

1787 Nematode Management using Remote Sensing Technology, Self-Organized Maps and Variable Rate Nematicide Applications

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Remote sensing technology is being examined as a field worthy means for predicting reniform nematode population densities in the cotton rhizosphere using hyperspectral reflectance data and self-organized maps. Our objectives were to determine 1) the hyperspectral reflectance of cotton plants affected by different reniform nematode population levels in controlled microplot tests and in fields naturally infested with the reniform nematode, 2) to determine spatial distribution of the nematodes in the field and establish zones for variable rate nematicide applications, and 3) to determine the cotton yield improvements utilizing variable rate nematicide applications. Hyperspectral reflectance data was collected from reniform infected cotton plants growing in controlled microplots and from naturally infested field locations. Hyperspectral signatures have been identified which correspond to reniform nematode colonization and level of infection of cotton plants. These signatures and reniform populations were classified using a supervised artificial neural network Self Organized Map architecture. Nematode contour maps were developed to demonstrate the spatial distribution of the nematode and to establish zones of differing nematicide rate. Cotton yield results were higher in the site-specific application treatments compared with the conventional single rate applications. The combination of hyperspectral reflectance and VRT applications of nematicides may someday provide our producers with a rapid nematode prediction method and reduce nematicide applications to specific infield sites.

Keywords: *cotton, remote sensing, reniform nematode, site-specific applications*

The reniform nematode *Rotylenchulus reniformis* Linford & Oliveria) has become an economically serious pest to cotton production in the southeast United States. This nematode has increased from a relative unknown nematode pest to a major production constraint in Alabama, Louisiana, and Mississippi (Lawrence *et al.*, 2005). In these three states the reniform population numbers are higher compared with the other southeast cotton growing states. In fact nematode numbers in the other southeast states rarely at any time of the growing season reach the levels that Alabama, Louisiana, and Mississippi experience at planting in the spring. Over 328,073 bales of cotton are estimated to be lost due to the reniform nematode valued at over \$128 million dollars in these three states alone (Blasingame, 2007). On a per farm bases it has been estimated that the reniform nematode costs each of our producers 100 to 500 lb of lint per acre or \$55 to \$275 per acre each year.

The primary means to manage plant-parasitic nematodes include the use resistant varieties, rotation with crops that are considered a non-host to the nematode and the use of nematicides. A cotton cultivar with resistance or tolerance to the reniform nematode would be a profitable solution-however; resistance has not been incorporated in *Gossypium hirsutum* commercially available cultivars (Usery *et. al.* 2005). The uses of crop rotations with non-host plants are effective in reducing the reniform nematode numbers but are often

economically prohibitive (Usery *et al.* 2005). This leaves the use of nematicide as the most widely used means of reniform nematode management in the southeast cotton belt. The applications of nematicides have proven effective at lowering reniform numbers and increasing yields but can be expensive and offer short term protection (Lawrence and McLean, 2000).

To implement a successful reniform management program, the cotton producers must identify the nematode present in a field and determine the actual numbers present in specific locations of the field. This requires the collection of numerous nematode samples and laboratory analysis for identification and enumeration. This is a time-consuming and costly process. Currently nematicides are applied as a single rate across the field without regards to the spatial distribution of the in-field variation in reniform numbers. The spatial distribution of the reniform nematode is in a scattered pattern across an infested field and areas exist where there are no nematodes present. This is an ideal situation for site-specific applications of nematicides using variable rate technology. The application of the chemical only where needed and at rates necessary to manage the nematode would be more efficient and environmentally safer. To refine nematode management the authors investigate 1) the use of Remote Sensing Technology for predicting the in-field locations and reniform nematode present in the plant's rhizosphere based on the plant's reflectance, and 2) evaluate the efficacy of site-specific variable rate nematicide applications.

Remote Sensing Technology: Remote Sensing is a process of measuring characteristics of a target without physically coming in contact with the object. The basic idea behind this method is that a source of light emits energy (radiation) (Doshi, 2007a). This emitted energy that passes through atmosphere interacts with the target of interest. Some part of the energy is reflected back by the target after the interaction. This reflected energy is collected by a receiver then analyzed using various methods to estimate/measure the characteristic features of the target (Doshi, 2007a). ASD spectroradiometer is used in conjunction with an artificial light placed on the target and measures the reflected light energy from the target. ASD spectroradiometer collects and measures hyperspectral reflectances (signatures) at wavelengths ranging from 350nm to 2500nm. Reflectance in the range of 350nm-1300nm that includes both Visible and NIR regions provide information regarding cellular structure of the plant's leaves. Mid-IR region which includes bands from around 1301nm-2500nm provides information regarding moisture content of the plants. NIR regions (650nm-1300nm) provide information about the stress-factor in the plants (Doshi, 2007a, 2007b, 2007c; Kelley, 2003; Lillesand and Kiefer, 2000). Apart from this ranges, there also exists certain unique bands in the EM spectrum that contains vital information regarding the plant's health (Null, 2002).

Background: Gausman (1976) first looked into the idea of utilizing reflectance obtained from remote sensing technology to observe the effect of reniform nematodes on cotton leaves. Kelley (2003) furthered this concept by estimating various populations infested in cotton by providing remotely sensed hyperspectral data to Self-Organizing Maps. Doshi (2007a) expanded the above work in trying to find a correlation with the reniform population present in plant's rhizosphere with the corresponding plant's reflectance using Self-Organized maps. Doshi *et al.* (2007b) also introduced the idea of directly predicting the population range of nematode population present in the cotton plant's rhizosphere by collecting hyperspectral reflectance from the corresponding plant's leaves and providing it to the supervised Self-Organized Maps. The primary reason of using Self-Organized maps for estimating, classifying and predicting nematode populations is that remotely sensed hyperspectral signatures are large in dimensions with bands ranging from 350 nm- 2500nm. Identifying a change in reflectance resulting from different threshold levels of nematode

populations at 2151 different bands is practically infeasible (Doshi, 2007a; Kelley, 2003; Lawrence et al, 2004, 2006). Another reason includes that the nematode may cause variable effects at different wavelengths (Doshi, 2007a). Furthermore, large numbers of high dimensional hyperspectral signatures are not only difficult to process but also cause storage problems (Doshi, 2007a; Null, 2002). Hence, it is vital to reduce the data set in such a way that it retains maximum relevant information required for proper estimation of *Rotylenchulus reniformis* numbers. In order to avoid the problems of pattern recognition and data compression of high dimensional data, an unsupervised artificial neural network known as Self-Organized maps was chosen (King et. al, 2000; Doshi et al, 2007a, 2007d).

What are Self-Organized Maps? Self-Organized maps are 'unsupervised, competitive, and self-learning' neural maps that iteratively adapt themselves based on the input data provided to the network (Doshi, 2007a, King et al, 2000, 2005; Kohonen, 1990). Self-Organizing maps identifies the similarities among the high-dimensional data presented to them, clusters data samples based on the similarity, and projects them onto a low dimensional (rectangular or hexagonal) map grid. Thus, SOM performs both the function of pattern recognition and data compression (Doshi, 2007a, 2007d; King et al, 2000, 2005; Null, 2002). The working of the SOM is discussed as follows (Kohonen, 1990; Null, 2002; SOM toolbox Documentation):

For every d -dimensional input vector, there exists d -dimensional codebook vector (also known as the reference vector) $m_i = [m_{i1}, m_{i2}, m_{i3}, \dots, m_{id}]$ on the map, where i is the neuron on the map (Doshi, 2007a, 2007c, 2007d, Kohonen, 1990; Null, 2002; SOM toolbox Documentation; Vesanto et al, 1999). During the training, Euclidean distances are computed between the randomly selected input data sample and all its corresponding codebook vectors. Number of codebook vectors associated with each input sample vector depends on the size of the map. The minimum distance between the input sample vector and one of its corresponding codebook vectors becomes the winning neuron and is widely known as Best-Matching Unit (BMU) (Doshi, 2007a; King et al, 2000, 2005; Kohonen, 1990; Null, 2002;). The input sample is then mapped on to the location of the BMU (Doshi, 2007a). The neighboring neurons of the BMU are then moved closer toward or furthered away from it based on the update rule (King et al, 2000, 2005). This procedure is repeated for the entire data set of d -dimensional input vectors. Hence, for our research, the input sample is the hyperspectral signature with its bands taking the place of dimensions. In our case, we used supervised SOM, a variant of original SOM.

Supervised-SOM works in similar fashion as the original SOM, but the only difference between the original SOM and supervised SOM is the utilization of class information for creation of the map (Doshi, 2007a, 2007b; Hannula, et al, 2003; Xiao et al, 2006; Null, 2002; SOM Toolbox Documentation). In the supervised SOM algorithm, the class vector is appended to the d - dimensional feature vector (training vector). The size of the binary class vector depends on the number of class. The class vector has only two values '0' or '1'. The location of value '1' in class vector indicates the class of the feature vector. The class vector is not used in determination of BMU but only used during the 'representation and ordering' of BMU's on the map (Doshi et al, 2007a, 2007b; Hannula, et al, 2003; Xiao et al, 2006; SOM Toolbox Documentation). This causes the formation of 'class-clustered maps' (Null, 2002). According to SOM Toolbox documentation, "The class of each map unit is determined by taking maximum over these added components, and is labeled accordingly". Once the map is created, the class vector is removed. The supervised SOM map is then provided with the d -dimensional feature vector (test vector) of unknown class for class estimation (Xiao et al, 2005, 2006). Based on the similarities of features in d -dimensions

between the training and testing samples, the test sample is placed on a particular map unit. The location of the d -dimensional test sample on the labeled (class) map unit indicates the predicted class of the test sample (Doshi et al, 2007a, 2007b).

Procedures: Remote sensing: Tests were conducted over the years from 2001 to 2006. Cotton plants were grown in microplots, small fiber glass cylinders, located in the R.R. Foil Plant Science Research Farm located at Mississippi State University. There were 25 microplots filled with a sandy loam (61.25%, 31.25%, 7.5%, S-S-C, pH 6.4) soil. At cotton planting, each microplot was artificially infested with five initial (P_i) levels the reniform nematode. The initial inoculum population levels of $P_i=0, 500, 1000, 1500$ and 2000 juveniles and vermiform adult life stages per 100cc of soil was added to the appropriate microplot and incorporated into the soil (Doshi, 2007a; Kelley, 2003). Parameters of plant height, soil temperature, canopy temperature, average relative humidity were recorded bi-weekly throughout the cotton growing season (Kelley, 2003). The ASD hyperspectral data was collected for each microplot using a Fieldspec Pro Spectroradiometer with a 1.4 m fiber optic cable and 25° Field Of View (FOV). The ASD hyperspectral data consists of 2151 bands ranging from 350-2500 nm. For the year 2001, the first hyperspectral readings were taken on 6th June 2001; 25 days after planting (DAP) (Doshi et al., 2007b; Kelley, 2003). The hyperspectral readings were taken from single cotton leaves, each located at "3 nodes basal from the apical portion of the plants in all microplots" (Kelley, 2003). Hyperspectral reflectances were collected by using a clamp-on tungsten filament (i.e., an artificial light source) used to minimize any variability caused from natural light and to eliminate unwanted atmospheric effects. For each ASD reading, corresponding soil samples were collected from the cotton plant roots in each microplot. Samples consisted of six soil cores 2.5 cm in diameter and 20 cm in depth. A 100 cm^3 sub-sample was extracted and reniform numbers were enumerated using the methods previously described. The hyperspectral readings along with their respective soil samples were taken bi-weekly from all microplots. These dates include 19 June (33 DAP), 25 June (39 DAP), 10 July (54 DAP), 6 August (81 DAP), and 20 August (95 DAP) in 2001. Hyperspectral signatures were also collected for the year 2006. For the year 2006, the same microplots were used to conduct the tests and the same procedure was used to calculate the nematode numbers and its corresponding hyperspectral readings. The dates for the year 2006 include: 14th June (44 DAP), 21st June (51 DAP), 28th June (58 DAP), 5th July (65 DAP), 12th July (72 DAP), 26th July (86 DAP), and 2nd August (93 DAP) (Doshi et al, 2007a, 2007c).

Site-Specific Variable Rate Applications: Tests were conducted in producer fields currently in cotton production and naturally infested with the reniform nematode. Each field was mapped using a Tremble Ag132 GPS unit with OmniSTAR differential correction. A 0.1 ha grid size was established and used for soil sample locations. All sample points have GPS coordinates to allow data to be imported into GIS and analyzed. Soil samples were collected prior to planting to determine initial nematode numbers. The reniform nematode was extracted from 500cm^3 using the gravity screening and centrifugal flotation method. A nematode distribution map was generated from the initial nematode numbers which served as the prescription map for the variable rate applications. Nematode threshold classes were developed from the interpolated maps. These thresholds indicated which level of nematicide would be applied to the different zones. All experiments were conducted as a completely random design with nematicide rates as treatments and replicated three times. The plots were 12 rows, spaced 96.5cm apart. 1, 3 D (Telone II) (1, 3-dichloropropene) was applied at the uniform rates of 14.3, 18.9, 28.4, 37.8 and 47.9 l/ha and a variable rate application of 0 - 47.3 l/ha. Vapam (Metam sodium) was applied at the uniform rate of 28.4, 47.3 and 75.7 l/ha and a variable rate application of 0 - 75.7 l/ha. In all tests aldicarb (Temik 15G) was also included at 5.7 kg/ha to serve as a control treatment. The variable rate equipment was obtained from Raven Industries. The system consisted of a GPS, computer, flow meter,

control valve and boom valve. All the components communicated to dispense the desired rate of the nematicide at the appropriate locations with each test area. The nematicides were injected in the row using a four-leg Bigham Brothers paratill and the row immediately hipped to prevent any loss of the nematicide. Aldicarb was applied at the time of planting. All plots were maintained with standard production practices recommended by the Mississippi State Extension Service commonly used in the test area. Plots were harvested with a cotton picker equipped with a yield monitor.

Results and Discussion: Remote Sensing: The hyperspectral signatures collected from ASD spectroradiometer ranges from 350–2500 nm. Bands from 350 to 450 nm were discarded to account for sensor and atmospheric conditions as well as to take into account the large amount of scattering that occurs at these wavelengths (Doshi et al, 2007a, 2007c). Hyperspectral signatures were divided into three classes based on nematode numbers: Class 1 (0-1500 nematodes per 100 cm³ of soil), Class 2 (1501-4000 nematodes per 100 cm³ of soil), and Class 3 (above 4001 nematodes per 100 cm³ of soil). Hyperspectral signatures for each class were assigned different labels. Label for class 1 was 'Raaa', Class 2 was 'Raac' and Class 3 was 'Raca' The labels assigned for the different classes are for visualizing the location of the classes on the map. Factors such as days after planting, plant's growth stage, etc. were not taken into consideration in the analysis. For the year 2001, there were 1107 hyperspectral signatures with 653 hyperspectral signatures in Class 1, 167 hyperspectral signatures in Class 2 and 287 hyperspectral signatures in Class 3. For year 2006, there were 551 hyperspectral signatures with 234 hyperspectral signatures in Class 1, 138 hyperspectral signatures in Class 2 and 179 hyperspectral signatures in Class 3. Hyperspectral signatures were divided into 75% training data and 25% testing data for each of the three classes. Classification was performed using the supervised-SOM classification method.

In 2001, the overall classification accuracies between hyperspectral signatures and reniform nematode classes were around 70%. Individual classification accuracies for Class 1, 2, and 3 were 85%, 50% and 70%, respectively. Classification accuracies were consistent for the year 2006. This indicates that there exists a correlation between the nematode present in cotton plant's rhizosphere and its corresponding hyperspectral reflectances. These results were highly encouraging given the fact that the economic threshold values for implementing a nematode management program lies in the range of the nematode Class 1 (Doshi et al, 2007a, 2007b; Kelley, 2003). For this research, we also tried to investigate the classification accuracies in visible region, NIR region and Mid-IR regions of the electromagnetic spectrum. It was seen that classification accuracies were higher in NIR and Mid-IR region compared to accuracies found in Entire Spectrum, indicating that nematode affects the cellular structure and moisture content of the plants (Doshi et al, 2007a, 2007b). In the supervised SOM classification method, the nematode class of both the trained and test samples was known before analyzing them with SOM. The authors expanded the concept by introducing a predictor concept to estimate/predict reniform nematode numbers by providing hyperspectral signatures of the plant without the nematode numbers to the supervised SOM (Doshi et al, 2007b). The supervised SOM is created from the hyperspectral signatures affected from known nematode numbers. For the predictor concept, authors used two different years of data. During training, the combined hyperspectral signatures used were from year 2001 and 2006, while for estimating/predicting nematode numbers, hyperspectral signatures were collected from a field located at Natchez and Belzoni, Mississippi, in 2004 and year 2005, respectively. Twelve field samples were randomly selected from the field (for both years) along with their corresponding soil samples collected near the roots of the plants. The soil samples were analyzed in the lab to obtain an estimate of the actual nematode populations based on 100 cm³ sub-sample of soil and then verified with the nematode populations estimated by SOM based on hyperspectral reflectances. The

nematode population was predicted in the entire EM spectrum. In the lab analysis of soil samples, it was found that five samples had nematode populations less than 1500 reniform/100cm³ and were classified as Class 1, five samples had nematode populations less than 4000 cm³ but more than 1500 cm³ and were classified as Class 2, and 2-samples had nematode populations greater than 4000 cm³ and belonged to nematode Class 3. Hyperspectral signatures of the samples were provided to the supervised SOM map (created from the combined hyperspectral signatures of different classes for the year 2001 and 2006) for predicting the actual reniform nematode population. Additionally, U-matrix was built from the map supervised-SOM map unit to visualize the distance between the neighboring neurons. U-matrix computes the distance between a given codebook vector and its adjacent neighbors and color-codes this distances onto a map (Ultsch et.al., 1990; SOM Toolbox Documentation). It was found that from the unknown test samples 75 % were predicted while 25 % were wrongly predicted. The samples that were incorrectly predicted were from Class 2 and from Class 3, thereby matching the result of classification accuracies (Table 1). It was also seen that from the samples wrongly predicted, the samples were off by only one range. We believe this may be due to the fact that the populations of the samples were on the boundary between the two classes and also given the fact that the nematode classes were randomly divided in terms of reniform population numbers.

The same proctor analysis was also performed using hyperspectral reflectances from a harvested field in which the cotton stalks had been cut and the stalks were exhibiting re-growth in 2006. We collected 150 hyperspectral reflectance samples and their corresponding nematode soil samples. From the lab analysis, it was seen that most of samples had reniform population numbers less than 1500 nematodes per 100cm³ of soil. Thus all the samples belonged to nematode Class 1 (Fig 1). When the hyperspectral signatures collected from the cotton plants with re-growth were provided to the supervised SOM map (created from the combined hyperspectral signatures from the year 2001 and 2006) for class recognition and estimation, it was found that nearly 70% of the samples were predicted correctly. An accuracy of 70% from 150 unknown nematode samples within an 8 hectare field is very acceptable. Basic nematode sampling practices oftentimes used in agriculture would result in only 5 nematode samples within this same field area which will not provide an accurate indication of the spatial distribution of the reniform nematode in a field. The authors feel the error accounting for the 30% of the samples predicted wrongly may be from the laboratory extraction and enumerating techniques and human error

Site Specific Variable Rate Applications: The variable rate site-specific applications of metam sodium in reniform nematode infested fields improved the yield of cotton in two separate locations. In the first location cotton lint yields were 1125, 1070, 1046, 1034, and 1053 kg/ha lint in the treatments that received metam sodium at 28.3-75.7 (variable rates), 75.7, 47.3, and 28.3 l/ha and aldicarb (2580 kg/ha), respectively. The variable rate application used a total of 54.1 l/ha compared with the conventional single rate applications. At the second location total lint yields were higher. Cotton yields were 1944, 1790, 1940, and 1556 kg/ha of lint where metam sodium was applied at 0-75.7 (variable rate), 75.7, 47.3, and 28.3 l/ha, respectively The variable rate site-specific application **produced** the highest yields with a total volume of 18.9 l/ha of metham sodium compared to the conventional single rate application.

In the third test we used 1, 3-D as our nematicide of choice. The conventional rate of 1, 3-D at 47.3 l/ha produced the highest lint yields of 1019 kg/ha. The conventional rate of 28.3 l/ha and the variable rate application were very similar, yielding 960 and 953 kg/ha of lint, respectively. The variable rate application also averaged 28.3 l/ha of 1, 3-D. This may explain the similarity in the yields between the two treatments. We view the variable rate

application as being successful because specific locations within the field were treated with rates as high as 47.3 l/ha in high nematode number locations and net returns were still comparable to the 28.3 l/ha single rate application.

The site-specific applications were based on contour maps developed from the initial nematode numbers found in the field. The greater the numbers of nematode sample points that are collected increase the precision application of the specific rate of the nematicide to the specific field target. Contour maps were then used to create the metham sodium and 1, 3-D prescription maps. Both of these maps were based on placing the nematode numbers in various classes or ranges. Often times the nematode classes did not follow the threshold values that were previously developed for single rate nematicide applications. As an example in test location two the in-field nematode numbers ranged from 0-19900 vermiform life stages per 500cm³ soil. Reniform population numbers were then arranged into three classes, low (0-4000 nematodes), medium (4000-8000 nematodes) and high (8000 + nematodes). Only three nematode classes were developed in that we choose to use only three application rates of the nematicide. In later test where we used six rates of 1, 3-D and we developed six nematode classes. As additional data is generated the optimum nematode numbers/nematicide rate can be more precisely determined to provide the maximum benefit from the nematicide and subsequent returns to the producers.

Conclusions: Results from this study demonstrated it was possible to use remotely sensed data (hyperspectral reflectances) with Self-Organized maps in predicting nematode numbers in a field situation. With additional testing this technique may provide our cotton producers the ability to accurately predict how many reniform numbers are present and where they are located in a field. More tests in different soil types and in scaling these leaf level measurements into a commercially viable orbital or suborbital system are necessary to validate the robustness of this approach. The key to site-specific VR nematicide applications is to have a good representation of the nematode infestation and spatial distribution in the field. The combination of hyperspectral reflectance and variable rate applications based on nematode numbers will allow our producers to target the areas of the field that are a problem while reducing the total amount of pesticides that are applied. This will maximize the greatest yield returns to our agriculture producers while reducing the hazardous impact of pesticides on the environment.

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Table 1. Original class of 12 samples from lab analysis of soil samples as well as the classes predicted by Self-Organized maps

Sr. No	Labels Assigned	Range Predicted from lab analysis	Range Predicted from remotely-sensed hyperspectral data provided to Self-Organized maps	
			Class from Map	Label from map (Figure 2)
1	SM01	Class 1	Class 1	Raaa
2	SM02	Class 1	Class 1	Raaa
3	SM03	Class 1	Class 1	Raaa
4	SM04	Class 1	Class 1	Raaa
5	SM05	Class 1	Class 1	Raaa
6	SM06	Class 3	Class 1¹	Raaa
7	SM07	Class 3	Class 3	Raca
8	SM08	Class 2	Class 2	Raac
9	SM09	Class 2	Class 2	Raac
10	SM10	Class 2	Class 3²	Raca
11	SM11	Class 2	Class 1³	Raaa
12	SM12	Class 2	Class 2	Raac

Fig.1. Illustration of predictor concept using map grid and U-matrix. The map was trained using hyperspectral signatures obtained from cotton plants grown in microplots for the year 2001 and 2006 but the twelve samples were randomly taken from the field data for the year 2004 and 2005.

