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Development and Assessment of Nematode Management Zones in Cotton: *Gossypium hirsutum*

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DEVELOPMENT AND ASSESMENT OF NEMATODE MANAGEMENT ZONES IN COTTON: *GOSSYPIUM HIRSUTUM*

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Abstract

Populations of plant-parasitic nematodes are difficult to manage due to their inherently sporadic nature and uneven distribution throughout a field. Soil sampling accompanied by laboratory extraction is the preferred method for estimating densities and locations of nematodes within a field. The uneven and sporadic nature of nematodes make them well suited for zone management in row crops, provided that effective zones can be defined. Effective zone definition for precision agriculture requires that differences in factors between zones are large and differences within zones are small.

This study compared methods of defining zones based on physical soil properties, soil SSURGO data, and grids of similar area to cost-effectively direct nematode sampling efforts. Twenty-six methods of zone definition were investigated based on soil electrical conductivity (EC), physical soil properties and relative nematode index predictions in various combinations. For each zone definition method, the fitness of models used to define zones was evaluated using the Davies-Bouldin Index (DBI) for measuring cluster separation where effectiveness of zone definitions decrease as the DBI increases. The DBI range for all zone methods investigated was 24.918, with a minimum of 5.086 and maximum of 30.004. The most effective zone was created by contouring relative weighted nematode index predictions, with predictions based on soil EC, with a delineation range of one standard deviation, which returned the lowest DBI.

Zones created based on a three equal range division of field silt levels returned the highest DBI indicating the least effective zone method. Using silt content in any range delineation showed to be inappropriate for zone definition. The two highest DBI values returned were when silt was used at a range delineation of 0.5 standard deviation, DBI of 29.0399, and a three equal division range, DBI of 30.004. Use of SSURGO soil data was also found to be

significantly less effective for defining zones with a DBI of 27.155 compared with zones definitions based on soil EC. Zones defined using soil EC as a contributing factor demonstrated significantly effective zones. Of the nine zone definitions that were significantly effective, seven were defined using soil EC as some factor.

A second goal of this project was to asses multi-hybrid planting technology as a tool for the management of nematodes. Cotton varieties are now available that are resistant to Southern root-knot nematode, the most common and important species on cotton. For this study, a field was chosen based on the ability to grow two consecutive years of cotton within a two-year cotton to one-year peanut crop rotation and an unknown distribution of nematode density and species. This field did not return Southern root-knot nematode densities in adequate quantities for any solid conclusions to be made as to the use of resistant cotton varieties for determination of Southern root-knot nematode aggregations to be used as a basis for multi-hybrid planting or variable rate application for nematode control. The cost of this approach can be prohibitive as it can include higher seed costs, planter upgrades, and creation of planting prescriptions, which may be based on costly nematode sampling. If accurate nematode sampling zones can be determined, the overall cost of implementing this technology can be reduced.

Dedication

The following thesis is dedicated to my beautiful wife Michele “Shelly” Eubank and our two children: Aubree Mae Eubank and James Caleb Eubank. You all have endured this journey with me and have been a true source of strength since the beginning. Without you, none of this would have happened. Also, to my parents Wendell and Nancy Griggs and Kenneth Eubank. Your support, love and encouragement through the years have helped to mold and shape me into the man that I am today.

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Introduction and Related Work

Cotton Production in the Southeastern United States

The agricultural landscape of the southeastern United States is extremely diverse. Many different crops and production practices can be found across the region. Crops grown range from unique crops such, as olives in Georgia and rice in the Mississippi River Delta, to the more familiar crops such as corn, wheat, and soybeans that can be found across the Southeast. Cotton (*Gossypium hirsutum)* has been a staple of the southeastern agricultural landscape since Eli Whitney submitted a patent application for the cotton gin in 1794.

Cotton production is a vital part of the economy of the southeastern United States. In 2018, planted cotton exceeded 5.2 million hectares in the 14 cotton-producing states. Harvested hectares in the Lower Southeast (Alabama, Florida, Georgia, and South Carolina) exceeded 930,000 hectares producing over 640,000 metric tonnes of lint (National Agricultural Statistics Service, 2019). For the same year, South Carolina producers planted over 121,000 hectares of cotton with an average lint yield of 816 kg/ha totaling over 97,900 metric tonnes (National Agricultural Statistics Service, 2019). Economically, cotton ranks second in row crop production value for South Carolina with a value in excess of $172 million ($157 million in lint and $15.8 million in seed) (National Agricultural Statistics Service, 2018).

Production Inputs for Southeastern Cotton Production

As compared with some other major southeastern crops, cotton requires intensive management. For cotton to achieve an optimal yield, management of a wide variety of pests, including weeds, nematodes, diseases, insects, wildlife, and even growth rate is necessary. These issues are typically managed by some form of chemical application. For this reason, a sprayer is often the most used piece of machinery on a cotton farm.

Fertility

Soil fertilization for cotton is generally made in split application timings. Base fertilizations of phosphorus, potassium, calcium and any deficient micronutrients are applied in the spring prior to planting. If these rates, especially potassium, are not sufficient, foliar diseases can arise later in the season. Application rates are based on the results of soil sampling. An in-season application of nitrogen is needed prior to first bloom to ensure ongoing adequate nutrient supply for growth.

Most of the nitrogen required by cotton is applied in a side- or top-dress application. Recommended rates of nitrogen for cotton in South Carolina are 78 and 112 kg/ha [WE1]for dryland and irrigated fields, respectively (Jones, et al., 2019). Too much nitrogen can cause excessive growth and require increased use rates of plant growth regulators.

Diseases

From the time the cotton seedling emerges from the ground it is at risk from diseases caused by pathogens such as *Rhizoctonia solani* and *Pythium* spp. These seedling diseases are present in almost every cotton field and occur primarily in cool and wet conditions (Jones, et al., 2019). If these conditions are present, a fungicide application at planting or supplemental fungicide seed treatments may be warranted.

Pathogens such as *Stemphylium* spp., *Alternaria* spp., and *Cercospora* spp. can cause foliar symptoms. Often, expression of these leaf spots are enhanced by insufficient levels of potassium, shallow root systems, or drought (Dodds & Allen, 2017). Foliar fungicide applications in these situations are typically not cost effective.

Cotton is also host to a wide range of other pathogens that are expressed as foliar diseases. A new fungal leaf disease, areolate mildew *(Ramularia areola),* has recently become common in South Carolina (Jones, et al., 2019). Like many of the other foliar diseases, expression is often favored by very wet environmental conditions including heavy rains and heavy dews that remain until late in the morning for several consecutive days. Fungicide efficacy and cost effectiveness is determined by multiple environmental and crop stage factors.

Weed Control

Weeds compete with cotton for available nutrients and water, reducing yield and fiber quality. Weed management requires multiple herbicide applications per season to control a wide spectrum of broad-leaf weeds and grasses. A typical cotton herbicide program in the Southeast can require up to six applications (Jones, et al., 2019). Applications are typically made prior to planting, at planting, and at multiple times during the growing season.

Regulation of Plant Growth

When cotton is actively growing, plant-growth regulators (PGRs) are used to control growth. If vegetative growth is too fast, more of the plant's energy is diverted away from the reproduction processes and directed to stalk growth. The result is a tall plant with fewer bolls. The most common PGR, mepiquat chloride, is typically applied in one to four applications. The first application is typically made during early reproductive growth, when pre-floral buds (squares) and initial blooms are produced. Subsequent PGR applications are made based on

observed growth rates and environmental conditions. Herbicides, fertilizers, insecticides, and fungicides are often tank-mixed with PGRs for applications when environmental and crop conditions warrant their use.

Insect Control

Insect management is another critical component of cotton production. Cotton is susceptible to a wide range of insect pests throughout most of the crop cycle. Effective, season-long control typically requires multiple applications of insecticides applied when insects exceed economic thresholds.

Insects feed on above-ground vegetative tissues (leaves, stems, apical meristems, etc.) and/or reproductive tissues (squares, blooms, or bolls). Insects are problematic from seedling emergence to physiological maturity of the bolls. In the seedling stage (cotyledon to roughly five true leaves), plants are susceptible to thrips, primarily tobacco thrips (*Frankliniella fusca*) (Wang, et al., 2018; Reay-Jones, et al., 2019). Thrips are tiny insects that feed on the tender new growth destroying leaf cells and disrupting water and nutrient movement throughout the plant.

Other arthropods such as spider mites, whiteflies, and aphids can feed on leaf tissue causing economic damage throughout the crop life cycle. These piercing and sucking arthropods remove plant juices from leaves and stems, and, in heavy infestations, can cause yield and economic losses (Greene, 2017).

Various species of stink bugs and bollworm (*Helicoverpa zea)* feed directly on young cotton bolls. As a result, bolls are either aborted or partially damaged, resulting in yield losses. Stink bugs use piercing and sucking mouthparts to feed on the developing seed inside young bolls. Chewing caterpillar pests, such as bollworm, feed on both young and mature bolls. These

pests cause yield loss, stained lint, poor color grades, and reduced fiber quality (Harrell, May 2018).

Nematodes

In addition to the pests already mentioned, plant-parasitic nematodes must be considered in any cotton management program. Nematodes are microscopic round worms that live in the soil with some species being parasitic on the root systems of cotton. Nematodes rely on root cells for nutrition. They obtain it by puncturing the root cell wall with their stylet and extracting the cytoplasmic contents. This parasitic relationship results in potential significant yield loss for producers across the Cotton Belt. Koenning et al. (2004) classified the reniform nematode (*Rotylenchulus reniformis)*, southern root-knot nematode (*Meloidogyne incognita)*, and Columbia-lance nematode (*Hoplolaimus columbus)* as the three species of greatest concern to cotton producers.

Strategies for Pest Management in Southeastern Cotton Production

For weed, insect, and disease management, chemical control has been the standard practice. When fungal diseases appear, fungicides containing active ingredients (AIs) such as pyraclostrobin, fluxapyrox, pyridinyl-ethyl-bensamide, or azoxystrobin can mitigate the damage. Fungicides can be applied as a seed treatment, in liquid form in the seed furrow at planting, or directly to the crop in a foliar application.

Weed control chemistries in cotton fall in one of two categories, pre-emergent or post-emergent herbicides. Pre-emergent herbicides prohibit undesired weeds from germinating while post-emergent herbicides kill weeds already established and growing. An effective weed control program in cotton often utilizes a combination of both pre and post-emergent type herbicides.

Cotton varieties have been developed through gene addition to provide tolerance to three popular types of post-emergent herbicides, glyphosate, dicamba, and 2,4-D. Each of these herbicides can be sprayed directly onto varieties containing tolerance for the chemistry, without injury to the crop. Herbicide-resistant weeds, such as Palmer amaranth (*Amaranthus palmeri)*, are a major concern in cotton production. Resistant weed species require overlapping use of herbicides with multiple modes of action (Ward, et al., 2013) for the most effective control.

Insect control is similar to weed control in that multiple applications are often needed. Insecticides, such as acephate, pyrethroids, sulfoxaflor, pyriproxyfen, chlorantraniliprole, and imidacloprid, are used regularly to control targeted pests. Often, when these insect pests are identified, one application may not result in sufficient control; multiple applications may be required. Insecticides can be costly, so economic factors must be considered prior to application.

In the mid 1990's a new biotechnology trait in cotton was introduced that contained genes found in the bacterium *Bacillus thuringiensis* (*Bt*) allowing for expression of Cry proteins. This pioneered a new method of biological control specifically targeting lepidopteran pests (Bravo, et al., 2007). This plant-incorporated protectant has allowed for the decreased use of broad-spectrum insecticides, such as pyrethroids (Shelton, et al., 2002; Manda, et al., 2006). Despite this genetic resistance provided by transgenic technology, supplemental bollworm control is often needed (Fleming, et al., 2018).

Historically, control of nematodes has relied heavily on the use of nematicides containing the AI aldicarb, applied in-furrow at-planting. Aldicarb also provided suppression of other pests, like tobacco thrips. Despite its high level of toxicity to humans, the relatively low cost of this nematicide gave rise to its widespread use across the Southeast. Due to manufacturing and political issues, production of aldicarb, as Temik 15G (Bayer Crop Science, St. Louis, MO), was

discontinued in 2010 when the registrant voluntarily withdrew the registration of the material from the Environmental Protection Agency. In 2016, aldicarb was returned to the market, as AgLogic (AgLogic Chemical LLC, Woodbine, GA), by another manufacturer but at a much higher price. The new cost precluded uniform application across all hectares. To be economically feasible, growers must now use it only where nematode or thrips populations are known to exceed damage thresholds. Other nematicides with AIs such as 1,3-Dichloropropene as Telone II (Dow Agrosciences, Zionsville, IN), fluopyram as Velum (Bayer CropSciences, St. Louis MO), and oxamyl as Vydate (Dupont Chemical Co., Wilmington DE) are either less effective or cost prohibitive with costs often exceeding $148-185 per hectare. In addition to high material costs, some nematicides, such as Telone II, requires special application equipment as well as an additional, pre-plant pass across the field, which increases production costs.

In recent years, cotton varieties with genetic resistance to the southern root-knot nematode (SRKN) have been introduced to the cotton market. These varieties were developed using selective breeding techniques for plants with a natural genetic resistance to nematodes. High nematode population densities or when fields contain multiple species at damaging levels, nematicides may still be required in conjunction with resistant varieties.

Nematode management decisions are based on established economic threshold for each nematode species. Economic thresholds are defined as the pest density at which management action should be taken to prevent an increasing pest population from reaching the economic injury level (Hunt, 2014). When a pest population reaches the economic injury level, the population density of a pest is such that the value of the damage caused is equal to the cost of control (Ferris, 1978). Producers determine nematode population densities by collecting soil samples and submitting them to a nematode assay laboratory. Results are reported as counts of

larvae by species per 100 cm^3 of soil. Economic thresholds differ among nematode species and within soil textures. For example, cotton grown in a sand or sandy loam soil texture has a threshold level for SRKN of 100; in clay soils the threshold number rises to 130 (Appendix 1) (Clemson Extension Service, 2000).

The cost to conduct a laboratory assay for a single nematode sample ranges from $15 to $20, as compared to fees for soil fertility samples at $6-10 each. Each nematode sample can represent an entire field or a portion of a field. With increased labor costs for sample collection, higher laboratory fees, and thin profit margins, growers often perceive sampling for nematodes as a cost prohibitive practice.

Nematodes Parasitic to Southeastern Cotton

Of the three main cotton-parasitic nematodes, SRKN and the reniform nematode are considered sedentary endoparasites. These nematodes have a complex interaction with their host and can be responsible for considerable damage to agricultural crops (Tygat, et al., 2000). They move through the soil rhizosphere to locate host plant roots. Once a host is found, these nematodes enter the root tissue and migrate to pro-vascular cells to establish a permanent feeding site. When the feeding site is established, the nematodes trigger the redevelopment of several cells into 'giant cells' that provide the nourishment required to complete their life cycles (Jones & Goto, 2011). Eggs are laid and hatched at these permanent feeding sites.

SRKN infection sites develop into the root galls that are the distinctive indicator of the presence of SRKN. Visual symptoms of the infection by reniform nematode, however, are not as easily identified. Reniform nematodes cause necrosis within the root that results in plant stunting, yellowing, and wilting. These symptoms can be mistaken for fertility deficiencies, drought stress, or other environmental issues. Unlike SRKN and reniform nematodes, Columbia

lance nematodes (CLN) are migratory and feed both endo- and ecto-parasitically. CLN feed on both the external root surfaces and internal root tissue. CLN do not permanently establish themselves within the root tissue of the host plant. Instead, CLN migrate through the root tissue feeding and laying eggs continuously. CLN may also leave the root at any time, migrate through the soil, and infect other roots. Because CLN feed on root tips, patterns of root growth can be altered, depending on nematode density. This feeding may cause a stunted tap root and an increase in secondary branching in the upper four inches of soil (Blasingame, et al., 2003).

Losses and Control Costs for Nematodes in Southeastern Cotton

In 2018, cotton lint yields for South Carolina averaged 816 kg/ha. For the same year, cotton lint yields in Georgia averaged 776 kg/ha. Average market price for cotton lint was $1.62/kg (USDA, 2018). Across the U.S. cotton belt nematodes annually cause an estimated 10% yield loss (Koenning, et al., 1999; Blasingame & Patel, 2005) for a potential lint yield loss of 32.6 kg/ha and $130/ha in South Carolina. Lint yield losses in Georgia are estimated at an average of 31 kg/ha and $126/ha. With a combined planted acreage in excess of 419,000 ha across both states and 5.67M ha planted across the Cotton Belt, nematodes are economically important pests.

Although sampling and analysis costs are perceived to be high, soil sampling is the only method for accurately estimating population densities of nematodes. The perceived high costs often result in less than suitable numbers of samples being collected for a given area. Typically, core samples from multiple areas within a field are combined into one or two composite samples and submitted for assay. Using one or two composite samples, a nematode management decision is made for an entire field. However, nematodes often are not uniformly distributed across a field but may be clustered, based on host type and soil texture. If the field is heterogeneous in its nematode distribution, then assay results may lead to inappropriate or inefficient management

decisions (Mueller, et al., 2010). When a small number of composite samples are used per field, there is a low level of precision in estimating the nematode populations present.

With the cost of cotton production rising each year, growers must maximize their net return on each hectare of cotton they plant. Integration of new technologies, such as global positioning systems (GPS), into production practices supports growers' efforts to improve their profit margins. Recent estimates are that some form of precision agriculture is being used by 73.5% of growers across the Cotton Belt as part of their current production practices (Zhou, et al., 2017).

GPS guidance systems automatically steer equipment along pre-defined guidance lines to reduce input overlap (seed, chemical, fertilizer), reduce waste, and maximize field hectares planted. Also, properly equipped tractors, sprayers, and other application machinery can utilize GPS technology, along with other sensor data, to precisely control input application rates. Inputs can be applied at a uniform rate or programmed for variable rate application (VRA).

When VRA technology is used, product application rates are adjusted to meet the varying requirements within a field. These rate changes are done automatically, utilizing GPS technology, while the application machinery remains in motion. The use of VRA can integrate several factors including yield zones from yield maps, soil fertility and texture maps, and visual observations by the grower. Many individual layers of spatially recorded data are used to create a VRA prescription. These prescriptions are not a "one size fits all" scenario. For example, a VRA prescription map for agricultural lime application may be different from a VRA map for potassium fertility application. Different VRA prescriptions may require different data layers for construction.

Growers are realizing the potential for increased returns on investment in these new and rapidly advancing technologies. Zhou et al. (2017) surveyed cotton growers specifically concerning their precision agriculture practice adoption and estimated that 25.3% of respondents had adopted VRA practices for at least one phase of their operation. Profitability was indicated by 37% of respondents as the most important reason for incorporating precision agriculture practices in their operations.

VRA technology for nematode management is not a new concept. Potential yield increases have been observed with VRA of aldicarb for control of SRKN on cotton in Texas (Wheeler, et al., 1999). Variable rate technology has shown potential for reducing overall inputs while controlling nematodes on cotton without a negative impact on yield (Overstreet, et al., 2014).

An emerging area of VRA technology gaining strong interest is multi-hybrid planting. Multi-hybrid planters offer the ability to switch seamlessly between two seed varieties with no interruption to the planting process (Jeschke & Shanahan, 2015). Varieties can be changed from within individual rows or to multiple rows in any pattern across the planter allowing producers to match seed varieties to their varying field requirements.

Multi-hybrid planting may have the potential to combat SRKN nematodes. This new planting technology can be used in conjunction with recently introduced SRKN cotton varieties, as well as with treated seed. For multi-hybrid planting to reach its full potential, accurate management zones must be defined. Zones can be divided in either a rigid geometric grid or a contoured pattern. Grid zones are often based on a specific area division, such as 2.5 ac. Contour zones are typically based on some form(s) of underlying georeferenced data such as yield or soil texture properties. Regardless of the method employed, for management zones to be effective,

each zone must minimize the variability within the zone while maximizing the variability between the zones.

Zone Management Approach to Nematode Control

Because nematodes have an uneven population dispersion with some correlations to soil texture, they may be suited for control through zone management. In a zone management approach, a field is not treated as a single, homogenous unit but subdivided into smaller, subunits. The goal of zone definition is to achieve a greater level of homogeneity in the subunits as compared with that of the field unit. These subunits are treated independently from each other based on desired control level in each subunit.

The first step in developing a nematode management strategy of any type is to determine the nematode species present in the field along with their relative densities, as this predicts the incidence and severity of plant damage (Barker & Olthof, 1976). Early season damage to roots can be exhibited by above ground damage such as stunting or chlorosis. These symptoms in turn are directly related to yield loss. Thus, at-plant densities can be used to predict yield losses and, when coupled with the costs of different control programs, to determine the economic threshold of each nematode species. The economic threshold for any nematode species can be defined as the nematode density at which management actions should be taken to prevent the nematode density from reaching the economic injury level (the level at which revenue from increased yields is equal to control costs) (Ferris, 1978).

The control measures most commonly employed by producers are crop rotation, host plant resistance, and nematicides. The nematicides aldicarb and 1,3-dichloropropene are considered the industry standards with fluopyram as another option currently being used on a minimal number of hectares. With the current political and social climate permeating agriculture,

pesticide usage is under intense scrutiny, especially the usage of those labeled "Restricted Use Pesticide" by the Environmental Protection Agency. With this intense public scrutiny, reductions in pesticide use are becoming a necessity for producers. By effectively employing VRA technology, producers can be better stewards of their resources. Wrather et al. (2002) reported a 46 to 61% reduction in total nematicide application using VRA while maintaining yield levels of cotton in SRKN infested fields, compared to uniform rates. It was concluded that, even though yields were similar between VRA and uniform rates, a higher overall net economic return could be achieved because of the reduction in the cost of nematicide applied, excluding costs for implementing variable rate technology.

Effectiveness of nematicide treatments can vary from year to year,d and VRA of nematicides have provided mixed results (Wheeler, et al., 1999). A comparison of uniform applications of aldicarb against VRA of aldicarb in cotton was conducted by Wheeler et al. (1999). In only three out of eight Texas fields studied did a VRA nematicide application return equal or higher yields when compared to fields that received a uniform application rate. However, VRA in these three fields did result in an overall reduction in the total amount of aldicarb applied. In contrast, two of the eight fields had a higher total usage of aldicarb with no yield difference as compared to the uniform application rate. Wheeler, et al. (1999) did not include any net economic returns in their conclusions.

In Georgia, Baird et al. (2001) concluded that VRA of nematicides can reduce the overall applied quantity of nematicides leading to increase profitability for growers. It was determined that a VRA of 1,3-dicholorporene had similar yields when compared to a single uniform rate of 1,3-dicholroproene in a field in southwest Georgia with natural infestation of SRKN. VRA of 1,3-dichloropropene also had the greatest economic return of any of the treatments investigated

when sampling, and chemical costs were included in the final cost analysis. Similar results were observed by Wrather et al. (2002) in VRA of aldicarb.

Soil properties have shown potential in associating field management zones to SRKN risk (Ortiz, et al., 2011). In management zone delineations based on terrain elevation, normalized difference vegetation index, and soil electrical conductivity (EC), Ortiz, et al. (2012) concluded that site-specific application of nematicides for control of SRKN on cotton in Georgia can be cost effective but efficacy, of nematicide rate and type can vary across management zones. In fields with little soil texture variability, a site-specific approach may not be economically beneficial (Oritz, et al., 2012).

For a field to be a candidate for VRA of nematicides, it must exhibit heterogeneity in its nematode populations, and the spatial clustering of these populations must be determined or, at least, predictable. Management zones based on variations in soil texture have resulted in differing management strategies for each zone. For Ortiz et al. (2012), an instance of no response to nematicide applications was observed in the field with the smallest zone differences in terrain and edaphic properties. Erwin et al. (2007) concluded that performance of the nematicide 1,3-dichloropropene varied according to soil texture; it was more efficacious in coarsely textured soils than in areas of more finely textured soils. This variation in effectiveness coupled with the aggregated population dispersions of nematodes make VRA a potentially profitable application practice.

Defining Management Zones

Much research has been done in the way of zone management practices and applications in row crop agriculture (Bongicoanni & Lowenberg-Deboer, 2000; Koch, et al., 2004). Although

zone management is most common in fertility applications, other agricultural inputs, such as nematicides, can benefit from zone management practices.

All VRAs are based on defined zone management maps. These management zones can be defined in numerous ways. The simplest method of zone definition is division into grids of equal size that are not based on any data layers. More complex zone maps are classified by data layers such as spatial yield maps, laboratory soil sample analyses, and soil EC. Zones that are classified by underlying data can be further defined by data range delineation; the overall number, shape, and size of zones can be influenced by range separations based on standard deviations (σ) or other user-defined criteria.

Soil characteristics are commonly used in zone management classifications. Soil sampling provides the most the accurate measure for any soil characteristic. The average cost of a soil sample analysis ranges from $6, for a routine fertility test, to $12, for a soil texture analysis. Soil fertility sampling is an investment for producers, and return on investment must be considered. Soil grid sampling, when used in conjunction with variable rate applications, has been shown to have a positive return on investment (Bongicoanni & Lowenberg-Deboer, 2000; Koch, et al., 2004). Grid sampling is accomplished by subdividing a field into smaller units and sampling those subdivided units separately. The advantage of a grid sampling technique, as compared to a single composite sample of a field, is that more soil variability within a field can be identified.

Another option for zone classification is estimation of soil texture through sensor-based soil EC. Sensor platforms along with their associated software, such as a Veris platform (Veris Technologies, Salina, KS) generate soil EC maps. These data can be an effective predictor of soil texture and allow a producer to generate a detailed map highlighting variations in soil texture

(Overstreet, et al., 2014). Soil EC mapping services are often readily available through many seed and chemical retailers and other private entities at economical rates. These private entities collect and combine the data into formats that can be uploaded into geographical information system (GIS) software programs available to growers and their consultants.

Soil EC measures the soil's resistance to electrical currents and can be used as an estimator of soil particle size. Soil particle size has been shown to have a high correlation with soil EC (Williams & Hoey, 1987). Sand correlates to a low EC; silt correlates to a medium EC; clay correlates to a high EC (Mueller, et al., 2010). Monfort et al. (2007) demonstrated that variations in soil texture, like those shown in soil EC maps and soil texture analysis, can be valid criteria for the creation of management zones. Soil EC has also been shown to be an effective predictor of nematode population densitites for certain species. Predictions of the relative densities of spiral nematodes (*Helicotylenchus* spp.), ring nematodes (*Criconema* spp.), and CLN in a South Carolina cotton field using soil EC data have been shown to be possible (Mueller, et al., 2010). Soil EC has also shown a strong correlation with spatial variabilities of CLN and SRKN (Wiatrak, et al., 2009).

One limitation to soil EC is that the measured value for a given position is not constant like other physically measured soil properties. Soil EC readings can vary even when collected in the same location because it is heavily influenced by soil moisture. As soil moisture rises, so do the overall soil EC readings. However, although actual soil EC measurements shift with soil moisture, the relative soil EC within a field does not. High EC zones will remain high and low EC zones will remain low when compared to the average soil EC of the field. This consistent relativity makes soil EC data a good, although not perfect, tool for predicting relative soil texture and relative variability within a field.

Generally, soil textures and soil organic matter (OM) do not change significantly from year to year. For general mapping of soil properties within a field, yearly sampling, either by soil samples or by sensor-based sampling, is typically not needed provided excessive soil amendments are not added or soil movement does not occur. Therefore, once soil properties are determined for a field, those values can be relevant across multiple crop years.

Soil Texture and Nematodes

Abundant research exists correlating nematode populations with soil type and particle size (texture) (Wyse-Pester, et al., 2002; Monfort, et al., 2007). Reproduction rates in SRKN have been shown to be greater in more coarse soil textures (Koenning, et al., 1996). The potential for yield suppression and crop damage, in cotton, has been shown to increase in areas where soil texture is defined by higher sand content (%) than compared to areas of soil texture defined by lower sand content (%) and higher silt content (%) (Monfort, et al., 2007). Reniform nematode has shown positive correlation to silt and clay content (Robinson, et al., 1987; Heald & Robinson, 1990; Koenning, et al., 1996; Davis, et al., 2013; Moore & Lawrence, 2013), but stronger correlations to sand content have been identified (Holguin, et al., 2015).

Nematode populations are influenced by edaphic as well as non-edaphic factors. The population of a species can be affected by the presence of another species. Distribution of CLN populations within a field have been shown to be influenced by the presence of reniform nematode as well as soil texture (Holguin, et al., 2015). Additionally, populations of SRKN can be suppressed in the presence of CLN. If populations of CLN are high enough, it can replace SRKN as the predominant parasitic species within a field (Bird, et al., 1976; Kraus-Schimdt & Lewis, 1981).

When population densities of the target nematode species are highly correlated to soil texture, soil texture can be used as the basis for creation of functional management zones. Unfortunately, research with nematodes is a challenging area because nematodes are unevenly distributed both vertically and horizontally within a field (Barker, & Campbell, 1981; Ferris, 1984). For this reason, nematologists and other researchers have difficulty determining the best method for characterizing nematode distributions (Barker, & Campbell, 1981). Although research has shown positive results in using VRA of nematicides, and that nematode populations are correlated to soil textures, the best method for zone management and classification is still unclear.

Management of nematodes has the potential to be coupled with precision agriculture technologies for more directed and cost-effective control. This research discusses the utility of selected methodologies for application of precision agricultural technologies for management of nematodes in cotton.

Study Objectives

Nematodes exist in aggregated distributions, which can be related to specific field conditions (Ortiz, et al., 2011; Holguin, et al., 2015; Holguin, et al., 2015). To improve zone management practices for cotton parasitic nematodes in cotton, this study was conducted to satisfy the following objectives.

1) Identify, using cluster analysis, the best method for delineating management zones based on, physical soil properties, a predicted relative weighted nematode index, and SSURGO soil data, to be used for directed nematode sampling.
2) Define a methodology to prescribe placement of commercially available SRKN susceptible and resistant cotton varieties using multi-hybrid planting technology for

control of Southern root-knot nematode using commercially available equipment and systems.

Assessment of Zone Management Delineations for Nematode Sampling in Cotton

Plant-Parasitic Nematodes and Site-Specific Zone Management

When crop production inputs, such as nematicides, are applied using VRA, they are applied at variable rates predetermined for specific locations within a field. These locations and rates are based on layers of georeferenced data that have been collected, analyzed, and assembled into application maps. Data layers used in VRA map construction are the result of either laboratory sample analyses or sensor-based data that are contoured into digital maps using commercial GIS software. Examples of data layers include crop yield, soil EC, soil texture, etc. (Mallarino & Wittry, 2001).

Adoption of VRA has allowed growers to improve input use efficiency and thus improve return on investment by application of the right product at the right rate in the right location. The foundation for any VRA is the development of accurate management zones based on georeferenced data. Management zones can be created by using data layers such as physical soil properties (sand, silt, clay, and OM content) (Mzuku, et al., 2005), soil EC (Lund, et al., 1999), yield (McGraw, 2016), and other factors that have an association with a pest or other input need. Within each zone classification, the separation range can impact the shape, size, and number of individual zones. For example, percent sand content can be used to create management zones within a field. When a more generalized sand map is desired, larger ranges for each division of sand content are used, resulting in a smaller number of divisions and zones; for instance, the range in each division of sand content could be set equal to one standard deviation of sand content. To capture the variability at an increased level of detail, smaller ranges for each division of sand content are used resulting in a larger number of divisions and zones; for instance, the range in each division of sand content could be set equal one half of the standard deviation of

sand content. The number of zones and zone granularity, distinguishable sections, can be adjusted to fit the specific application requirements.

Another source for zone classification of soils is the soil survey geographical database (SSURGO) maps (United States Department of Agriculture, 2019). SSURGO maps use the soil types defined by the National Resources Conservation Service. However, since the original intended use for SSURGO maps was in natural resource planning (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture, 2019), they have been shown to be inadequate in creating maps of soil zones for use in precision agriculture (Mausbach, et al., 1993; Franzen & Peck, 1995; Mallarino & Wittry, 1998; Kitchen, et al., 1999).

At the time of its collection, most of the soil information represented by SSURGO maps was collected between a scale of 1:12,000 and 1:63,360. At a scale of 1:12,000, 1 cm is equal to 120 meters. On a 1:63,360 scale map, 1 cm is equal to 633.6 meters. These large scales result in zones with areas too large to be used as a basis for field level precision agriculture practices. In practice, using SSURGO classifications may be suitable for county- or regional-level resource assessment but they capture very little of the field-level variability in soil texture.

To illustrate comparison of nematode management zones, consider an example field where soil samples were collected at random for nematode assays (Figure 2-1). The reported species count is listed on the map at the sample location and reported in nematodes/100 cm^3 of soil. Sample points highlighted in green indicate assay counts that are below a hypothetical economic threshold. Sample points highlighted in yellow represent assay counts in which a cultural control may be recommended. Sample points that are highlighted in red represent assay counts in which a nematicide application will be economically beneficial. For a 24-ha field, collecting19 nematode samples is not considered cost effective by most growers. With an

average cost of $15-20 per sample, sampling of this field would cost $285-380 for laboratory assays, amounting to $12-16 per hectare.

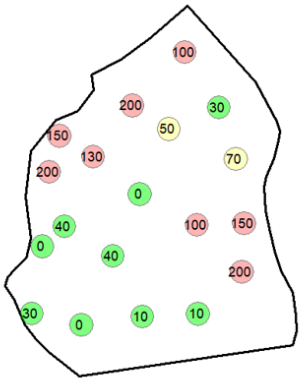

Figure 2-1. Numbers of nematodes per 100 cm^3 of soil at various locations in an example field.

If this field were divided into different zones that accurately clustered populations as either above or below published economic thresholds, the cost could be reduced significantly. By using management zones, multiple core samples collected from each zone can be combined into a single sample and submitted for assay (Figure 2-2).

Assuming an economic threshold of 100 nematodes/100 cm^3 of soil in our example field (Figure 2-1), effective use of managment zones for nematode sampling will require zones that return average densitities over 100 be separated from those under 100. Ineffective creation of zones (Figure 2-2a) can result in average densitities returned for all zones falling below 100, despite many sampling positions exceeding 100. This example would suggest that a nematicide application would not be ecnomically benefical. However, if zones were more carefully defined, some (3 and 4) would benefit from a nematicide application (Figure 2-2b). In this example, we

have the luxury of knowing population densitities at each of the 19 field positions; an objective of successful nematode management zone definition is to successfully aggregate the samples into representative, composite samples in the absence of population knowledge at each sample location.

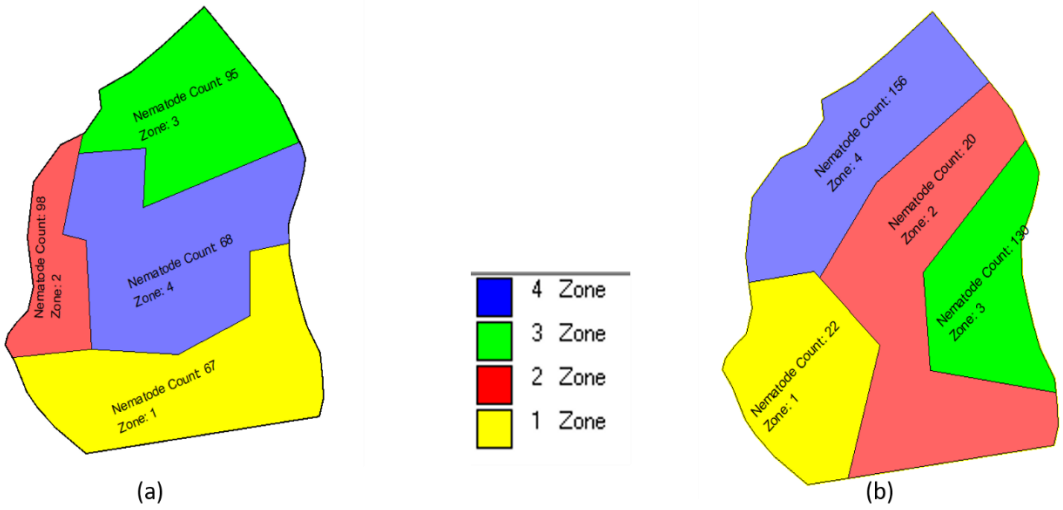

Figure 2-2: Examples of management zones based on nematode densities and locations from Figure 2-1, with an ineffective method (a) for defining management zones and an effective method (b) for defining zones for managing nematodes, assuming an economic threshold of 100 Southern root-knot nematodes per 100 cm^3 of soil.

To date, a method for creating nematode management zones has not been defined, such that the nematode population variability between zones is maximized while the population variability within zones is minimized. In an attempt to develop a method of zone definition for nematode sampling that meets these criteria, this study was conducted to satisfy the following objectives:

1) Evaluate the use of geospatial properties using cluster analyses for building optimum management zones for nematodes.
2) Develop predictive models for nematode populations as functions of soil and geospatial properties.
3) Evaluate the predictive models' abilities to build optimal management zones using cluster analysis.

The scope of this study was confined to the Coastal Plain of Georgia and South Carolina where soil textures are variable. These ultisol soils represent the majority of the approximately 690,000 hectares of cotton in South Carolina planted in 2018 (USDA, 2018). The nematode species of focus were those identified by Koenning et al. (2004) as the most common nematodes in cotton, those found in all cotton producing states, and those that were present in plot samples. These species were Columbia lance nematode (CLN) and Southern root-knot nematode (SRKN).

Materials and Methods

Zone Map Creation

Soil sample data were collected from five fields in Bamberg and Barnwell Counties in southeastern South Carolina. Trimble Farmworks (Trimble, 2019) was used to divide each field into 60- x 60-m grids (Figure 2-3), with sample locations at the center point of each grid cell. Samples were collected at a depth of 15–20 cm using a soil probe to extract a core diameter of 2 cm. For each grid cell, 10-16 core samples were randomly obtained at various distances surrounding the grid cell center. The core samples for each grid cell were thoroughly combined and divided into two separate samples and used for soil property and nematode analysis. Each bag containing a single, homogeneous, sample was labeled with field location and grid number.

The software program Soil Sampling Utility (Clemson, 2016) was used along with GPS position to guide sample collection at the center of each grid cell. A GPS-enabled tablet PC ran the software in the field to ensure samples were collected at precise locations within a field and labeled correctly.

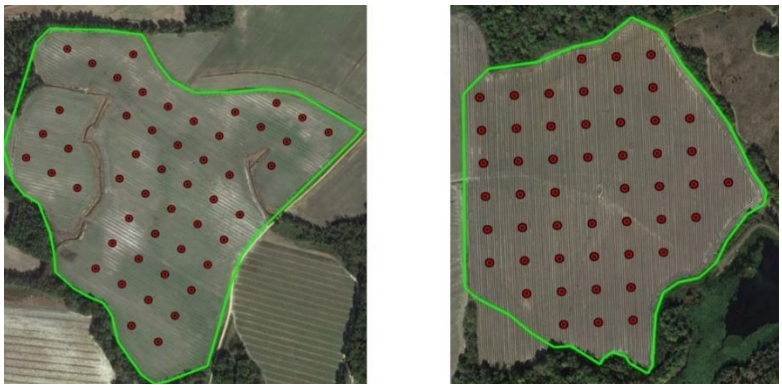

Figure 2-3. Soil sample locations on 60 x 60-m grid pattern.
Points shown are the central location of the defined grid.

Samples were analyzed for soil properties (soil texture and organic matter) at the Edisto Research and Education Center, using the methods and procedures defined by Huluka and Miller (2014). Nematode assays were conducted through the Clemson University Nematode Assay Lab. The result was a data set containing nematode population counts (#/100 cm^3), sand content (%), silt content (%), clay content (%), organic matter (%), and georeferenced field location. Appendix 2 contains the tabulated dataset for all fields, sample timings, and crop years. Soil texture and organic matter samplings for all fields in this study were collected prior to planting; Fields 1 and 2 were sampled in 2018, and Fields 3, 4, and 5 were sampled in 2017.

Nematode sampling for Field 1 was conducted in 2018, 6-8 weeks post planting (mid-season). Field 2 was sampled for nematodes mid-season 2019. In Field 3, nematode samples were collected at 3 different times: mid-season 2018, post-harvest 2018, post-harvest 2019. Fields 4 and 5 were sampled for nematodes post-harvest during 2018 and 2017, respectively.

Based on data generated in Georgia by Holguin et al. (2015) it is unlikely that both CLN and reniform nematode will be present above threshold levels in the same sample, so under the direction of Dr. John Mueller, of Clemson University, the decision to include stubby-root nematode (*Trichodorus* spp.*)* in place of reniform was made (Mueller, 2019).

Using the soil property results of the georeferenced soil samples, contour maps of sand, silt, clay, and OM content were generated for each of the fields. Utilizing the GIS software platform Trimble Farmworks (Trimble, 2019), separate contour maps for each physical soil characteristic using three different range delineations were constructed. The range delineations were one half standard deviation ($\sigma = 0.5$), one standard deviation ($\sigma = 1$), and 3 equal range divisions of each soil characteristic range. The contour maps of the physical soil properties were all generated using the Local Averaging interpolation (Average) method (Bolstad, 2008) with a cell size set to 30 m (representing one half of the sampling grid size) with 100% smoothing and a minimum contour area of 1 acre (Appendix 3).

Contour maps of soil EC were created utilizing the same parameters as the soil properties and were reported in milliSiemens per meter (mS/m). The EC data for the five fields were collected in parallel passes measuring 15 m from center of pass to center of pass using a Veris 3100 soil EC mapping system towed behind a tractor equipped with a GPS receiver with Real Time Kinematic (RTK) corrections. RTK correction ensures horizontal accuracy to within less than 2.5 cm. Data points were collected at a rate of 1 point per second for deep EC, shallow EC, and elevation. Deep EC represents soil EC at depths from 0 to 90.0 cm while shallow EC represents soil EC at depths from 0 to 30.0 cm. Average EC, as defined in this study, was calculated as the average of the shallow and deep EC values at any given position. Contour maps were generated for both deep and shallow EC across all five fields using the same range

parameters as the maps of physical soil properties. These contour maps were also created using the Average method in Trimble Farmworks, and the cell size was set to match the pass spacing at 15 m.

In addition to the contour maps discussed above, each study area was also divided into a 4-section grid (GRID), each grid cell being of similar area. These grids were created by dividing the study area in half by both longitude and latitude. These grids were not created based on any underlying physical soil data.

SSURGO data were downloaded from the Web Soil Survey (United States Department of Agriculture, 2019). Using the Area of Interest tool in the Web Soil Survey, field boundaries were drawn, and SSURGO data for the individual fields were downloaded in ArcView shapefile format. The SSURGO shapefiles were then imported into Trimble Farmworks. A total of 26 different contour, GRID, and SSURGO maps were created for each field. Each map was exported from Trimble Farmworks in shapefile format with attributes specifying associated polygon classifications.

Data composition and transformations

Using Excel (Microsoft, 2019), the data sets were combined, grouped, and separated by study area and sorted by crop year and sample timing. Each nematode species was weighted against its threshold level by dividing the assay count by the published economic threshold to return a weighted population (WP). By definition here, any WP in excess of 1 was in excess of the economic threshold for that species. The economic thresholds used in number of nematodes per 100 cm^3 were: 100 for SRKN, 75 for CLN, and 70 for stubby-root (Mueller, Personal Communication, October 22 2019). Once the WPs were calculated for each species present in each sample, the sum of those WPs was calculated for each sample to determine a weighted

nematode index (WNI) for the sample. By this definition, the WNI treats each nematode species as having an additive effect; for example, if a sample included 50 SRKN and 37.5 CLN per 100 cm^3, the WPs would be 0.5 and 0.5, respectively, and the WNI for the sample would be 1.0. The WNIs for each sample timing and field were averaged to calculate an average weighted nematode index ($\overline{WNI}$). Each WNI was then divided by the respective $\overline{WNI}$ to return a relative weighted nematode index (rWNI) for each sample, which represented the WNI for a given sample as compared to the average for the field and sample timing. For example, an rWNI value of 1 indicates that the WNI is equal to the average WNI, or $\overline{WNI}$, for the field and sample timing; values less than one are proportionately below average and values greater than one are proportionately above average.

To associate the EC data to each sample, Circular Polygon Generator (Clemson, 2018) was used to draw a circular polygon around each data point with a 23-m diameter. The software labeled the generated polygons with the same sample identification as the original data point and saved the output as shapefiles. These shapefiles were then used in Point Polygon Merge Utility (PPMU) (Clemson, 2019) with the shallow EC, deep EC, and elevation point data from each field. PPMU appends a point dataset with selected attributes from a polygon dataset in which each point resides. So, the EC and elevation values residing within each circular polygon were appended with an attribute specifying sample point ID associated with the circular polygon. This data file for each field was then used in JMP 14 (SAS, 2018) to determine the average of each attribute by its polygon location. These averages were then used as the shallow EC, deep EC, and elevation attributes for each data point in the compiled dataset.

Next, using the compiled nematode and soil property dataset, the relative value of each soil property was calculated as:

$$y = x / [\Sigma x / n], \quad (1)$$

where *x* is the soil property value for which a relative value, *y*, is being calculated and the average of all the data points for a given property in the same field is determined by $[\Sigma x / n]$. This relative return of each soil property allowed for a normalized comparison of the data attribute points.

Each absolute and relative associated data point was then transformed using the following functions: reciprocal, square, square root, natural log, log, and exponent. These transformations were added to the data set as additional data attributes for a total of 112 attributes associated with each nematode assay count.

Data Modeling

After compiling the data, approximately 20% of the data points were randomly selected as a testing group. The Random Number Assignment tool in Microsoft Excel (Microsoft, 2019) was used to assign a number between 0 and 1 to each data point. All data points with an assigned value of 0.2 or less were assigned to the testing group, with remaining points assigned to the training group.

Using JMP, stepwise regression models were constructed to predict rWNI using the remaining 80% of the dataset (training group). Stepwise regression models were constructed using the following stopping rules: Bayesian information criterion forward (BIC), Akaike information criterion forward (AICc), and p-Value stopping (Ott & Longnecker, 2016). The BIC and AICc were set to forward only regression. The p-Value was a mixed direction model with probabilities to enter and leave set to 0.25. All models treated the rWNI as the response and the soil characteristic or transformation thereof as the construct model effects. Prior to finalization of each regression model, a regression outlier check was performed using Cook's D Influence (Ott

& Longnecker, 2016). Points returning a Cook's D value ≥ 1 were excluded from the consideration for that regression model (Ott & Longnecker, 2016).

When the models were computed, a collinearity check was conducted on any significant ($p < .05$) factors. To perform a collinearity check using JMP, the Variance Inflation Factor (VIF) was examined for each model (Ott & Longnecker, 2016). For this study, VIF = 5 was used as the collinearity threshold. If any model returned a VIF for any factor above 5, that factor was removed from the model and the model was recomputed. For models with multiple VIFs greater than 5, the highest VIF factor was removed first, the model recomputed, and the process repeated until all VIFs returned were less than 5.

This regression analysis was repeated using only the EC data and associated transformations. This was conducted to determine if, in the absence of physical soil texture data, sensor-based EC data could be used to validate soil sampling zones. The VIF constraints were not applied to models constructed only from the EC data and transformations because the data set inherently exhibited collinearity.

Using JMP, a predicted rWNI was calculated for each of the data points in the testing group using Models 1 through 4 (Equations 2 through 5). The absolute error was determined for each model prediction in the testing group, as compared to the actual rWNI. For comparison of model accuracy, the data were subjected to a one-way analysis of variance (ANOVA) using the model type as the factor and absolute error as the response.

Cluster Analysis

Using Excel, the overall data set was separated into seven groups, each group representing a single sampling instance for each field (three groups for Field 3 and one group for

each of the other four fields). Using each zone map, PPMU was used to append these datasets with the zone classification from each of the maps (contour, GRID, and SSURGO soil type), a separate attribute being appended for each map. For example, the separated dataset for Field 3 in 2017 mid-season was appended with the zone ID in which each sample resided for each of the 25 contour maps and 1 GRID map. This process was repeated for all seven sampling instances.

Once the zone classifications were appended to all data points, the data sheets were sorted by the zone classification value and the Davies-Bouldin Index (DBI) for cluster analysis was used to determine the appropriateness of the clustering by each map. Each zone classification value within the classifications for a given map was used to define each cluster and the rWNI within those classification values were the cluster values used in calculating the DBI. This process was repeated using the rWNI values as data points that were clustered into zones.

The DBI score is a measure that is used to infer the appropriateness of data partitions. It can assist in comparing relative appropriateness of various divisions of a dataset and does not depend on either the number of clusters being analyzed nor the method of defining the clusters (Davies & Bouldin, 1979). The DBI score is defined as a function of the ratio of the sum of within cluster scatter to between cluster separation. The scatter within each cluster was calculated as follows (Davies & Bouldin, 1979):

$$S_i = \sqrt{\frac{\sum_{j=0}^{n}(X_j - A_i)^2}{n}}, \qquad (2)$$

where, S_i represents the within cluster scatter of the i th cluster, n represents the number of values in the cluster, X_j represents the j th value in the cluster, and A_i represents the centroid of cluster i. The Minkowski metric of the centroids for each pair of clusters was then calculated as (Davies & Bouldin, 1979):

$$M_{ij} = \left|A_i - A_j\right|, \tag{3}$$

where, M_{ij} represents the between cluster separation, or the distance between cluster *i* and *j* centroids. The cluster similarity measure, R_{ij}, which represents the ratio of the sum of the within cluster scatter to the between cluster separation for each pair of clusters was calculated as:

$$R_{ij} = \frac{S_i + S_j}{M_{ij}}, \tag{4}$$

Finally, the DBI is calculated using the following formula:

$$DBI = \bar{R} = \frac{1}{N}\sum_{i=0}^{N} R_i, \tag{5}$$

where: *DBI* and $\bar{R}$ represent the cluster measure or Davies-Bouldin Index, *N* represents the number of clusters, and R_i represents the maximum of all R_{ij} such that $i \neq j$. Essentially *DBI* represents the average of the maximum R_{ij} for each cluster. Calculation of *DBI* using the equations shown above was completed using Davies Bouldin Index Calculator (Clemson University, 2019).

Once the *DBI* were compiled for each method of zone contour definition, ANOVAs were conducted to individually compare the components of each zone contouring method. ANOVAs for the classification basis, separation ranges, number of clusters, field number, and sample timing were conducted. An ANOVA of the full system was also conducted in addition to the individual component ANOVAs. This individual component analysis as well as the full system analysis helps to validate the full system analysis as well help to identify areas of the full system in which this process may not apply.

Results and Discussion

No populations of reniform nematode were identified in any of our plot samples, thus, reniform nematode was not included in the models and analyses presented here. For the data set containing both the soil texture and the EC data, stepwise regression models using the AICc and the p-Value stopping rules returned significant models ($p < 0.05$). The AICc stopping rule, Model 1 (Equation 6), was defined by:

$$rWNI = 2.871 - \frac{0.766}{rOM} - 0.058 \cdot e^{OM} - \frac{0.357}{rSEC} - 0.112 \cdot rAEC^2, \quad (6)$$

where: *rOM* represents relative organic matter (unitless), *OM* represents organic matter (%), *rSEC* represents relative shallow EC (unitless), and rAEC represents relative average EC (unitless). The p-Value stopping rule, Model 2 (Equation 7), was defined by:

$$rWNI = 1.036 - 4.794 \cdot 10^{-42} \cdot e^{Sd} - \frac{0.611}{rOM} - 0.058 \cdot e^{OM} + \frac{0.655}{SEC} - \frac{0.850}{rSEC} - 0.106 \cdot rAEC^2 + \frac{133.99}{EL}, \quad (7)$$

where: Sd represents sand content, *SEC* represents shallow EC (mS/m), *EL* represents elevation (m), and other terms have been previously defined.

For the models constructed using only the EC data, the AICc and the p-Value stopping rules again, both returned significant models. The model constructed using the AICc stopping rule, Model 3 (Equation 8), for the EC data set, was defined as:

$$rWNI = 0.948 - 1.480 \cdot SEC + \frac{3.89}{SEC} + 5.404 \cdot ln(SEC) - \frac{0.515}{rDEC} - 1.160\, AEC^2 + 2.75 \cdot log10(AEC) + 0.014 \cdot e^{AEC}, \quad (8)$$

where: *rDEC* represents relative deep EC (unitless), *AEC* represents average EC (mS/m), and all other terms have been previously defined. The p-Value stopping rule, Model 4 (Equation 9), for the EC data set, was defined by:

$$rWNI = 2.453 + \frac{1.474}{SEC} - \frac{1.125}{rSEC} + 1.662 \cdot log\,10(rDEC) - 2.175 \cdot rAEC + 2.822 \cdot log10(\,AEC) + \,.061 \cdot e^{rAEC}, \quad (9)$$

where: *rAEC* represents relative average EC (unitless) and all other variables have been previously defined.

Table 2-1 shows the ANOVA results of the derived models using the testing group. Absolute error in rWNI prediction was calculated from application of the models to the testing group, where the absolute error for any given sample in the testing group was calculated as the absolute value of the difference in model-predicted rWNI and actual rWNI. No significant differences were found between the mean absolute errors for the four models.

Table 2-1. Regression statistics of predicted rWNI separated by model type and reported in descending order of R^2

Model Name	R^2 [e]	N [e]	p-Value [e]	Mean Abs. Err. [f]	Connecting Letter [f]
Model 3[c]	0.080	294	0.0075	1.348	A
Model 2[b]	0.059	290	0.0156	1.303	A
Model 4[d]	0.057	294	0.0092	1.310	A
Model 1[a]	0.044	290	0.0124	1.271	A

[a] R^2, N, and p-Value calculated using the training data set
[b] Mean Abs. Error and Connecting letter report calculated using the test data set
[c] Equation 8: rWNI prediction model using soil EC data only with the AICc stopping rule
[d] Equation 7: rWNI prediction model using physical soil properties and soil EC data using the p-Value stopping rule
[e] Equation 9: rWNI prediction model using soil EC data only with the p-Value stopping rule
[f] Equation 6: rWNI prediction model using physical soil properties and soil EC data with the AICc stopping rule

The models that returned the highest R^2 value for both the full data set, Model 2 (Equation 7), and the EC only data set, Model 3 (Equation 8), were used to calculate the predicted

rWNI for the overall dataset. These predictions were used as a data layer to generate three additional contour maps per model to be used in the cluster analysis. The methodology for creation of these contour maps was the same as described for the physical soil properties.

Sample timing was also evaluated to determine if any differences in prediction accuracy were related to the stage in the growing season at which the samples were collected. For the at-harvest samples, the mean absolute error trends downward as compared to the mid-season samples (Table 2-2). In an ANOVA comparison, a significant difference ($p<.001$) in mean absolute error was observed. The mid-season observations (n=124) returned a mean absolute error of 1.74 compared to a mean absolute error of 1.08 for the at-harvest sample timing observations (n=240). This could be attributed to the larger number of at-harvest samples, which may have improved at-harvest model predictions.

Table 2-2. Comparison of mean absolute errors (Mean Abs. Err.) by sample collection timing.

Model Name	Mid- Season n	Mid-Season Mean Abs. Err.	At-Harvest n	At-Harvest Mean Abs. Err.
Model 1[a]	31	1.70906	60	1.045
Model 2[b]	31	1.74084	60	1.077
Model 3[c]	31	1.74552	60	1.142
Model 4[d]	31	1.76976	60	1.072

[a] Equation 6: rWNI prediction model using physical soil properties and soil EC data with the AICc stopping rule
[b] Equation 7: rWNI prediction model using physical soil properties and soil EC data with the p-Value stopping rule
[c] Equation 8: rWNI prediction model using soil EC data only with the AICc stopping rule
[d] Equation 9: rWNI prediction model using soil EC data only with the p-Value stopping rule

A non-normal distribution was observed in the *DBI* values; they were normalized using a Box Cox transformation with lambda = -0.3. The transformed *DBI* values *(tDBI)* were used in subsequent ANOVA comparisons. Individual ANOVAs were conducted using *tDBI* as the

response and separation range, classification basis, number of clusters, field number, sample timing, and a combination of classification and separation range as the factors.

As discussed, *DBI* reflects the appropriateness of each partitioning method (zone classification methodology) relative to clustering rWNI. *DBI* was calculated for each zone methodology at each sampling instance, with a smaller *DBI* indicating a more appropriate data cluster (Pakhira, et al., 2004). Because there were seven sampling instances across the five fields, this provided seven *DBI* values for each zone classification methodology. A complete list of transformed and untransformed *DBI* values for this study can be found in Appendix 4. Using JMP, Student's t-tests were conducted to indicate significant differences between factors (α=0.05). Tables 2-3 through 2-7 show the ANOVA results for each component and the full system comparison.

The data presented in Table 2-3 show that a separation range of $\sigma = 1$ clusters the rWNI significantly better than $\sigma = 0.5$. When seeking to maximize population differences between zones and minimize differences within zones, larger zones may be more appropriate for zone management of nematodes based on the data considered here. A separation range of $\sigma = 1$ generally results in three zones for a field and a separation range of $\sigma = 0.5$ generally results in five zones for a field. Maps based on SSURGO classifications returned the least numerically appropriate range separation.

Table 2-3. Average DBI values as a function of separation range delineations. T-tests were conducted on tDBI using α = 0.05. Factors not connected by the same letter are significantly different.

Separation Range	DBI	T-test, α =0.05
σ= 1	11.6103	B
Equal Ranges x3	12.3742	AB
σ= 0.5	18.1941	A
Grid	21.7798	AB
SSURGO	27.1554	AB

When mean tDBI scores were compared across each individual zone classification basis (sand, silt, clay, EC, etc.), zone contours based off wRNI predictions from Model 3 (Equation 8) returned the most appropriate clustering ($p < 0.5$) basis (Table 2-4); with the model construction based on soil EC properties only, without physical soil properties, tDBI averages returned were significantly lower than any other classification basis. Like the separation range ANOVA, a classification based on SSURGO data returned the one of least numerically appropriate clustering bases.

Table 2-4. ANOVA calculation of tDBI values as a function of soil classification basis' (α=.05). Factors not connected by the same letter are significantly different.

Classification Basis	DBI	T-test, α =0.05
Model 3 (Equation 8)	6.5034	C
SH EC	12.3317	B
DP EC	12.7538	B
Soil OM	14.0169	AB
Clay	15.1001	AB
Sand	15.1774	AB
Model 1 (Equation 6)	16.2493	AB
Grid	21.7798	AB
SSURGO	27.1554	AB
Silt	27.3862	A

Not all fields were equally suited for zone management of nematodes (Table 2-5). The data considered here, or any of the soil properties examined in this study, did not suggest a reason as to this lack of suitability. Soil properties are not the only influence on nematode presence and population densities. Agronomic production practices and environmental conditions must be considered. With the fields under investigation in this study being in different counties (growing environments) and non-identical agronomic practices across each field, there were many unknown variables outside of soil properties.

Table 2-5. ANOVA calculation of tDBI values as a function of field number. Factors not connected by the same letter are significantly different.

Field Number	DBI	T-test, α =0.05
4	9.36201	C
2	10.5423	BC
5	13.7921	ABC
3	17.1272	A
1	18.0129	AB

The separation range and soil classifications were examined together as a system (Table 2-6), to look at specific methods of zone construction inclusive of basis and separation range. A contour zone based on predictions of the rWNI using only soil EC data, at a separation range of $\sigma = 1$, returned the most appropriate zone definition. Relative to basis for zone development, three of the nine methods in the best grouping used Model 3 (Equation 8), and two of the nine methods used Deep EC. Relative to range separation, five of the nine methods in the best grouping used $\sigma = 1$. The data presented in Table 2-6 supported the findings shown in Tables 2-3 and 2-4 in that larger zones tended to cluster nematode populations more appropriately along with using the contours based on the rWNI predictions from Model 3 (Equation 8). Management

zones based on SSURGO classifications along with silt returned the least appropriate zone classifications and definitions (numerically).

Table 2-6. ANOVA calculation of tDBI values as a function of zone classification + separation range. T-tests were conducted using $\alpha = 0.05$ and $\alpha = 0.1$. Factors not connected by the same letter are significantly different.

Classification + Separation Range	DBI	T-test, α =0.05
Model 3 (Equation 8) + σ= 1	5.0860	F
Model 3 (Equation 8) + σ= 0.5	7.0045	EF
SH EC + Equal Ranges x3	7.8349	DEF
Model 3 (Equation 8) Equal Ranges x3	7.8394	DEF
DP EC + Equal Ranges x3	8.8193	CDEF
Clay + σ= 1	9.6099	BCDEF
Soil OM + σ= 1	10.0996	ABCDEF
Model 2 (Equation 7) + σ= 1	11.9028	ABCDEF
DP EC + σ= 1	12.4416	ABCDEF
Sand + Equal Ranges x3	12.8495	ABCDE
Soil OM + Equal Ranges x3	13.2715	ABCDE
SH EC + σ= 1	14.5065	ABCDE
Clay + Equal Ranges x3	15.2787	ABCDE
Model 2 (Equation 7) + Equal Ranges x3	15.9869	ABCDE
Sand + σ= 1	16.3038	ABCDE
Sand + σ= 0.5	16.7977	ABCDE
SH EC + σ= 0.5	17.4048	ABCDE
DP EC + σ= 0.5	19.8647	ABCD
Soil OM + σ= 0.5	21.4515	ABCD
Grid + Grid	21.7798	ABCD
Model 2 (Equation 7) + σ= 0.5	23.3261	ABC
Silt + σ= 1	23.6892	ABC
Clay + σ= 0.5	25.1257	ABC
SSURGO + SSURGO	27.1554	ABC
Silt + σ= 0.5	29.0399	AB
Silt + Equal Ranges x3	30.0040	A

Conclusions

For field zones to successfully direct sampling or VRA treatment efforts for nematodes considered detrimental to cotton, zones must be defined in a way that maximizes the variability of the nematode densities across zones while minimizing the variability within each zone. By using

cluster analysis, significant differences in zone definition methods were able to be determined in this study. Larger zones with a separation range of $\sigma = 1$ with contours based on the predicted rWNI from soil EC data only, as shown in Model 3 (Equation 8), tended to cluster nematode populations most appropriately.

Although the best zone development methods are suggested here, zone management for nematode control is not equally suited across all fields and conditions. Significant differences were found in tDBI across field locations. Further investigation into environmental and agronomic differences and their impacts to nematodes across fields is needed to determine when fields are best suited for zone management for nematode control.

Soil EC was described as an effective predictor of nematode populations previously (Mueller, et al., 2010). This study supports those findings and further demonstrates that EC can be useful for developing contour zone maps for effective nematode sampling and VRA of nematicides. This study also found that zone definitions based on the rWNI prediction, as a function of EC, (Model 3 - Equation 8), clustered nematode densities more appropriately than EC alone or the rWNI prediction, as functions of EC and physical soil properties, (Model 2 - Equation 7).

The differences in the strength of the model's basis of construction (all soil properties or EC only) were not significant, but significant differences were observed between the models' ability to appropriately cluster nematode populations. Model 2 (Equation 7) was built with EC factors as well as soil OM and elevation. When these factors were added, the clustering ability of Model 2 (Equation 7) was reduced. The correlation of these additional factors would warrant further investigation.

The ability of soil EC and the predicted rWNI to most appropriately cluster the rWNI may be economically beneficial to producers wanting to employ zone management for nematode control. Soil EC is relatively inexpensive to collect and often faster to generate than physical soil sampling. A single operator can cover in excess of 60 hectares in a single day. Mobile sensor platforms allow for many hectares to be mapped quickly with typically fast data turn-around times, e.g., 24 hours. Soil sampling for physical soil properties analysis is a comparatively slower, more labor intensive, and more costly process. Pre-sample mapping is needed to generate the sample position, more labor and supplies are needed to collect samples, and laboratory turn-around times usually require 7 to 10+ days.

In addition to the economic advantages, soil EC readings are taken at a much higher density than traditional soil sampling. With a higher volume and continuous nature of data collection, EC data are more effective at capturing soil variability with a greater level of precision as compared to physical soil sampling. This allows for more separations within the data to be observed and more precise contours to be generated.

For practical applications of these findings by growers, historical field knowledge should be considered when defining zones for nematode management. If nematodes are known to be a problem, application of these findings in conjunction with VRA of nematicides may result in reduced costs for nematode control. If a field is known to have relatively homogeneous nematode populations, cost savings may not be realized using these methods. Also, it was shown in this study that zone management for nematode control is not equally suited for all fields. While soil EC and models to predict rWNI as a function of soil EC were shown to be the optimal for classifying zones of nematode management, these results may vary in different conditions; further investigation is needed to determine the viability of the methods presented here across a wider

range of soil conditions and nematode populations. Capture of environmental factors may increase the explanation of these findings and provide more insight as to which field and environmental conditions must be met in order for these methods to be implemented successfully. Generalization of these results to nematode species other than SRKN, CLN, and stubby-root are not supported by this study.

Multi-Hybrid Planting Technology for Management of Plant-Parasitic Nematode Management in *Gossypium hirsutum*

Introduction

Since its inception, the overall goal of precision agriculture has been to increase economic returns or reduce environmental impacts by placing inputs where and/or when they are needed at the rates needed to gain the maximum benefit. When input products are precisely placed where they are needed in the required quantities, the use efficiency of the product is increased. Koch, et al. (2004) demonstrated the capability of VRA to provide positive economic advantages.

Multi-hybrid planting (MHP) technology is a category of VRA that utilizes defined management zones for seed planters to seamlessly change between two varieties without interruption of the planting process (Jeschke & Shanahan, 2015). One potential area of application for MHP is minimizing yield losses due to southern root-knot nematode (SRKN). Recent introduction of cotton varieties that are genetically resistant to SRKN are typically priced higher than susceptible varieties. Multi-hybrid planting can place these resistant varieties where needed and place higher yielding susceptible varieties elsewhere in a field.

MHP technology is not just limited to different seed genetics. Multiple species of nematodes may be present in each field along with SRKN. Nematode species such as reniform and Columbia lance can have negative effects on cotton yields when population densities are above damage thresholds (Ferris, 1978; Clemson Extension Service, 2000). The only commercially available genetic resistance, in any cotton variety to date, is for SRKN. MHP of a resistant variety with a susceptible variety can be combined with VRA of an in-furrow nematicide. This MHP technology can offer growers multiple options in nematode control.

The following study outlines and describes one method for creating a MHP prescription for minimizing yield losses due to southern root-knot nematode, using a cotton variety resistant to SRKN compared with a commercially available, high yield potential variety that is susceptible to SRKN. The overall goal of this study was to define and demonstrate a methodology that can enable cotton producers to utilize commercially available systems and equipment to assist in seed placement prescription for MHP in fields where SRKN distributions may not be known.

The field selected for this study was in a two-year cotton and one-year peanut rotation for more than 10 years. This rotation is representative of typical crop rotation practices across central and southern Georgia. Crop history is a key factor in nematode population densities and crop rotation, using host plant resistance can be an effective control measure for SRKN (Hague & Overstreet, 2002). Peanuts are typically considered a non-host plant for SRKN, potentially resulting in a negative effect on overall SRKN population densities, especially for the crop season following the peanut crop.

Materials and Methods

Study Area Classification

A field with an unknown distribution of SRKN was selected that was located approximately 10 km southeast of Hawkinsville, Georgia, in Pulaski County, for a two-year study. Year one, two cotton varieties were planted in alternating strips across a field for determination of highest net profit in a defined management zone. In year two, an MHP

prescription was created and executed for each defined management zone based on the highest profiting seed variety in year one.

Irrigation was supplied to the study area by an overhead center pivot irrigation system. Irrigation applications were made to supplement natural rainfall to maintain a minimum of 1.25 cm of water per week. Irrigation applications were terminated when the cotton reached an open boll percentage of 60%.

In year one, the first year that cotton was planted after peanuts, the entire field was soil sampled on a grid pattern. Using Trimble Farmworks, the field was divided into grids measuring 60 x 60 m. Using the central point of each grid, soil sample cores were collected at a depth of 15 to 20 cm using a soil probe to extract a core diameter of 2.54 cm. Multiple sample cores were collected within a 23-m radius around each central grid point. The cores were then combined to create a homogenous composite sample for that grid. The collected samples were analyzed at the Clemson University Edisto Research and Education Center for soil texture: percent content of sand, silt, and clay. Texture determination was made using the particle size determination methods and procedures defined by Huluka and Miller (2014).

Georeferenced soil texture data were used to create a soil texture contour map of the study area using Trimble Farmworks. The contour map was divided into 3 zones based on total

sand, silt, and clay content percentages. This 3-division zone map covered the high, medium, and low sand content zones and served as the basis for nematode sampling regions (Figure 3-1).

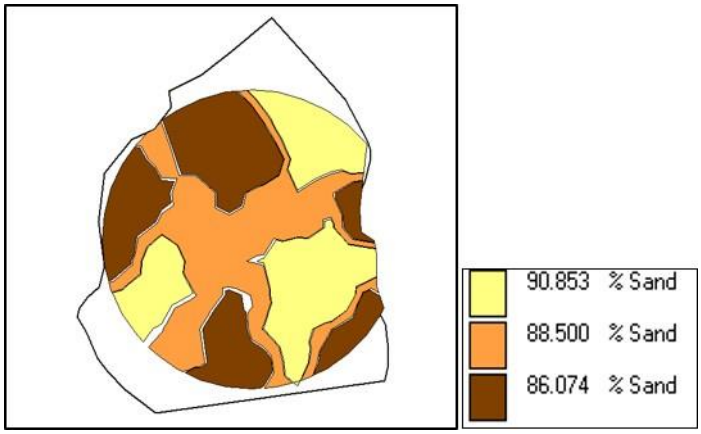

Figure 3-1. Equal range division of sand content (%) of the irrigated portion of the study area. These ranges cover the high, medium, and low sand content zones of this field and were determined by soil sample analysis.

In addition to dividing the study area into zones based on sand content, the field was divided into similar area grids of 0.2-hectares (GRIDS). These grids were equal sized squares and not based on any georeferenced data layers. The hypothesis behind using GRIDS, was that using a comparatively smaller area might enhance the ability of spatial yield response to seed variety to determine the presence of SRKN. Population densities of SRKN can be highly variable and spatially aggregated within fields (Barker & Olthof, 1976; Starr, et al., 1993; Wrather, et al., 2002; Wyse-Pester, et al., 2002; Monfort, et al., 2007). In larger zones, these potential problem areas may be masked by higher yields from lack of SRKN presence in the majority of the zone. For this reason, a smaller area concentration may allow a higher probability identifying areas with SRKN present.

Maps of the GRIDS and sand contour zones were used to create individual ESRI shapefiles in Trimble Farmworks that were exported for use in Point Polygon Merge Utility

(PPMU) (Clemson University, 2016). These shapefiles served as the classification datasets for the end of season yield data analysis. Using these classification zones, yield projections were created to assess if a MHP prescription could have been applied to increase net profit returns.

Planting

In year one of this study, the trial was planted as an alternating strip test. Two different varieties of cotton were planted across the study area in 12-row plots with a row spacing of 0.97 m using a 12-row commercial planter. The planter was split evenly with each seed variety. Planter rows 1-6 were filled with the SRKN-resistant variety, and rows 7-12 were filled with a SRKN-susceptible variety (Figure 3-2). A serpentine planting pattern resulted in 12-row strips of each variety filling the study area (Figure 3-3).

Figure 3-2. Commercial planter used to plant alternating 12-row strips across the entire study area in year one. Planter rows 1-6 were filled with DP 1558 and rows 7-12 were filled with DP 1555. Planting was done in sequential passes to acheive 12-row (15m) wide plots.

Deltapine (Bayer Crop Science, St. Louis MO) varieties 1555 B2RF (DP 1555) and 1558 B2RF NR (DP 1558) were selected in year one. DP 1555 was a common high yield potential variety that was popular across the region. DP 1558 was a complimentary variety in terms of relative maturity and geographic adaptation. The only differentiating factor in this trial was the

two varieties. Agronomic management of the trial was consistent across both varieties and conducted by the cooperating grower.

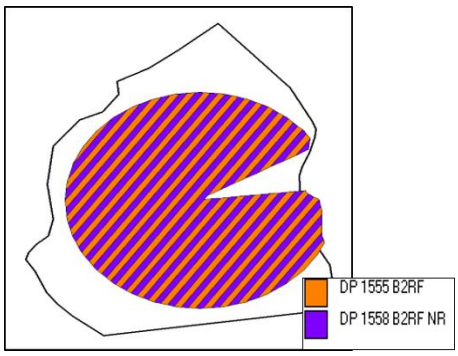

Figure 3-3. Alternating strip pattern of DP 1555 and DP 1558 cotton varieties in year one. The Study area focused on the irrigated portion of field only in an effort to control environmental variability.

In year two, the cotton market underwent a shift in herbicide technologies. With this technology shift, seed for DP1555 was not available in year two. For this reason, the varieties investigated were changed to Deltapine varieties 1646 B2XF (DP 1646) and 1747 NR B2XF (DP 1747). The SRKN-susceptible variety, DP1646, was selected due to its high yield potential and popularity with growers across the region. The SRKN-resistant variety, DP 1747, was selected to compliment DP 1646 in relative maturity and geographic fit. Again, as in year one, a comparison of a variety with a genetic resistance to SRKN to a variety that is susceptible to SRKN was the overall goal.

Planting in year two was done with a 6-row planter equipped with Precision Planting vSet Select seed meters and a Seed Sense 20/20 control display (Precision Planting, Tremont, IL). The

vSet Select planting system is a commercially available system from Precision Planting that enables multi-hybrid planting capabilities.

Harvest

In year one, harvest was conducted using commercial yield monitoring systems on separate John Deere (John Deere Manufacturing Company, Moline, IL) round baling cotton pickers. In the first year, a John Deere 7760 and a John Deere CP690 were used. Both cotton pickers were equipped with John Deere Green Star 3 2630 yield monitoring (cotton mass flow) systems. Each variety was harvested by a single picker allowing for accurate yield monitor calibration for each cotton picker to a single variety. The John Deere CP690 was used to harvest DP 1555, and the John Deere 7760 was used to harvest DP 1558.

During harvest, when a round bale (roll) was ejected from a picker, it was immediately labeled with its corresponding variety. Rolls were grouped and separated by variety, and this separation was maintained throughout the ginning process to obtain variety specific lint percentages (LP). Each variety was ginned separately, and, once ginning was complete, a LP of 0.423 was returned for DP 1555 and an LP of 0.41 was returned for DP 1558.

To determine an accurate lint yield, each "Ctn Ms Yld" datum point, as reported by the yield monitor, was multiplied by the average LP returned by the gin. This LP was a combination of both varieties, as it was not possible to keep varieties separated in year two due to variety changes throughout a strip. Once the LP was applied to the "Ctn Ms Yld," a corrected lint yield (kg/ha) was defined for each datum point. Using the same process as in year one, the corrected lint yield was used to determine the RASC ($/ha) for each treatment.

In the second year, a single John Deere CP690, with a Green Star 3 2630 spatial yield monitoring system was used. Yield monitor calibration was completed prior to the harvest of this field using the same varieties that were in the study. Variety separation was not possible for calibration due to the MHP planting prescription implemented.

Statistical Analysis of Yield Data

Year One

To create an MHP map, the first step was to classify each yield data point as a function of the GRIDS and sand content zones. After harvest, spatial yield data were downloaded from each cotton picker. Each separate yield file was uploaded into Trimble Farmworks. Once in Trimble Farmworks, separated yield data were exported in a common separated values (CSV) spreadsheet format. In each yield data spreadsheet from year one, a "Variety" column was manually appended, and the respective variety was added to each data row in the "Variety" column. Once the variety was added to each yield sheet, PPMU was used to append each yield data sheet with the appropriate GRIDS and sand zone within which each yield data point resided.

Prior to any statistical analysis, yield data were first normalized through JMP. Normalization was applied due to the unequal number of yield points in each GRIDS or sand content zone. To normalize both appended yield data sets from year one, a Box-Cox Univariate transformation was applied to the "Ctn Ms Yld" data attribute for each variety. When the transformation was applied, the Optimum Power was determined to be *lambda* (λ) = 1.5. Transformed yield data were appended to the data set and reported in a new data column.

Once each YLD data point was associated with its GRIDS and sand zone location, outlier YLD points were identified and excluded from the data set using Tukey's rule. Specifically, a

datum point was considered to be an outlier if it was more than 1.5 times the interquartile range from the quartiles. Once outliers were excluded, mean yields for each variety were calculated for each GRIDS and sand zone.

For each variety, seed costs per hectare were determined by dividing the cost per bag of seed by the number of thousand seeds (ksd) per bag (250 ksd bag^{-1}). This quotient was then multiplied by the number of ksd planted per hectare (17 ksd/ha) to give the seed cost per hectare. Next, gross receipts (excluding any discounts or premiums) in $/ha were calculated for each sand zone and GRIDS. This was done by multiplying the LP by the mean yield (kg/ha) for each GRIDS and sand content zone and then multiplying this product by the market price. The market price at time of harvest in year one was $1.60 per kg of lint. Returns above seed costs (RASC) were determined by subtracting the seed costs from the gross receipts ($/ha). For each sand zone and GRIDS, the seed variety that demonstrated the highest RASC dictated the seed variety prescribed for that zone or GRIDS in an MHP application. The methodology discussed here for prescription plan development is also known as Directed Prescriptions or Directed R_X (Barnes & Kirk, 2017).

Year Two

In the second year, two treatments were added based on the results of the profit analysis in year one. The following four treatments were examined in year two: 1) Sand zones MHP based on year one RASC, 2) GRIDS MHP based on year one RASC, 3) uniform planting of DP 1646, and 4) uniform planting of DP 1747. These treatments were planted as strips in a randomized complete block design with 11 total replications. Net profits were calculated using the same methods from year one.

Figure 3-4. Year two MHP application map. Treatments (4) were planted in a randomized complete block design with 11 replications across the irrigated portion of the field. Year two planted varieties were DP 1646 B2XF and DP 1747 NR B2XF.

Results and Discussion

Year One

In year one when the yield results from both DP 1555 and DP 1558 were compared against the sand zone content, lower yield trends were observed in the DP 1558 variety (Figure 3-5). The only sand content zone that showed a yield advantage utilizing DP 1558 was the lowest sand content zone of 82.64% sand, constituting 1.2 ha (5%) of the test area. If an MHP were conducted based on sand content zones, DP 1558 showed an average increase of $37 ha in the lowest sand content zone over DP 1555. This would equate to an overall profit gain of approximately $93.23 total for the irrigated portion of this field, ($4.49/ha).

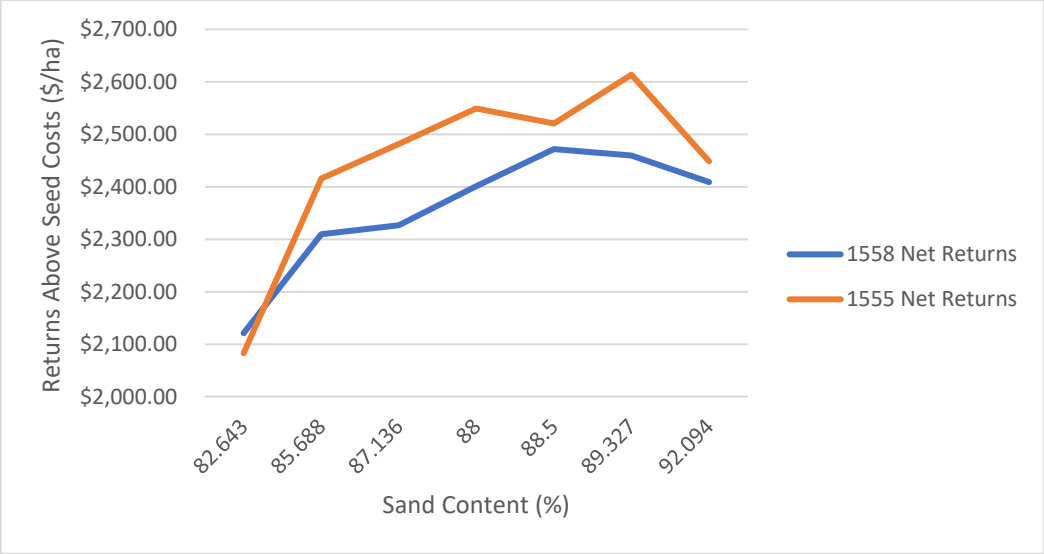

Figure 3-5. Returns above seed costs (RASC) of each variety as a function of sand content zone in year one.

The same analysis process was conducted with the GRIDS grid pattern. These results returned more yield and net profit separations. A total of 32 GRIDS, totaling 6.2 hectares, demonstrated a net profit advantage using the SRKN resistant variety (Appendix 5). If the results of the GRIDS field division were to be implemented in an MHP application using DP 1555 and DP 1558, it would return a potential RASC of $2,609/ha. When compared to a uniform planting of DP 1555, a potential $135/ha increase in RASC could be achieved (Appendix 6). When compared to a uniform planting of DP 1558, a potential increase of $232/ha could be achieved (Appendix 6).

These RASC increases are based on a single season of observed yield results and conditions for this field. Not all grid areas were used in these calculations. Any grid that did not contain yield data from both varieties was excluded from the analysis. To control environmental variability, any area outside of the center pivot irrigation system was also excluded from the study area.

Year Two

In year two, Hurricane Irma passed through the region 6 weeks prior to harvest. The cotton at this point was nearing maturity with bolls nearing 60% open. This storm impacted the study area with wind speeds in excess of 33 m/s, resulting in severe plant lodging and storm fallout of lint across the entire field that impacted harvest. The extent of yield loss could not be measured, but harvest of the trial was conducted.

An ANOVA comparison of results from year two showed that a uniform planting of DP 1646 returned a significantly higher *($p<.05$)* RASC as compared with all other treatments (Table 3-1). Neither MHP planting prescription based on year one data returned higher RASC's than a uniform planting of DP 1646. In all sand content zones, DP 1646 outperformed DP 1747 (Table 3-2). In the GRIDS MHP treatment, DP 1646 returned a higher RASC in all but 4 GRIDS (Appendix 7), which was 5% of the total number of GRIDS considered. Due to the additional two treatments, total strip widths, and width of each GRIDS, both varieties were not planted in all GRIDS. Any GRIDS not planted with both seed varieties were excluded from analysis. Of the four treatments, the strips with a uniform planting of DP 1747 returned the lowest RASC.

Both MHP prescriptions applied in year two resulted in a lower RASC when compared to DP 1646 but a higher RASC when compared to DP 1747 (Table 3-1). The MHP prescription created from RASC data from year one for the GRIDS resulted in a decrease in RASC of $91.18/ha, as compared to a uniform planting of DP 1646, but an increase in RASC of $242.57/ha as compared to a uniform planting of DP 1747. The MHP prescription based on sand content (%) divisions resulted in a $56/ha loss in RASC as compared to a uniform field planting of DP 1646 but a $278/ha gain when compared to a uniform planting of DP 1747.

Table 3-1. Return above seed costs results of all planted treatments in year two. Treatments not connected by the same letter are significantly different.

Treatment	RASC ($/ha)	T-test (α=.05)
Uniform Planting of DP 1646	$ 2,915.41	A
MHP based on Sand (%) content	$ 2,859.51	B
MHP Based on GRIDS	$ 2,824.23	B
Uniform planting of DP 1747	$ 2,581.66	C

Table 3-2. Returns over seed costs (RASC) results for sand content (%) zone for each variety in year two.

	DP 1646	DP 1747
Sand Content (%) Zone	**Mean RASC ($/ha)**	**Mean RASC ($/ha)**
82.643	$ 1,663.69	$ 1,256.48
85.688	$ 1,880.42	$ 1,470.88
87.136	$ 2,041.53	$ 1,526.50
88	$ 2,019.24	$ 1,531.33
88.5	$ 2,037.30	$ 1,572.37
89.327	$ 2,264.83	$ 1,654.52
92.094	$ 2,182.47	$ 1,774.06
Averages	$ 2,012.78	$ 1,540.88

In addition to the MHP prescriptions developed from year one data, two MHP projections were constructed based on year two data to suggest the value of MHP. As previously stated, DP 1747 did not return a higher RASC in any of the seven sand content (%) divisions. So, an MHP prescription developed for sand content (%) zones, based on results demonstrated in year two, would have prescribed a uniform planting of DP 1646. However, an MHP prescription for the GRIDS based on RASC returns for year two would have out-profited a uniform planting of DP 1646, resulting in a projected increase in RASC of $10/ha (Appendix 7). This demonstrates that

there were areas of this field in which DP 1747 outperformed DP 1646, with regards to RASC, that are not identified when using sand content as a basis of zone definition.

The original intent of this study was to define a methodology for identifying SRKN densities within a field, but this methodology was neither validated nor nullified due to lack of SRKN densities. In year one, no SRKN were detected in soil or in roots; no galling was observed. Sampling in year two was inconclusive due to extreme heat conditions and delays in shipping of nematode samples that rendered any assay counts unreliable. The lack of SRKN presence in the samples in year one would tend to predict a lack of SRKN presence above any thresholds levels in year two. While this study does not demonstrate a benefit of MHP to address SRKN pressure, it still provides a meaningful demonstration of how the Directed Prescriptions methodology can be used for development of MHP prescriptions for nematode management.

Yield is influenced by numerous environmental and genetic factors. The main influencing factor for yield in this study was intended to be the response of the SRKN resistant cotton to presence of SRKN. With this determining influence not present, the yield differences between the two varieties could not be attributed to SRKN. Without being able to attribute any differences in yield or RASC to known populations of SRKN no solid conclusion could be made. Further testing and vetting of this methodology in a field with a known history of SRKN should be conducted before commercial implementation.

Conclusions

Utilization of a crop rotation scheme of 2 years of cotton with one year of peanuts by growers is a viable strategy to reduce the incidence of fields with severe SRKN-induced yield losses in Georgia. Although cotton yield levels are still impacted by SRKN presence, crop rotation to include non-host plant types, such as peanuts, have affected overall SRKN levels. The

field in this study was a part of this typical crop rotation, and its lack of SRKN presence could be attributed to this. While fewer fields have severe damage due to SRKN, significant yield losses in fields that are in continuous cotton production are still evident. Across Georgia, these fields are typically non-irrigated fields that are not sampled for nematodes due to perceived costs versus benefits.

For multi-hybrid planting to be a feasible option for cotton producers, it must be able to show a positive return on investment. Given the young age of this technology (<6 years), research in its application has been very limited. To date, published research in multi-hybrid planting has primarily focused in corn, with no published research being conducted in cotton.

As a result of the young age of MHP technology, a lack of any clearly defined methodologies in determining field classifications have been described. Because the foundation of many precision agriculture applications is zone definition, how the zones are defined is critical. Currently, many methods for zone classifications within a field are being used. The best zone classification method for a specific application is an area of precision agriculture that has not been fully addressed.

The data and results presented in this study highlight a potential methodology (Directed Prescriptions) that, after further vetting and investigation, can be implemented by growers interested in MHP technology or VRA applications of nematicides. These tests can be conducted to assess projected outcomes of MHP without the need for a multi-hybrid planter. The classification layers can be collected, zones can be created, and spatial yield data from multiple years can be analyzed, as a function of each classification zone, and a potential or projected net profit return can be calculated as demonstrated in this paper. This can allow growers to decide if a multi-hybrid planter might be profitable in their operation, if more return on investment can be

gained in utilization of VRA of nematicides, or if a combination of the two technologies would be most economical.

Conclusions

Predictive Management Zone Definitions

Previous studies examining distributions of nematodes in varying soil textures reported associations of southern root-knot nematode (SRKN) and Columbia lance nematode (CLN) with soil particle size (Khalilian, et al., 2001; Koenning, et al., 2004; Mueller, et al., 2010; Ortiz, et al., 2010; Holguin, et al., 2015). Sandy loam soils have been shown to induce large and rapid increases in populations of stubby-root nematode that result in severe crop injury (Thomason, 1959). Our investigations showed that management zones created from predicted relative weighted nematode index (rWNI), based on soil EC alone, cluster nematode populations more appropriately than rWNI predictions based on physical soil textures. Our findings support these previous works because soil EC correlates highly to soil texture and particle size (Williams & Hoey, 1987).

Soil EC is been a major focus of study for nematode population distributions. Implements, such as the Veris 3100, allow for a much more economical data generation at a higher density of data points than typical soil texture sampling. The typical cost for soil EC data generation is approximately $30 per ha or less, with data points collected in parallel passes with a center to center spacing of 18 m and recorded in one second intervals (pricing and collection methods for EC mapping obtained through conversations with local retailers offering EC mapping services). Typical travel speed for soil EC data collection is 6-8 km/h. At this travel rate, data points are collected every 1-2 m resulting in approximately 247 to 333 data points per hectare. This high density of sampling points creates a higher definition map of soil texture changes throughout a field compared with composite soil texture samples, for example, collected on a 50 x 50 m (0.25 ha) grid pattern, which would result in four data points per hectare.

In using a very large data set of nematode assay counts and their associated physical soil properties and other geospatial attributes, instructive nematode management zone maps were defined. After calculating the Davies-Bouldin Index (DBI) for the rWNI for all zone creation methods, the most appropriated method was achieved when soil EC was used to predict rWNI and contouring those predictions at a range of $\sigma = 1$. The studies conducted here strive to more accurately direct nematode sampling efforts and more effectively utilize zone management of nematode control measures by categorizing those areas that are at a higher risk potential of nematode damage.

The soil types of fields examined in this study were representative of the Coastal Plain region of Georgia and South Carolina. This region encompasses a large percentage of the cotton production in those states. Application of these findings outside of these high sand soil textures is unsupported by this study.

Previous studies have indicated that SRKN and CLN have a strong correlation to sand content, but no studies have used the Davies-Bouldin index as a measure of management zone adequacy. In use of factorial and indicator kriging, Ortiz et al. (2010) found that SRKN populations increased in more coarsely textured soils. They also concluded that EC data alone may not capture total spatial distribution of SRKN. It was suggested that use of slope or elevation may increase the precision and accuracy of mapping. Our data helps support this hypothesis. Our data show that in calculating the DBI values for each method of zone definition, the prediction model that incorporates elevation, physical soil properties, and soil EC returns a numerically higher DBI value (less appropriate clustering) than that of the zone definition method using rWNI predictions based on soil EC alone. Although these models differ numerically, the only significant differences between the models is the model that is based on soil EC data only at

a range separation of σ =1 and the model based on physical soil properties and soil EC at a range of σ =.05. From an economic perspective, there is no additional benefit or indication of any increased return on investment to using physical soil properties with soil EC data.

Slope was not examined in this study, but it is be hypothesized that calculation of slope between EC points could improve sample clustering accuracy. Soil moisture content, being affected by field slope and soil texture, has been shown to be an important factor in the distribution of nematodes (Herring, et al., 2010; Davis, et al., 2013; Moore & Lawrence, 2013; Petersen, et al., 2013). Slope determination for an area is sometimes calculated from digital elevation models (DEMs) published by the United States Geological Survey (USGS). Like SSURGO maps, USGS DEMs are reported at a large scale in order to encompass a large area in a single map. DEMs are produced corresponding to 7.5 minute quadrangles, and complete coverage of the United States is available at a 30 m resolution, with some areas available at a 10 m resolution (DiBiase, et al., 2018). This broad area scale may lack precision in determining slope variability within a field in the accuracy needed for precision agriculture implementation. If a process was developed to accurately determine slope between elevation point data from EC mapping, planting, or harvest, then a field level slope profile, combined with EC data, could possibly result in a more appropriate zone definition for nematode clustering.

Historical spatial yield data for the study areas were not available. Spatial yield monitoring in cotton has not been as widely adopted as it has been in grain production. Trying to use yield variations to determine distributions of nematode populations within a field has many inherent challenges. When using yield in any sort of predictive analytics, not all variables can be quantified. Most often, these variables may be attributed to either 1) environmental factors or 2) genetic factors. However, in the future if multiple years of spatial yield data are captured, they

could be compiled to determine yield trends. Future studies could include those for more precise zone delineations.

Soil clay content and soil OM content were, numerically, two of the leading zone delineation bases for nematode clustering in our study. Khalilian et al. (2002) reported that, when OM was added (compost) to their experimental plots, the densities of CLN significantly decreased. If CLN densities are affected negatively by increasing OM levels, applications of composts such as manure and chicken litter may impact the clustering ability of zones defined by soil OM. Applications of any compost to the investigated fields were not recorded as part of this study. This negative correlation to OM, could explain differences in CLN response between work done by Khalilian et al. (2001), where it was shown that a high correlation existed in CLN populations to clay content (%), $R^2 = 0.916$.

Soil OM and clay content have a positive correlation (Burke, et al., 1990). As clay content increases, soil OM tends to increase as well due to slower decomposition rates of OM on the surface of clay particles and increased potential for aggregate formation (Bot & Benites, 2005). This relationship helps partially explain the similarities of clay and soil OM in their ability to cluster nematode populations since the larger the clay aggregates in soil, the more limited the mobility is for nematodes in the soil. This lack of mobility can restrict movement of nematodes through the soil in search of food sources.

The trial conducted by Khalilian et al. (2001) was conducted under conventional tillage production practices. This tillage program utilized numerous pre-planting tillage operations (disking, field cultivation, row bedding) and one in-season cultivation for weed control. Tillage operations have been shown to increase the breakdown and subsequent loss in soil OM (Reicosky, et al., 1995). The fields investigated in our study utilized strip tillage at planting. with

weed control accomplished through chemical applications. This reduction in number of tillage events can lead to increases in soil OM levels over time which could lead to a decrease in CLN densitities, making spatial correlations more difficult. Overall, agronomic practices have changed since the publication of Khalilian et al. (2001) findings. Repeating of this study under current production practices (tillage, weed control, and crop rotation) could produce different results that are more representative of the current production practices.

Future research in this area can include investigation of fields that receive applications of compost, such as chicken litter, as part of their fertility program and its impact on nematode populations. Slope within a field as a basis for nematode management zone development should be investigated. For example, pooling from run-off of applied compost material could affect nematode population levels in low lying areas of a field.

Future work could also be directed at examining the relationship to fertility applications, soil texture, and nematode populations. Ortiz et al. (2010) stated that SRKN population densities increase in areas of more coarsely textured soils. These areas are often more prone to fertilizer leaching and drought stress, and the relationship between nematodes and fertility levels, if any, is still not fully understood. A study that examines change in identified populations of SRKN and CLN over time as compared to soil nutrient levels may bring new insight to nematode control. In addition to fertility, the relationship between soil chemical properties, soil texture, and nematode reproduction and population densities need to be examined. Identifying this relationship may help in understanding what fields are better suited for defining nematode management zones with the methods described in our study.

Multi-Hybrid Planting

The Directed Prescription (Barnes & Kirk, 2017) methodology of the 2-year field study could potentially predict areas of nematodes that are above threshold levels. The methodology can be amended to using alternating strips of nematicide, and then applying the data analysis described in Chapter 3, year one, to a field with a known nematode problem. This can reduce the variability induced when seed varieties of differing genetic backgrounds are used. After an initial investigation with alternating strips of nematicides, a hypothetical or projected return above variable input cost analysis can then be conducted to determine if a return on investment for nematode control is great enough to offset the cost of variable rate technology, whether that technology be a multi-hybrid planter or variable rate application (VRA) equipment for nematicide application.

Practical application of this methodology can also be accomplished using either pre-plant or in-furrow nematicides in lieu of different seed varieties. Variable rate application systems are typically less costly and less complex in their operation than multi-hybrid planters. Most of these systems can be installed on planters and implements already in use by growers reducing overall costs. If the methodology from year one returns areas that are potentially above threshold for nematodes and encompass only a portion of a field, VRA of nematicides may be economical. Consideration must be given to desired yield goals along with the relative costs of nematicide, resistant varieties, application system costs, and cost of time in generating and analysis of the required data.

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Appendices

Appendix 1

Threshold levels of plant-parasitic nematode by species, soil texture, and pre plant sample timing in conventional tillage agronomic systems

CROP TO BE PLANTED IS COTTON				
	ACTION LEVELS			
	Nematodes per 100 cc of soil			
Nematode	Sand to sandy loam	Clay loam to clay	Preplant: turned/ disced*	Control options**
Columbia lance	1-49	1-99	1-16	A,E
Hoplolaimus columbus	50-99	100-149	17-33	B,C
	100+	150+	34+	B,C,D
Lance	1-199	1-249	1-69	A,E
Hoplolaimus galeatus	200-249	250-349	70-89	B,C
		250+	350+	90+ B,C,D
Reniform	1-49	1-49	1-15	A,E
Rotylenchulus reniformis	50-749	50-749	16-149	B,C
	750+	750+	150+	B,C,D
Ring	1-399	1-599	1-139	A,E
Criconemella spp.	400+	600+	140+	B,C,D
Root knot	1-49	1-99	1-16	A,E
Meloidogyne incognita	50-99	100-129	17-39	B,C
	100+	130+	40+	B,C,D
Lesion	1-49	1-79	1-16	A,E
Pratylenchus spp.	50-99	80-149	17-32	B,C
	100+	150+	33+	B,C,D
Spiral				
Scutellonema spp. &	1-799	1-999	1-264	A,E
Helicotylenchus spp.	800+	1,000+	265+	B,C,D
Sting				
Belonolaimus longicaudatus	10+	NA	1+	B,D
Stunt	1-599	1-799	1-199	A,E
Tylenchorhynchus spp.	600+	800+	200+	B,C,D

*When soil is prepared for planting, nematodes become scattered and will be fewer when compared to samples taken from about living plant roots.

A - Nematodes at this level are not likely to cause a problem. B - Nematodes at this level are likely to cause a problem. C - Apply cultural controls. See crop recommendations*. D - An approved nematicide can be of value***. E - Continue to monitor populations periodically.

***For management options, see the Clemson Extension publications, *South Carolina Cotton Growers Guide* (EC 589), and *Pest Management Handbook, Volume 1* (EC 670), available from county Extension offices or the Clemson Extension Bulletin Room (864-656-3261).

Appendix 2

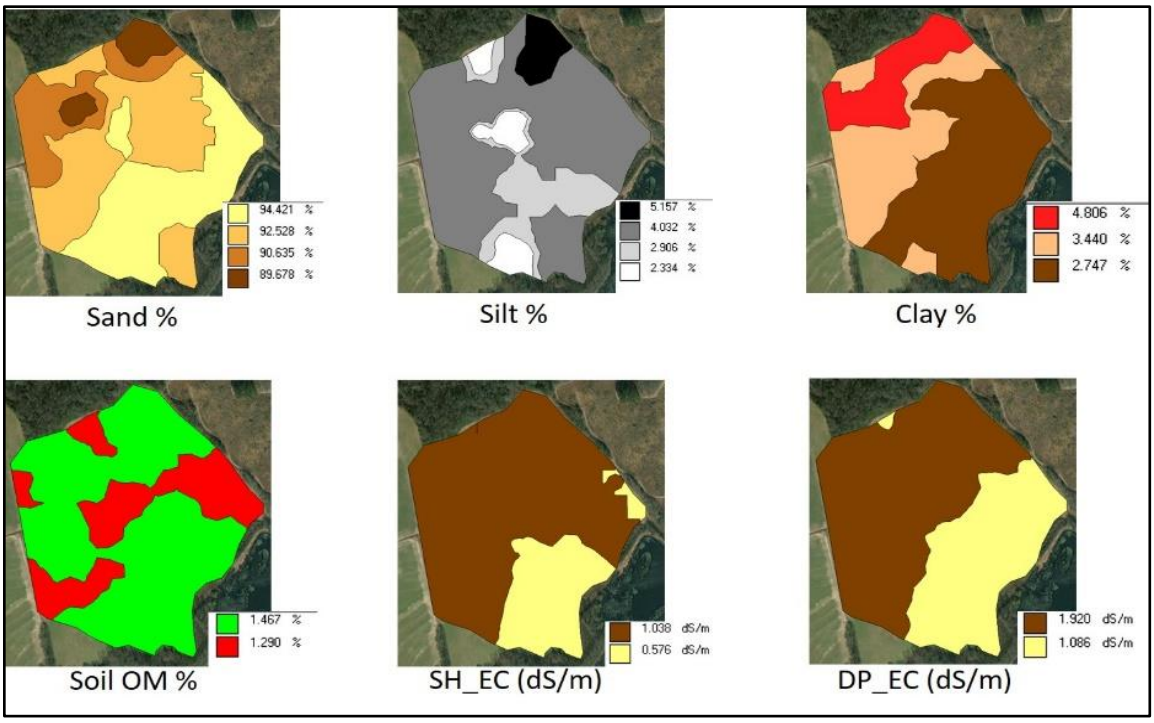
Tabulated dataset of nematode counts and soil textures each of study area.

		Field 1	Field 2	Field 3	Field 4	Field 5
USDA Soil Txt. Class		Loamy Sand	Sand	Sand	Sand	Sand
CLN (#/100 cm^3 of soil)	N	49	38	150	50	50
	Mean	0.61	0.00	5.20	1.20	0.80
	Std Dev	2.42	0.00	12.99	3.28	3.40
	Min	0.00	0.00	0.00	0.00	0.00
	Max	10.00	0.00	80.00	10.00	20.00
	Range	10.00	0.00	80.00	10.00	20.00
Stubby root (#/100 cm^3 of soil)	N	49	38	150	50	50
	Mean	2.86	9.21	5.60	0.80	2.20
	Std Dev	6.77	20.05	12.01	4.44	7.08
	Min	0.00	0.00	0.00	0.00	0.00
	Max	30.00	90.00	70.00	30.00	30.00
	Range	30.00	90.00	70.00	30.00	30.00
SRKN (#/100 cm^3 of soil)	N	49	38	150	50	50
	Mean	0.41	3.42	83.13	1.80	0.00
	Std Dev	2.86	13.81	182.67	7.48	0.00
	Min	0.00	0.00	0.00	0.00	0.00
	Max	20.00	70.00	1120.00	40.00	0.00
	Range	20.00	70.00	1120.00	40.00	0.00
Sand (%)	N	48	37	150	49	50
	Mean	86.10	88.63	92.53	91.64	89.50
	Std Dev	6.63	4.68	1.89	1.80	3.70
	Min	65.00	74.00	86.00	87.00	76.00
	Max	93.00	93.50	95.00	95.00	94.50
	Range	28.00	19.50	9.00	8.00	18.50
Silt (%)	N	48	37	150	49	50
	Mean	7.89	5.32	4.03	2.48	6.44
	Std Dev	5.08	2.11	1.12	1.55	2.41
	Min	0.50	1.50	1.50	0.50	3.00
	Max	25.00	10.50	7.00	6.00	17.50
	Range	24.50	9.00	5.50	5.50	14.50
Clay (%)	N	48	37	150	49	50
	Mean	6.01	6.05	3.44	5.88	4.06
	Std Dev	3.36	3.47	1.36	0.98	1.86
	Min	2.00	2.00	2.00	2.00	1.00

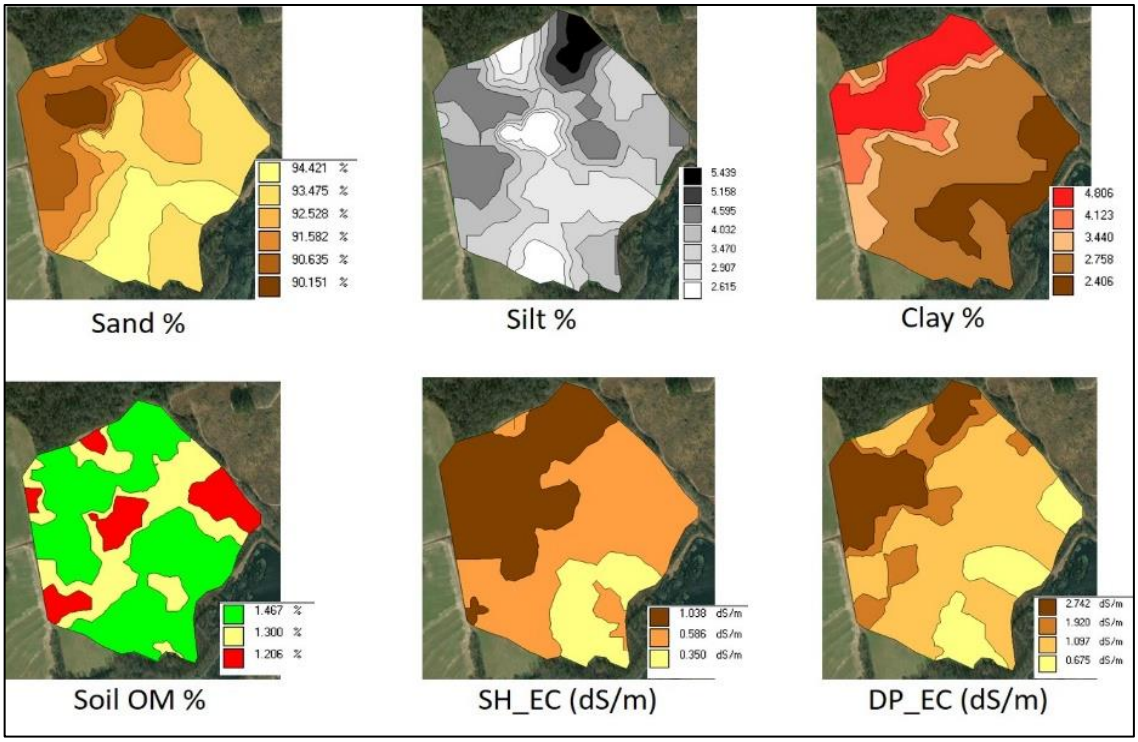
		Field 1	Field 2	Field 3	Field 4	Field 5
	Max	18.00	16.50	8.00	7.50	12.00
	Range	16.00	14.50	6.00	5.50	11.00
Soil OM (%)	N	47	38	150	44	50
	Mean	1.60	1.59	1.47	1.56	1.40
	Std Dev	0.77	0.65	0.33	0.50	0.64
	Min	0.81	0.62	1.08	0.94	0.66
	Max	3.74	3.21	2.60	3.64	3.78
	Range	2.93	2.59	1.52	2.70	3.11
SH EC	N	49	38	150	50	50
	Mean	1.80	2.03	1.04	0.92	1.61
	Std Dev	1.27	1.37	0.90	0.96	1.31
	Min	0.60	0.41	0.33	0.22	0.36
	Max	6.34	6.30	5.23	5.08	5.88
	Range	5.74	5.90	4.90	4.86	5.51
DP EC	N	49	38	150	50	50
	Mean	3.64	1.99	1.92	2.21	2.98
	Std Dev	1.18	1.11	1.64	2.45	1.50
	Min	1.38	0.31	0.43	0.18	0.64
	Max	6.22	4.28	7.76	11.43	7.08
	Range	4.84	3.97	7.34	11.24	6.43

Appendix 3

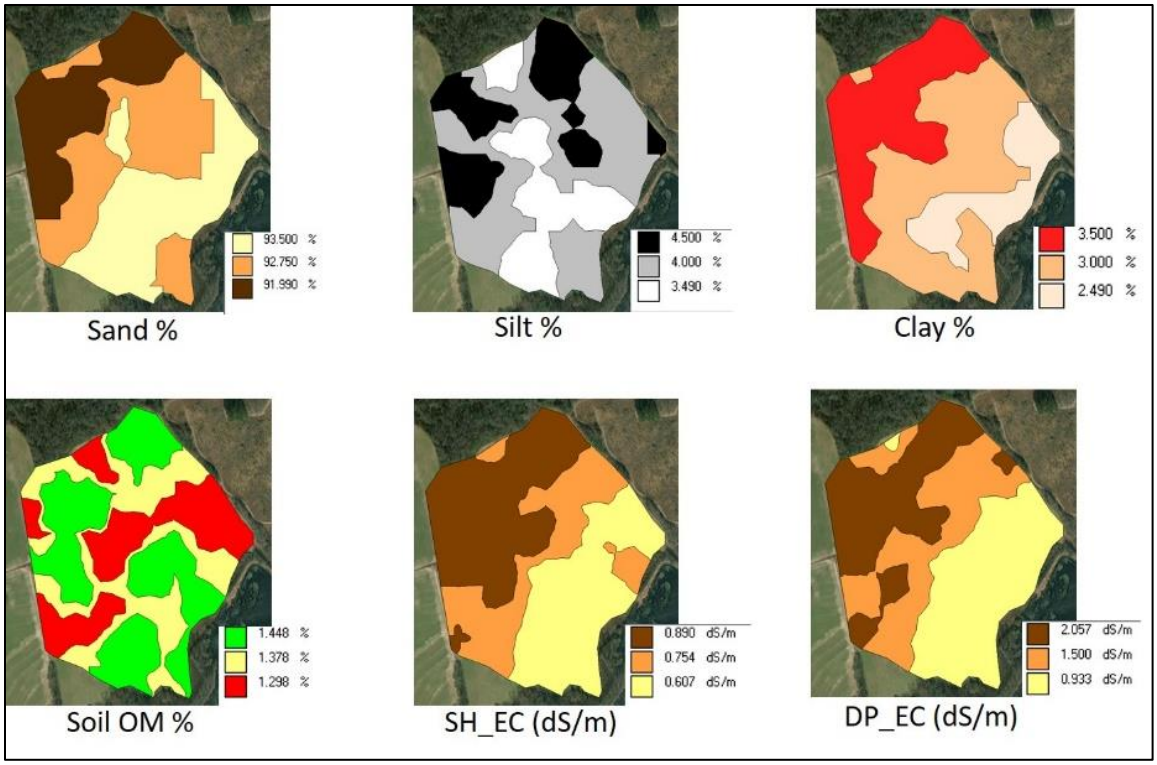
Field Contour Examples



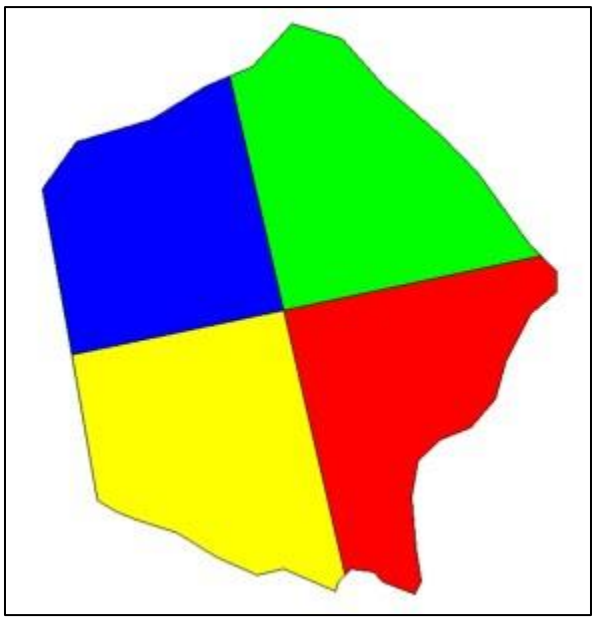
Appendix 0-1: Example of a fields soil characteristic contoured with a range division of 0.5 standard deviation ($\sigma = 0.5$)



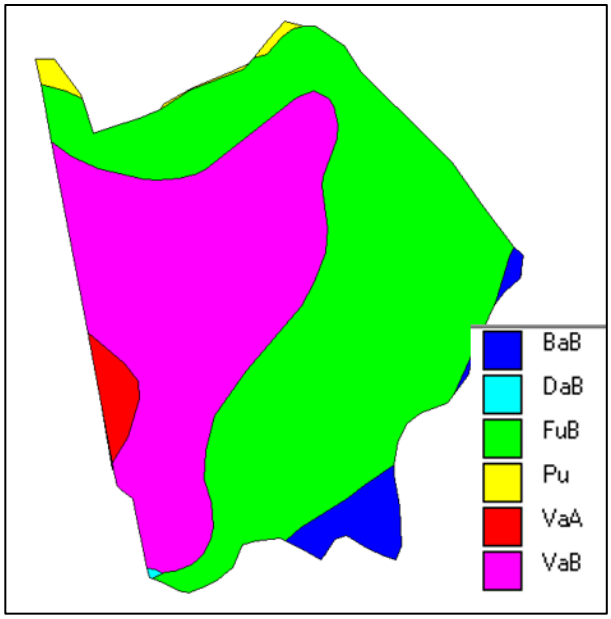
Appendix 0-2: Example of a field with a contour range of each soil characteristic contoured at a range division of 1 standard deviation ($\sigma = 1$).



Appendix 0-3: Example of a field with soil characteristics contoured with 3 equal range divisions.



Appendix 0-4: Example of a field divided into 4 zones of similar area. Divisions are arbitrary and not based on any underlying data.



Appendix 0-5: Example soil zones based on SSURGO soil type from the United States Department of Agriculture.

Appendix 4

DBI values of relative Weighed Nematode Indices

Trt. No.	Field Number	Year	Sample Timing	Soil Class	Separation Range	# Clusters	*DBI* Score	tDBI values
13	2	2017	Mid-Season	Clay	Equal Range x3	2	10.031	1.664
14	2	2017	Mid-Season	DP EC	Equal Range x3	3	5.159	1.296
15	2	2017	Mid-Season	Sand	Equal Range x3	4	3.878	1.114
16	2	2017	Mid-Season	SH EC	Equal Range x3	2	7.778	1.532
17	2	2017	Mid-Season	Silt	Equal Range x3	3	9.112	1.615
18	2	2017	Mid-Season	Soil OM	Equal Range x3	2	7.870	1.538
23	2	2017	Mid-Season	Min AIC	Equal Range x3	3	3.650	1.073
26	2	2017	Mid-Season	p-value	Equal Range x3	3	82.248	2.445
13	3	2017	At Harvest	Clay	Equal Ranges x3	3	24.348	2.054
13	5	2017	At Harvest	Clay	Equal Ranges x3	4	8.483	1.578
13	1	2017	Mid-Season	Clay	Equal Ranges x3	3	100.746	2.498
13	3	2017	Mid-Season	Clay	Equal Ranges x3	3	24.172	2.051
13	3	2019	At Harvest	Clay	Equal Ranges x3	3	8.835	1.599
13	4	2018	At Harvest	Clay	Equal Ranges x3	3	8.678	1.59
14	3	2017	At Harvest	DP EC	Equal Ranges x3	3	6.651	1.445
14	5	2017	At Harvest	DP EC	Equal Ranges x3	4	3.811	1.102
14	1	2017	Mid-Season	DP EC	Equal Ranges x3	3	54.556	2.329
14	3	2017	Mid-Season	DP EC	Equal Ranges x3	3	10.059	1.666
14	3	2019	At Harvest	DP EC	Equal Ranges x3	3	18.410	1.942
14	4	2018	At Harvest	DP EC	Equal Ranges x3	3	6.250	1.41
15	3	2017	At Harvest	Sand	Equal Ranges x3	3	10.833	1.702
15	5	2017	At Harvest	Sand	Equal Ranges x3	3	35.967	2.195

Trt. No.	Field Number	Year	Sample Timing	Soil Class	Separation Range	# Clusters	*DBI* Score	tDBI values
15	1	2017	Mid-Season	Sand	Equal Ranges x3	3	6.264	1.411
15	3	2017	Mid-Season	Sand	Equal Ranges x3	3	9.185	1.62
15	3	2019	At Harvest	Sand	Equal Ranges x3	3	77.983	2.431
15	4	2018	At Harvest	Sand	Equal Ranges x3	3	21.921	2.013
16	3	2017	At Harvest	SH EC	Equal Ranges x3	3	5.568	1.342
16	5	2017	At Harvest	SH EC	Equal Ranges x3	4	10.998	1.71
16	1	2017	Mid-Season	SH EC	Equal Ranges x3	3	25.313	2.069
16	3	2017	Mid-Season	SH EC	Equal Ranges x3	3	3.676	1.078
16	3	2019	At Harvest	SH EC	Equal Ranges x3	3	11.554	1.734
16	4	2018	At Harvest	SH EC	Equal Ranges x3	3	5.087	1.287
17	3	2017	At Harvest	Silt	Equal Ranges x3	3	776.113	2.881
17	5	2017	At Harvest	Silt	Equal Ranges x3	3	9.628	1.644
17	1	2017	Mid-Season	Silt	Equal Ranges x3	3	996.791	2.913
17	3	2017	Mid-Season	Silt	Equal Ranges x3	3	43.544	2.259
17	3	2019	At Harvest	Silt	Equal Ranges x3	3	9.986	1.662
17	4	2018	At Harvest	Silt	Equal Ranges x3	3	18.717	1.949
18	3	2017	At Harvest	Soil OM	Equal Ranges x3	3	8.853	1.601
18	5	2017	At Harvest	Soil OM	Equal Ranges x3	2	9.321	1.627
18	1	2017	Mid-Season	Soil OM	Equal Ranges x3	4	28.113	2.108
18	3	2017	Mid-Season	Soil OM	Equal Ranges x3	3	7.946	1.543
18	3	2019	At Harvest	Soil OM	Equal Ranges x3	3	23.351	2.038
18	4	2018	At Harvest	Soil OM	Equal Ranges x3	3	30.320	2.136
23	1	2017	Mid-Season	Min AIC	Equal Ranges x3	3	9.445	1.634
23	3	2017	At Harvest	Min AIC	Equal Ranges x3	3	52.746	2.319
23	3	2017	Mid-Season	Min AIC	Equal Ranges x3	3	5.665	1.352

Trt. No.	Field Number	Year	Sample Timing	Soil Class	Separation Range	# Clusters	*DBI* Score	tDBI values
23	3	2019	At Harvest	Min AIC	Equal Ranges x3	3	5.765	1.363
23	5	2017	At Harvest	Min AIC	Equal Ranges x3	3	10.436	1.684
23	4	2018	At Harvest	Min AIC	Equal Ranges x3	3	5.447	1.329
26	1	2017	Mid-Season	p-value	Equal Ranges x3	3	8.719	1.593
26	3	2017	At Harvest	p-value	Equal Ranges x3	3	8.604	1.586
26	3	2017	Mid-Season	p-value	Equal Ranges x3	3	17.320	1.917
26	3	2019	At Harvest	p-value	Equal Ranges x3	3	8.629	1.587
26	5	2017	At Harvest	p-value	Equal Ranges x3	3	44.973	2.269
26	4	2018	At Harvest	p-value	Equal Ranges x3	3	12.688	1.778
19	3	2017	At Harvest	Grid	Grid	4	23.702	2.044
19	5	2017	At Harvest	Grid	Grid	4	18.768	1.95
19	1	2017	Mid-Season	Grid	Grid	4	15.789	1.877
19	2	2017	Mid-Season	Grid	Grid	4	49.847	2.302
19	3	2017	Mid-Season	Grid	Grid	4	11.458	1.73
19	3	2019	At Harvest	Grid	Grid	4	89.745	2.468
19	4	2018	At Harvest	Grid	Grid	4	10.876	1.704
20	3	2017	At Harvest	SSURGO	SSURGO	3	213.154	2.666
20	5	2017	At Harvest	SSURGO	SSURGO	6	39.464	2.227
20	1	2017	Mid-Season	SSURGO	SSURGO	4	22.116	2.017
20	2	2017	Mid-Season	SSURGO	SSURGO	7	17.116	1.911
20	3	2017	Mid-Season	SSURGO	SSURGO	3	6.013	1.387
20	3	2019	At Harvest	SSURGO	SSURGO	3	61.332	2.364
1	3	2017	At Harvest	Clay	$\sigma = 0.5$	5	29.071	2.12
1	5	2017	At Harvest	Clay	$\sigma = 0.5$	6	99.859	2.496
1	1	2017	Mid-Season	Clay	$\sigma = 0.5$	6	34.246	2.179

Trt. No.	Field Number	Year	Sample Timing	Soil Class	Separation Range	# Clusters	*DBI* Score	tDBI values
1	2	2017	Mid-Season	Clay	σ = 0.5	3	42.338	2.25
1	3	2017	Mid-Season	Clay	σ = 0.5	5	9.517	1.638
1	3	2019	At Harvest	Clay	σ = 0.5	5	7.366	1.502
1	4	2018	At Harvest	Clay	σ = 0.5	6	46.323	2.279
2	3	2017	At Harvest	DP EC	σ = 0.5	6	34.402	2.18
2	5	2017	At Harvest	DP EC	σ = 0.5	6	16.179	1.887
2	1	2017	Mid-Season	DP EC	σ = 0.5	5	19.464	1.965
2	2	2017	Mid-Season	DP EC	σ = 0.5	4	39.086	2.223
2	3	2017	Mid-Season	DP EC	σ = 0.5	6	10.827	1.702
2	3	2019	At Harvest	DP EC	σ = 0.5	4	27.093	2.094
2	4	2018	At Harvest	DP EC	σ = 0.5	6	12.281	1.763
3	3	2017	At Harvest	Sand	σ = 0.5	6	13.371	1.802
3	5	2017	At Harvest	Sand	σ = 0.5	3	46.018	2.277
3	1	2017	Mid-Season	Sand	σ = 0.5	5	8.287	1.566
3	2	2017	Mid-Season	Sand	σ = 0.5	5	28.526	2.113
3	3	2017	Mid-Season	Sand	σ = 0.5	6	100.194	2.497
3	3	2019	At Harvest	Sand	σ = 0.5	6	71.828	2.409
3	4	2018	At Harvest	Sand	σ = 0.5	5	2.089	0.661
4	3	2017	At Harvest	SH EC	σ = 0.5	5	73.376	2.415
4	5	2017	At Harvest	SH EC	σ = 0.5	4	5.841	1.37
4	1	2017	Mid-Season	SH EC	σ = 0.5	5	9.680	1.646
4	2	2017	Mid-Season	SH EC	σ = 0.5	3	8.617	1.586
4	3	2017	Mid-Season	SH EC	σ = 0.5	5	10.694	1.696
4	3	2019	At Harvest	SH EC	σ = 0.5	5	41.652	2.244
4	4	2018	At Harvest	SH EC	σ = 0.5	5	91.039	2.472

Trt. No.	Field Number	Year	Sample Timing	Soil Class	Separation Range	# Clusters	*DBI* Score	tDBI values
5	3	2017	At Harvest	Silt	σ = 0.5	7	16.330	1.891
5	5	2017	At Harvest	Silt	σ = 0.5	4	82.090	2.445
5	1	2017	Mid-Season	Silt	σ = 0.5	7	40.709	2.237
5	2	2017	Mid-Season	Silt	σ = 0.5	4	14.501	1.839
5	3	2017	Mid-Season	Silt	σ = 0.5	7	207.226	2.66
5	3	2019	At Harvest	Silt	σ = 0.5	7	23.045	2.033
5	4	2018	At Harvest	Silt	σ = 0.5	6	11.579	1.735
6	3	2017	At Harvest	Soil OM	σ = 0.5	3	6.056	1.391
6	5	2017	At Harvest	Soil OM	σ = 0.5	4	73.158	2.414
6	1	2017	Mid-Season	Soil OM	σ = 0.5	6	59.044	2.353
6	2	2017	Mid-Season	Soil OM	σ = 0.5	4	11.949	1.75
6	3	2017	Mid-Season	Soil OM	σ = 0.5	3	8.799	1.597
6	3	2019	At Harvest	Soil OM	σ = 0.5	3	32.243	2.157
6	4	2018	At Harvest	Soil OM	σ = 0.5	7	62.634	2.37
21	2	2017	Mid-Season	Min AIC	σ = 0.5	6	7.624	1.521
21	3	2017	At Harvest	Min AIC	σ = 0.5	5	16.419	1.894
21	3	2017	Mid-Season	Min AIC	σ = 0.5	5	3.971	1.129
21	3	2019	At Harvest	Min AIC	σ = 0.5	5	10.826	1.702
21	5	2017	At Harvest	Min AIC	σ = 0.5	7	7.698	1.526
21	4	2018	At Harvest	Min AIC	σ = 0.5	6	3.926	1.122
21	1	2017	Mid-Season	Min AIC	σ = 0.5	5	6.437	1.427
24	2	2017	Mid-Season	p-value	σ = 0.5	6	15.860	1.879
24	3	2017	At Harvest	p-value	σ = 0.5		157.687	2.603
24	3	2017	Mid-Season	p-value	σ = 0.5	4	55.294	2.333
24	3	2019	At Harvest	p-value	σ = 0.5	4	32.186	2.157

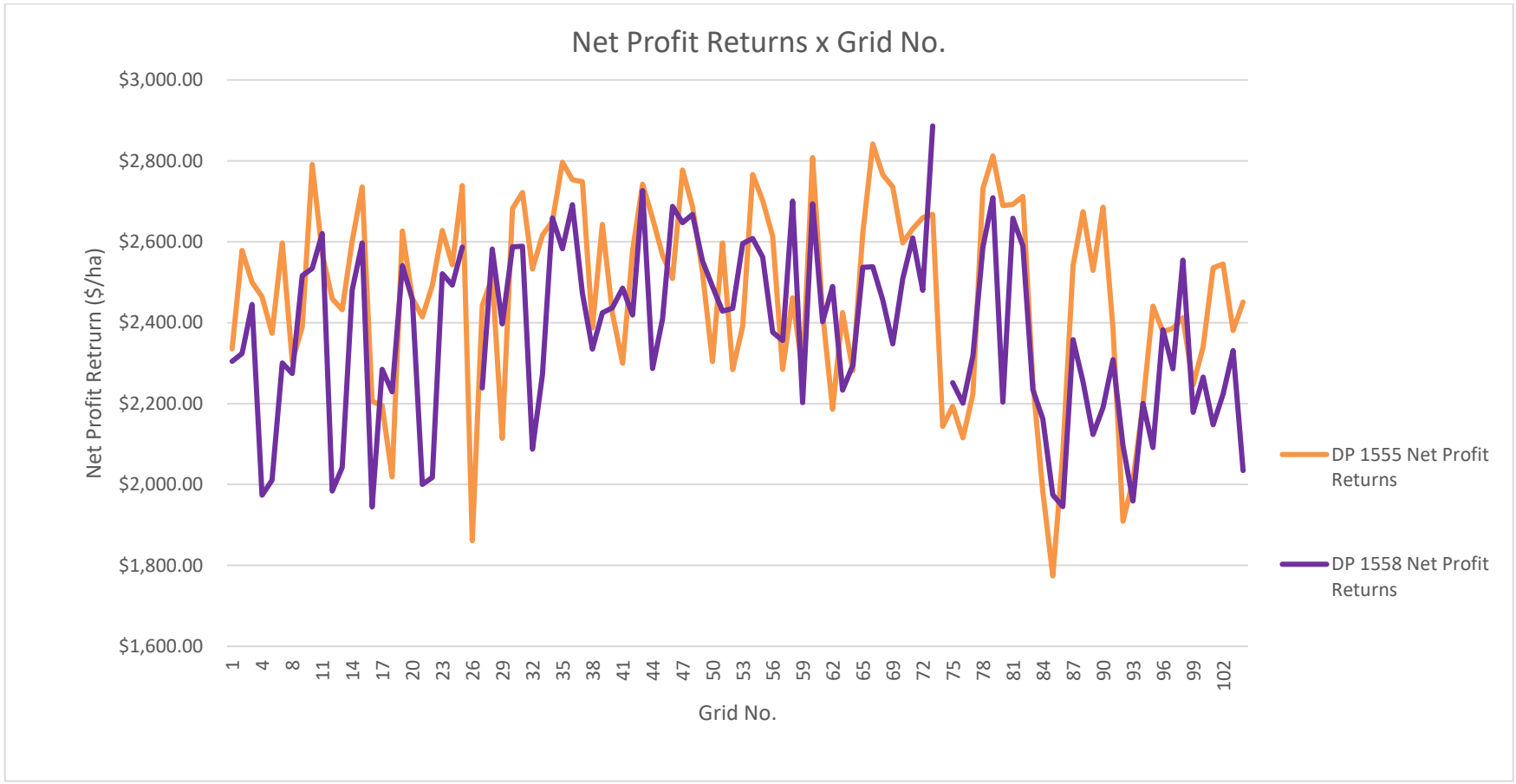
Trt. No.	Field Number	Year	Sample Timing	Soil Class	Separation Range	# Clusters	*DBI* Score	tDBI values
24	5	2017	At Harvest	p-value	σ = 0.5	6	14.046	1.825
24	4	2018	At Harvest	p-value	σ = 0.5	6	8.429	1.575
24	1	2017	Mid-Season	p-value	σ = 0.5	6	16.357	1.892
25	1	2017	Mid-Season	p-value	σ = 1	3	7.685	1.525
7	3	2017	At Harvest	Clay	σ = 1	3	100.769	2.498
7	5	2017	At Harvest	Clay	σ = 1	4	8.483	1.578
7	1	2017	Mid-Season	Clay	σ = 1	4	5.311	1.313
7	2	2017	Mid-Season	Clay	σ = 1	2	10.031	1.664
7	3	2017	Mid-Season	Clay	σ = 1	3	10.940	1.707
7	3	2019	At Harvest	Clay	σ = 1	3	34.695	2.183
7	4	2018	At Harvest	Clay	σ = 1	4	1.834	0.555
8	3	2017	At Harvest	DP EC	σ = 1	4	16.647	1.9
8	5	2017	At Harvest	DP EC	σ = 1	4	3.811	1.102
8	1	2017	Mid-Season	DP EC	σ = 1	3	8.929	1.605
8	2	2017	Mid-Season	DP EC	σ = 1	3	5.159	1.296
8	3	2017	Mid-Season	DP EC	σ = 1	4	856.173	2.894
8	3	2019	At Harvest	DP EC	σ = 1	4	29.509	2.126
8	4	2018	At Harvest	DP EC	σ = 1	4	6.815	1.459
9	3	2017	At Harvest	Sand	σ = 1	4	11.962	1.75
9	5	2017	At Harvest	Sand	σ = 1	4	10.998	1.71
9	1	2017	Mid-Season	Sand	σ = 1	4	178.839	2.63
9	2	2017	Mid-Season	Sand	σ = 1	4	3.878	1.114
9	3	2017	Mid-Season	Sand	σ = 1	4	581.158	2.839
9	3	2019	At Harvest	Sand	σ = 1	4	100.626	2.498
9	4	2018	At Harvest	Sand	σ = 1	3	2.176	0.693

Trt. No.	Field Number	Year	Sample Timing	Soil Class	Separation Range	# Clusters	*DBI* Score	tDBI values
10	3	2017	At Harvest	SH EC	σ = 1	4	16.459	1.895
10	5	2017	At Harvest	SH EC	σ = 1	4	10.998	1.71
10	1	2017	Mid-Season	SH EC	σ = 1	4	11.785	1.743
10	2	2017	Mid-Season	SH EC	σ = 1	2	7.778	1.532
10	3	2017	Mid-Season	SH EC	σ = 1	4	23.171	2.035
10	3	2019	At Harvest	SH EC	σ = 1	4	10.444	1.684
10	4	2018	At Harvest	SH EC	σ = 1	4	45.839	2.275
11	3	2017	At Harvest	Silt	σ = 1	4	3.231	0.989
11	5	2017	At Harvest	Silt	σ = 1	2	59.117	2.353
11	1	2017	Mid-Season	Silt	σ = 1	4	449.100	2.8
11	2	2017	Mid-Season	Silt	σ = 1	3	9.112	1.615
11	3	2017	Mid-Season	Silt	σ = 1	4	259.819	2.705
11	3	2019	At Harvest	Silt	σ = 1	4	10.076	1.666
11	4	2018	At Harvest	Silt	σ = 1	4	34.083	2.177
12	3	2017	At Harvest	Soil OM	σ = 1	2	13.843	1.818
12	5	2017	At Harvest	Soil OM	σ = 1	2	9.321	1.627
12	1	2017	Mid-Season	Soil OM	σ = 1	4	25.531	2.072
12	2	2017	Mid-Season	Soil OM	σ = 1	2	7.870	1.538
12	3	2017	Mid-Season	Soil OM	σ = 1	2	4.973	1.273
12	3	2019	At Harvest	Soil OM	σ = 1	2	11.499	1.731
12	4	2018	At Harvest	Soil OM	σ = 1	5	9.081	1.614
22	2	2017	Mid-Season	Min AIC	σ = 1	4	3.042	0.946
22	3	2017	At Harvest	Min AIC	σ = 1	3	419.632	2.789
22	3	2017	Mid-Season	Min AIC	σ = 1	3	4.851	1.258
22	3	2019	At Harvest	Min AIC	σ = 1	3	4.981	1.274

Trt. No.	Field Number	Year	Sample Timing	Soil Class	Separation Range	# Clusters	*DBI* Score	tDBI values
22	5	2017	At Harvest	Min AIC	σ = 1	5	5.313	1.314
22	4	2018	At Harvest	Min AIC	σ = 1	4	1.868	0.57
22	1	2017	Mid-Season	Min AIC	σ = 1	4	2.701	0.859
25	2	2017	Mid-Season	p-value	σ = 1	4	128.146	2.556
25	3	2017	At Harvest	p-value	σ = 1	3	3.710	1.084
25	3	2017	Mid-Season	p-value	σ = 1	3	14.373	1.835
25	3	2019	At Harvest	p-value	σ = 1	3	6.239	1.409
25	5	2017	At Harvest	p-value	σ = 1	4	8.939	1.606
25	4	2018	At Harvest	p-value	σ = 1	3	38.652	2.22

Appendix 5

Year one net returns by.2 ha grid zone.



Appendix 6

Year one net profit returns by variety ($/ha) and projected multi-hybrid planting (MHP).

Grid No.	DP 1555 Net Profit Returns ($/ha)	DP 1558 Net Profit Returns ($/ha)	Projected MHP Net Profit Returns ($/ha)	MHP Directed Seed Variety
1	$ 2,335.69	$ 2,304.39	$ 2,335.69	DP 1555
2	$ 2,578.40	$ 2,323.55	$ 2,578.40	DP 1555
3	$ 2,499.84	$ 2,444.71	$ 2,499.84	DP 1555
4	$ 2,464.42	$ 1,973.53	$ 2,464.42	DP 1555
5	$ 2,373.86	$ 2,010.48	$ 2,373.86	DP 1555
6	$ 2,597.15	$ 2,300.39	$ 2,597.15	DP 1555
8	$ 2,304.03	$ 2,274.19	$ 2,304.03	DP 1555
9	$ 2,389.71	$ 2,516.34	$ 2,516.34	DP 1558
10	$ 2,790.77	$ 2,534.38	$ 2,790.77	DP 1555
11	$ 2,571.20	$ 2,620.65	$ 2,620.65	DP 1558
12	$ 2,459.26	$ 1,983.92	$ 2,459.26	DP 1555
13	$ 2,432.15	$ 2,041.99	$ 2,432.15	DP 1555
14	$ 2,601.92	$ 2,480.95	$ 2,601.92	DP 1555
15	$ 2,735.69	$ 2,597.41	$ 2,735.69	DP 1555
16	$ 2,205.39	$ 1,944.57	$ 2,205.39	DP 1555
17	$ 2,195.63	$ 2,285.01	$ 2,285.01	DP 1558
18	$ 2,018.99	$ 2,229.42	$ 2,229.42	DP 1558
19	$ 2,626.90	$ 2,541.20	$ 2,626.90	DP 1555
20	$ 2,460.05	$ 2,457.57	$ 2,460.05	DP 1555
21	$ 2,414.31	$ 2,000.09	$ 2,414.31	DP 1555
22	$ 2,492.34	$ 2,017.32	$ 2,492.34	DP 1555
23	$ 2,627.81	$ 2,520.78	$ 2,627.81	DP 1555
24	$ 2,543.36	$ 2,493.13	$ 2,543.36	DP 1555
25	$ 2,739.36	$ 2,586.68	$ 2,739.36	DP 1555
27	$ 2,442.28	$ 2,238.98	$ 2,442.28	DP 1555
28	$ 2,512.90	$ 2,582.40	$ 2,582.40	DP 1558
29	$ 2,113.73	$ 2,397.31	$ 2,397.31	DP 1558
30	$ 2,681.97	$ 2,587.83	$ 2,681.97	DP 1555
31	$ 2,721.81	$ 2,588.72	$ 2,721.81	DP 1555

Grid No.	DP 1555 Net Profit Returns ($/ha)	DP 1558 Net Profit Returns ($/ha)	Projected MHP Net Profit Returns ($/ha)	MHP Directed Seed Variety
32	$ 2,532.77	$ 2,087.25	$ 2,532.77	DP 1555
33	$ 2,616.60	$ 2,271.82	$ 2,616.60	DP 1555
34	$ 2,651.29	$ 2,658.50	$ 2,658.50	DP 1558
35	$ 2,796.46	$ 2,582.60	$ 2,796.46	DP 1555
36	$ 2,753.53	$ 2,691.61	$ 2,753.53	DP 1555
37	$ 2,748.32	$ 2,473.12	$ 6,898.70	DP 1558
38	$ 2,387.77	$ 2,335.01	$ 2,387.77	DP 1555
39	$ 2,642.83	$ 2,424.66	$ 2,642.83	DP 1555
40	$ 2,424.82	$ 2,437.00	$ 2,437.00	DP 1558
41	$ 2,299.54	$ 2,485.77	$ 2,485.77	DP 1558
42	$ 2,577.82	$ 2,419.02	$ 2,577.82	DP 1555
43	$ 2,742.51	$ 2,726.43	$ 2,742.51	DP 1555
44	$ 2,658.27	$ 2,286.89	$ 2,658.27	DP 1555
45	$ 2,566.91	$ 2,409.35	$ 2,566.91	DP 1555
46	$ 2,509.12	$ 2,687.51	$ 2,687.51	DP 1558
47	$ 2,777.17	$ 2,647.28	$ 2,777.17	DP 1555
48	$ 2,687.15	$ 2,667.80	$ 2,687.15	DP 1555
49	$ 2,524.41	$ 2,552.34	$ 3,864.97	DP 1558
50	$ 2,303.60	$ 2,489.71	$ 2,303.60	DP 1555
51	$ 2,597.31	$ 2,428.47	$ 2,597.31	DP 1555
52	$ 2,284.09	$ 2,435.73	$ 2,435.73	DP 1558
53	$ 2,391.76	$ 2,595.69	$ 2,595.69	DP 1558
54	$ 2,766.27	$ 2,608.38	$ 2,766.27	DP 1555
55	$ 2,701.85	$ 2,561.99	$ 2,701.85	DP 1555
56	$ 2,613.41	$ 2,376.41	$ 2,613.41	DP 1555
57	$ 2,284.86	$ 2,355.92	$ 2,355.92	DP 1558
58	$ 2,461.61	$ 2,700.48	$ 2,700.48	DP 1558
59	$ 2,277.56	$ 2,202.14	$ 2,277.56	DP 1555
60	$ 2,808.12	$ 2,694.05	$ 2,808.12	DP 1555
61	$ 2,423.75	$ 2,402.64	$ 2,423.75	DP 1555
62	$ 2,185.95	$ 2,489.43	$ 3,357.84	DP 1558
63	$ 2,425.40	$ 2,233.00	$ 2,425.40	DP 1555
64	$ 2,281.74	$ 2,295.11	$ 2,295.11	DP 1558
65	$ 2,621.54	$ 2,536.53	$ 2,621.54	DP 1555
66	$ 2,841.70	$ 2,538.03	$ 2,841.70	DP 1555
67	$ 2,765.88	$ 2,456.31	$ 2,765.88	DP 1555
69	$ 2,735.56	$ 2,347.61	$ 2,893.56	DP 1558
70	$ 2,597.40	$ 2,508.81	$ 2,597.40	DP 1555

Grid No.	DP 1555 Net Profit Returns ($/ha)	DP 1558 Net Profit Returns ($/ha)	Projected MHP Net Profit Returns ($/ha)	MHP Directed Seed Variety
71	$ 2,632.16	$ 2,609.63	$ 2,632.16	DP 1555
72	$ 2,659.30	$ 2,479.75	$ 2,659.30	DP 1555
73	$ 2,667.91	$ 2,886.36	$ 2,886.36	DP 1558
75	$ 2,193.60	$ 2,252.12	$ 2,252.12	DP 1558
76	$ 2,115.43	$ 2,200.93	$ 2,115.43	DP 1555
77	$ 2,223.42	$ 2,318.17	$ 2,318.17	DP 1558
78	$ 2,731.37	$ 2,586.91	$ 2,731.37	DP 1555
79	$ 2,812.37	$ 2,709.01	$ 2,812.37	DP 1555
80	$ 2,689.72	$ 2,203.56	$ 2,689.72	DP 1555
81	$ 2,692.18	$ 2,658.18	$ 3,612.39	DP 1558
82	$ 2,712.33	$ 2,589.57	$ 2,934.84	DP 1558
83	$ 2,254.04	$ 2,234.45	$ 2,254.04	DP 1555
84	$ 1,982.16	$ 2,161.83	$ 1,982.16	DP 1555
85	$ 1,774.18	$ 1,974.49	$ 1,937.23	DP 1558
86	$ 2,074.66	$ 1,945.63	$ 2,074.66	DP 1555
87	$ 2,541.30	$ 2,357.69	$ 2,541.30	DP 1555
88	$ 2,674.61	$ 2,253.83	$ 2,674.61	DP 1555
89	$ 2,529.26	$ 2,123.67	$ 3,126.51	DP 1558
90	$ 2,685.82	$ 2,189.94	$ 4,464.12	DP 1558
91	$ 2,385.17	$ 2,308.93	$ 2,385.17	DP 1555
92	$ 1,908.84	$ 2,096.01	$ 1,908.84	DP 1555
93	$ 2,010.24	$ 1,958.86	$ 2,010.24	DP 1555
94	$ 2,198.61	$ 2,200.17	$ 2,241.69	DP 1558
95	$ 2,440.59	$ 2,091.48	$ 2,440.59	DP 1555
96	$ 2,378.09	$ 2,382.83	$ 2,378.09	DP 1555
97	$ 2,386.24	$ 2,285.74	$ 2,386.24	DP 1555
98	$ 2,412.07	$ 2,554.65	$ 2,654.83	DP 1558
99	$ 2,246.56	$ 2,178.72	$ 2,384.26	DP 1558
100	$ 2,340.54	$ 2,265.20	$ 2,340.54	DP 1555
101	$ 2,535.39	$ 2,147.66	$ 2,535.39	DP 1555
102	$ 2,545.33	$ 2,223.36	$ 2,545.33	DP 1555
103	$ 2,380.33	$ 2,331.44	$ 2,574.30	DP 1558
104	$ 2,451.39	$ 2,034.81	$ 2,919.51	DP 1558
Average	$ 2,484.63	$ 2,377.74	$ 2,623.14	

Appendix 7

Year two returns above seed costs by grid number and projected multi-hybrid planting (MHP).

Grid No.	1646 Net Returns ($/ha)	1747 Net Returns ($/ha)	MHP Directed Planting Potential Net Returns ($/ha)	MHP Directed Variety
1	$ 1,950.29	$ 1,541.58	$ 1,950.29	DP 1646
3	$ 2,209.84	$ 1,586.82	$ 2,209.84	DP 1646
4	$ 1,973.49	$ 1,337.83	$ 1,973.49	DP 1646
6	$ 1,694.47	$ 1,484.26	$ 1,694.47	DP 1646
8	$ 1,467.73	$ 1,391.09	$ 1,467.73	DP 1646
9	$ 1,954.61	$ 1,474.11	$ 1,954.61	DP 1646
11	$ 2,290.16	$ 1,685.01	$ 2,290.16	DP 1646
12	$ 2,027.12	$ 1,258.15	$ 2,027.12	DP 1646
14	$ 2,188.80	$ 1,606.91	$ 2,188.80	DP 1646
15	$ 2,224.96	$ 1,837.76	$ 2,224.96	DP 1646
16	$ 929.35	$ 655.04	$ 929.35	DP 1646
17	$ 1,373.52	$ 1,348.74	$ 1,373.52	DP 1646
18	$ 1,504.69	$ 1,350.40	$ 1,504.69	DP 1646
20	$ 2,118.48	$ 1,596.88	$ 2,118.48	DP 1646
21	$ 1,563.69	$ 870.88	$ 1,563.69	DP 1646
23	$ 2,292.94	$ 1,628.94	$ 2,292.94	DP 1646
24	$ 2,620.26	$ 1,913.77	$ 2,620.26	DP 1646
25	$ 2,363.60	$ 1,620.60	$ 2,363.60	DP 1646
27	$ 1,197.76	$ 1,679.80	$ 1,679.80	DP 1747
28	$ 1,826.89	$ 1,652.07	$ 1,826.89	DP 1646
29	$ 1,326.96	$ 1,223.14	$ 1,326.96	DP 1646
31	$ 2,174.82	$ 1,579.94	$ 2,174.82	DP 1646
32	$ 956.80	$ 1,034.34	$ 1,034.34	DP 1747
34	$ 2,401.81	$ 1,753.26	$ 2,401.81	DP 1646
35	$ 2,559.76	$ 1,932.58	$ 2,559.76	DP 1646
36	$ 2,445.52	$ 1,771.43	$ 2,445.52	DP 1646
37	$ 2,385.42	$ 1,772.19	$ 2,385.42	DP 1646
38	$ 1,275.37	$ 1,004.00	$ 1,275.37	DP 1646
39	$ 1,725.12	$ 1,558.24	$ 1,725.12	DP 1646
40	$ 1,840.55	$ 1,568.55	$ 1,840.55	DP 1646
41	$ 1,532.39	$ 1,232.12	$ 1,532.39	DP 1646
43	$ 2,351.36	$ 1,515.14	$ 2,351.36	DP 1646
44	$ 1,341.29	$ 1,326.29	$ 1,341.29	DP 1646
46	$ 2,282.45	$ 1,862.59	$ 2,282.45	DP 1646

Grid No.	1646 Net Returns ($/ha)	1747 Net Returns ($/ha)	MHP Directed Planting Potential Net Returns ($/ha)	MHP Directed Variety
47	$ 2,405.31	$ 1,843.85	$ 2,405.31	DP 1646
48	$ 2,526.34	$ 1,872.64	$ 2,526.34	DP 1646
49	$ 2,487.84	$ 1,676.23	$ 2,487.84	DP 1646
51	$ 1,935.34	$ 1,615.32	$ 1,935.34	DP 1646
52	$ 2,098.27	$ 1,470.31	$ 2,098.27	DP 1646
53	$ 1,603.76	$ 1,467.37	$ 1,603.76	DP 1646
55	$ 2,259.44	$ 1,557.44	$ 2,259.44	DP 1646
56	$ 1,885.40	$ 1,071.28	$ 1,885.40	DP 1646
57	$ 2,410.84	$ 1,819.59	$ 2,410.84	DP 1646
58	$ 2,574.73	$ 1,992.51	$ 2,574.73	DP 1646
59	$ 2,587.20	$ 1,868.16	$ 2,587.20	DP 1646
60	$ 2,567.82	$ 1,928.60	$ 2,567.82	DP 1646
63	$ 1,804.05	$ 1,410.50	$ 1,804.05	DP 1646
64	$ 1,672.94	$ 1,507.07	$ 1,672.94	DP 1646
65	$ 1,810.31	$ 1,475.27	$ 1,810.31	DP 1646
67	$ 2,132.83	$ 1,480.28	$ 2,132.83	DP 1646
69	$ 1,941.77	$ 1,131.87	$ 1,941.77	DP 1646
71	$ 2,588.12	$ 1,792.32	$ 2,588.12	DP 1646
72	$ 2,552.67	$ 2,140.26	$ 2,552.67	DP 1646
73	$ 2,348.37	$ 1,890.57	$ 2,348.37	DP 1646
75	$ 1,615.30	$ 1,296.56	$ 1,615.30	DP 1646
76	$ 1,301.38	$ 1,393.15	$ 1,393.15	DP 1747
77	$ 1,737.04	$ 1,240.97	$ 1,737.04	DP 1646
79	$ 2,295.48	$ 1,619.27	$ 2,295.48	DP 1646
80	$ 2,128.00	$ 1,340.16	$ 2,128.00	DP 1646
81	$ 2,711.75	$ 1,822.43	$ 2,711.75	DP 1646
82	$ 2,375.96	$ 1,974.31	$ 2,375.96	DP 1646
83	$ 1,669.97	$ 1,371.04	$ 1,669.97	DP 1646
84	$ 1,373.28	$ 1,160.92	$ 1,373.28	DP 1646
86	$ 1,971.84	$ 2,078.13	$ 2,078.13	DP 1747
87	$ 2,384.17	$ 1,806.24	$ 2,384.17	DP 1646
88	$ 2,256.88	$ 1,460.24	$ 2,256.88	DP 1646
89	$ 2,114.40	$ 1,566.93	$ 2,114.40	DP 1646
90	$ 2,120.51	$ 1,458.86	$ 2,120.51	DP 1646
91	$ 1,893.56	$ 1,345.70	$ 1,893.56	DP 1646
92	$ 1,469.65	$ 1,239.68	$ 1,469.65	DP 1646
94	$ 2,342.11	$ 1,766.77	$ 2,342.11	DP 1646
95	$ 2,106.29	$ 1,689.13	$ 2,106.29	DP 1646
97	$ 2,025.45	$ 1,442.93	$ 2,025.45	DP 1646

Grid No.	1646 Net Returns ($/ha)	1747 Net Returns ($/ha)	MHP Directed Planting Potential Net Returns ($/ha)	MHP Directed Variety
98	$ 2,108.78	$ 1,464.51	$ 2,108.78	DP 1646
99	$ 2,247.07	$ 1,580.56	$ 2,247.07	DP 1646
100	$ 2,277.76	$ 1,776.28	$ 2,277.76	DP 1646
101	$ 2,162.82	$ 1,704.74	$ 2,162.82	DP 1646
103	$ 2,266.36	$ 2,070.80	$ 2,266.36	DP 1646
104	$ 2,117.22	$ 1,615.94	$ 2,117.22	DP 1646
Averages	$ 2,020.74	$ 1,556.36	$ 2,030.33	

Appendix 8

Year two returns over seed costs by .2 ha grid zone.