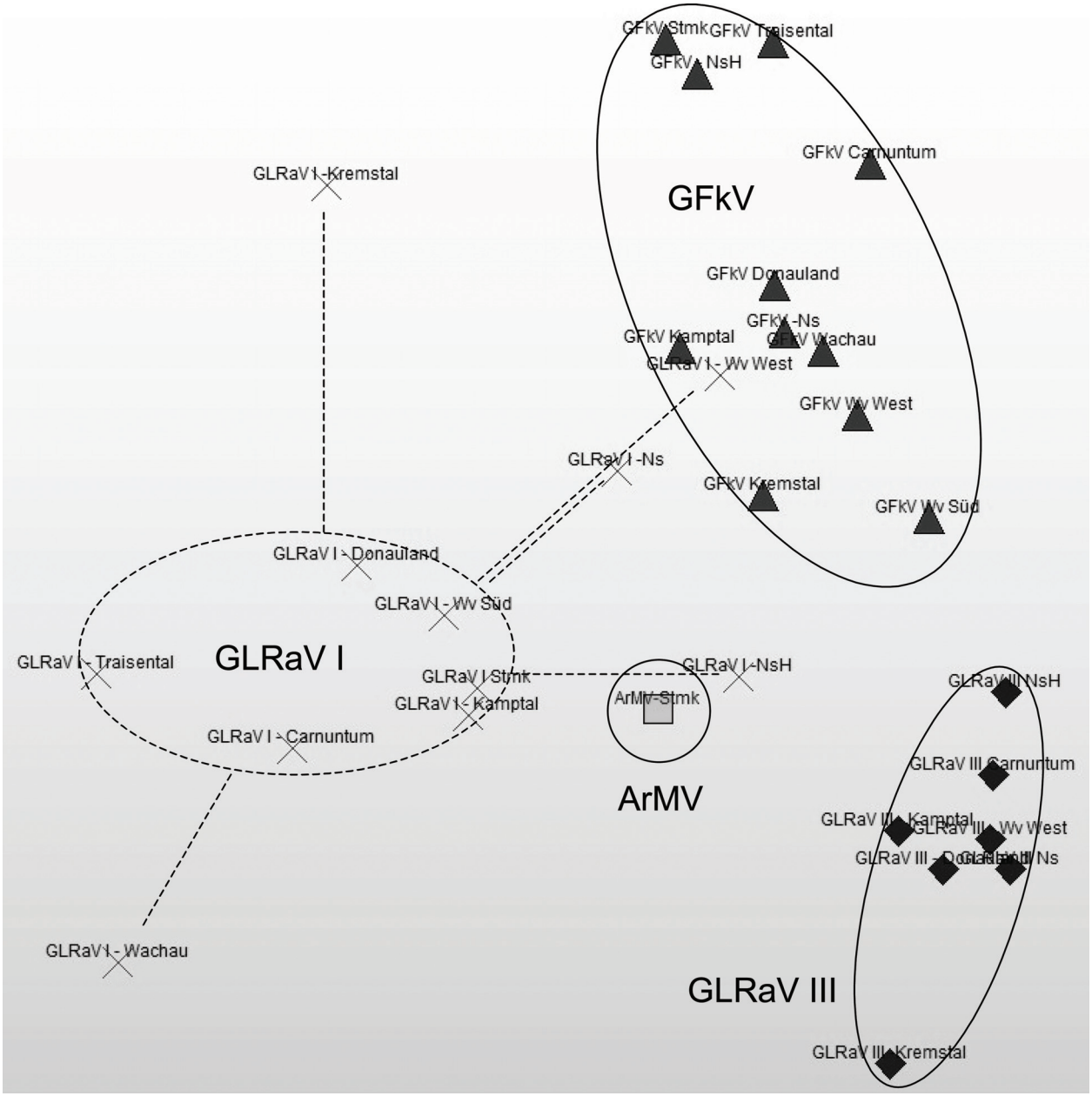


Fig. 4: Principal Component Analysis of the frequency distributions of the infection classes. Clustering can be observed concerning virus type, but not concerning region.

this for Austrian vineyards. In GLRaV-1 the highest degrees of clustering occurs in the regions Wachau (11), Carnuntum (5), Southern Weinviertel (13) and Styria (15-18).

Fitting the data to a negative binomial distribution

With the exception of GFkV from the Traisental all observed infection class distributions showed higher

variance values than mean ones. This is a feature of a negative binomial distribution (but of course not only of this kind of distribution). It can be utilized to measure the degree of clustering.
The infection classes do not fit well to the negative binomial distribution. The quality of fit was poor and thus the two approximation procedures lead to different results. We conclude that the infection classes are not negatively binomially distributed.

Comparison of infection patterns in real and simulated vineyards

Longevity and infectivity of the vector in the simulated vineyard were varied by increasing both values by 1, from 1 to 100. All possible values were combined so that 10,000 different infection class distributions were created. These distributions were compared with the observed ones. The result is shown exemplarily for the region Neusiedlersee-Hügelland in Figure 6.

Obviously the result is not a dot that shows an area where the combination of longevity and infectivity explains the observed data best. A curve appears instead. It is possible to fit this trajectory with very high quality to functions like:

6) $y=a+b/x$, or
7) $y=a+b/(x)^{1/2}$, or something in between.

Thus we get two parameters, a and b, that describe the result and are interpretable. Apparently "a" is the minimum infectivity that is necessary to be in accordance with the data. Furthermore we can perform a coordinate transformation of the kind x' = x and y' = y-a, so that b = x'y' for eq. 6. In this new coordinate system "b" is simply the product of longevity and infectivity which is the mean number of infected vines per vector. So "b" gives approximately this number, especially if "a" is low. Even if this is not the case "b" gives an imagination of the magnitude of the number of infected vines per transmitter. In the case of a spatial random distribution of infected vines we assume a = 0 and b = 1. If the number of infected vines per vector "b" equals 1 (one vine), the infection of any two vines is completely independent and thus at random if the movement of the transmitters occurs randomly. Both values get higher, if the spatial distribution of the infected vines is not at random.
We performed this analysis for all available virus/(sub-)region combinations and compared the results for the different virus types using eq. 6.
The minimum infectivity "a" is with 44 % the highest for ArMV, so the vector of this pathogen must be very infectious and the one of GLRaV-1 with 31 % must be too. The infectivity of GLRaV-3 is significantly lower, 18 % on the average. Lowest is the one of GFkV with 12 %. However in any case (with the exception of ArMV, where only one value is available) the deviation from the mean is high.
The value of "b" for ArMV, 9.5, is highest. It is likely that this overestimates the number of infected vines per vector because "a" for ArMV is also high and a vector would have to visit 22 vines for infecting 9.5 of them, if infectivity is at minimum (44 %). For GLRaV-1 "b" is relatively high, too, with a value of 6.3, but reaches only 2.1 for GLRaV-3 and 1.2 for GFkV, respectively. Especially for GFkV the value is within the range assumed for a spatial random distribution.
We performed ANOVA to decide whether there is a significant difference of the "a" and "b" values concerning the virus types. In fact this is the case for both parameters (ANOVA for "a": P = 0.0001, Levene's test P = 0.1; ANOVA for "b": P = 0.0001, Levene's test P = 0.056). The multiple range test (95 % LSD) shows that there is no significant difference between GFkV and GLRaV-3, whereas the difference between GLRaV-1 and GFkV and GLRaV-1 and GLRaV-3 is significant concerning "a" and "b" as well.

As can be seen from Figure 7 "a" and "b" are correlated.

Correlation between vines of the sampling unit

Different vectors produce dissimilar infection patterns not only because they differ in infectivity and longevity. Mealybugs for example tend to crawl along the branches and thus spread a pathogen along the vine row more easily than vertically on a vine. Longidorid nematodes on the other hand should not have such a directional preference. In order to find out whether there is a preferential spreading of the pathogens along the vine row we calculated the correlation of any two vines within the sampling unit concerning infection using the contingency coefficient. This coefficient shows a zero value if no correlation exists and is one in the case of perfect correlation.The results are shown in Table 1.
The average contingency coefficient is highest for ArMV (0.37), followed by GLRaV-1 (0.32), GLRaV-3

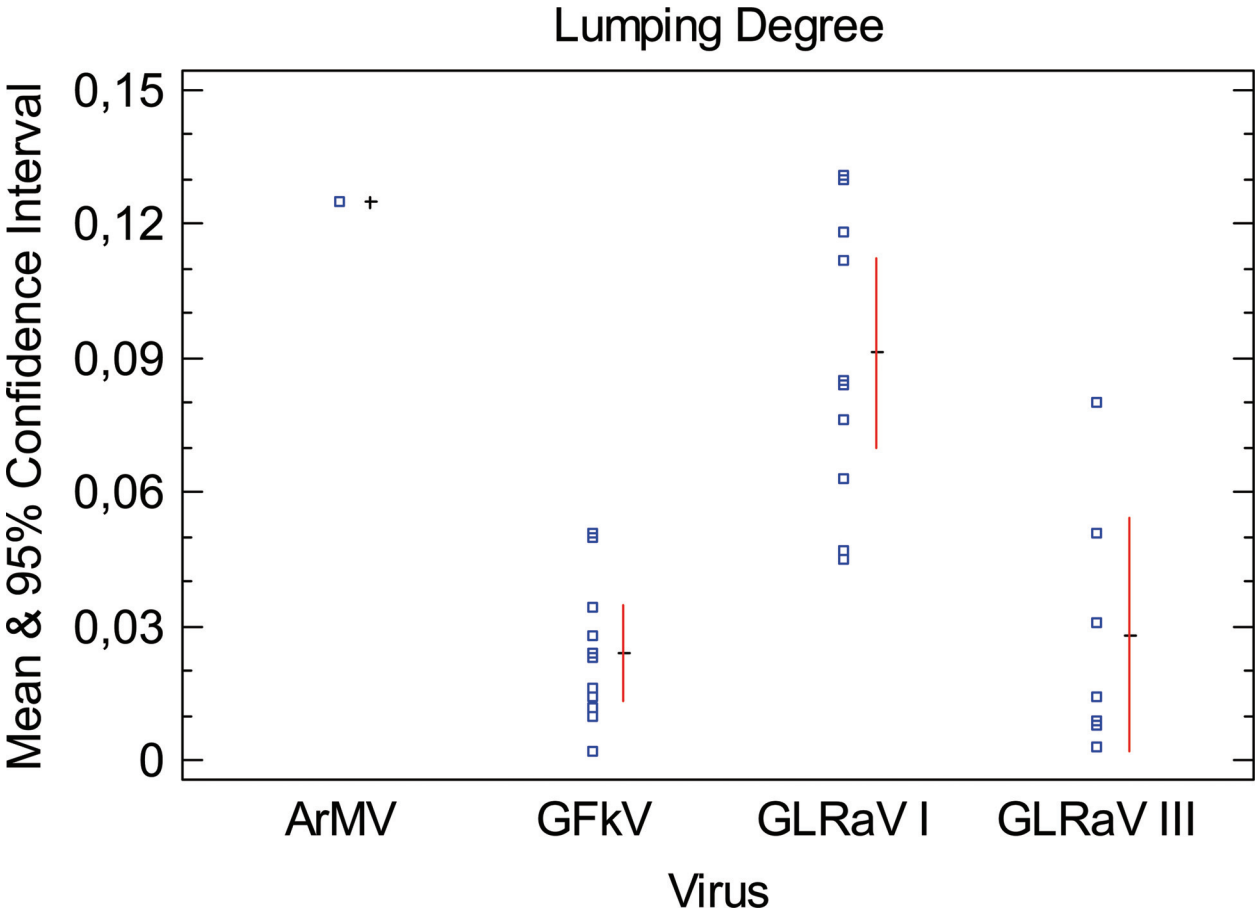


Fig. 5: Comparison of the distribution of virus infected vines per sampling unit with a binomial distribution. The higher the "lumping degree" D, the less the pattern of infection is at random.

(0.22) and GFkV (0.16). It is zero in the case of a random distribution and is higher as a consequence of clustering, so once again we see that infected vines with ArMV and GLRaV-1 cluster more than those with GLRaV-3 and GFkV. In comparing the coefficients of the vine pairs of the three vines that lie in the central row with the others, we may find out whether there is a preferred pathogen spreading along the row. In the case of ArMV the central line coefficients (0.42 to 0.46) lie outside the range of the others (0.26 to 0.38), indicating that there is indeed a higher pathogen spreading within the row than vertically to it. Concerning all other viruses the ranges overlap and hence there is no clear indication of a preferred direction of infection. This result is unexpected because it is generally believed that the transmitter of ArMV is a soil nematode, whereas the ones of the leafroll diseases are Coccidae and Pseudococcidae.

Discussion

Using a computer simulation of vectors with determined features moving in an artificial vineyard for comparison with our data, we hoped to find a clear impression of the abilities of the real world vectors. Especially we wanted to get a consistent picture of their infectivity and of the number of host plants each vector visits. This number depends on both, the mobility and the longevity, because even a very slow vector is able to visit a lot of hosts if it lives long enough and we are not able to separate these two factors simply by looking at spatial infection patterns. Comparable patterns also occur if a low infectivity vector visits every plant on its trajectory or if only some hosts are visited and the vector has a high infectivity. If these factors could be separated clearly, we would see dots instead of curves in

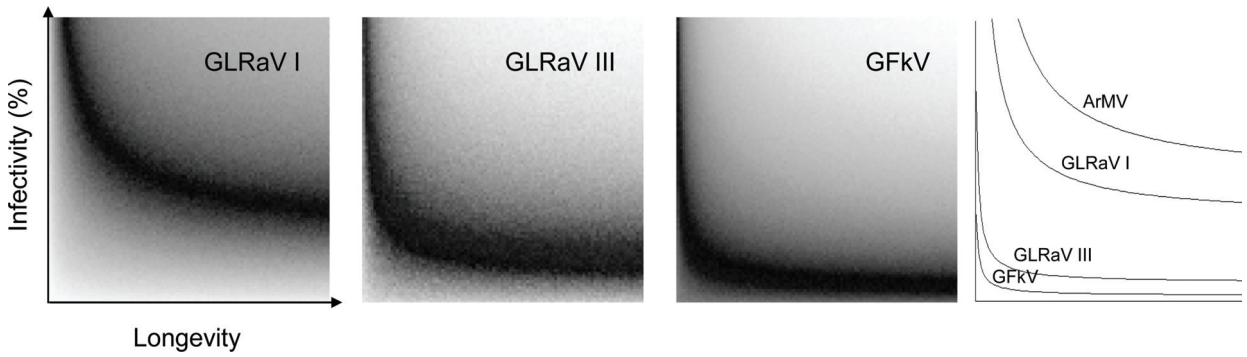


Fig. 6: Comparison of simulated and observed infection class distributions for three types of virus in one winegrowing region (Neusiedlersee-Hügelland). The darker, the better the match. For the approximation of the right most graphic equation 6 was used. Here to allow comparison of all analyzed virus types ArMV from Styria was added.

Figure 6 and maybe in Figure 7 "a" and "b" would not be correlated. As a consequence, we didn't get information about infectivity and number of visited hosts (longevity * mobility) but about minimum infectivity - that is infectivity under the assumption that the number of visited hosts is high. If this number is low, infectivity may be much higher than approximated.
The second value we got can be interpreted as number of infected hosts per vector, but the quality of this interpretation depends on the minimum infectivity, which must be small. Thus the result is not satisfying. In future analyses it may be necessary to use more vines per sampling unit. Whether a study with more vines per unit enhances the interpretability will be analysed in a separate simulation (Tiefenbrunner et al., 2010).
Worldwide GLRaV is the most common grapevine virus. In Austria GLRaV-1 is the dominating virus species, but we detected GLRaV-3 and GLRaV-6, too. GLRaV-3 is frequent (about 5 % of the tested plants). Since Dimitrijevic (1973) it has been known that the leafroll disease is spread in the field and in the 80ies the importance of pseudococcid mealybugs for the transmission was recognized (*Pseudococcus longispinus*: Rosciglione et al., 1983; Tanne et al., 1989; Planococcus ficus: Rosciglione and Gugerli, 1989; Engelbrecht und Kasdorf, 1990). *Heliococcus bohemicus*, *Phenacoccus aceris* and *Parthenolecanium corni*, a soft scale (Coccidae), transmitted GLRaV-1 (Sforca et al., 2003). *Pulvinaria vitis*, *Pseudococcus calceolariae*, *Ps. longispinus*, *Ps. maritimus*, *Ps. viburni*, *Planococcus citri*, *Pl. ficus*, *Heliococcus bohemicus* and *Phenacoccus aceris* are known vectors of GLRaV-3 (Charles et al., 2006). The vector spectrum of both virus species overlaps and hence some similarity of distribution and clustering in the field could be expected. However, our data do not support the conclusion that both virus species are transmitted by the same vector in Austrian vineyards since GLRaV-1 clusters, and GLRaV-3 does not. Even if the transmission ability of the two virus species is different we would expect more similarity in the distribution patterns in the case that both species are transmitted exclusively by the same vector. Furthermore GLRaV-1 is more widespread than GLRaV-3 that does not occur e. g. in Western Styria, whereas GLRaV-1 is very frequent in this winegrowing region. The vectors for GLRaV-6 are unknown. In Austria the frequency is very low and in some winegrowing regions this species does not occur or is very seldom.
GFkV was first characterised by Boulila et al. (1990). Worldwide it is a widespread virus in vines with limited economical significance. Serious damages are caused only in the presence of other grapevine viruses (Walter and Martelli, 1997). A vector of this virus is not known and it is even doubted that one exists. Our observations are in congruence with the assumption that the virus is transmitted by a very mobile vector with probably low infectivity. Within the vineyard this virus is the most randomly distributed and thus does not aggregate.
The occurrence of the virus is not homogenous in Austria with "hot spots" in the west of the wine growing area Neusiedlersee and in the Wachau and the Kamptal. We interprete this inhomogenity in occurence as a hint for the existence of one or more vector species and as refutation of the assumption of spreading by human activity alone.

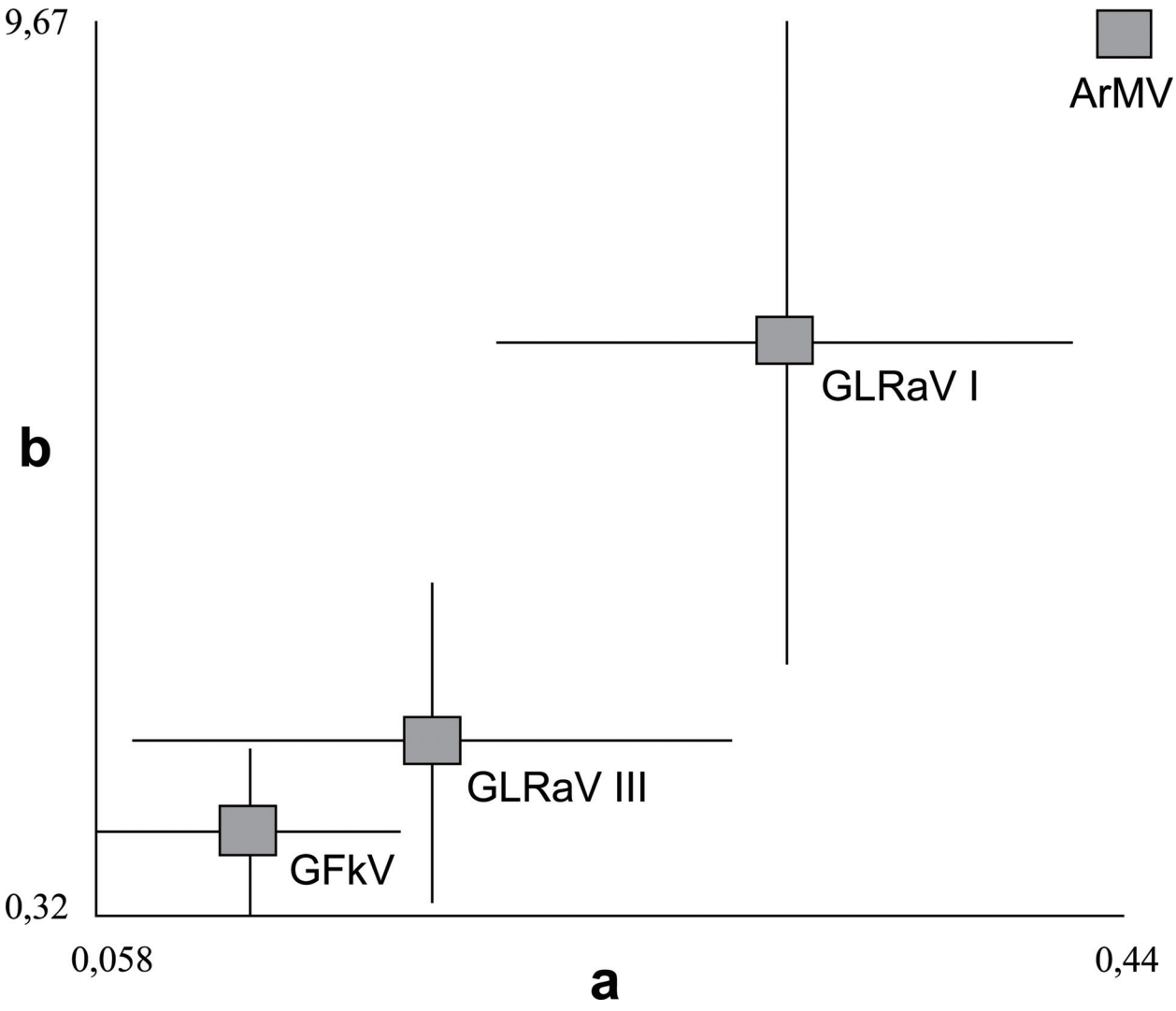


Fig. 7: Estimated "a" and "b" values (from eq. 6) of the comparisons of simulated and observed infection class distributions (mean ± standard deviation). These values are indicative for minimum infectivity ("a") and number of infected vines per vector ("b"), respectively.

ArMV, a comoviridae nepovirus, is associated with relatively cool-climate viticulture and is mostly distributed over Central Europe (Abelleira et al., 2009). In Austria it occurs mainly in the south. It is transmitted by *Xiphinema diversicaudatum*, a soil nematode with low mobility. Our observation that this virus tends more to aggregate than other Austrian viruses therefore does not come as a surprise. However, the fact that this virus is more frequent in the vine row than at right angle to it is unexpected because soil organisms should not distribute preferentially along the rows. This observation may be an artefact caused by the different distance of neighbouring vines in the row and right-angled to it. The distance between the vine rows is much higher – two to three times that of the neighbouring distances within the row. However, if this is the right explanation, one wonders why this effect cannot be seen in GLRaV, where it should be

Table 1: Contingency coefficients for infection of any two vines of the sampling unit. The results for the pairs of vines where both belong to the middle row are highlighted

ArMV	in front	behind	lower row	higher row
center	0,42	0,46	0,31	0,26
in front		0,46	0,30	0,38
behind			0,38	0,35
lower row				0,36

GFkV	in front	behind	lower row	higher row
center	0,20	0,15	0,12	0,15
in front		0,20	0,17	0,16
behind			0,20	0,10
lower row				0,16

GLRaV-1	in front	behind	lower row	higher row
center	0,35	0,33	0,29	0,27
in front		0,35	0,30	0,30
behind			0,36	0,30
lower row				0,32

GLRaV-3	in front	behind	lower row	higher row
center	0,26	0,28	0,19	0,25
in front		0,22	0,21	0,16
behind			0,27	0,14
lower row				0,18

expected and even be enhanced, because the vectors distribute preferentially along the rows.
Although we think that our method is a useful tool in virus transmission research we are aware that further development is needed.

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