J. Agr. Sci. Tech. (2013) Vol. 15: 1523-1536

Spatial Distribution of *Macrophomina phaseolina* and Soybean Charcoal Rot Incidence Using Geographic Information System (A Case Study in Northern Iran)

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ABSTRACT

Charcoal rot caused by *Macrophomina phaseolina* is an important disease of soybean throughout the world. To understand the spatial distribution of soybean charcoal rot incidence and *M. phaseolina* populations in Golestan Province, 172 soybean fields were surveyed for population density, in two successive years, and integrated with Geographic Information System (GIS). Each year, 60 fields were also surveyed for disease incidence. Propagule density was determined by assaying five 1-g subsamples of soil from each field using a size-selective sieving procedure. In the seasons of 2009-2010 and 2010-2011, disease incidence ranged from 0 to 97% and 3 to 91% with the highest in Gorgan and Aliabad, respectively. Total mean of disease incidence were 21.01 and 35.84 percent in the province. In the two sampling years, Sclerotia were recovered from 73.33 and 93.57% of the total fields. The average population density per gram of soil ranged from 0.65 to 14.31 and 4.7 to 16.9, respectively, with the highest levels in Aliabad in both years. Charcoal rot incidence was positively correlated with soil populations of *M. phaseolina* (r= 0.61 and r= 0.47, P= 0.01). Geostatistical analyses of the survey data showed that the influence range of propagule density and disease incidence was between 8,000 to 14,000 m. In general, no significant correlation could be found between soil factors and sclerotia numbers. But, higher average air temperatures and decreased precipitation may have a significant effect on disease intensity.

Keywords: Geostatistics, GIS, Golestan province, IRAN, Plant disease.

INTRODUCTION

Charcoal rot of soybean [*Glycine max* (L.) Merr.] is a disease of economic significance and occurs throughout the north regions of Iran (Rayatpanah and Alavi, 2006) as well as in tropical and subtropical regions of the world (Dhingra and Sinclare, 1978; Wyllie, 1988). The charcoal rot disease is caused by soil borne fungus *Macrophomina phaseolina* (Tassi) Goid. with a wide variety of hosts. The disease reduces both soybean yield and seed quality (Smith and Wyllie, 1999). Generally, combination of heat stress, soil-water deficit, light-textured soil, previously sensitive crops, and the stress site associated with host reproduction is considered to enhance disease severity in the field (Dhingra and Sinclair, 1978). Severity of charcoal rot on soybean increases with increasing temperature (28 to 35°C) and limited soil moisture (Smith and Wyllie, 1999). Severity of damage to soybean due to *M. phaseolina* is strongly correlated with occurrence of drought (Manici *et al*., 1995). Sclerotia are the most important propagules for the survival of this pathogen in soil (Smith and Wyllie, 1999). Mixing of the

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[DOR: 20.1001.1.16807073.2013.15.7.7.6]

infected and colonized crop debris in the soil by tillage leads to an increase in inoculum density in the soil (Mihail, 1989). Several factors appear to influence the persistence of microsclerotia in soil, therefore, the survival of microsclerotia is variable in soil (Dhingra and Sinclair, 1974; Dhingra and Sinclair, 1975; Olanaya and Campbell, 1988).

The study of spatial distribution of diseases provides important information on where a disease is occurring and effects of environmental factors on plant disease epidemics. Based on that, preventive and control measures can be taken. Several spatial statistical techniques have been employed to characterize the distribution of plant pathogens and diseased plants (Wu *et al.,* 2001). Geographic information systems (GIS) can describe, manipulate, analyze, and display the data of most variables referenced by geographic coordinates (Star and Estes, 1990). GIS can be adapted to any size operation, and data can be incorporated at any scale from a single field to an agricultural region to describe the spatial relationships and interactions between pathogens, hosts and environmental variables (i.e., soil type, temperature) in relation to plant disease epidemics (Nelson *et al*., 1999). Various analyses can be performed in GIS environment and maps can be derived for an effective and comprehensive management of plant disease.

GIS have been applied in plant pathology for the spatial analysis of plant diseases epidemics (Nutter *et al.,* 1995; Orum *et al.,* 1997) and most extensively for mapping distributions of disease or specific genotypes of plant pathogen (Nelson *et al.,* 1994). It has also been used in the plant disease epidemiology and management (Nelson *et al*., 1999). Thomas *et al.* (2002) geo-referenced ground-based weather, plant-stage measurements, and remote imagery in GIS software using an integrated approach to determine 6 insect pests and 12 disease risk map of various crops in northern California and Washington. Jaime-Garcia *et al*. (2001) spatially analyzed genetic structure of *Phytophthora infestans*, the causal agent of late blight in a mixed potato and tomato production area in Mexico. Furthermore, GIS has been applied to determine the spatial relationship among soil texture, crop rotation and Aspergillus community structure (Jaime-Garcia and Cotty, 2006). In order to compare the effect of planting density on the distribution of Basal Stem Rot of oil palm, a GIS-based study was done on distribution pattern of the disease in an area about 10.88 ha in Malaysia from 1993 to 2005 (Azahar *et al.,* 2011).

The objectives of the current study were (i) to describe the distribution of soybean charcoal rot in the Golestan Province, which is the major region of soybean production in Iran; (ii) to identify regions with different charcoal rot risk within the province using GIS; and (iii) to quantify the spatial dependence between some soil properties and population density of the fungus *M. phaseolina*.

MATERIALS AND METHODS

Study Area

The present study was done in the Golestan Province (36º.24 N, 38º.05 E), in northeastern Iran, from 2009 to 2011. The study area is approximately 267 km long and 95 km wide (Figure 1). The area receives no uniformly distributed rainfall with an average of 450 mm. The annual rainfall and evaporation in the area varied from 200 to 700 mm and 800-2000 mm, respectively. Area climate is variable because of natural and geographical conditions. Southern areas are covered by forests, but northern parts have semi-arid and dry weather. Northern and southern nonagricultural lands were deleted by overlaying the province map with a land-use map obtained from Soil and Water Research Institute. The boundary of the middle part of the region included farms suitable for soybean cultivation. The most populous sites were identified (Figure 1-B).

[DOR: 20.1001.1.16807073.2013.15.7.7.6]

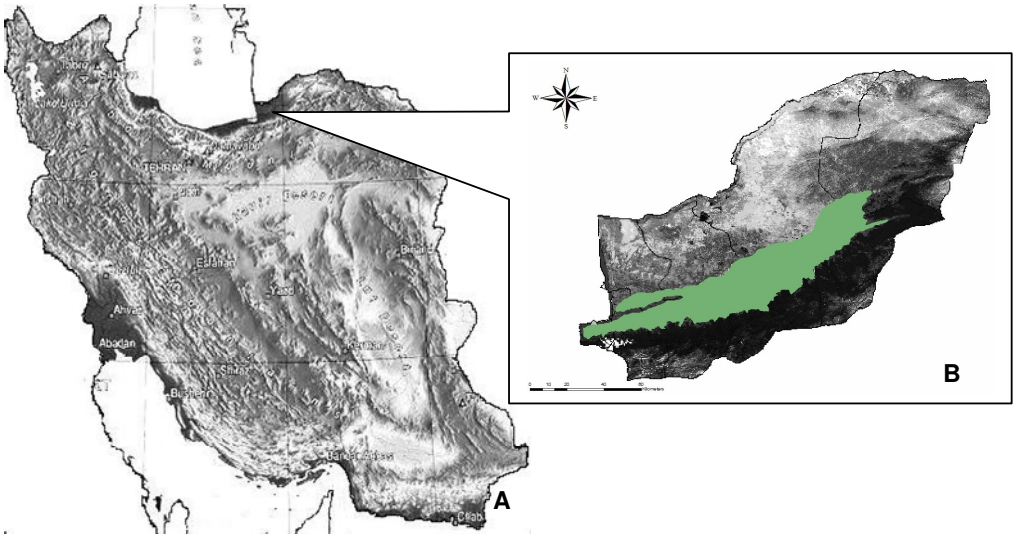

Figure 1. Maps of the study area: (A) Location of the study area on the northern part of Iran map, and (B) Enlarged map of Golestan Province, including 11 cities, area colored with green is the study area.

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Data Collection

In 2009-10 and 2010-11, respectively, 60 and 172 (including the first 60 fields) soybean fields (with the area of 5,000 to 10,000 m^2) were surveyed. Each sampled location within the province were geo-referenced with a hand-held global positioning system (GPS) unit (Model ETREX VISTA HCX, GARMIN) in decimal degree. To determine soil population in each field before harvest (at growing stages R7 or R8), ten soil samples were collected from the upper 0-15 cm depth, after removal of plant debris. Soil sampling was done in a "X" pattern with the length of approximately 100 m in each direction. Samples were thoroughly mixed and transported to the laboratory, air dried, passed through 2 mm sieve, and assayed for the *M. phaseolina* using Campbell and Nelson (1986) method with some changes. Five 1-g subsamples were taken from each soil sample to estimate the sclerotia population. Subsamples were mixed with 100 ml of 0.525% NaCLO solution for 8 minutes and then washed through a 250 µm sieve in tandem with a 38 µm sieve with running tap water for 1 minute. The material on the 250 µm sieve was discarded and the material on the 38 µm was washed with sterile distilled water for 3 minutes and then poured into a sterile 250-ml flask with 40-50 ml sterile distilled water and 100 ml of molten cooled (50-55ºC) potato dextrose agar medium amended with chlortetracycline at 50 mg L^{-1} and streptomycin sulfate at 8 mg L^{-1} was added. The mixture was distributed evenly into five Petri dishes and incubated for 4-5 days at 30°C, in dark. Colonies of *M. phaseolina* were counted and the number expressed as CFU (colony forming units per gram of dry soil sample). Identification of *M. phaseolina* colonies was based on characteristically black sclerotia submerged in the medium.

Each year, 60 fields were assessed for disease incidence. In each field, plants were sampled in a "X" pattern with 0.5×0.5 m^2 quadrates and the number of soybean plants showing charcoal rot symptoms on root was

counted. The percentage of infected plants were then calculated as disease incidence (DI).

Statistical and Spatial Analysis

Analysis of variance (ANOVA) was used to assess differences among cities in the CFU g^{-1} of soil, using the General Linear Model (GLM) procedure of SPSS (SPSS 18.0 for Windows). Duncan's multiple comparison procedure (α= 0.05) was performed for means separation of CFU g^{-1}. Pearson's correlation coefficient was calculated to examine the relationships between disease incidence and soil population.

Base map of the Golestan Province was georeferenced in the Universal Transverse Mercator (UTM) coordinate system and digitized using ArcGIS (version 9.3 for Windows; ESRI, Redlands, CA). Sample sites were also geo-referenced in the UTM coordinate system. The maximum distance between sample locations was ≈ 27 km and the minimum distance was ≈ 91 m. Geostatistical analyses were performed on CFU g^{-1} and DI to describe patterns of soybean charcoal rot disease and *M. phaseolina* in soils throughout Golestan Province. First CFU data for each field were normalized with Log transformation as follows:

Ln (CFU)= Ln (CFU+1)

According to the *x, y* coordinates of the fields, a point-coverage was generated for all these fields in geographic information system. To produce the Models that best represent the variability of the variables in the province and interpolate values in unsampled areas, the performance of two spatial interpolation techniques, Inverse distance weighting and Kriging were compared. All interpolation methods have been developed based on this theory that closer points to each other have more correlations and similarities than farther points. Geostatistical analyses were made using the Arc GIS 9.3 software. Views and layouts of the interpolated values were also created with the best selected method.

Inverse distance weighting functions (IDW) is a nearest neighbor interpolation technique that combines both the neighborhood and gradual change notions (Burrough and McDonnell, 1998). It is assumed substantially that the rate of correlations and similarities between neighbors is proportional to the distance between them that can be defined as a distance reverse function of every point from neighboring points. The inverse distance function is expressed by Equation (1):

$$z = \frac{\sum_{i=1}^{N} z_i . d_i^{-p}}{\sum_{i=1}^{N} d_i^{-p}} \qquad (1)$$

Where, *Z* is the estimated value, *Zi* is the variable value calculated at the location *i, d* is the separation between the estimated point and the sampled location, *p* is an analysis-defined power parameter and *N* represents the number of sampling points used for estimation. The main factor affecting the accuracy of inverse distance interpolator is the value of the power parameter *p* (Isaak and Srivastava, 1989). In this study, the inverse distance weighting method (search radius of 15 km, minimal point number 15 and optimized power) were compared with the ordinary kriging method using fitted variogram.

Kriging is considered an optimal estimation method as it estimates values for unsampled locations without bias and with minimum variance (Mohammadi, 2002). Ordinary kriging is a stochastic spatial interpolation technique based on the spatial structure of sampled points. Estimates of values at unsampled locations are obtained from the information provided by the structures of spatial variability, as depicted by an autocorrelation function, using semi-variogram as a measure of dissimilarity between observations. The structure of data

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may be described by four parameters: the *sill, range, nugget* and *anisotropy*. The variance value at which the curve reaches the plateau is called the *sill*. The total separation distance from the lowest variance to the sill is known as *range*. The *nugget* refers to variance at separation distance of zero. In theory, it should be zero. However, noise or uncertainty in the sample data may produce variability that is not spatially dependent. *Anisotropy* of the dataset describes spatial continuity with respect to the defined direction. It may be equal in all directions, which is known as an omnidirectional semivariogram (Goovaerts, 1997). The semivariogram is computed by using Equation (2):

$$\gamma(h) = \frac{1}{2n}\sum_{i=1}^{n}\left\{Z(x_i) - Z(x_i + h)^2\right\} \quad (2)$$

Where, n is the number of pairs of data point, which are separated by h distance; $Z(x_i)$ and $Z(x_i+h)$ are the amounts of the variable Z at x_i and x_i+h locations.

Model semivariograms were developed for transformed CFU data and non-transformed disease data. Experimental variograms were obtained using geostatistical analyst tool of ArcGIS for CFU g^{-1} soil and DI. Variogram models (either linear, circular, spherical, exponential, or Gaussian) were tested to determine their relative fit to data and the best one was selected based on cross-validation procedure (Isaaks and Srivastava, 1989). Omnidirectional semivariograms were applied for further analysis because the effect of changes in the directions was not found to be significant.

RESULTS

Statistical Analysis

Distribution map of sample sites, CFU g^{-1} soil and observed disease incidence for each survey in the two study years are depicted in Figure 2. The results showed that most of

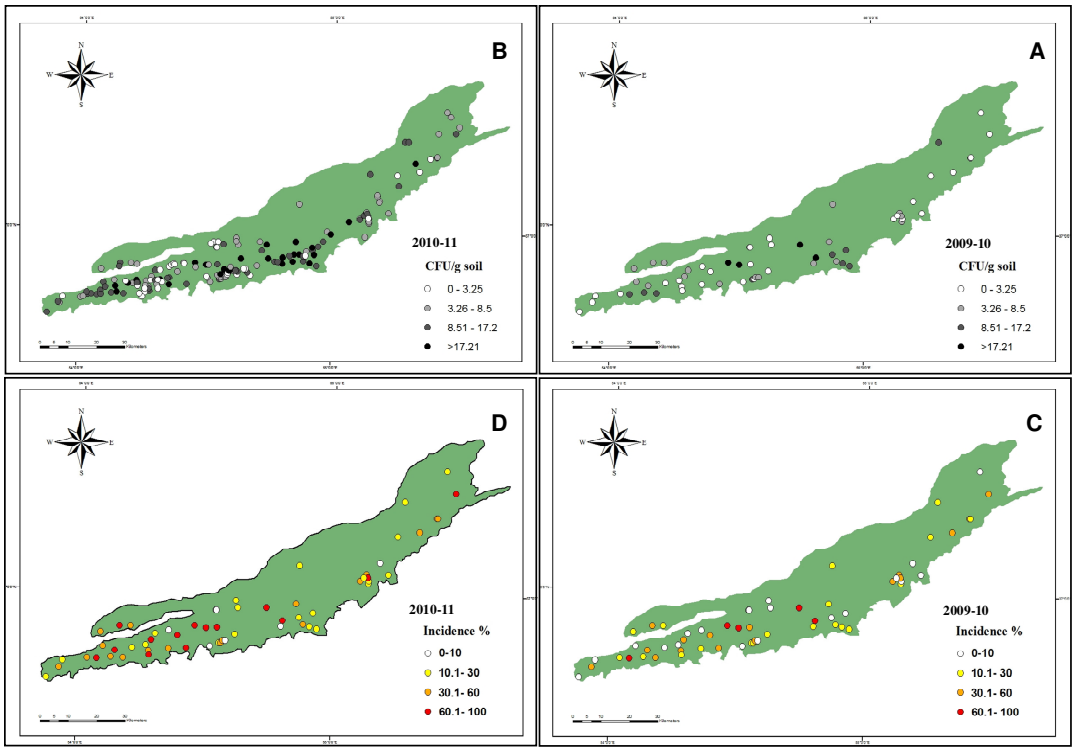

Figure 2. Distribution of soybean fields: CFU extracted from 1-g soil sample (A and B) and observed charcoal rot incidence (C and D) during the seasons of 2009-2010 and 2010-2011 in Golestan Province.

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the soybean fields in the Golestan Province were infected. Microsclerotia were recovered from most of the sampled fields, but significant differences in population density occurred among the different sites in Golestan Province. The average population density per gram of soil, from the eleven sites ranged from 0.65 to 14.31 and 4.7 to 16.9 for the first and second year, respectively (Table 1).

On a site-by-site basis, sclerotia were recovered from a minimum of 50% of the sampled fields and a maximum of 100% of the total fields. Total sclerotia recovered during the seasons 2009-10 and 2010-11 ranged from 0 g^{-1} in Agh-Qale and Kalale to 30 g^{-1} in Gorgan soil and 0 g^{-1} in Agh-Qale and Gorgan to 39 g^{-1} in Aliabad soil, respectively. At least one microsclerote was recovered from 73.33% of the total 60 fields and 93.57% of the total 172 fields sampled in the two years. Four fields in 2009 and seventeen fields in 2010 had CFU exceeding 20 per g of soil, and all were in the central part of the region. In general, during two sampling years, a greater average number of sclerotia were extracted from fields in the middle of province, ranged from less than one to 39 per g soil sample (Table 1).

In 21 and 32 of the 60 fields sampled, disease incidence exceeded 30% in the first and second sampling years, respectively (Figures 2-C and -D). In most of the fields in 2009-10, disease percent was not more than 60%, except in six fields located in the western and central part of the province (Figure 2-C). Incidence of charcoal rot increased in the second year. As before, soybean charcoal rot was generally more severe near Gorgan, Aliabad and Kordkoy in the western and central part of the province (Figure 2-D). In 2009, microsclerotial populations ranged from less than one g^{-1} dry soil in two fields, where symptoms of charcoal rot were not found, to 30 g^{-1} soil in one field in the Gorgan site where the incidence of soybean charcoal rot was 96.8%. Incidence of charcoal rot in the 60 soybean fields was positively correlated with soil populations of *M. phaseolina* (r= 0.61 and r= 0.47, P= 0.01 for the first and second years, respectively).

Spatial Analysis

The performance of the two interpolation methods, in terms of the accuracy of estimates, was evaluated by comparing deviations of the estimates from the

Table 1. Number of sampled fields and soil populations of *Macrophomina phaseolina* (cfu) in soybean fields in Golestan Province from 2009 to 2011.

	2009-2010			2010-2011		
Site name	No. fields[a]	cfu range[b]	cfu mean[c]	No. fields	cfu range	cfu mean
Gorgan	18	0.25-30	6.21(2.08) ab	64	0-31	8.44 (0.91) a
Ali-Abad	9	6-25.1	14.31(2.35) b	24	2-39	16.9 (1.97) b
Kordkoy	6	0.2-12	7.97 (1.87) ab	22	0.1-21	10.23 (1.44) ab
Ramian	6	0.6-3.75	2.5 (0.57) a	18	0.1-34	10.51 (1.96) ab
Agh-Qale	4	0-2	0.65 (0.43) a	9	0-10.75	5.46 (1.33) a
Bandar-Gaz	4	0.2-1.6	0.74 (0.3) a	9	2-14.25	8.08 (1.28) a
Minoodasht	3	0.4-4	2.34 (1.05) a	6	0.75-18.25	7.71 (2.89) a
Bandar-Turkmen	3	3.5-8.5	5.27 (1.62) ab	5	6.25-15	8.8 (1.6) a
Gonbad	3	2-10	5.5 (2.36) ab	5	3-14.75	10.75 (2.61) ab
Azadshahr	2	2.5-2.8	2.65 (0.15) a	5	2-8	4.7 (1.14) a
Kalale	2	0-3	1.5 (1.45) a	5	5-11.75	7.2 (1.19) a
Total	60	…..	5.95 (0.91)	172	----	9.86 (0.59)

[a] Number of sampled fields, determined based on areas under soybean cultivation in each site.
[b] Number of Microsclerotia g^{-1} dry soil recovered from fields soil, are presented in numerical range.
[c] Average number of Microsclerotia g^{-1} dry soil, with the standard error in parentheses. Means with the same letter are not significantly different by Duncan multiple comparison test (α= 0.05).

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measured data through the use of cross-validation technique (Isaak and Srivastava, 1989). In this procedure, the comparison of performance between interpolation techniques was achieved by using error statistics including the mean biased error (MBE), the mean absolute error (MAE) and the root mean square error (RMSE). The value of these criteria should be close to zero if the algorithm is accurate.

Equations 3-5 were used to calculate error parameters.

$$MAE = \frac{1}{n}\sum_{i=1}^{n}\left|\hat{Z}(x_i) - Z(x_i)\right| \quad (3)$$

$$MBE = \frac{1}{n}\sum_{i=1}^{n}\left(\hat{Z}(x_i) - Z(x_i)\right) \quad (4)$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\hat{Z}(x_i) - Z(x_i)\right)^2} \quad (5)$$

Where n is the number of observation, $\hat{z}(x_i)$ and $Z(x_i)$ are predicted and observed data respectively (Isaaks and Srivastava, 1989).

Table 2 summarizes the equations of error statistics and the performance of the interpolation methods for the two sampling years. The values of *MAE*, *MBE* and *RMSE* for each survey on charcoal rot incidence and *M. phaseolina* propagule density in the two years are generally lower for kriging methods as interpolators. The results suggest that the accuracy of estimates and, therefore, the accuracy of mapping variables were improved by using kriging. Furthermore, it must be taken into account that the kriging technique has an intrinsic additional advantage over the other interpolation method since its estimates are unbiased and with minimum variance (Mohammadi, 2002). Therefore, ordinary kriging method was selected to generate an interpolated map in subsequent analyses.

Experimental semivariograms were generated from the transformed population density data (Figure 3) and disease incidence data (not shown) during 2009-2010 and 2010-2011. Samples were spatially dependent when a semivariance began small and increased with an increasing distance (Francl and Neher, 1997). Specific data of variogram construction and modeling are presented in Table 3. Both semivariograms had approximately the same range (of ≈13 km), indicating the underlying spatial dependence of the population density within this range. Spatial dependence for the pathogen was calculated based on the ratio of nugget (C_0) to the sill (C_0+C), which is referred to as the "relative nugget effect". From the result, the spatial structure calculated for the *M. phaseolina* was found to be relatively strong with the relative nugget effect value of 19.3 and 36.2% (Table 3) for the first and second years, respectively. The results of semivariograms constructed for charcoal rot incidence was similar and presented the same type of variogram model, i.e. the spherical, for the two study years. The values of $(C_0)/(C_0+C)$ were also near 25% for disease incidence in both sampling years (Table 3).

Because of the spatial continuity, models obtained by the variograms can be used in

Table 2. perfomance of kriging and IDW methods for spatial interpolation of soil population density of *Macrophomina phaseolina* and soybean charcoal rot incidence during the seasons 2009-10 and 2010-11 in Golestan Province.

		2009-10			2010-11		
		Error parameter			Error parameter		
	Method	MAE	MBE	RMSE	MAE	MBE	RMSE
CFU [a]	IDW	0.704	0.172	0.873	0.744	0.110	0.934
	Kriging	0.649	0.105	0.779	0.691	0.063	0.855
DI [b]	IDW	0.187	0.013	0.234	0.211	0.025	0.265
	Kriging	0.187	0.011	0.233	0.184	0.010	0.229

[a] Population density of *M. phaseolina* in soil samples.
[b] Charcoal rot disease incidence.

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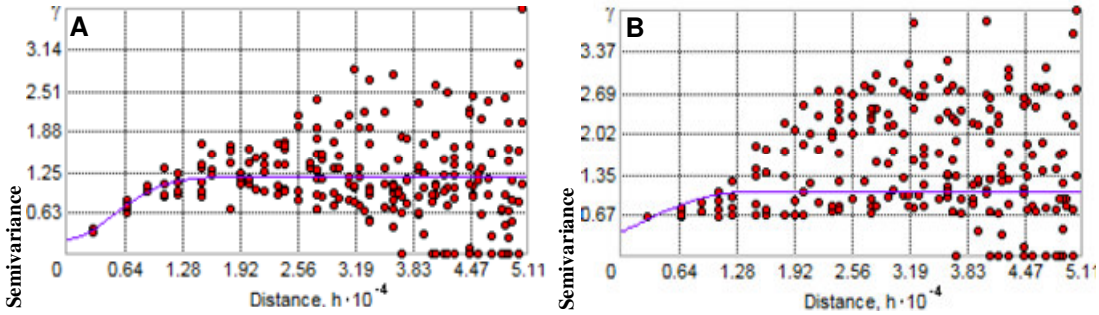

Figure 3. Semivariograms of soybean charcoal rot fungus, *Macrophomina phaseolina* sampled in 2009-10 (A), and 2010-11(B).

Table 3. Selected variogram models and their main features for population density of *Macrophomina phaseolina* and soybean charcoal rot incidence during the seasons 2009-10 and 2010-11 in Golestan Province.

		Model [a]	Range (m)	Partial sill (C) [b]	Nugget (C_0)	Nugget/Sill (%)
CFU [c]	2009-10	Gaussian	13285.7	0.949	0.226	19.3
2010-11		Spherical	13353.2	0.666	0.378	36.2
DI [d]	2009-10	Spherical	8968.3	0.073	0.025	25.5
2010-11		Spherical	8246.7	0.101	0.033	24.6

[a] Models selected based on comparison of error parameters using cross validation technique; [b] This value represent the sill minus the nugget; [c] Population density of *M. phaseolina* in soil samples, [d] Charcoal rot disease incidence.

the estimation of data in unsampled areas using kriging. Semivariograms were subjected to kriging to construct contour maps for population density of *M. phaseolina.* Although the maps changed from 2009 to 2010, the locations of the high *M. phaseolina* population density areas remained stable (Figure 4).

Interpolation results for the two surveys showed population density status in the province. In the first year, a major propagule focus was detected in the middle of the region, two minor foci in the west of the province, and one smaller focus in the eastern part (Figure 4-A). A similar trend was detected in the second year (Figure 4-B). The second year data of propagule density validated the result of the first year analysis. But, the level of *M. phaseolina* population density was slightly higher. The minor propagule focus in the eastern province developed into a major focus and the central large foci encompassed larger area after a year. It could be the result of the greater number of sampled fields in the second year.

Disease incidence during 2009-10 (mean 21.01%) was lower than during 2010-11 (mean 35.84%). In 2009-10, the disease levels were not generally higher than 50% throughout the whole province, except in Aliabad and Gorgan. But, a considerable increase in disease incidence occurred in all of the areas in 2010-11 (Figure 4). Disease foci in the western and central part of the province spread out and merged into a large area (Figures 4-C and -D).

GIS representations of the results of kriging showed that Gorgan and Aliabad were higher in both *M. phaseolina* propagule density and charcoal rot incidence than other areas of the province during the study years.

DISCUSSION

Results suggest that charcoal rot was widespread throughout the region. *M. phaseolina* was recovered from soils of

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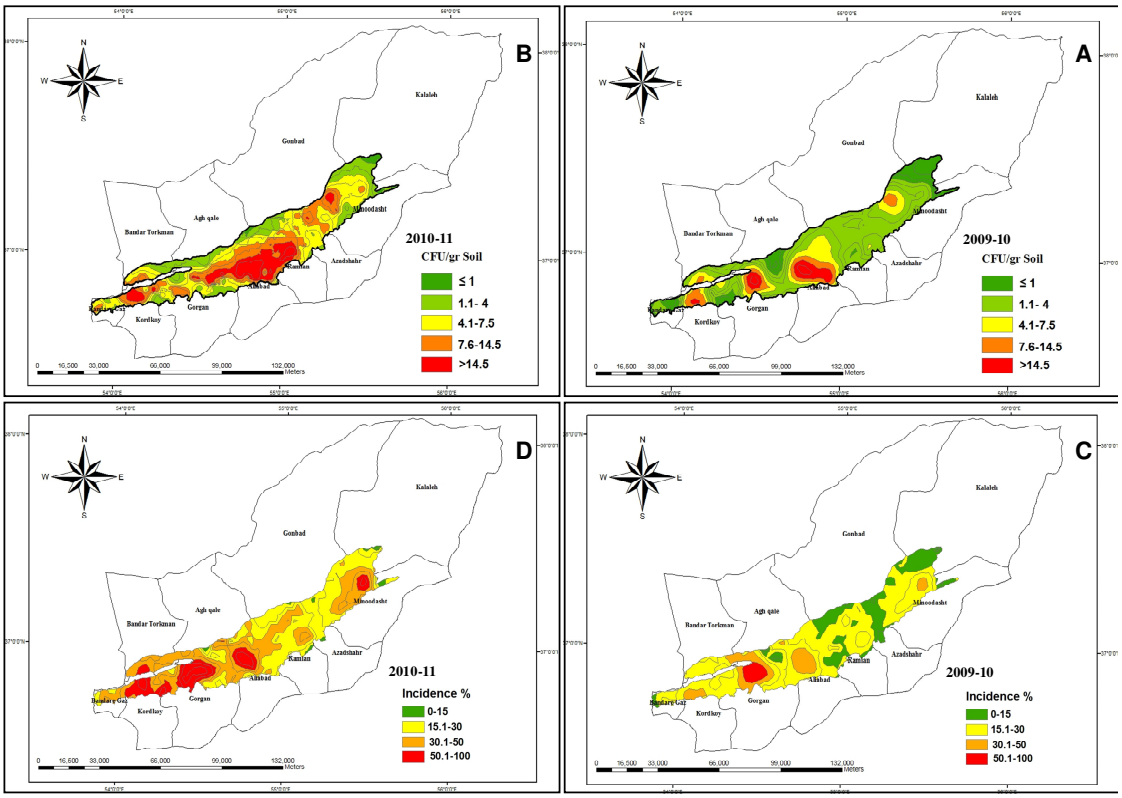

Figure 4. Interpolation maps of *Macrophomina phaseolina* propagule density (A and B**),** and soybean charcoal rot incidence (C and D), using kriging method in geographic information systems, for the surveys conducted in 2009- 20-11.

almost all sampled fields and, for the first and second years, respectively, 91.7 and 100 percent of the surveyed soybean fields had visible charcoal rot,. Since *M. phaseolina* was detected in soil from several field crops, this pathogen appears to be well established in the region. This agrees with previous reports of *M. phaseolina* from Mazandaran and Golestan (Rayat panah *et al.,* 1993; Rayatpanah and Alavi, 2006).

The analysis of the spatial structure of both the charcoal rot and its pathogen data indicates spatial autocorrelation of the variables. Both variables showed spatial autocorrelation with the range of 8,000 to 14,000 m in the area that is 175,000 by 34,000 m. This suggests localized sources of the pathogen and its restricted spread. Microsclerotia can disperse by mixing of the infected and colonized crop debris in the soil by tillage and subsequent movement of soil by disking, leveling, flooding, and wind at small scales (Campbell and Gaag, 1993; Mihail, 1989; Mihail and Alcorn, 1987; Olanya and Campbell 1988).

Variogram models were validated through the relation $(C_0)/(C_0+C)$. This ratio provides an estimation of the amount of randomness that exists. If the spatial class ratio was < 25%, the variable was considered strongly spatially dependent; and if it was > 75%, the variable was considered weakly spatially dependent (Cambardella *et al.,* 1994). The value of nugget/sill (%) for population density and disease data showed that the studied phenomenon is tending towards patchiness through the province. This is in agreement with the past findings of Mihail and Alcorn (1987) and Olanaya and Campbell (1988), in which propagules of *M. phaseolina* were found to have aggregated spatial pattern in several fields. This is generally found for propagules of most

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soilborne pathogens (Griffin and Baker, 1991).

Populations of microsclerotia of *M. phaseolina* in the fields in the middle of the province were significantly higher than those in the east, but similar to those in the west of the province. This is the result of cropping history in these areas. Aliabad, Ramian, Gorgan and Kordkoy, located in western and central part of the province, had the largest area under soybean cultivation, long-term history of soybean cultivation, and higher level of *M. phaseolina* population density. Although soil populations of *M. phaseolina* would be expected to increase slowly over time, the population of microsclerotia was reported to increase in soybean monoculture (Francl *et al.,* 1988) or after continuous cropping of sensitive crops (Lodha and Singh, 1985; Mihail, 1989). However, population density was not increased significantly in some cases. Young and Alcorn (1984) suggested that there were (biotic or abiotic) factors that impose a limit on the increase of microsclerotia of *M. phaseolina* in soil. It could be a subject for further investigations.

Since these critical disease areas are identified, control programs, which could reduce the number of microsclerotia, must be focused on these particular areas. Because *M. phaseolina* has a wide host range, selecting or developing host resistance or tolerance is not easy. Chemical management is often uneconomical or infeasible. But the combination of tillage, crop rotation, solarization, changes in planting date, using organic amendments with nitrogen-enriched materials, more frequent irrigation, use of biocontrol agents, and late maturing cultivars could reduce the number of microsclerotia in soil (Francl *et al.,* 1988; Lodha and Singh, 1985; Young and Alcorn, 1984; Ndiaye *et al.,* 2007).

The interpolation maps indicated that charcoal rot severity was not uniformly distributed across the region. Although information on the land use and number of soybean production years was not available, results showed a positive association between soil population of *M. phaseolina* and incidence of charcoal rot. Populations of microsclerotia in soil were directly related to the severity of charcoal rot (Short *et al.,* 1978), but in one experiment, a population of *M. phaseolina* of less than one microsclerotia g^{-1} was sufficient to cause greater than 90% mortality (Young and Alcorn, 1984). This suggests that factors other than population density of microsclerotia in soil have influence on disease incidence. Dhingra and Sinclair (1978) showed that the development of disease is also associated with conditions that accelerate host maturity at high temperatures and during drought stress. Data gathered on climatic conditions at the five research stations in the province (Table 4) indicated that eastern area of the province receive lower precipitation and have higher average air temperatures than the other areas during July through September. It could be a reason for the occurrence of high charcoal rot incidence and severity, despite of the low number of *M. phaseolina* microsclerotia population in soil.

It appeared that charcoal rot incidence was higher in 2010 than 2009. This could be explained by the climatic differences between the two study years. There was a 7.6-8.6% increase in air temperature and also a reduction of 66.7-100% in precipitation during July through September for 2010

Table 4. Mean values of mean daily air temperature, relative humidity, precipitation, pan evaporation, and solar radiation from 2009, 2010 for the critical months of July, August, and September.

	Air temperature (°C)		Relative humidity (%)		Precipitation (mm)		Solar radiation (Hour)		Pan evaporation (mm)	
Station	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Aliabad	25.5	27.6	68.7	55.8	2.5	0.4	5.1	8.9	5.7	7.1
Bandar-Turkmen	26.2	28.2	73.5	64.6	2.7	0.2	7	9.6	6.5	8.8
Gorgan	26.6	28.9	66.2	55.3	2.3	0.3	6.4	9.5	5.1	8.1
Gonbad	27.6	29.7	62.5	50.6	0.9	0	7.6	10	5.8	7.3
Kalale	26.7	28.8	65.1	60.3	1.5	0.5	8.3	10.2	6.5	9.3

[DOR: 20.1001.1.16807073.2013.15.7.7.6]

compared to 2009, in the five stations. Decreased precipitation and higher average air temperatures during the critical months of July, August, and September in 2010 (Table 4) could have put more stress on the plants, leading to conditions that raised the level of disease incidence significantly.

There were no significant correlations between numbers of *M. phaseolina* microsclerotia and soil properties (soil particle size, organic matter%, pH and EC) in the studied fields. But, some trends were found. For instance, the percentage of organic matter in the middle of the province (including Aliabad and Ramian) was significantly greater than the other parts. There was also a high level of *M. phaseolina* microsclerotia in that part. Wrather *et al,* (1998) demonstrated that organic matter content and *M. phaseolina* population density in the 0-7.5 cm soil layer were significantly and positively correlated. More in-depth studies on the effect of soil characteristics and population density of *M. phaseolina* are needed.

CONCLUSIONS

To our knowledge, this is the first work to provide a systematic analysis of the distribution of soybean charcoal rot and its pathogen *M. phaseolina* in Iran. This research documented the aggregated spatial pattern of soil borne microsclerotia and its widespread distribution in soybean fields of Golestan Province. The knowledge of spatial pattern of a disease is useful for making management decisions, especially for application of site-specific management as in precision agriculture. Demonstration that disease incidence and population density is a spatially dependent variable on a regional scale has implications for crop loss assessments strategies. The results indicate that the integration of GIS and geostatistics provides a powerful tool for analysis of plant disease. The resulting maps can be used and updated for further studies.

ACKNOWLEDGEMENTS

We thank Mr. Mohammad Ali Kolasangiani and Mrs. Leila Torbati (Department of Plant Pathology, Agriculture and Natural Resources Research Center of Golestan) for their field assistance.

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[DOR: 20.1001.1.16807073.2013.15.7.7.6]

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بررسی پراکنش مکانی قارچ *Macrophomina phaseolina* و وقوع بیماری پوسیدگی ذغالی سویا با استفاده از سامانه‌ی اطلاعات جغرافیایی(مطالعه موردی درشمال کشور)

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چکیده

پوسیدگی ذغالی که بوسیله قارچ *Macrophomina phaseolina* ایجاد می‌شود، یکی از مهم ترین بیماری‌های سویا در سراسر جهان است. با هدف بررسی پراکنش مکانی جمعیت قارچ *M. phaseolina* و وقوع بیماری پوسیدگی ذغالی سویا در استان گلستان، جمعیت قارچ در ۱۷۲ مزرعه سویا طی دو سال متوالی اندازه‌گیری شد و پایگاه اطلاعاتی مبتنی بر سامانه‌ی اطلاعات جغرافیایی تهیه گردید. همچنین درصد وقوع بیماری در ۶۰ مزرعه در هر سال تعیین شد. تراکم جمعیت قارچ با بررسی پنج نمونه یک گرمی از خاک هواخشک مزارع و با استفاده از روش الک‌های متوالی با اندازه‌های انتخابی تعیین گردید. دامنه تغییرات درصد وقوع بیماری در سال های ۱۰-۲۰۰۹ و ۱۱-۲۰۱۰ به‌ترتیب از ۹۷-۰ ٪ و ۹۱-۳ ٪ بود که بیشترین مقدار آن به‌ترتیب در شهرستان‌های گرگان و علی‌آباد می باشد. میانگین درصد وقوع بیماری در دو سال مورد بررسی به‌ترتیب ۲۱/۰۱ و ۳۵/۸۴ بدست آمد. میکروسکلرت‌های قارچ از ۷۳/۳۳ درصد کل مزارع سال اول و ۹۳/۵۷ درصد کل مزارع سال دوم جداسازی شدند. بیشترین میزان جمعیت قارچ در هر دو سال در شهرستان علی‌آباد مشاهده شده است که مقدار متوسط آن در سال اول ۱۴/۳۱-۰/۶۵ و در سال دوم ۱۶/۹-۴/۷ عدد میکروسکلرت بر گرم خاک

[DOR: 20.1001.1.16807073.2013.15.7.7.6]

در نوسان بود. همچنین همبستگی نسبی بین میزان جمعیت قارچ در خاک و وقوع بیماری در هر سال مشاهده شد (r=۰/۶۱ و r=۰/۴۷ ، p=۰/۰۱). نتایج تحلیل‌های زمین‌آماری نشان داد که شعاع تاثیر جمعیت قارچ در خاک و میزان وقوع بیماری ۱۴۰۰۰-۸۰۰۰ متر می باشد. به طور کلی رابطه معین و معنی‌داری بین خصوصیات خاک و تعداد میکروسکلرت موجود در خاک یافت نشد اما نتایج نشان می‌دهد که دمای بالا و کاهش بارندگی تاثیر معنی‌داری بر افزایش میزان بیماری داشته است.

[DOR: 20.1001.1.16807073.2013.15.7.7.6]