

## **The spatial pattern of “Mal de Río Cuarto del Maíz” (Rio Cuarto Corn disease) in corn fields**

**Eduardo V. Trumper<sup>1</sup>; David E. Gorla<sup>2</sup> and Mariano P. Grilli<sup>2</sup>**

*1. Sección Entomología, E.E.A. Manfredi - I.N.T.A., Ruta Nacional Nro. 9, Km 636, (5988) Manfredi, Córdoba, Argentina*

*2. Laboratorio de Ecología de Insectos, Fac. de Cs. Agropecuarias, Universidad Nacional de Córdoba, Avda. Valparaíso s/n (Campo Experimental), (5000) Córdoba, Argentina*

**Abstract.** *The spatial pattern of Mal de Río Cuarto del maíz (Rio Cuarto Corn Disease) was studied during three growing seasons in Córdoba Province, Argentina. The study combined ELISA determinations in experimental plots outside the endemic area during the first growing season with symptom assessments in that situation and in commercial plots in or near the endemic area during the two subsequent growing seasons. For the experimental plots studied during the first growing season, ELISA data indicated a random spatial pattern of the disease, whereas symptoms data, analyzed by ordinary runs test, showed only 13 out of 120 samples with clumped pattern. Symptom analysis during the next two seasons revealed only 3 of 23 samples with significant aggregation. No edge-to-center disease gradients were identified, suggesting that the vector does not disperse from nearby sources. Our results agree with the hypothesis of a random spatial pattern of this disease, consistent with its monocyclic nature.*

**Resumen.** *Se estudió el patrón de disposición espacial del “Mal de Río Cuarto del maíz” durante tres campañas agrícolas en la Provincia de Córdoba, Argentina. El trabajo combinó estimaciones por la prueba de ELISA en un campo experimental durante la primera campaña, con datos de síntomas microscópicos tomados en la misma situación y en campos comerciales durante dos campañas posteriores. Las muestras analizadas por medio de ELISA mostraron un patrón espacial de la enfermedad indistinguible del esperado por azar. Los datos de síntomas, analizados mediante el “test común de runs”, mostraron que sólo 13 de las 120 muestras estudiadas en la primera campaña en el campo experimental tenían disposición espacial agregada y que sólo 3 de las 23 muestras analizadas en los campos comerciales durante dos campañas posteriores mostraron agregación. No se identificó ningún gradiente de incidencia de Mal de Río Cuarto desde el perímetro al centro del cultivo, observación que sugiere que el vector no se dispersa a lotes de maíz desde fuentes cercanas.*

### **Introduction**

The Mal de Río Cuarto del Maíz (MRC), (Rio Cuarto Corn Disease), is caused by the Mal de Río Cuarto Virus (MRCV, Fijivirus)(Conci and Marzachi 1993) which is transmitted by the planthopper *Delphacodes kuscheli* Fennah (Homoptera: Delphacidae) (Remes Lenicov et al. 1985). The epidemiology of MRC closely resembles Maize Rough Dwarf Disease in countries around the Mediterranean (Conti 1984). MRC represents one of the major constraints to corn production in Argentina as it is spread over a large area in Central Argentina and particularly in the southwest of Córdoba Province.

Epidemics of plant disease vary both in time and space (Madden et al. 1988) and there can be a heterogeneous distribution of disease within the affected crop (Madden 1988, Madden et al. 1990). In general, spatial patterns of disease can be classified into three categories: uniform, random, and clustered or clumped (Madden and Campbell 1986). In a uniform pattern there is a regular arrangement of infected and healthy plants. In a random pattern every plant in the crop has a similar probability of being infected. Finally, a clumped pattern is represented by groups of infected plants whose frequency of occurrence is

significantly greater than that of groups occurring by chance.

The identification of disease spatial patterns in the field can be used to develop an efficient sampling plan, to understand its dynamics, and to provide evidence for the mechanisms of disease spread (Madden and Campbell 1986). For example, a random pattern of infected plants would suggest that the pathogen does not spread from plant to plant within the crop (i.e. no secondary transmissions occur, and hence the disease is monocyclic). Tomato spotted wilt, strawberry mild yellow-edge (Madden and Campbell 1986) and tobacco black shank (Campbell et al. 1984) behave this way. This observation would draw the attention of researchers to certain types of analytical models to describe the dynamics of disease epidemics (van der Werf and Riesbos 1990).

When an epidemic starts from infected plants in a single focus, a gradient of infected plants away from the focus often is observed (Campbell and Madden 1990). These gradients can be described by simple empirical equations, modelling disease incidence as a function of distance to a focus from which the pathogen progresses. This kind of approach aims at quantifying the slope of the disease gradient which, in turn, can be used to compare it among various plant genotypes and to draw conclusions about relevant features of the disease epidemiology. With MRC, one such feature is the role of surrounding weeds as a source of MRC virus spread.

Many diseases show clumped patterns, which can be the result of different phenomena: 1) the disease vector tends to be aggregated; 2) secondary spread occurs from infected to adjacent or nearby uninfected plants; 3) individual infective vectors infect groups of plants. Valdivia et al. (1990) claimed that corn plants showing symptoms of MRC occur in groups. On the other hand, Conti (1984) states that in the MRDV-Maize Rough Dwarf system, the virus reaches corn crops from surrounding strips or patches of grass weeds. Several unpublished reports indicate that weedy edges of corn fields in Argentina could act as sources from which the MRC virus spreads into plots to give an infection gradient. The aim of this work has been to verify these assumptions and previous occasional observations.

## Materials and Methods

### *Sampling*

The study was carried out during three agricultural seasons (1990-91; 1992-93 and 1993-94) in experimental and commercial corn fields in Córdoba Province, Argentina. Results for the 1991-1992 season were discarded because of the very low incidence of MRC in the available corn fields. All the maize fields studied were sown between October and December. During the 1990-91 season, the spatial pattern of Rio Cuarto Corn Disease was studied in two 6-ha plots at Manfredi Agricultural Experimental Station in the central area of Córdoba province, outside the endemic region, using two different methods: 1) estimation of infection by ELISA (Clark and Adams 1977) and 2) assessment of presence or absence of disease symptoms (Giménez Pecci and Truol 1991). The latter procedure also allowed for the analysis of the relationship between disease incidence and distance to field edge. During the 1992-93 and 1993-94 seasons, records of the spatial pattern of disease were made in ten commercial plots alongside the National Route 8, and intensively in one commercial field at Espinillo, Provincial Route 36, respectively. All these fields were located in or near the endemic area of MRC.

*Identification of infected plants through ELISA (1990-91 season).* Sampling was carried out weekly from October 30 to December 26 in two fields. The samples were collected systematically along the main diagonals of each plot. Ten sample points were identified with flags in each diagonal. All the sampling points were equally spaced. On the first sampling date, one plant (pivot plant) was identified where each flag had been set previously, along the four diagonals. A leaf sample was taken from each selected pivot plant and from two additional plants, in adjacent rows at both sides of the pivot plant. Each sample consisted of a 10-cm segment of the third newest leaf of the plant. During the successive sampling dates, samples were taken from three plants adjacent to one of the three plants selected (and from which a sample had been taken) the previous date. This procedure was repeated weekly on nine occasions. Thus, 27 samples were taken from each of the 40 sampling points giving a grand total of 1180 samples during the entire study.

*Identification of plants with Rio Cuarto Corn Disease symptoms (1990-91 season).* The same diagonals

mentioned in the previous section were used to sample plants for macroscopic symptoms assessment. Each flag was taken as the starting point of continuous series of ten plants in the same row, which were observed carefully in order to identify Rio Cuarto Corn Disease symptoms. For each series, the sequence of plants with and without symptoms was recorded. This procedure was repeated three times in the third month of plant development. The total number of sampled plants was 1200.

*Identification of plants with Rio Cuarto Corn Disease symptoms (1992-93 and 1993-94 .season).* During the 1992-93 season, ten corn plots were studied. On different dates at the end of the epidemic, one series of approximately 100 contiguous plants selected at random was sampled in each plot. During the 1993-94 season, a single intensive sampling was carried out following a systematic design, when 36 sample points were located in a 6x6 grid. Each point was separated from each other by ten meters. Each sampling unit consisted of a series of maize plants within ten meters along a row (at an average rate of four plants per meter). This scheme was designed originally for a spatial autocorrelation analysis, which was eventually discarded due to uniformly high levels of disease incidence.

### Statistical analysis

In general, two approaches were used to study the spatial patterns of infected plants. One technique used the ordered sequence of plants in a row, and the other used the number of infected plants per sample or section (quadrat) of a field (Madden and Campbell 1986). Here, the first approach was chosen because it is less time-consuming and interpretation of the data is usually straight-forward. Of the three techniques based on ordered sequences of rows, ordinary runs analysis was found to be the most reliable for detecting clustering of infected plants (Madden et al. 1982). A run is defined as a succession of one or more infected or healthy plants. As an example, consider the following pattern of ten symbols representing a crop row with infected (+) and uninfected (-) plants: + - - - + + - - +, where five runs are present. Reading right to left, the runs are '+', '- - -', '+ +', '- -' and '+ +'. The ordinary runs test quantifies the difference between observed and expected runs under the null hypothesis of randomness, given by

$$E(U) = 1 + \frac{2m(N-m)}{N}$$

where  $E(U)$  is the expected number of runs  $m$ : is the number of infected plants, and  $N$  is the total number of plants in the sample. The observed number of runs will be less than  $E(U)$  if there is clustering of infected plants. The standard deviation of  $U$  is given by

$$s(U) = \sqrt{2m(N-m) \frac{2m(N-m) - N}{N^2(N-1)}}$$

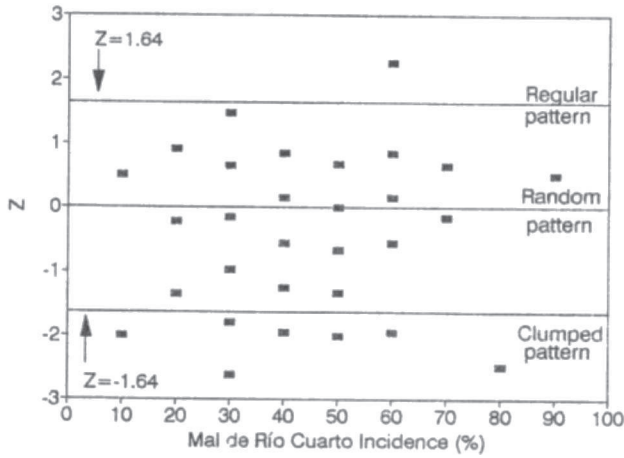
A normal test is used to determine clustering, with a value of  $Z$  less than -1.64 indicating clustering ( $P < 1.05$ ). Ordered sequences recorded during the three seasons were analyzed with this technique.

If there is no secondary transmission or contagion effect, the probability of finding an infected adjacent plant beside an uninfected pivot plant, should not differ from the probability of finding an infected adjacent plant beside an infected pivot plant. A chi-squared test for proportion comparison was used to determine whether or not there is clustering of infected plants around previously infected ones (Mead et al. 1993).

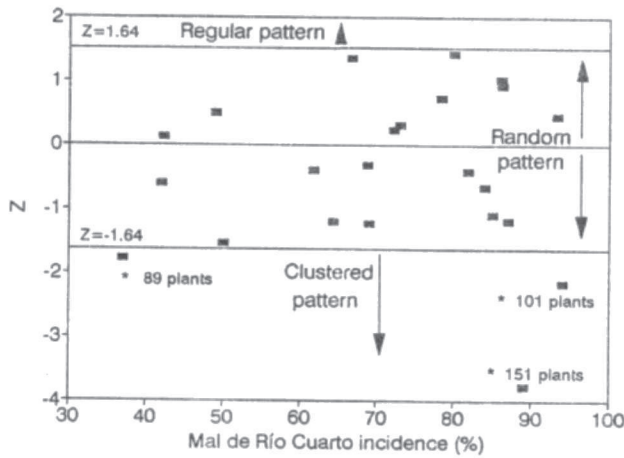
## Results

During the first growing season, the regression between Rio Cuarto Corn Disease incidence and distance to the field border did not show significant associations with distance to the north, south, east or west plot border. The probability of finding an infected plant adjacent to a previously infected one was not significantly greater than the probability of finding an infected plant besides a previously uninfected one. According to the ordinary runs test, the observed number of nets was significantly less than that expected under the null hypothesis of randomness in 10% of the cases ( $P < 0.05$ ).

In 70% of the cases studied in the 1992-93 season, the observed number of runs was not significantly less than the expected under the null hypothesis of randomness. From the 36 samples assessed for



**Figure 1.** Relationship between Z and Rio Cuarto Corn Disease incidence during the 1990-1991 season.

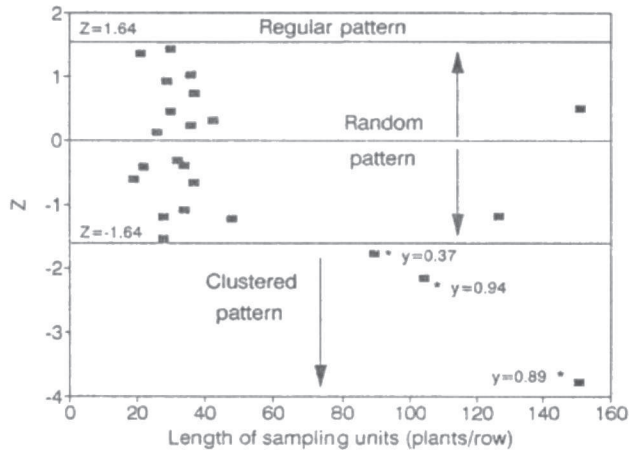


**Figure 2.** Relationship between Z and Rio Cuarto Corn Disease incidence during the 1992-1993 and 1993-1994 seasons.

symptoms during the 1993-94 season, 23 showed nearly 100% of infected plants. Consequently, the ordinary runs test was not used, as the results would have been meaningless. In the 13 remaining samples, the null hypothesis was never rejected.

The value of  $Z ((U - E(U))/s(U))$ , the normal distribution statistic on which the null hypothesis of randomness is tested, was not affected by disease incidence during the 1990-91 season. For each sample, Z was calculated and then plotted against the corresponding levels of disease incidence. Observed values of Z lower than expected under the null hypothesis occurred along a wide range of incidence values (Figure 1). However, the frequency of rejections of the null hypothesis of randomness was greater at low and high incidence values, compared to intermediate values.

When data sets from the last two seasons (from the endemic area of MRC) were pooled, neither incidence (Figure 2) nor the length of sampling units (Figure 3) significantly affected the results of the runs test, even though the samples identified as significantly deviated from randomness (i.e., clumped) corresponded to long sampling units.



**Figure 3.** Relationship between  $L$  and the length of the sampling unit (number of plants per series) during the 1992-1993 and 1993-1994 growing seasons.

## Discussion

The absence of a gradient of MRC incidence from the plot border to its center found in this work agrees with the most frequent observation by Valdivia et al. (1990) and is consistent with the lack of association between vector density and distance to the plot border observed in previous work (Trumpet, unpublished data). Interestingly, researchers in the endemic area claimed to have observed that the disease progressed along a gradient decreasing from the perimeter of the plots to its center. However, the present work was conducted outside the endemic area. While the disease vector, *D. kuscheli*, was frequently found on weeds around corn fields at different locations within the endemic area (Ornaghi Pers. Coin., Remes Lenicov et al. 1991), the same species was rare on the weedy edges of corn fields at Manfredi Experimental Station, outside the endemic area (Trumper et al. 1991). Thus, it seems probable that a generally more abundant population of the vector in the endemic area makes it more abundant on corn-related weeds. Hence, due to the occurrence of a clear and nearby focus of vector dispersal, it would be more probable to identify both vector and disease gradients in maize plots (Trumpet et al. 1991).

The approach involving conditional probabilities showed clearly that MRC has a random spatial pattern. Ordinary runs test results were less obvious, since a part of the whole data set showed evidences of a clumped spatial pattern. Most rejections of the null hypothesis of randomness occurred when small samples were taken (1990-91 season).

Madden et al (1982) compared the sensitivity of ordinary runs test and two other ordered sequence techniques through simulation of random and clumped spatial patterns. They concluded that the runs test correctly classified all the samples generated by simulation of a randomly distributed disease, while 5 % of the samples generated by simulation of an aggregated spatial pattern were incorrectly identified as random. According to these findings, if MRC were randomly distributed, no samples should be misclassified as clumped. However, in this work, the null hypothesis was rejected in up to 11 % of the cases. On the contrary, if the disease had a clumped spatial pattern, only 5 % of the cases should be regarded as random, far less a figure than that actually found in our work (always above 70%). Although this weakness in the ordinary runs tests suggested by Madden et al (1982) may render it insufficiently reliable to draw a clear conclusion from the data presented in this work, it should be noted that our results show agreement with the hypothesis of a random spatial pattern of Rio Cuarto Diseases Corn Disease. This is consistent with the view that MRC is a monocyclic disease (Trumpet 1996).

In the work of Madden and co-workers, random patterns were generated on the basis of two probabilities of infection ( $p=0.25$  and  $p=1.75$ ), while clumped distributions were generated assigning a probability,  $p=11.75$ , of a plant being infected if the adjacent plant was infected and  $p=0.25$  if it was

uninfected. This means that only one “clumping power” was simulated. It is reasonable to assume that the sensitivity of the test changes according to this “clumping power” of infected plants. The same authors pointed that in field studies, the frequency of rejection of the null hypothesis increases positively with disease incidence. This trend was not verified clearly in our work. Further simulations of spatial patterns including different scenarios likely to be found in the field, particularly different length of sampling units and disease incidence, should be completed to improve the sensitivity analysis of the ordinary runs test. Acknowledgments. This work was initiated at the Centro de Investigaciones Entomológicas (U.N.C.), whose support is gratefully acknowledged. The project was partially supported by CONICOR and CONICET (through scholarships to EVT and grants to DEG), INTA-PROMARC, U.N.C. and I.F.S. (through grants to DEG). We thank Irma Laguna and Guillermo March for advice and Claudia Altamira, Claudia Sutter, Patricia Rodriguez Pardina, Maria Gimenez Pecci, and Beatriz Valdivia for field assistance and ELISA tests.

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