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Cotton Leaf Curl Disease: Measuring and Analyzing its Epidemics Using GIS

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Abstract: Cotton leaf curl disease occurred throughout the province of Punjab during the year 1996, but there were patches where it was less severe and other patches where it was very severe. Nine varieties were planted in Mian Channun project area. CIM-240 was predominant variety through out cotton growing region of the Punjab There were varietal differences in both incidence and severity. To eliminate the possibility of varietal effects on the spatial pattern, kriging was done with only those fields planted with CIM-240. There were not enough points to give a broad coverage of the area, but the basic spatial pattern of incidence was the same as when all varieties except C1M-1100 are included in the analysis. Any influence of variety was evidently randomly distributed across the landscape so that the spatial pattern of the disease on CIM-240 was similar to the spatial pattern on all the varieties combined. This might not always be so in years when there are strong regional differences in the selection of varieties. There was a strong correlation (r = 0.962) between incidence and severity and the spatial pattern of severity was quite similar to that of incidence.

Key words: Cotton Leaf Curl Disease (CLCuD), CLCuV, GIS, Geostatistics, Cotton, whitefly

Introduction

The cotton leaf curl disease (CLCuD) has devastated the economy of Pakistan during the last few years. The cause of the disease is at least two whitefly-transmitted geminiviruses (WTGs) collectively named as cotton leaf curl virus (CLCuV). CLCuV, which has been the focus of study in several virology laboratories in Europe and North America, was first sequenced at the University of Arizona (Hameed *et al*., 1994; Harrison *et al*., 1997; Nadeem *et al*., 1997). DNA analyses of virus collections of plants exhibiting leaf curl symptoms from different cotton growing areas suggest that the genetic composition of these viruses is very diverse (Personal communication, D. Robinson July 1997 and C. Fauquet, June 1998). These recent research results underscore the need to monitor the genetic composition of the population of the viruses in the field. The monitoring will aid breeders and strengthen virus management planning.

The CLCuD has continued to appear in new areas of Pakistan since it was recognized as a major problem in 1991. It was first reported from Pakistan in 1967 (Hussain and Ali, 1975). At present, the disease can be found in all cotton growing areas of the Punjab. The first report from Sindh did not occur until 1996. The area under CLCuD in Sindh is increasing every year. The cotton growing areas of Pakistan represent a wide variety of agro-ecological regions. The ecosystem diversity includes size of fields, distances from rivers, crop composition and sequencing, planting schedules, climatic variability, elevation and soil types. Because of this ecological diversity, individual farmers alone cannot manage a disease that is present on a regional scale. Therefore, a regional approach to disease management is required. The present study recognizes the necessity of a broad approach to address the problem and incorporates modern spatial analysis technology into management planning i.e. Geographic [Information System (GIS) based spatial analysis programs. The long-term goal is to examine the incidence and severity of the leaf curl disease in relation to cultural management practices and distribution of alternate hosts, insect vectors etc. To develop an understanding of spatial patterns of landscape characteristics and how this influences disease in an area the size of a Tehsi. The Mian Channun area was selected for special attention as a model system in this study. In previous cotton growing seasons the Mian Channun area has a history of spatial variability in the severity and incidence of CLCuD (high and low areas). The goal is to use what is learnt in the Mian Channun area as the basis for the design of a management program throughout the cotton-growing regions of Pakistan. Beside Mian Channun weekly data on incidence and severity of CLCuD were also collected from about 160 points through out main cotton growing regions of Punjab and were included in the present study (Fig. 1).

Materials and Methods

Data Collection: Data on CLCuD incidence and severity were collected from Mian Channun area during the month of October 1996 from about 100 fields. Disease incidence was recorded by walking into the field to an arbitrary point and closely observing plants in a 10 to 15 meter length of row. The field was rated based on what was observed in the field as well as plants in the selected part of the row as follows:

Rating	Description	Estimated Incidence
0	No plants with symptoms observed either in or outside the selected row	0%
1	A few scattered plants with symptoms but none in the selected row	Up to 1%
2	One to 3 plants in the selected row showing symptoms	1% to 10%
3-	More than 3 and less than 6 plants in the selected row showing symptoms	10% to 90%
3	More than 6 and less than 10 plants in the selected row showing symptoms	
3+	More than half of the plants in the selected row showing symptoms	
4	All of the plants in the selected row showing symptoms	90%-100%

Disease severity was rated using a six-point scale. Zero (0) represented plants with no symptoms, one (1) very mild symptoms, up to five (5) indicating symptoms so severe that it was anticipated that yields of cotton would be zero. For severity, the field was rated as a whole rather than as an average of the ratings of individual plants. The effort to standardize and communicate the severity rating system is ongoing. Another data set was collected in the same year by the Pest Warning and Quality Control of Pesticides (PWandQCP) Department. They conducted weekly surveys of four fields in each of approximately forty tehsils in the cotton growing districts of Punjab (Fig. 1). Beside PW and QCP Plant Pathology sections at Ayub Agricultural Research Institute, Faisalabad and Central Cotton Research Institute, Multan contributed data from approximately 10 sites. From mid-June to late September, the same 150 plants in each field were rated each week for severity of CLCuD on a scale of 0 (no symptoms) to 5 (severe symptoms). CLCuD incidence was calculated as the percentage of plants rated above 0. The project recorded farmer name, variety, and a brief description of the location for each field. Dominant landscape features and weeds in fields surrounding the selected field were also recorded. These data were entered using ACCESS, (Microsoft Corp., Redmond, WA). A handheld Global Positioning System (GPS), (Garmin 45XL), was used using the Universal Transverse Mercator (UTM) coordinate system to record the X and Y coordinates at each field location where observations were made to facilitate spatial analysis of the data. The GPS recorded points in the western part of the province in the UTM zone 42 coordinate system and points in the eastern part of the province in the UTM zone 43 coordinate system. Prior to analysis of Pest Warning data, coordinates for all points were transformed to the zone 43 coordinate system using Arc/Info. For simplicity in presentation, monthly averages of the weekly data on incidence and severity were calculated.

Geostatistical analysis: Fields in Mian Channun were rated for incidence and severity in a qualitative manner. Ratings were translated to indicator variables that are scored 0 or 1 depending on whether the field ranked above or below a given cut-off. The spatial autocorrelation of the indicator variables were assessed by variogram analysis and indicator kriging was used to create regional probability maps. This procedure translates qualitative data at the scale of a field into quantitative estimates at the scale of a region. In the Mian Channun case, data were analyzed using three selected indicator variables based on the following cut-off ratings: (a) Symptoms easily found in the field (rating of more than 1: i.e. more than a few scattered plants with symptoms; (b) Symptoms very common in the field (a rating of more than 2: i.e. many plants showing symptoms, more than three or four plants in 10 meters of an arbitrarily selected row would show symptoms); (c) Symptoms on almost all of the plants (rating of 4: i.e. all plants in 10 meters of an arbitrarily selected row show symptoms; finding a plant without symptoms is not easy). Maps for sample variogram analysis of these indicator variables were generated. Variogram models of these autocorrelations were used in the computation of the probability maps for the three variables. Geostatistical analyses were done using GeoEAS, from the U.S. Environment Protection Agency (USEPA, EMSL-LV, EAD, Las Vegas, NV) (Journel, 1989; Issaks and Srivastava, 1989). Zero-nugget variogram model was used to the kriging and to produce the probability maps without overemphasis on variogram modeling. Minimum of seven data points within a circular 15-km radius were used for the neighborhood search parameters for kriging. PC ARCVIEW (ESRI, Redlands, CA) (Version 3.0) and PC ARC/INFO (Version 3.5) were used to manage and display the spatial data including the output of the geostatistical analyses (Mayers, 1991).

For Pest Warning data variogram analysis indicated that variables for both severity and incidence are patchy with ranges of spatial autocorrelation exceeding 50 km. To obtain an overview of the data, moving spatial averages were calculated for 10 km by 10 km grid cells using the GeoEAS krige program. As with the Mian Channun data, a simplified variogram model of the spatial structure was used in kriging. A zero nugget spherical model with the sill equal to the sample variance and a range of 30 km was used for kriging instead of a carefully fitted variogram model. A search radius of 30 km was selected. It was specified that at least seven fields must be located within the search radius of a cell in order to calculate an estimate for that cell. Fields planted with CIM1100 were omitted from the kriging because fields planted in this variety were rated 0 in incidence and severity regardless of location and this is evidence in support of reported tolerance or resistance in this variety. To eliminate the possibility of varietal effects on the spatial pattern, kriging was also done with only those fields planted with the most common variety, CIM-240.

Results

Sample variogram analysis of indicator variables shows spatial autocorrelation (patchiness) for all three variables (Fig. 2). The variograms, together with point plots of the variables (Fig. 3), provide a picture of the patchiness. The point plots of the indicator transformation of the incidence data (Fig. 4) and plots of data points based on incidence and severity (Fig. 5) indicate that the leaf cur! disease has a pattern. Histograms of the incidence and severity ratings (Fig. 6) give an idea of frequency distributions.

About nine varieties were planted in the project area. The spatial distribution of these varieties among the fields surveyed is shown in Fig. 7. All three CIM-varieties were rated 0 for incidence and severity. One of the CIM-1100 fields rated 0 for incidence was in an area where the probability that it would be rated 4 for incidence was 80% based on indicator kriging of varieties other than CIM-1100 (Fig. 8). Except for CIM-1100, CIM-443 and CIM-435, almost all fields contained plants with CLCuD symptoms.

The probability of finding a field with at least some plants with CLCuD symptoms was uniformly high throughout the study area (Fig. 4). However, the probability of finding a field completely infected with CLCuD was patchy. In some areas, the probability of finding such a field was greater than 75%, whereas in other parts of the study area, the probability was less than 25% (Fig. 4). The regional overview of the CLCuD situation in Mian Channun is obtained by comparing the three maps in Fig. 4. The overview is that the disease occurred everywhere (top figures), with patches in which it was less severe (light areas of middle figures), and patches in which the disease was very severe or almost complete (dark areas of bottom figures) (Fig. 4).

A map of the spatial distribution of varieties planted in 1996 in main cotton region shows that CIM-240 was the predominant variety throughout the Punjab (Fig. 9). Both point

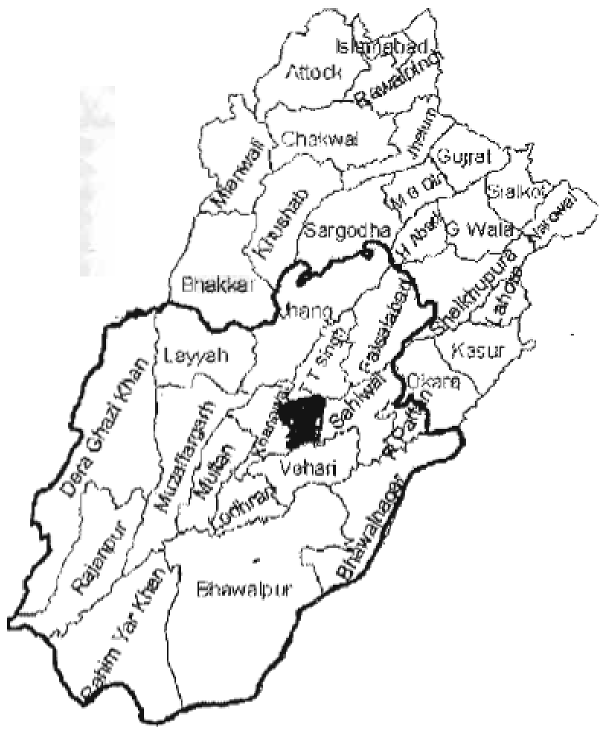

Fig. 1: Map of Punjab showing project area boundaries. Dark area represents Mian Channun project area. Dark line shows the data collection area of Pest warning department within Punjab

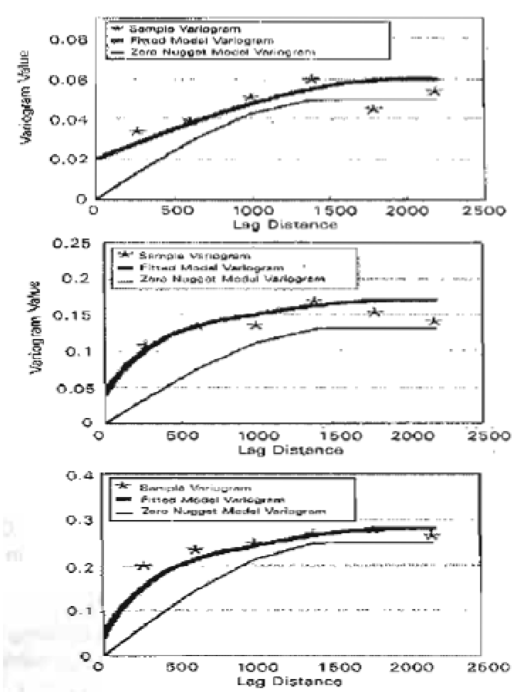

Fig. 2: Vriogram analysis of indicator variables in Mian Channun study area

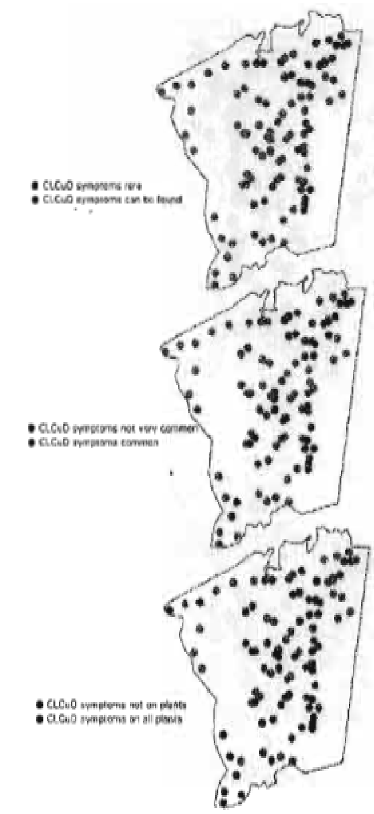

Fig. 3: Fields classified by indicator variables based on symptom categories

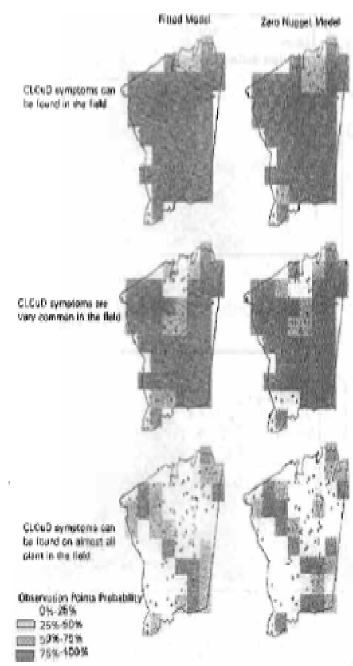

Fig. 4: Comparison of kriging generated probability maps using fitted Vs "zero nugget" variogram models

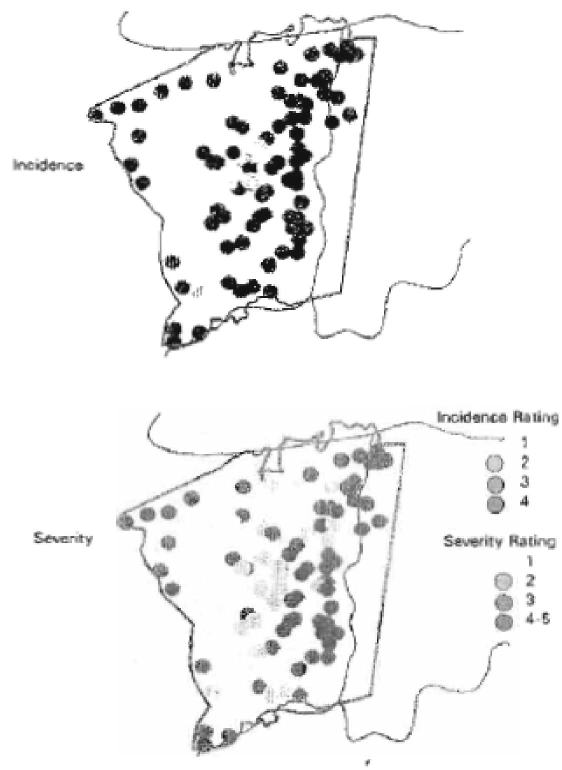

Fig. 5: Cotton leaf Curl Disease incidence and severity in Mian Channun Project area during 1996

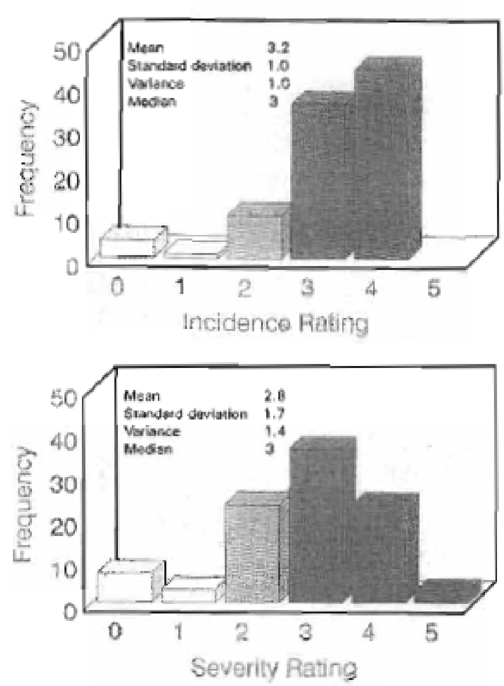

Fig. 6: Histogram of severity and incidence ratings on CLCuD in Mian Channun Project area during 1996

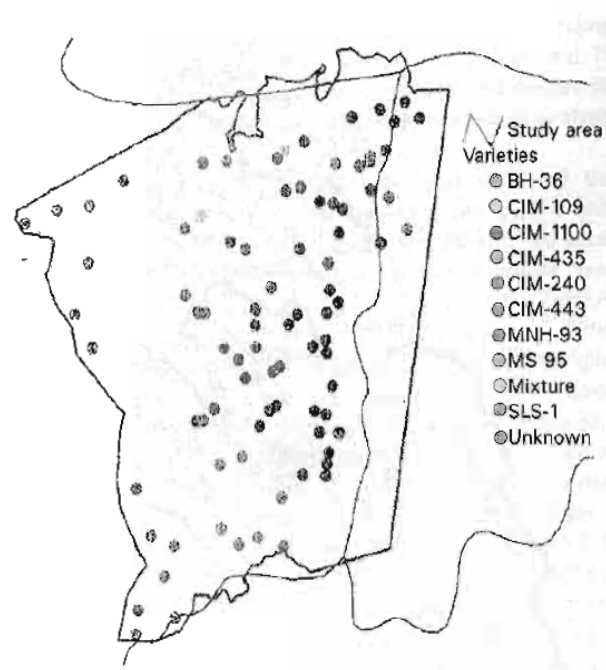

Fig. 7: Spatial pattern of cotton varieties in Mian Channun Project area during 1996

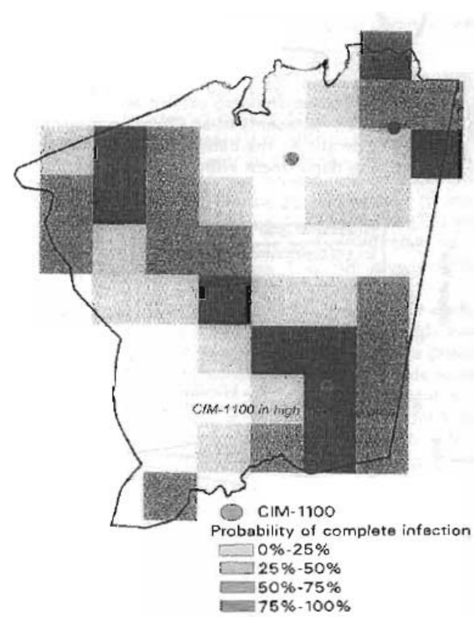

Fig. 8: Cotton variety CIM-1100 in relation to CLCuD incidence in other varieties during 1996 in Mian Channun area

maps (Fig. 10, 11) and overview maps created by kriging (Fig. 12-14) show areas of high and low incidence and severity. There were not enough points to give a broad coverage of the area, but the basic spatial pattern of incidenceis the same as when all varieties except CIM-1100

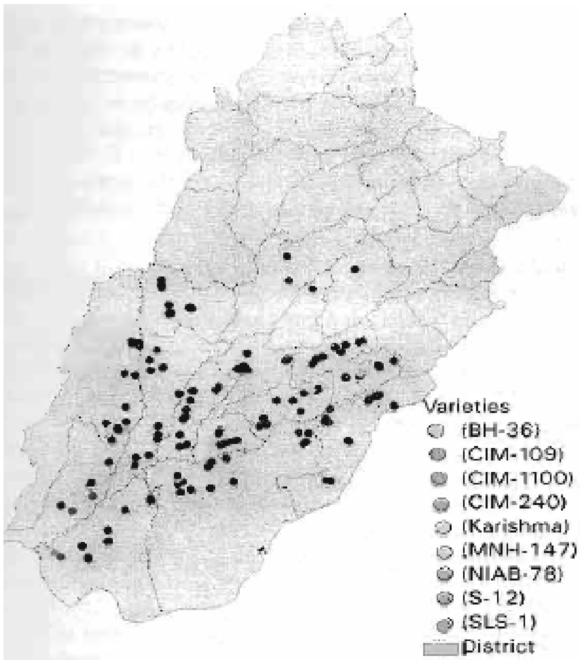

Fig. 9: Cotton varieties in the field surveyed during 1996 in cotton groeing region of Punjab

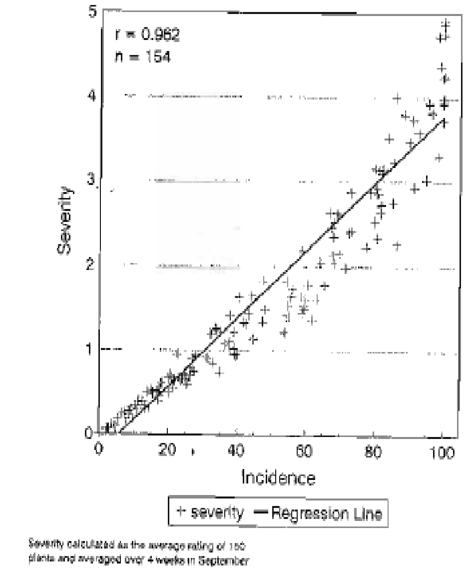

Fig. 11: Correlation of severity with incidence. September 1996 data from PW and QCP department. Severity was calculated as the average rating of 150 plants and averages over 4 weeks in September

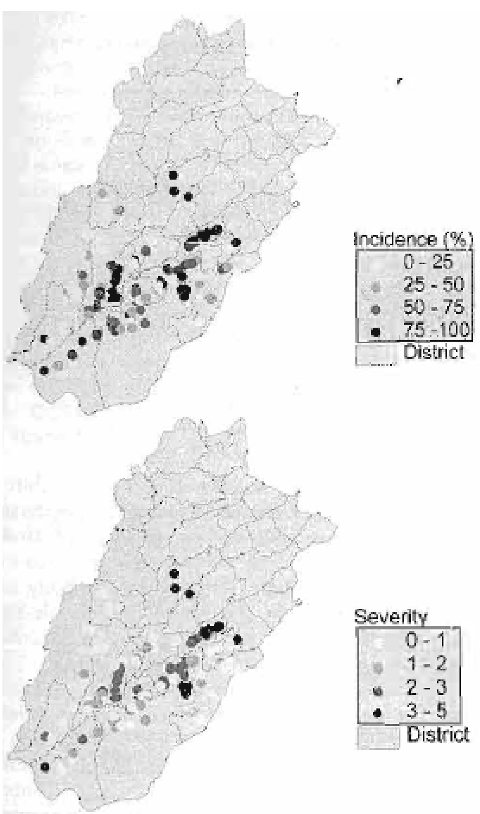

Fig. 10: Incidence and severity of CLCuD during 1996 survey of the Pubnjab cotton areas

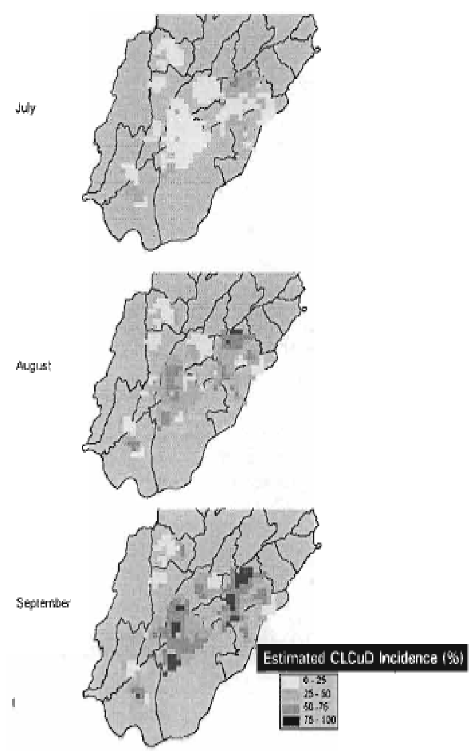

Fig. 12: Spatial moving average of CLCuD incidence on all avarieties excluding CIM-1 100 during 1996

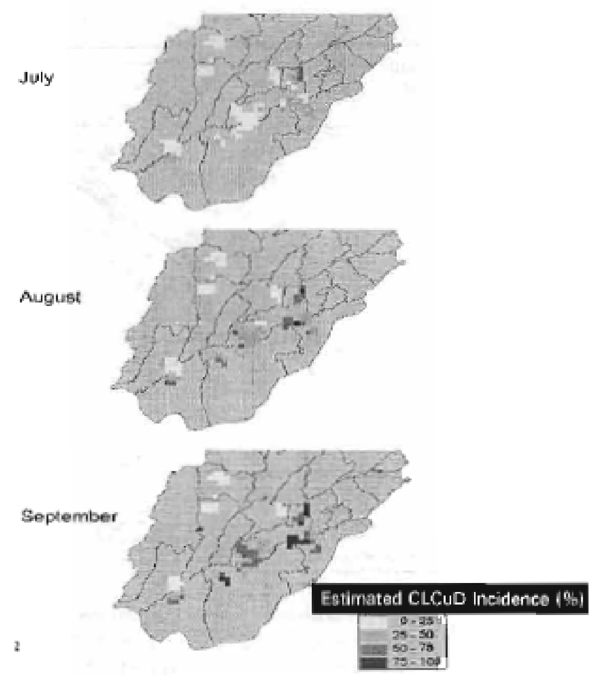

Fig. 13: Spatial moving average of CLCuD incidence on CIM-240 during 1996

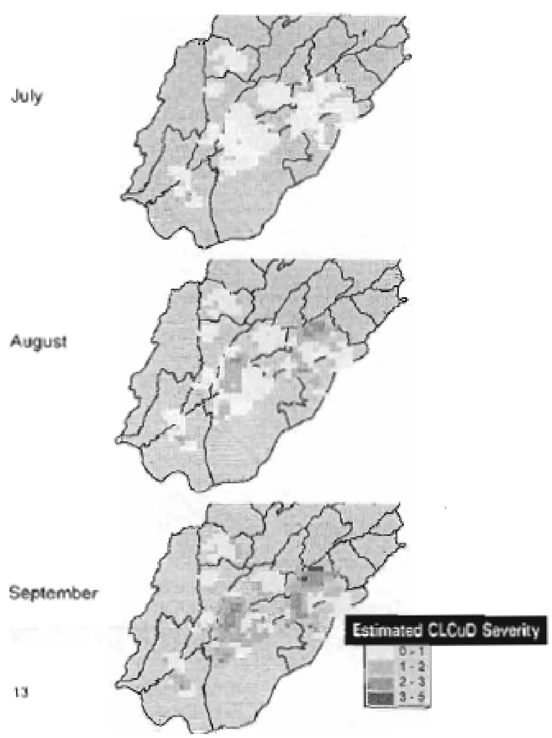

Fig. 14: Spatial moving average of CLCuD severity on all varieties except CIM-1100 during 1996

are included in the analysis (Fig. 12). Any influence of variety was evidently randomly distributed across the landscape so that the spatial pattern of CIM-240 was similar to the spatial pattern of the varieties combined. This might not always be so in years when there are strong regional differences in the selection of varieties. There was a strong correlation ($r = 0.962$) between incidence and severity in the data and the spatial pattern of severity (Fig. 11) is quite similar to that of incidence (Fig. 10).

Discussion

CLCuD was observed throughout the Punjab during 1996, but the areas of highest incidence and severity were patchy (Barnes *et al*., 1999). The reasons behind the patchiness are not known but landscape analysis planned for future might help to understand observed patterns and provide guidance for CLCuD management (Nelson *et al*., 1999). Using the UTM coordinates obtained with the GPS units, the observations could be displayed on maps and analyzed geostatistically. Having the spatial coordinates and storing the information in GIS format strengthens the historical record for the year 1996. Geostatistical analysis of the data indicates a strong spatial autocorrelation for CLCuD incidence and also documents the patchiness of high incidence fields (Orum *et al*., 1999). Varietal differences were also noted. Nearby fields tended to be similar in incidence unless there was a strong difference in the resistance of the cotton varieties (such as a field in CIM-1100. CIM-1100 is resistant to CLCuD. In order to avoid its influence it was excluded in all analyses of regional spatial patterns. In future analysis, we would like to summarize data by Tehsil. The observed spatial autocorrelation means that for such summaries, better estimates can be made by selecting fields as widely separated as possible within a given Tehsil.

In comparing the temporal progression of the overall spatial patterns between July, August, and September (Fig. 12-14), some caution is required because the same plants in the same fields were repeatedly sampled. Independent samples were not taken each week. Therefore, the temporal pattern looks more consistent that it would appear if independent samples had been taken. One way to take advantage of the repeated sampling is to model each field with a mathematical growth equation such as the logistic model and compare the growth rate parameter from the model for each of the 160 fields. A point map and a kriged map of the growth parameter can then be displayed to see if there are regional patterns in the rate of within-field increase in CLCuD. This is a time consuming process because each field needs to be modeled separately.

For the coming years, it is recommended that a shift away from weekly monitoring of individual fields and emphasize the need for pre-plant and post-harvest surveys of landscape characteristics that make some areas more conducive to virus disease epidemics than others. Replacing the weekly surveys of the same 150 plants in the same fields with monthly surveys of more fields using the rating system described for the Mian Channun project would be more beneficial. The idea is to rate more fields qualitatively and then to use geostatistics to translate the qualitative ratings at the scale of a field into quantitative estimates at the scale of a region (Nelson *et al*., 1994). This is analogous to the common practice of translating qualitative ratings of individual plants into quantitative averages for fields. In the assessment of incidence, particular emphasis will be given during the month of September when we recommend that as many fields as of September when we recommend that as many fields as possible widely spaced within the tehsils are rated to give a final regional estimate of CLCuD incidence. Finally, after most of the cotton has been picked and fields plowed under, we recommend that a post-harvest landscape survey be done in the month of December. For CLCuD to continue to be a problem each season, the virus needs to have a living host to bridge between the cotton growing seasons. The purpose of the post-harvest survey is to identify plants that can serve as bridge. An important source- quite possibly may be cotton plants themselves that either are in unplowed fields or that survive plowing. By comparing results of a post-harvest landscape survey in December with results of a pre-planting survey in April, the importance of surviving cotton plants can he compared with other surviving host plants in determining regional patterns. By organizing the landscape data in a GIS, the results can be compared with regional patterns of incidence in years to come. Such data can form the basis of a regional management program that focuses on landscape elements conducive to disease with a special emphasis on the off-season host plants for the virus. The patchiness already observed in the higher levels of CLCuD incidence suggests that coordinated action at the local level has the potential to bring benefits in a regional context. The goal in using GIS is to inspire coordinated good virus disease management at local level by giving easy to visualize regional perspective. Recent information suggests that the cotton leaf curl virus in Pakistan has more genetic diversity that was previously known (Hameed *et al*., 1994; Harrison *et al*., 1997; Nadeem *et al*., 1997). It is important to understand the temporal and spatial characteristics of that diversity because it will have a major impact on the long-term success of varietal resistance developed either through traditional breeding programs or through molecular engineering approaches (Nelson *et al*., 1999). By combining the tools of GIS with the tools of molecular analysis it will be possible to explore the extent of the diversity (Nelson *et al*., 1994). PCR-amplified geminiviral DNA from symptomatic plants will be characterized molecularly using a variety of techniques. GIS software will allow us to link images of DNA gels to locations and collection dates in a way that is easy to retrieve and display for future reference. Samples for a preliminary analysis of this question were taken in 1996. A more ambitious plan for the collection of samples for DNA analysis in coming years has been planned. Results of DNA analysis will be managed and displayed in a GIS database for comparison with other data including disease incidence and severity as well as crop variety.

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