Conditional Probability and Integrated Pest Management
Using a Nonlinear Kriging Technique to Predict Infectious Levels of Verticillium dahliae in Michigan Potato Fields

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Abstract: A recent survey of potato (*Solanum tuberosum)* growers in the state of Michigan identified that soilborne pathogens were causing concerns as to whether growers would be able to continue to meet the high demands for marketable potatoes. Of these soilborne pathogens, *Verticillium dahliae* is one of the most concerning due to its direct correlation with yield decline and its persistence in the soil. Following the survey a statewide soil study was conducted to study soilborne pathogens and their interactions with multiple abiotic and biotic factors. The use of geostatistics and geographical information systems (GIS) were incorporated into this study to assess the spatially distribution of colonies of *V. dahliae* across a field and to use geostatistical methods to determine *V. dahliae* inoculum levels throughout the entire field from 20 soil samples. Furthermore, the research team incorporated the use of a nonlinear indicator Kriging method to create conditional probability maps of soilborne pathogen inoculum levels and predict where inoculum levels would be high enough to result in infection. The methods presented in this paper evaluated conditional probability mapping of soilborne plant pathogens for the potential to become a practical crop management tool for commercial potato growers.

1 INTRODUCTION

In 2012, a team comprised of potato growers and university researchers was formed to address the issue of declining yields and decreased tuber quality in some areas in Michigan dedicated to potato production. The goals of the research were 1. to better understand the spatial variability of soilborne pathogen inoculum levels in potato fields; 2. to better understand the soil biology and quantify soil microbial diversity and 3. to predict where in the field an infection may occur based on pathogen levels determined by conditional probability.

Verticillium dahliae is a soilborne pathogen that is particularly significant and, in conjunction with *Pratylenchus penetrans* (root-lesion nematode), can cause potato early die (PED) (Stevenson et al., 2001). *Verticillium dahliae* has a wide host range including bell pepper, eggplant, mint, potato, and tomato. Potato plants are infected directly via penetration of root hairs by the fungus. Once the fungus has penetrated the root cortex it enters the xylem where it quickly plugs the vascular system leading to premature senescence (Figure 1). PED is an annual production concern for commercial potato growers and impacts plant health and subsequently, crop yield. The Ascomycota fungus *Verticillium dahliae* is a well-documented pathogen of potato plants (Martin et al., 1982, Nicot and Rouse, 1987b, Powelson and Rowe, 1993). The use conditional probability may better determine where infection by *V. dahliae* might occur based on inoculum levels at sampled locations.

This research used geographic information systems (GIS) and geostatistics to create predictive maps of entire fields from known sample points. The use of linear Kriging methods in soil science has been well documented (Kerry et al., 2012, Kravchenko and Bullock, 1999, Mueller et al., 2004, Yost et al., 1982). This project evaluated a nonlinear Kriging model to interpolate the data for *V. dahliae*. Nonlinear Kriging techniques have advantages over linear Kriging techniques due to their ability to account for uncertainty and therefore are often used to predict the conditional probablity for categorical data at non-sampled locations (Eldeiry and Garcia, 2013, Goovaerts, 1994). Indicator Kriging is a nonlinear Kriging technique that is flexible and can

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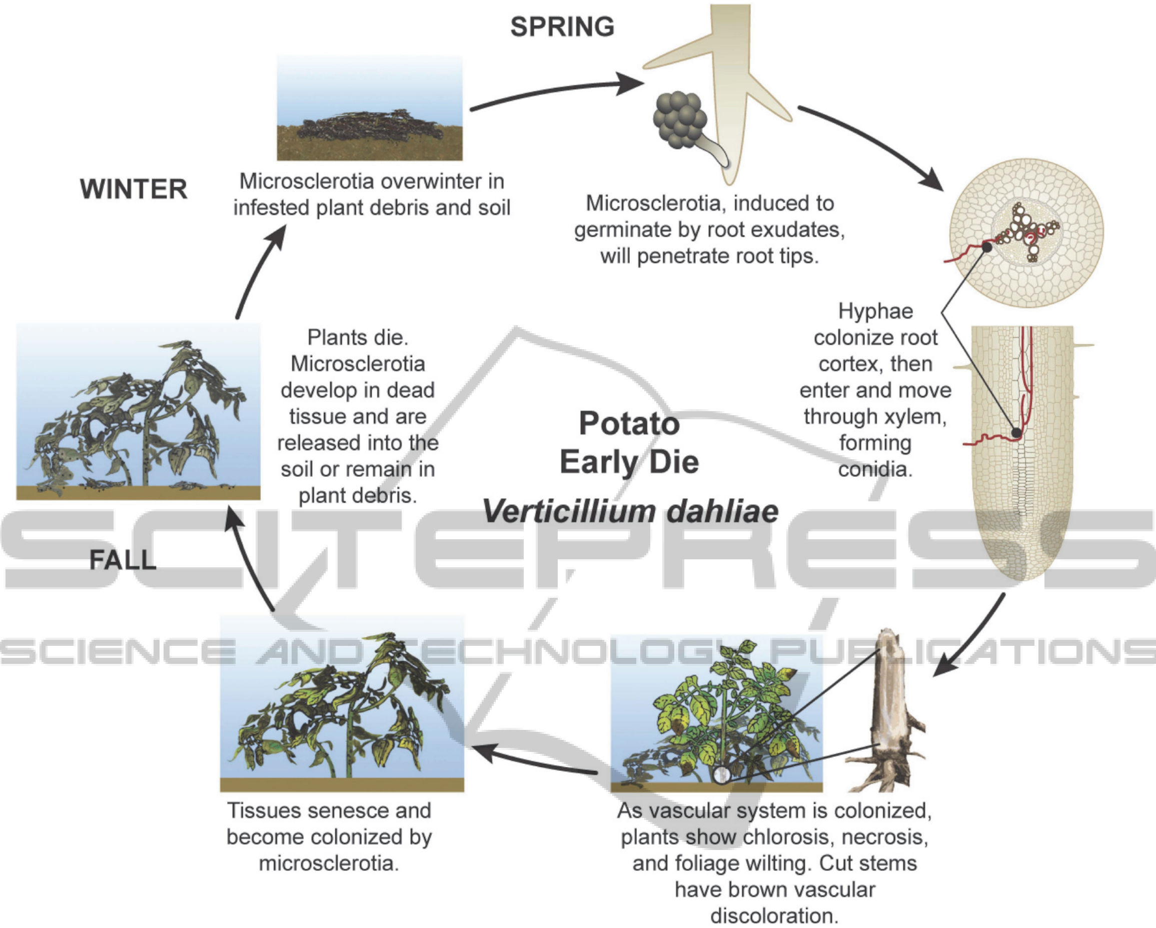

Figure 1: The disease cycle for potato early die shows how direct penetration of the root cortex leads to vascular blockage and plant death. The dead plant tissue serves as an overwintering structure for new microsclerotia. Image is reproduced with permission, from Steere and Kirk © 2013 Michigan State University. All rights reserved.

be modified to fit specific management or research goals by modifying the critical threshold criteria (Smith et al., 1993). Conditional probability maps generated using indicator Kriging can be used to visualize the probability of any point in space (within the field of interest) being greater than a set threshold. When known threshold values are available for certain pathogens and insects, a conditional probability map can be a valuable agronomic crop management tool.

2 MATERIALS AND METHODS

2.1 Study Area and Collection of Data

Three field sites located in a commercial potato production area were established for this study in Saint Joseph County in the Southwestern corner of Michigan. Each field was ~30 ha. Each field was on a two-year rotation, alternating between round white potatoes used for chipping and seed corn (*Zea mays*). 20 soil cores were collected from each field, on a grid-sampling scheme to obtain samples proportionally throughout the entire field, with a 25 mm JMC soil corer (Clements Assoc., Newton, IA) to a depth of ~100 mm around a central point in each grid (10 cores and mixed). The position of each point was recorded using a Trimble Juno 3D Handheld GPS device (Trimble Navigation Limited, Sunnyvale, CA). Soil samples were placed in separate labelled plastic bags and stored at 4°C pending further analysis. Soil data were entered relative to their geographical coordinates and plotted and analysed using ArcGIS 10.1 (ESRI Inc., Redlands, CA).

2.2 Quantification of *Verticillium dahliae* Colony Forming Units

To estimate *V. dahliae* colony forming units (CFU), 10 g of soil from each sample point was prepared using the wet sieving method (Nicot and Rouse, 1987a). Soil left in the 37μm sieve was plated onto an NP-10 medium (Kabir et al., 2004) which served as a selective nitrogen source and promoted the development of CFU of *V. dahliae* while inhibiting the growth of other soilborne fungi and bacteria. Isolates were stored at 20°C for 14-21 days and observed at 4x magnification under a dissecting microscope (Leica Microsystems Inc., Buffalo Grove, IL) and the number of microsclerotia (CFU) were recorded. Each sample point was replicated five times to confirm the accuracy of the initial CFU enumeration.

2.3 Data Interpolation

2.3.1 General Interpolation

In most interpolation methods, predicted values can be estimated by weighted averages from the surrounding areas. The general equation for the interpolation of non-sampled locations is computed as follows:

$$Z^*(x_0) = \sum_{i=1}^{n} \lambda_i Z(x_i) \tag{1}$$

where $Z^*(x_0)$ is the non-sampled location that is being predicted, $Z(x_i)$ are the values at n sampled locations and λ_i are the weights assigned to each sampled data point (Goovaerts, 1997). The difference between interpolation methods is dependent on how λ_i is calculated and what their respective values are.

2.3.2 Indicator Kriging Interpolation Method

The indicator Kriging model assumes an unknown, constant mean. The technique has been well documented (Journel, 1983; Solow, 1986) and the general form can be computed as follows (Eldeiry and Garcia, 2013)

$$I(s) = \mu + \varepsilon(s) \tag{2}$$

where μ is an unknown constant and $I(s)$ is a binary variable. The indicator function under a desired cut-off value z_k is computed as

$$I(x, z_k) = \begin{cases} 1, if\ z(x) \geq z_k \\ 0, otherwise \end{cases} \tag{3}$$

The indicator Kriging model estimator $I(x_i,z_k)$ at the location can be calculated using

$$I^*(x_o; z_k) = \sum_{i=1}^{n} \lambda_i I(x_i; z_k) \tag{4}$$

and the indicator Kriging, given Σλ=1, is

$$\sum_{j=1}^{n} \lambda_j \gamma_I(x_j - x_i) = \gamma_I(x_o - x_i) - \mu \tag{5}$$

Where λ_j is the weight coefficient, γ_I is the semivariance of the indicator kriging codes at the respective lag distance, and μ is the Lagrange multiplier. These steps transform the data set into values between 0 and 1 based on the probability of that point in space being above the set threshold value. Based on previous work done on the number of *V. dahliae* CFU needed to promote PED (Nicot and Rouse, 1987b), the threshold value for this interpolation method was set at 5 CFU/10 g of soil.

2.3.3 Model Evaluation

The accuracy of the indicator Kriging model was evaluated by using the root mean square error (RMSE) cross-validation calculated as (Ramos et al., 2008)

$$RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^{N} [\hat{Z}(x_i) - Z(x_i)]^2} \tag{6}$$

where $\hat{Z}(x_i)$ is the predicted value at the cross-validation point, $Z(x_i)$ is the measured value at point x_i and N is the number of data sets measured. The successfulness of the model in assessing the variability was evaluated by using the root mean squared standardized error (RMSSE) cross-validation statistic calculated as (Ramos et al., 2008)

$$RMSSE = \sqrt{\frac{1}{N} \sum_{i=1}^{N} \left[\frac{\hat{Z}(x_i) - Z(x_i)}{\sigma^2(x_i)}\right]^2} \tag{7}$$

where $\hat{Z}(x_i)$ is the predicted value at the cross-validation point, $Z(x_i)$ is the measured value at point x_i, N is the number of data sets measured, and $\sigma^2(x_i)$ is the variance at cross-validation point x_i.

3 RESULTS AND DISCUSSION

Cross-validation statistics analysis was performed on data for the three fields with a low-, high- and variable-risk based on spatial distribution of CFU (Table 1). These cross-validation statistics are used to determine how well the indicator Kriging equation interpolated the *V. dahliae* CFU numbers for each of the three fields. The closer the RMSE is to zero, the closer the prediction is to the measured values (Robinson and Metternicht, 2006). All three fields had RMSE values relatively close to zero meaning that the model derived from the data points in each of the respected fields accurately predicted the probability of any point in space within the field being greater than the threshold of 5 CFU/10 g of soil.

Table 1: Cross-validation parameter root mean squared error (RMSE).

Field	RMSE[a]	RMSSE[b]
1	0.1133264	0.953032
2	0.3442308	1.145598
3	0.4960541	1.034625

[a] Root mean squared error, the root value of the mean squared error

[b] Root mean squared standardized errors The closer to 1, the more accurate the prediction of variability for that model

The RMSSE shows the model's successfulness in assessing variability. The closer the RMSEE is to 1, the more successful the prediction of variability for that model was (Robinson and Metternicht, 2006). The calculations using the indicator Kriging equations above for each of the three fields of interest showed high levels of accuracy in predicting and assessing variability. Each of the three equations preformed well in regards to how accurate the predictions of the established threshold probability (CFU > 5 CFUs/10 g of soil) at points that were not sampled.

Conditional probability maps were generated for the three individual fields (Figure 2). These maps spatially represented the probability of PED incidence based on a 5 CFU/10 g of soil threshold. A conditional probability map was generated of the low-risk field (Figure 2A). Based on the 20 original *V. dahliae* CFU values and a threshold value of 5 CFU/10 g of soil, the indicator Kriging model developed for this field predicts a low incidence of PED. The small portion of the field colored red had a probability from 0.95 to 1 of PED. The majority of the field, colored in blue had a probability between 0 and 0.1 for PED. A conditional probability map was generated of the high-risk field (Figure 2B). The majority of this field had a probability between 0.95 and 1 for PED. This is quite a contrast from the low-risk field. Finally a conditional probability map was generated of the variable-risk field (Figure 2C). The result is a map where the probability of being above the established PED threshold varied throughout the field.

The visualized differences among these three maps shows how the use of conditional probability can be used to predict the spatial distribution of plant diseases in the soil and provide and informational tool for commercial potato growers. In an effort to help reduce inoculum levels of *V. dahliae* and other soilborne pathogens, growers will often elect to use soil fumigants. For many years, soil fumigants such as methyl bromide were used, with great effectiveness, to eliminate soilborne plant pathogens such as *V. dahliae* (Wilhelm and Paulus, 1980, Wilhelm et al., 1961, Ebben et al., 1983). More recently, the commercial agriculture industry has phased out the use of methyl bromide due to its negative effect on the environment (Thomas, 1996). New soil fumigants such as metam sodium and chloropicrin have taken the place of methyl bromide but as researchers begin to better understand the role of beneficial soil microorganism related to plant health (Hayat et al., 2010) the use of any broad-spectrum fumigant is being re-evaluated in a new context. While these soil fumigants may control soilborne pathogens, they may be, in effect, reducing the beneficial soil microorganism populations that assist in plant growth and natural defence against plant pathogenic bacteria and fungi.

The accessibility of conditional probability maps could become a useful informational tool for growers implementing integrated pest management. Rather than making crop management decisions for a field's acreage as a whole, a grower would be able to assess each field individually, or even at the sub-field level to determine problem fields or areas of the field that would benefit from soil fumigation. If the grower maintained a low-risk field (Figure 2A), they could use conditional probability as a holistic management tool to determine no need for fumigation in that field based on the PED risk. Conversely, if the grower assesses the conditional probability for PED and the results indicate a high-risk for PED above the established threshold (Figure 2B), the grower may elect to treat with applications of soil fumigants. Lastly, if a grower is managing a variable-risk field for PED (Figure 2C), this would allow the grower to make decisions based on a sub-field management approach and only apply fumigant to the portions of the field that present a greater

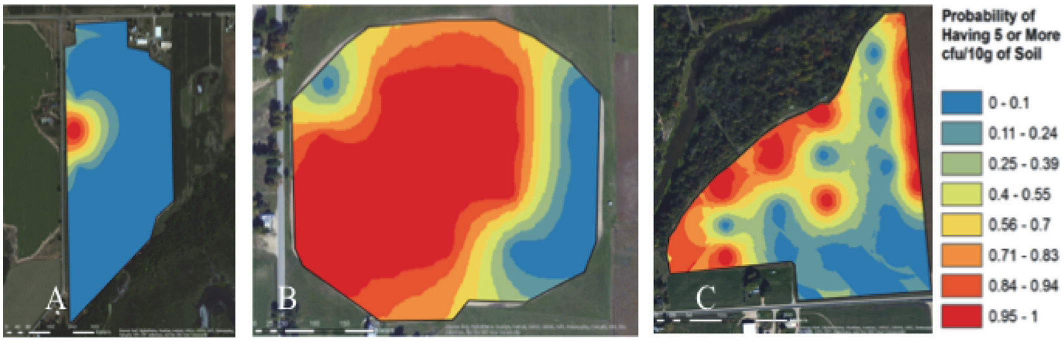

Figure 2: Conditional probability maps developed for low-risk field (A), high-risk field (B), and variable-risk field (C) using the indicator Kriging method of interpolation with the threshold set at 5 CFUs/10 g of soil. The conditional probability map for each field represents the risk for the development of potato early die (PED) based on the probability of that area in space having greater than 5 CFUs/10 g of soil with the color red representing a high probability and the color blue representing a low probability based on predicted values of *Verticillium dahliae* CFUs at that location in the field.

probability of PED. By moving away from generalized, large-scale management practices and into single field and sub-field management strategies with the incorporation of geostatistics and GIS, growers have the potential to greatly decrease input cost and negative environmental effects brought on by heavy regimens of soil fumigants and pesticides, and other inputs.

4 CONCLUSIONS

The results of this research show how the incorporation of conditional probability into an integrated pest management system has the potential to inform management decisions that can decrease the amount of soil fumigants applied on commercial potato fields. Though this study had a narrow focus looking at only one soilborne pathogen in one cropping system, the methods described above are adaptable and flexible enough to be easily incorporated into integrated pest management programs across cropping systems and for other soilborne pathogens. From an agronomic perspective, having the ability to sample a relatively small amount of data points and use those points to predict values for an entire field could greatly influence how integrated pest management is conducted in the future. Research going forward will be geared towards the geospatial interactions of soil pathogens and soil microbial populations in hopes of reducing the use broad-spectrum soil fumigants.

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