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Comparative Within Field Dispersal Patterns of Aphid and Whitefly Transmitted Viruses

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Abstract: Within field dispersal characteristics of an aphid-transmitted potyvirus (water melon mosaic 2) and a whitefly transmitted geminivirus (cotton leaf crumple) were compared. Despite differences in the virus, the insect vector and the host plant, within-field patterns of infection were similar for both systems studied at two spatial scales. Ordinary runs analysis and sample variogram analysis of individual plant data suggest plant to plant spread within rows over a range of 3 to 5 plants (0.5 to 2.5 m) and variogram analysis of quadrats suggests spatial structure (non-random spatial patterns) of incidence over a range greater than 15 meters. Sample variogram values in the direction perpendicular to row orientation (north-south) were higher than sample variogram values in the direction of row orientation (east-west) at both scales in both virus/vector/host systems. These observations are consistent with spread of the virus occurring faster within rows than between rows in a field.

Key words: Aphid, whitefly, cotton leaf crumple virus (CLCrv), virus transmission, water melon mosaic virus 2, potyvirus, geminivirus

Introduction

Characterizing the spatial pattern of infected plants within a field is important in understanding the epidemiology of plant virus diseases (Madden and Campbell, 1986). A wide variety of techniques and indices have been developed for the analysis of spatial patterns of diseased plants within a field. Ordinary runs analysis (Madden *et al*., 1982) has been found superior to doublet analysis (Van der Plank, 1947) for detection of clustering within rows. Further work on the spatial analysis of virus disease epidemics suggested the importance of multiple assessments of fields to understand how spatial patterns shift over time (Madden *et al*., 1987). Distance class (Gray *et al*., 1986) and spatial autocorrelation (Madden *et al*., 1987) techniques have been used for within field analysis of plant virus epidemics. In geostatistics, spatial autocorrelation is most frequently assessed using variograms (Isaaks and Srivastava, 1989). Geostatistics can be used for the analysis of the spatial patterns of plant diseases both within field (Chelleini *et al*., 1988; Johnson *et al*., 1991; Lannou and Savary, 1991; Lecoustre *et al*., 1989; Rupe *et al*., 1991; Todd and Tisserat, 1990; Webster and Boag, 1992) and regionally (Nelson *et al.*, 1994). Recently published results of a stochastic simulation model of four generalized types of vector transmission of plant viruses suggest the need for comparative analysis of spatial patterns of virus diseases within fields (Ferris and Berger, 1993). In this paper, the aphid transmitted non-persistent watermelon mosaic virus 2 (WMV2) on zucchini is compared with the whitefly-transmitted semi-persistent cotton leaf crumple virus (CLCrV) on cotton. WMV2 is an epidemiologically well characterized potyvirus (Adlerz, 1978; Gray *et al*., 1986; Nelson and Tuttle, 1969) a group whose members among the most important of plant viruses (Gibbs and Harrison, 1976). Whitefly-transmitted geminivirus diseases have received attention in recent years as changes in biotypes, host ranges and population densities of whiteflies have made an impact on a many crops (Cohen *et al*., 1992). The silverleaf whitefly (*Bernisia argentifolia*) replaced the sweetpotato whitefly (*Bemisia tabaci*) in the southwestern United States agroecosystems between 1988 and 1992 (Cohen *et al*., 1992). The silverleaf whitefly is a poor vector of lettuce infectious yellows closterovirus, a serious disease problem in the 1980's (Cohen *et al*., 1992), that has virtually disappeared from Arizona. However, there is no evidence to suggest that the new strain is less efficient than the previous strain in transmitting CLCrV. During the time of these experiments, the changeover from one biotype to the other was taking place, so, it is likely that a mixture occurred at least during some of the experiments reported here. Previous work with some of the aphid-transmitted potyviruses has shown that movement within a field is primarily from plant to plant (Gray *et al.*, 1986; Madden *et al.*, 1982; 1987; Nelson and Campbell, 1993). This is probably a reflection of the fact that when an aphid leaves a plant it is most likely to move to the next nearest plant. The result is a clustered pattern of infected plants. A preliminary report on the dispersal characteristics of cotton leaf crumple virus indicates such a pattern (Nelson and Stowell, 1989). A study of the spread of the whitefly-transmitted African cassava mosaic virus concluded that spread of the virus within field was not evident over distances exceeding a few meters and that the dominant spatial pattern in fields was determined by outside sources of the virus (Fargette *et al.*, 1990).

Materials and Methods

The spatial patterns of symptomatic plants were studied in one WMV2-zucchini and three CLCrV-cotton experiments conducted on the University of Arizona, Yuma Agricultural Center, Citrus Station near Yuma, Arizona. This station is isolated from the main agricultural crops of Yuma, except citrus. In all experiments, epidemics were initiated by introducing infected plants during periods of increasing insect vector populations. The zucchini field was planted in late April 1989 to match the rising levels of the two aphid vectors of watermelon mosaic 2 in the Yuma region *Aphis gossypii and Myzus persicae* (Nelson and Tuttle, 1969). In this experiment, inoculum was introduced into the center of the field by transplanting a block of eight WMV 2 infected greenhouse grown squash plants approximately 4 weeks after planting.

The cotton fields were planted in August of 1988, 1989

and 1990 to bring plants to their peak growth rate during the period of maximum increase of migrating whiteflies. These conditions assured optimum conditions for disease expression among young plants following introduction of the inoculum. Because the timing of planting and the placement of the two crops on the citrus station, the experimental field inoculations had little possibility of affecting the commercial crop. In two cotton experiments (1988; 1990) and in the zucchini experiment inoculum was introduced by transplanting infected plants into the field in a grid pattern. In 1989, inoculum was introduced by bringing in mature symptomatic cotton plants and associated whiteflies collected from cotton fields over 5 miles away from the Yuma Agricultural Center. CLCrV symptoms are very characteristic and CLCrV is the only virus known to occur on cotton in Arizona.

Following introduction of virus inoculum, fields were monitored for symptom development. The zucchini field was evaluated in each of four successive weeks beginning May 23, 1989 by walking the rows, counting plants and recording the sequence numbers of plants showing symptoms. Each week plants were scored 1 if symptoms were present and 0 if symptoms were not present. All plants in 64 adjacent rows were evaluated. However, because seedling mortality was high in the western third of the field due to flooding, only data from the eastern two-thirds of the field were used in the data analysis. The cotton fields were evaluated only once (in mid-October). Crumple leaf symptoms on young cotton are easy to identify and, because only the new growth following infection shows symptoms, the location of infected leaves indicates in a general way the time of infection. Plants were rated from 1 to 4 based on the relative portion of the plant showing symptoms. The rating system was as follows: 4: no visible symptoms, 3: symptoms only on the youngest leaves, 2: symptoms present on many leaves but not present on the oldest leaves and 1: symptoms on most or all leaves. For each of the three cotton experiments, three data sets were then derived from the ratings based on the symptoms. In data set "early", plants were scored 1 if they had symptom category 1, otherwise they were scored 0. This data set represents an estimate of the level of early infection. In data set "inter", plants were scored 1 if they had symptom category 1 or 2 and 0 otherwise. In data set "late", plants were scored 1 if they had symptom category 1, 2, or 3. This represented our estimate of the level of infection late in the experiment.

In the 1988 cotton experiment, plants were spaced approximately 10 cm apart whereas in the 1989 and 1990 experiments plants were thinned to a 30 cm spacing. In 1988, 14 rows were evaluated and in 1989 12 rows were evaluated. In 1990, the cotton field was divided into 10 experimental units, half of which received a stylet oil spray, and the other half received no treatment according to a randomized complete block design. Analysis of variance of data showed no oil spray treatment effect and thus, both treated and untreated portions of the field were used for the analysis of spatial patterns reported here.

Statistical Analysis: Runs analysis was done separately for each row using the formula (Madden *et al.*, 1982). Except for the 1990 cotton experiment, all plants in a row were scored for symptoms (100 ~ 550 plants per row,

depending on the crop and year). In the 1990 cotton experiment, row segments 6 m long were scored. Usually there were between 20 and 25 plants per segment. Row segments containing fewer than 20 plants were not used for runs analysis. Data from the rows were then combined using the formula of Johnson *et al.* (1991) to get a single value characterizing runs for each time in each experiment. Geostatistical analysis was done using GeoEAS (USEPA EMSL-LV, EAD, LasVegas, NV) and subroutine GAM2M from GSLIB (Deutsch and Journel, 1992). For geostatistical analysis of quadrats of the squash data and the 1988 and 1989 cotton data, plants were assigned to quadrats under the assumption that the counted plants were evenly distributed throughout the row. This assumption was necessary because the location of skips within rows were not mapped. In the 1990 cotton experiment, data from 60 pairs of adjacent 3x3 m quadrats were collected as part of the experimental design. The coordinates of the center of the quadrat were used for geostatistical analysis of quadrat counts with the southwest corner of the field serving as the origin. Quadrat sizes reported here were zucchini, 4 x 5 m (404 plants), cotton 1988, 1 x 2 m (20 plants), cotton 1989, 1 x 3 m (10 plants), cotton 1990, 3 x 3 m (30 plants). Quadrat sizes were selected based on a convenient subdivision of the field in such a way that the quadrat length was larger than the range of influence detected in the variogram analysis of the individual plant data.

Results

The observed number of runs were significantly smaller than the expected number of runs in all of the experiments (Table 1). This indicates within row clustering of plants showing symptoms and suggests plant to plant spread. Also, the absolute value of the z score and the percentage of rows in which runs analysis indicated clustering increased between the first and second time sequence in both the zucchini WMV2 experiment and the cotton-CLCrV experiments (Table 1). This suggests an intensification of clustering over time and is additional evidence for plant to plant spread with both systems. The z value levelled off or decreased in the third time sequence when almost all plants were infected. Sample variograms, generated from geostatistical programs, also suggest a similar type of patchiness within-row for both the squash-WMV 2 system and the cotton-CLCrV system (Fig. 1). In every case except for the intermediate symptom category of CLCrV in 1989 (Fig. 1F), the sample variogram values increased steadily over the first 3 to 5 lags until reaching a plateau. This provides an estimate of average within-row patch size 3 to 5 plants. The estimate of patch size in metres depended on plant spacing but ranged from 5 m (Fig. 1H) to 2.5 m (Fig. 1B). Sample variogram analysis of quadrats demonstrated a second scale of spatial pattern in all fields (Fig. 2). The sample variogram values rose over a range of more than 15 m in each case. In the squash experiment and the 1990 cotton experiment sufficient data permitted the comparison of directional variograms. In both cases the north-south sample variogram values exceeded the east-west sample variogram values throughout the range of distance observed (Fig. 2A and 1B). Since the rows ran in an east-west direction, this suggests a greater intensity of the spatial pattern (greater differences in values at any given lag) in

Table 1: Runs analysis of symptomatic plants comparing a squash watermelon mosaic virus 2 field and cotton-cotton leaf crumple virus field at various stages of disease progress

Crop Side of Field	Stage	Number of Plants	Plants Infected (%)	Rows Clustered (%)	Z
Squash					
South	Week1	4196	4	16	-5.4[1]
	Week2	4196	25	78	-15.6[1]
	Week3	4196	95	59	-15.6[1]
North	Week1	3793	2	19	-5.8[1]
	Week2	3793	13	50	-8.9[1]
	Week3	3800	61	84	-23.7[1]
Cotton 1990[2]	Early	4963	4	9	-4.8[1]
	Inter	4963	17	17	-5.9[1]
	Late	4963	48	15	-5.0[1]
Cotton 1989[2]					
Field A	Late	7703	94	39	-7.2[1]
Field B	Early	1941	9	50	-4.3[1]
	Inter	1941	48	100	-11.4[1]
	Late	1941	95	71	-6.2[1]
Cotton 1988[2]	Early	7666	2	67	-11.3[1]
	Inter	7666	27	100	-31.6[1]
	Late	7666	81	100	-24.8[1]

[1]A Z score less than -2.33 indicates the observed number of runs is significantly less, p = 0.01) than the expected number of runs in a one-tailed test.

[2]Time of infection was estimated based on the severity of symptom expression. Plants with severe symptoms and with older leaves with symptoms were assumed to have been infected earlier than plants with only young leaves showing symptoms. The categories intermediate and late represent cumulative levels of infection and thus include plants infected earlier
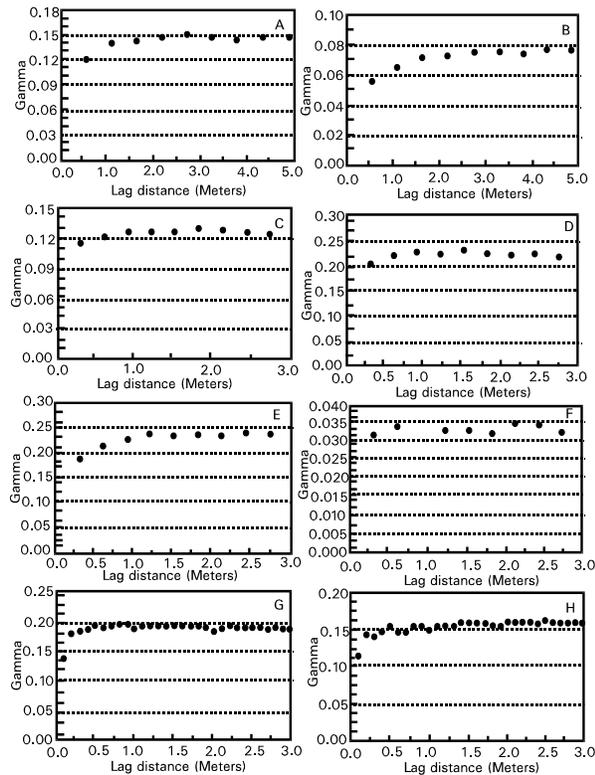

Fig. 1: Sample variograms showing the spatial dependency of infection of individual plants of zucchini by MWV2 at: (a) Week 2 and (b) Week 3 following inoculation and of cotton by CLCrV for years (C) 1990; Intermediate, (d) 1990: Late. The terms "intermediate" and "late" refer to the time sequence of accumulated infection based on symptom expression and are explained in the text
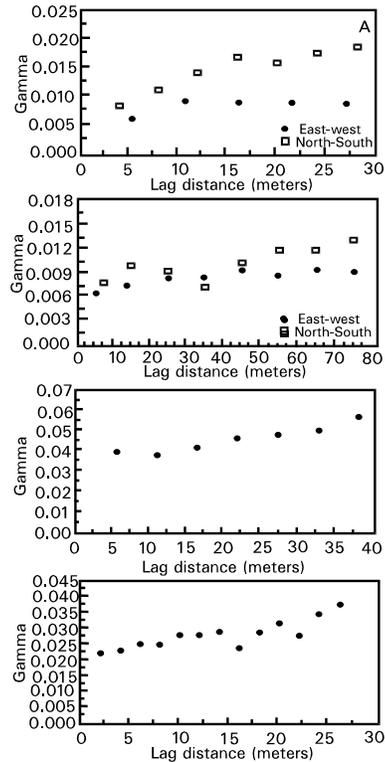
Fig. 2: Sample variograms showing the spatial dependency of percent virus incidenc ein quadrats for: (a) WNV2 on zucchini (4 × 5 m quadrats, 40 plants/quadrat), (b) CLCrV on cotton 1990 (3 × 3 m quadrats, 30 plants/quadrat), (c) CLCrV on cotton 1989 (1 × 3 m quadrats, 10 plants/quadrat) and (d) CLCrV on cotton 1988 (1 × 2 m quadrats, 20 plants/quadrat)

the direction perpendicular to the rows. This again is consistent with a dominance of within row plant to plant dispersal of the virus in both systems.

Discussion

Similar spatial Patterns were found at two scales for both the whitefly and aphid-transmitted viruses. Campbell and Madden (1990, page 321) and others have used the terms "true contagion" and "apparent contagion" to describe these different scales of pattern. In the case of viruses, true contagion describes patterns derived from the plant to plant spread within a field whereas apparent contagion describes field patterns ("hot spots" or gradients) that can be the consequence of factors outside of the field such as the pattern of sources of inoculum immediately surrounding the field, wind direction and patchiness of nearby vector sources. The spatial structure described as apparent contagion can also result from extensive plant to plant spread resulting in relatively large overlapping clusters (Madden *et al.*, 1987). In field experiments reported here, the greater intensity of pattern perpendicular to row direction at the scale of apparent contagion suggests that within-row vector movement may be influencing pattern at this scale. The observed spatial patterns suggest that most virus dispersal in the fields was plant to plant within row over a range of 3 to 5 plants (detectable with variogram analysis). The intensification of clustering between the first and second time period is also consistent with plant to plant spread (Madden *et al.*, 1987). There is possibility of much rarer movement within field at a larger scale (10 to 30 m or more) and/or a coalescence of clusters that still tends to be along rows, because the quadrat sample variogram values along rows are less than the sample variogram values across rows. These patterns were observed in both the aphid-transmitted WMV2 and whitefly-transmitted CLCrV systems. In a study of leafhoppers, another Homopteran vector of plant virus diseases, Power (1992) emphasized movement between adjacent plants within a row and concluded that leafhoppers were much more likely to move along rows than across rows. Power's conclusions with respect to leafhopper dispersal are consistent with the observed spatial patterns of the whitefly- and aphid-transmitted viruses studied here. The similarity of the within-field spatial patterns of the two viruses provides evidence that at least from the perspective of within field dynamics, cultural management techniques developed from an understanding of the epidemiology of aphid-transmitted viruses might well be extended to the management of whitefly-transmitted diseases. A final conclusion is that patterns of virus spread by aphid and whitefly vectors are more similar than they are different despite differences in the virus type and mechanisms of transmission. This similarity encouraged us to lump whitefly and aphid-transmitted viruses in simplifying risk assessment procedures in the design of a regional management plan for a complex of tomato viruses in Sinaloa, Mexico.

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