INTEGRATION OF NOVEL MANAGEMENT TECHNOLOGY FOR VERTICILLIUM WILT AND OTHER SOILBORNE DISEASES IN POTATO CROPPING SYSTEMS

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ABSTRACT

INTEGRATION OF NOVEL MANAGEMENT TECHNOLOGY FOR VERTICILLIUM WILT AND OTHER SOILBORNE DISEASES IN POTATO CROPPING SYSTEMS

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Potato production systems have been long plagued by soilborne diseases which can compromise tuber quality and yield. Verticillium wilt caused, by *Verticillium dahliae* Kleb., is a widespread disease of potatoes (*Solanum tuberosum* L.) and is an annual problem for potato growers throughout the USA. When not-treated, severely infested fields can lose marketable yield from 30-50%. The cost of pre-plant fumigation, new restrictions imposed by government agencies on the application of soil fumigants, and demand for high quality tubers has led to a renewed focus on management of Verticillium wilt in potato production in Michigan and across the USA.

Experimental field trials were conducted to determine efficacy of several commercially available fungicides applied in-furrow at-planting to decrease *V. dahliae* propagules in the rhizosphere and wilt disease in plant stems. Additional trials were conducted based on preliminary results to determine the effects of fluopyram at three application times; at-planting, at-emergence, and at-tuber initiation, for management of Verticillium wilt. At-emergence application of fluopyram or any combination of application times coupled with an at-emergence application led to a reduction in *V. dahliae* propagules in stem sap and subsequent yield increase, but had no effect on *V. dahliae* propagule levels in the rhizosphere. Finally, the effects of seven different at-planting and/or foliar treatment programs for control of Verticillium wilt were evaluated with special attention to root-lesion nematode (RLN) levels in the soil. Significantly lower levels of Verticillium wilt symptoms at the end of the growing season were seen in

treatments when compared to the non-treated control, however, no significant difference was observed in tuber yield, *V. dahliae* propagule levels in the soil, or RLN populations in the soil.

In addition to the work on Verticillium wilt, an approach integrating traditional soil sampling and GIS mapping systems was developed to analyze edaphic qualities and quantify soil microbial diversity across entire fields in order to examine correlations between yield and soil biological factors on a spatial scale. The automated geostatistical interpolation of soil properties has greatly reduced the cost and time of the statistical methods utilized in predictive statistics. The incorporation of inoculum levels along with the use of geostatistical analysis led to a methodology of sub-field management strategies based on conditional probability and binary mapping to predict where Verticillium wilt may occur based on pathogen populations and disease thresholds. Sub-field management strategies allow for more accurate management of problem areas in fields with fewer inputs. The methods presented are a novel approach to analyzing and predicting potato crop health, soil abiotic factors, and soilborne pathogens throughout an entire field with low error. Incorporation of these methods into potato integrated disease management may reduce management costs while simultaneously reducing the environmental impact of management inputs and increasing profitability.

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1.1.1 ORIGIN AND GLOBAL IMPORTANCE

The potato (*Solanum tuberosum* Linnaeus) is indigenous to the Andean highlands of South America and has been a cultivated crop for approximately 8,000 years (188). Potatoes are the fourth most important food crop grown worldwide and the most important non-cereal food crop. Potatoes are grown on every continent except Antarctica and are an important part of the diet of over 1.5 billion people across the globe (150). During the Age of Discovery (15^{th}-18^{th} centuries), potatoes were introduced to North America, Australia, Europe and Asia from South America as a valuable staple, fueling armies, improving diets, and changing economies (186). Potato tubers are low in fat, contain vitamin C, potassium, and antioxidants and are a good source of carbohydrates. Potato plants produce up to 78% more protein than rice and up to 54% more protein than wheat on equal amounts of land (5, 200). The potato is an essential dietary staple worldwide, however it's importance goes beyond the feeding of nations. For example, potato starch is used in the production of paper, adhesive and textile goods (47, 87).

1.1.2 GROWTH AND DEVELOPMENT

Potatoes are most often propagated from seed tubers which are vegetative, either as whole tubers or cut sections containing meristematic tissue known as eyes or buds (150). In many developing countries where maintenance of seed tubers is difficult, true potato seed (TPS) is used, however, as TPS progeny are a collection of genotypically different individuals which show considerable diversity in many of the important quality traits of potatoes in developed economies, TPS is not used commercially in the US (6). After a period of dormancy in cold storage, the dormant sprouts on the seed tuber respond to a thermal induced hormonal response

and resume development of meristematic leaves on the sprout initials (107). The sprouts then elongate between internodes and emerge through the soil. Throughout the growing season, the plant matures and lateral shoots with elongated internodes called stolons form from the most basal nodes of the plant below the soil (35). The terminal portion of the stolon becomes a carbohydrate sink and tubers are formed (200). Approximately 100 days following planting, the potato plant begins to die and the skins on the potato tubers begin to thicken (99). Once tuber skin has set, mechanical harvest occurs and tubers are shipped to chip manufacturers for processing directly from the field, or held in long-term storage bins in which temperature and humidity can be controlled.

1.1.3 GLOBAL PRODUCTION, PRODUCTION IN THE US, AND PRODUCTION IN THE STATE OF MICHIGAN

The global production of potato is approximately 385 million t. As of 2014, the top five potato producing countries were China, India, Russia, Ukraine, and the US producing 96.1, 45.4, 31.5, 23.7 and 20.0 million t, respectively per the Food and Agriculture Organization (FAO) of the United Nations (http://faostat3.fao.org/ browse/Q/QC/E, accessed March 9, 2016). The US is ranked 5th in total potato production worldwide (http://faostat3.fao.org/browse/Q/QC/E, accessed March 9, 2016) and average production per year from 2009-2014 was approximately 20 million t. Idaho, Washington, and Wisconsin are the top three production states according to the most recent estimations (2013) with 6.7, 4.9 and 1.3 million t produced annually (National Potato Council, 2015). Potato production in Michigan is valued around $207 million and ranks seventh nationally with approximately 0.80 million t produced annually (http://www.nass.usda.gov). Approximately 70% of the potatoes grown in the state of Michigan are using in the chip processing industry (83).

1.1.4 SOILBORNE POTATO DISEASES

According the American Phytopathological Society, there are 35 economically important bacterial, fungal, and oomycete pathogens of potato worldwide (108) which can be air or soilborne and cause damage on all parts of the plant (60). The potato crop is faced with a broad diversity of fungal, bacterial, and fungus-like pathogens that persist in association with soils. These pathogens cause diseases that affect the health of the seed piece, the growth of the crop, and tuber quality and yield. The pathogens that cause these diseases occur commonly throughout potato-producing regions of the US and Canada. The significance of the diseases they cause vary in importance, depending on the geographic location, the cultivar grown, and the intended use of the crop (169).

Several key potato pathogens originate from soilborne inoculum. Soilborne pathogens can be separated into two primary groups; those that cause disease during crop development and those that cause disease on tubers, although some may do both (78). Soilborne pathogens that affect crop development include *Rhizoctonia solani* Kühn which causes stolon canker and stem canker (55), *Colletotrichum coccodes* (Wallr.) S. Hughes which causes black dot (20), *Verticillium* spp. which cause Verticillium wilt (115), and many species of nematodes (129). *Streptomyces* spp. which cause common scab (181), *Sporangospora subterranea* (Wallr.) Lagerh which causes powdery scab (142), *Rhizoctonia solani* which causes black scurf (55), *Meloidogyne* spp. (root-knot nematodes) which cause tuber galls (129), and *Fusarium* spp. which cause Fusarium dry rot (34) are examples of soilborne pathogens that cause disease on potato tubers. One pathogen of significance is the soilborne fungus *Verticillium dahliae* Kleb. The fungal pathogen causes a disease called Verticillium wilt, also known as early die, early

maturity wilt, and potato early die (PED) (187). Verticillium wilt is arguably the most economically damaging soilborne disease in potato production (78).

1.2 Verticillium Wilt

1.2.1 IMPORTANCE OF VERTICILLIUM WILT OF POTATO

Wilt disease of potato in the US was first described in Oregon in the 1920s (138) and even then, wilt caused by *Verticillium* spp. was a major contributor to reductions in yield (138). During the 1970s and 1980s in areas with long-established potato production (North Dakota, Minnesota, Idaho) wilt developed slowly over the course of many years (170). Growers considered early maturity a "normal" situation on their land and did not realize that yield potential on their land was not being achieved (170). The recognition of Verticillium wilt in long-established areas of potato production was further complicated by the trend during the 1970s and 1980s of increasing yields due to improved cultivars, cultural practices, and improvements in crop disease management (170). In contrast to long-established potato production areas, growers in new production areas in the states of Washington and Oregon recognized the problem more quickly than those who dealt with Verticillium wilt on a chronic basis for many years (170, 185). After high initial yields were achieved on "virgin" soils, the early dying pattern developed quickly with subsequent cropping systems (170).

Verticillium wilt develops throughout a field mid-way through the growing season and then becomes severe during the period of maximum tuber bulking. A significant reduction in tuber size and total marketable yield can result with yield reductions of 10-15% and 30-50% in moderately and severely diseased fields, respectively (186). The economic impact of Verticillium wilt is significant due to the direct impact on yield and the significant cost of pre-

plant fumigation which has become routine (187). Restrictions on soil fumigants have become increasingly stringent making this management practice less attractive to growers (130). Increased restrictions of soil fumigants (50, 130) and increased tuber quality standards (186) have led to an increase of importance of Verticillium wilt in potato production.

1.2.2 *VERTICILLIUM DAHLIAE*, THE CAUSAL AGENT OF VERTICILLIUM WILT

1.2.2.1 Taxonomy

The genus *Verticillium* was first described by Nees von Esenbeck in 1816 (91, 164) and approximately 190 species have since been described (90). *Verticillium* Nees is a genus in the Ascomycota but was formerly characterized as a genus in Deuteromycota (164) due to the fact that no teleomorph has been identified for plant pathogenic species (10). Species share the characteristic *Verticillium* conidiophore that is comprised of narrow flask-shaped spore-forming cells (phialides) that are assembled into whorls (verticils) and attached along a main axis (91, 164). The type of resting structure formed is a determinant in *Verticillium* taxonomy with the three resting structures described as dark mycelium, chlamydospores, and microsclerotia (10, 90, 91, 164).

Verticillium albo-atrum Reinke & Berthold was first described in 1879 as the causal pathogen of potato wilt (90). Reinke and Berthold found that the diseased potato tissue became black or dark brown due to a blackening of the fungal hyphae. Furthermore, septation in the hyphae increased and short cells were formed, slowly increasing in width, and black masses of varying sizes and shapes appeared in the diseased tissue (91). Klebahn first isolated *Verticillium dahliae* from Dahlia sp. cv. Geiselher in 1913 (10). The principle point of difference between *V. albo-atrum* and *V. dahliae*, per Klebahn, was that *V. dahliae* formed microsclerotia. These microsclerotia arose from irregular multilateral septation, and budding of cells of one hypha or of

a number of contiguous hyphae (91). Controversy arose from Klebahn's proclamation of a new species. Aside from the production of microsclerotia, which *V. albo-atrum* does not produce, the two species differed in geographic distribution, optimal pH, optimal temperature range, host range, conidiophore shape, and conidial size (90, 91, 164, 195). In recent years, the species differentiation was confirmed using molecular markers (10, 58, 90, 145, 163, 175).

1.2.2.2 Host range

Verticillium dahliae and *V. albo-atrum* cause vascular wilt in more than 200 dicotyledonous plants (2, 164, 191). Host range includes important agricultural crops such as potato, tomato, lettuce, spinach, cotton, olive, and strawberries (164). *Verticillium dahliae* also colonizes numerous weed species which can lead to increased dissemination of the pathogen (56, 218). *Verticillium dahliae* is the primary cause of Verticillium wilt of potatoes in Michigan; however, *V. albo-atrum* can cause wilt at conducive temperatures. *Verticillium albo-atrum* grows optimally at about 20°C, whereas optimal growth of *V. dahliae* occurs at about 27°C. In areas where Verticillium wilt is more severe, *V. dahliae* is the dominant species. In arid climates, *V. dahliae* is predominant, whereas in cooler climates *V. albo-atrum* is often observed (41).

1.2.2.3 Fungal morphology

Verticillium dahliae produces vegetative hyphae that are 2 - 4μm in diameter, septate, hyaline, and thin-walled (195). The verticillate (whorled) conidiophores are branched, septate, hyaline, and 80 – 160 μm in diameter on which one to five phialides (flask-shaped projections) are formed (195) however, phialides are frequently borne directly from long horizontal hyphae, and are straight to slightly curved with a septum at the base, and produce single-celled conidia in a wet mucilage (164, 195). The first formed conidium is holoblastic and each successive conidium is enteroblastic (4). Conidia are continuous (rarely have one septate), hyaline, elliptical

and approximately 3 - 5.5μm x 1.5 - 2μm in size (164, 195). *Verticillium dahliae* forms clusters of thick-walled, heavily melanized cells (30 - 60μm in diameter) which separate as discrete bodies from the parent mycelium (164, 195). These structures are called microsclerotia. The number of microsclerotia formed is directly proportional to the number of hyphal fusions (13). Microsclerotia are the primary inocula of *V. dahliae* and can survive in the soil for 10-15 years (41, 76, 141, 164). The microsclerotia remain dormant in the soil until host root exudates stimulate germination (76, 141, 144, 191). Root colonization occurs following the germination of the fungus and penetration of the root cortex leads to an invasion of the xylem (9). Once the fungus reaches the xylem, conidia are produced and transported systemically throughout the host (205). As the host senesces, microsclerotia are produced and are incorporated back into the soil from decaying plant material.

1.2.2.4 Vegetative compatibility groups

There is no known telelomorphic stage of *V. dahliae* (10) yet genetic recombination can occur when fungal hyphae of the same or different species undergo anastomosis to produce a heterokaryon (190). This anastomosis determines vegetative compatibility (VG) and relies on the matching of specific loci in both individuals (124). Fungi use VG as mechanisms of self-recognition (147). In 1979, Vegetative Compatibility Groups (VCGs) were used to split 19 separate isolates of *V. dahliae* isolated from cotton into four groups (172). Expanding on this work, 250 *V. dahliae* isolates were separated into four VCGs with most isolates falling into VCG4. VCG4 was subsequently divided into two sub-VCGs, VCG4a and VCG4b, based on pathogenicity experiments performed on potatoes (97). All four VCGs of *V. dahliae* will colonize potato, yet most pathogenic infections occur from *V. dahliae* isolates belonging to VCG4 (41).

1.2.3 INTERACTION WITH ROOT-LESION NEMATODE

Symptoms of Verticillium wilt are often exacerbated in the presence of root lesion nematodes (*Pratylenchus* spp.) (19, 184). *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven, is most commonly associated with *V. dahliae* but at least four other *Pratylenchus* spp. have been known to co-occur (19, 134, 178). *Pratylenchus penetrans* can survive in the soil in the absence of a host, but similar to *V. dahliae*, has a wide host range (186). *Pratylenchus penetrans* can directly cause significant losses to potato, but the most severe losses result from the synergistic interaction with *V. dahliae* (62, 113, 134, 178, 184). Feeding injury by *P. penetrans* does not provide a direct avenue of entry for *V. dahliae* into the root system (19). While the exact mechanism for the synergism is not known, one hypothesis is that when *P. penetrans* feeds, it has a physiological effect on the potato root, perhaps increasing the amount of root branching, and subsequently more exposure to *V. dahliae* in the rhizosphere (19, 170). Alternatively, nematode feeding may increase root exudation and increase rhizosphere width, which in turn may increase the number of root contacts with microsclerotia and result in a higher percentage of root tips infected (19). *Verticillium dahliae* acting synergistically with *P. penetrans* has been shown to increase losses by 25% in comparison to when *V. dahliae* infects plants in the absence of the nematode (184).

1.2.4 LIFE CYCLE AND EPIDEMIOLOGY

The seasonality of *Verticillium dahliae* is characteristically monocyclic between germination of microsclerotia and formation of daughter microsclerotia, and due to the ability of microsclerotia to survive for extended periods of time in the soil, disease caused by the pathogen is polyetic (127). *Verticillium dahliae* can be introduced to a production field via infected seed tubers (161, 170), or on animals, equipment or water movement (185). Upon the introduction of

the fungus into a new field, little disease is observed but continuous planting of susceptible crops can eventually build up the amount of inoculum in the soil, to a threshold that may result in Verticillium wilt and reduce yield (151, 185). However, new inoculum is relatively unimportant compared to inoculum naturally present in soil (48, 49). When a susceptible host is present, exudates from the roots of that host stimulate the microsclerotia to germinate and root colonization begins (44). Hyphae then enter the root tissue via direct penetration (19, 134). Once inside the root tissue, the fungus enters the root cortex and begins producing conidia which then spread throughout the plant through the xylem (41, 78, 170, 185-187, 191, 198) (Figure 1.1). However, successful vascular infection is not guaranteed when cortical tissue is colonized as only 0.02% of cotton (*Gossypium hirsutum* Linnaeus) root penetration events end in systemic infection (65). Epidermal and cortical cells of the root respond rapidly to the presence of pathogens by the deposition of material similar to lignin over the inner surfaces of cell calls, and around penetrating hyphae to form lignotubers. Penetration hyphae pass through the cell wall and into the newly formed deposits, but continued accumulation around the hyphae of vesicular material extruded through the host plasma membrane results in formation of lignotubers. Eventually the hyphal tip enclosed in the lignotuber undergoes lysis and no further development occurs (201). Formation of lignotubers in potato root tissue has been shown to stop direct penetration of some hypha, but may be unable to halt penetration of multiple hyphae at different points of infection (166, 167). Additionally, formation of tyloses within the xylem may reduce the effective radius available for transport. If tyloses are formed rapidly, well before the spread of the pathogen, they will confer resistance to the host, but if they are formed after the

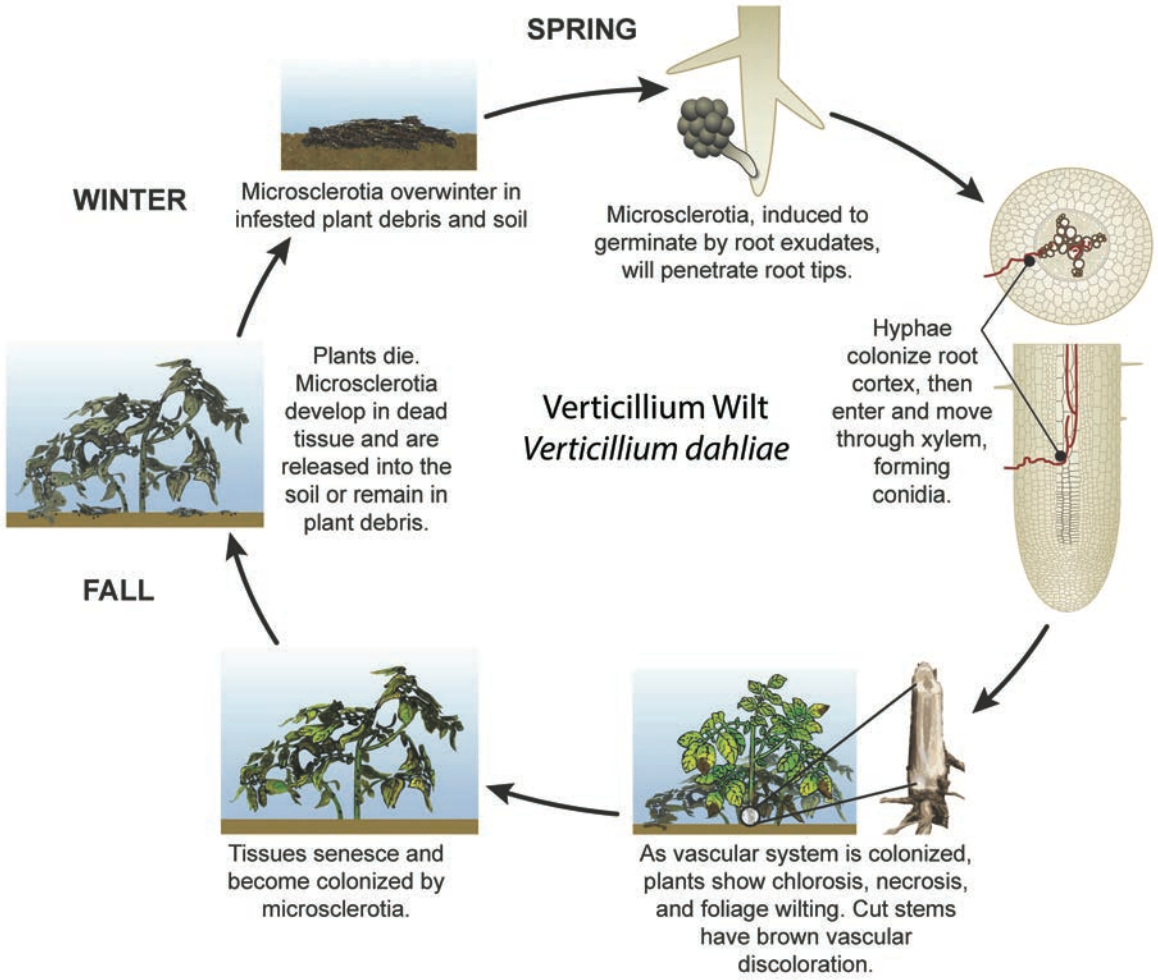

Figure 1.1. The disease cycle for Verticillium wilt shows how direct penetration of the root cortex leads to vascular blockage and plant death. The dead plant tissue serves as an overwintering structure for new microsclerotia. Image is reproduced with permission, from Michigan State University Extension Bulletin E-3207 © 2015 Michigan State University. All rights reserved

colonization of the pathogen, they will themselves be instrumental in bringing about more wilt symptoms (139). Systemic colonization of the plant occurs by growth within the xylem vessels and by formation of conidia which are transported with the xylem fluid and germinate to form new infections at remote sites (185). Following systemic colonization, symptom development occurs as a result of toxin production and vascular dysfunction (185). Penetration by *V. dahliae* and subsequent root cortex infection stops apical cell growth and causes premature root maturation (134). As the host plant dies, the entire plant is colonized and new microsclerotia are formed within the dying tissue (185). The microsclerotia are eventually incorporated into the soil, increasing the level of inoculum in successive growing seasons (192).

1.2.5 SYMPTOMS OF VERTICILLIUM WILT

Verticillium wilt of potato is characterized by a general decline of plants four to six weeks earlier than normal maturity (92, 134, 170, 185-187). Overall plant wilting, foliar chlorosis and necrosis, vascular discoloration, and premature vine death are some of the commonly observed symptoms in potato plants affected by Verticillium wilt. Symptoms begin at the base of the plant and begin to move upward in three stages as the season progresses (18, 170, 185). In the first stage, or latent stage, *V. dahliae* is present in the stems, but no symptoms or gas exchange effects are detectable (18). In the second stage, or local stage, there is an acropetal procession of wilting, chlorosis, and senescence of leaves or parts of leaves, but young leaves have normal gas exchange and no visible symptoms (18). The third stage, or system phase, continues the acropetal defoliation into the young leaves and total plant wilt, chlorosis, and necrosis can be seen (18). The pathogen grows throughout the plant via the plants vascular system which can result in a unilateral, one-sided wilting of the plant, based on individual vascular bundles becoming colonized at different times (41, 200). A slight brown discoloration

of conducting tissue by the end of the growing season is associated with infection of *V. dahliae* (41, 186, 187, 200). In severe cases, tuber stem-end discoloration can occur (41, 200). Plants infected with *V. dahliae* have a tendency to die erect, which helps to distinguish Verticillium wilt from other wilt diseases caused by pathogens such as *C. coccodes* and *Fusarium* spp. (185).

1.2.6 MANAGEMENT OF VERTICILLIUM WILT

Soil type, crop rotation, irrigation, and other management practices have an effect on the level of *V. dahliae* primary inoculum (microsclerotia) in the soil (14, 57, 96, 202). The amount of damage caused to potato plants by *V. dahliae* is directly correlated with the amount of the pathogen in the soil (170), however temperature, moisture, fertility, organic matter, and host susceptibility are factors that contribute to disease severity in a given year (14, 39, 151, 153). An economic threshold of between 5 and 30 colony forming units (CFUs) or propagules per gram of soil (CFU/g) has been established under industry standard growing conditions (38, 170), however, as little as 3 CFUs in 5 grams of soil were sufficient to cause Verticillium wilt in the hot and arid conditions of Israel (14). Soil levels of *V. dahliae* have been found to have a direct correlation with stem colonization of the potato cultivar Russet Burbank (151) and an inverse relationship has been observed between *V. dahliae* CFUs in the soil and yield when growing cultivars Norgold and Norchip in Colorado (153). In Idaho, direct correlations between irrigation method and fertility, and yield have been observed (39). Accurate quantification of *V. dahliae* propagules in the soil is necessary to inform management practices (131, 132, 146). Until the development of molecular quantification methods, microsclerotia quantification was done by plating varying quantities of soil onto a *V. dahliae*-selective media, waiting for microsclerotia to germinate, then manually counting the number of colony forming units (8, 22, 80, 101). Though research on these methods has been extensive, the accuracy of this technique has been debated

(73, 126) because these methods use direct DNA-isolation which presumes that a known, equal amount of DNA is present in each microsclerotium and that a fixed fraction of the DNA is viable. In recent years, several methods which utilize polymerase chain reaction (PCR) assays as well as quantitative polymerase chain reaction (qPCR) assays have been employed to replace conventional soil plating assays (7, 16, 131, 132, 165, 214). Extraction of DNA from the melanized microsclerotia, viability of the sclerotia from which DNA is recovered, and inhibitors found naturally in the soil remain the greatest obstacles to the effectiveness and accuracy of these molecular methods (16, 33).

Complete elimination of *V. dahliae* from the soil is unrealistic therefore, consistent management of Verticillium wilt is not yet possible (115, 170, 186, 187). Management strategies for Verticillium wilt caused by *V. dahliae* are similar across the pathogen's herbaceous host range and include soil fumigation, soil solarization, use of disease free seed, crop rotation, cultural adjustments, and planting of resistant cultivars. Though *V. dahliae* can survive in the soil for 10-15 years, crop rotation with a non-host maintains levels of *V. dahliae* without adding new propagules to the soil for infection in subsequent years (119).

Soil fumigation using methyl bromide was an effective management strategy for decreasing *V. dahliae* propagules in the soil until it was phased out in the mid 1990s (50, 63, 133, 179). In potato production systems, the use of metam sodium as a soil fumigant proved somewhat effective at reducing the number of microsclerotia in the soil, however, this method of management is cost prohibitive and has unfavorable environmental effects (38, 130, 170, 186). Metam sodium also will likely be phased out, as the US Environmental Protection Agency (EPA) has already begun to restrict the use of this chemistry and thus will limit the availability of this management strategy for many growers (130).

Soil solarization uses the heat energy from the sun to manage soilborne pathogens and was often used by ancient Indian civilizations to disinfest soil (177). The first modern application of soil solarization was seen in Israel's arid climate in 1976 when soil was artificially inoculated with *V. dahliae* (103). The soil was covered with polyethelyne sheets and the upper 25 cm of topsoil was found to be pathogen-free after 14 days (103). From 1976-1986, soil solarization studies were done in 24 different countries and significant reductions in Verticillium wilt were observed (40, 104, 105, 173, 197). Though effective, this management practice is not widely used in commercial potato production in the US due to its high cost and labor-intensive implementation (104).

Green manures, liquid animal manure, and meat and bone meal are organic soil amendments that have been used to successfully decrease levels of *V. dahliae* in the soil (28, 43, 123, 156, 203). These types of organic amendments when applied to the soil kill microsclerotia by promoting the accumulation of ammonia and nitrous acid (27, 28, 123, 203). Liquid animal manure has been shown to reduce microsclerotia germination of *V. dahliae* in both acidic and basic soil, although the mechanisms of inhibition differ from acidic to basic soil conditions (27, 28, 123). Bone meal amendments to the soil have also been shown to reduce germination of microsclerotia, but effects were only seen in sandy soil (203). Previous studies have shown that *Brassicaeceae* crops (broccoli, cabbage, cauliflower, turnip, radish, canola, rapeseed, and various mustards), sorghum Sudan grass, oats, corn, and winter peas used as green manures have some effect on levels of *V. dahliae* in the soil (43, 69, 117, 120, 155, 156). Field trials conducted in Idaho and Oregon with the potato cultivar Russet Burbank showed significant increases in marketable yield and reduction in Verticillium wilt severity following the incorporation of various green manures (43, 155, 156). Increases in marketable yield were not attributed to

reduction in inoculum alone but also to changes in the diversity of the rhizosphere brought on by these amendments. Though microsclerotia density was decreased, root infections were not (155, 156). While organic soil amendments have shown promise, the variability associated with this management practice due to soil type and climate, will limit its integration into commercial potato production (155, 156).

Irrigation strategy, fertility management and tillage type are cultural practices that can impact the severity of Verticillium wilt (24, 25, 42, 202). Before tubers begin to form, keeping the soil slightly drier than normal may help in limiting the germination of microsclerotia (24, 25). However, this management practice may leave young tubers more susceptible to other soilborne pathogens such as *Streptomyces scabies* (Thaxt.) Waksman and Henrici which causes potato common scab (1). Later in the growing season, after tubers have initiated and begin bulking, maintaining well irrigated plants to decrease stress may help diminish vascular wilt symptoms (42). Irrigation method also may have an impact on Verticillium wilt severity. Compared to overhead sprinkler irrigation, furrow-irrigation has been shown to increase the incidence and severity of Verticillium wilt, possibly due to availability of nitrogen in the root zone (39, 42). In addition to irrigation, fertility management should targets reducing plant stress which may subsequently limit root colonization by the pathogen (39, 42). Previous studies, focused on the relationship between nitrogen availability and root colonization of *V. dahliae* on the potato cultivar Russet Burbank, showed a direct correlation of nitrogen to wilt development, tuber size, and marketable yield (39). Additionally, potassium and phosphorus may diminish wilt symptoms when adequate levels are available to the plant (39). Other cultural management techniques such as tillage methods which bury crop debris, or completely inverting the soil (moldboard plow) have been shown to reduce CFUs in the infection zone of the soil (75, 202).

Though chemical and cultural control methods can aid in decreasing propagules of *V. dahliae* in the soil, they do not adequately reduce propagules to a number that is low enough to prevent infection. Most commercial grown potato cultivars do not possess innate resistance to Verticillium wilt (186, 187) but when resistance is present it is stable and heritable (29, 86, 88, 94). Several wild *Solanum* spp. have resistance to Verticillium wilt (26, 30) as well as several breeding lines from the US (31, 93). In the past there have been commercially available cultivars, but many are no longer available due to the emphasis on yield and tuber quality for processing.

Though many proposed management strategies exist for reducing Verticillium wilt in potato, the goal of complete elimination of the disease has not been attained. Without reliable management or control practices for Verticillium wilt, the decision for growers is dictated by the cost of management compared to the potential benefit to their crop. As stated previously, levels of CFU in the soil have a direct correlation with amount of disease, so accurate quantification of soil microsclerotia must be accomplished before more effective management strategies are achieved. Recent technological advancements have allowed researchers to not only decrease the time it takes to quantify microsclerotia (16) but to quantify and visualize risk levels for entire fields based on a relatively low number of sample points using geographical information systems and geostatistical analysis (199). Furthermore, studies have shown that organic amendments (e.g. green manures) may have an indirect effect on Verticillium wilt via the promotion of "beneficial" soil microbes with pathogen inhibitory activity (217). Identification of "beneficial" soil microbial populations and their spatial relationship to *V. dahliae* CFUs may aid in decision making for management of Verticillium wilt.

1.3 Soil Microbial Communities

1.3.1 SOIL HEALTH AND QUALITY

The last two decades have seen an increased interest in understanding the effects that modern, large-scale agricultural practices have on soil microbial communities (45, 66, 79, 98). This interest and the subsequent research has been enabled by technological developments which have advanced knowledge of the soil microbial ecology and the link between above ground and below ground biodiversity (12). These links operate as a feedback system in which plants are influenced by the soil microbial community and vice versa depending on the host plant species (112). Soil microbial communities are sensitive not only to the species of plant occupying the soil, but also to a variety of ecological influences on the plants (45, 66, 98). The ecological influences include growth phenology, soil fertility, insect defoliation of aboveground biomass, total plant net primary productivity, carbon input chemistry and timing, as well as plant community diversity (54). Some rhizosphere microbes carry plasmids with genes responsive to plant exudates, suggesting an intimate functional connection (219). It is clear that plant biology affects microbial communities in many ways, but the effects of these variations on plant function are not well documented (54). Feedback between plants and microbes is perhaps best expressed in the sequence of signals that result in the establishment of symbionts and mutualists. Typically, the plant initiates a positive molecular feedback loop that results in enhanced nutrition, thus increasing fitness for both the plant and the microorganisms. A classic example of this mutualistic relationship is the role of arbuscular mycorrhizas symbiosis for plants in acquiring phosphorus (P). The arbuscular mycorrhizas provide a very effective pathway by which P is scavenged from large volumes of soil and rapidly delivered to cortical cells within the root, bypassing direct uptake. At the same time, plants provide all the organic carbon requirements of

the fungi (196). Not only do plant hosts and their symbionts communicate through a system of molecular and genetic feedbacks, but plants appear to be able to use similar molecular feedback systems to deter bacteria that are non-beneficial or pathogenic (54). The impacts of these feedback systems are vital for plant health therefore it is imperative that applied, extension-based science assesses the impact of agricultural practices on soil microbial communities.

In simplest terms, soil quality is "the capacity of soil to function" (102). This definition, based on function, reflects the living and dynamic nature of soil (102). Soil quality can be conceptualized as a three-legged stool, the function and balance of which requires an integration of three major components—sustained biological productivity, environmental quality, and plant and animal health (102). Soil quality and soil health may be used interchangeably but the term soil health has been preferred because it portrays soil as a living, dynamic system with functions mediated by a diversity of living organisms that require management and conservation (46).

1.3.2 AGRICULTURAL EFFECTS ON SOIL HEALTH

The quality of a soil includes an inherent component, determined by the soil's physical and chemical properties within the constraints set by climate and the ecosystem (46). Past management of agriculture and other ecosystems**,** however, has substantially degraded and reduced the quality of many soils throughout the world (162, 189). Mechanical cultivation and the continuous production of row crops has resulted in the physical loss of soil, displacement of soil through erosion, large decreases in soil organic matter content, and the concomitant increase of carbon dioxide released into the atmosphere (85). Furthering the issue, the human population is expected to double over the next century which further threatens the accelerated degradation of soils and other natural resources, coupled with a necessary global increase in agricultural food production to support the increased inputs such a global system demands (67).

Soilborne diseases of potato are difficult to control (95), especially without adequate knowledge of the distribution of disease propagules. Past and present management of potato soilborne diseases includes broad-spectrum chemicals such as methyl bromide and metam sodium. These products are non-selective, affecting the whole microflora: pathogenic, beneficial or mutualists (95). Newer and sustainable soilborne disease management strategies such as biological fumigants, green manures, crop rotation, and organic matter amendments have some efficacy in reducing soilborne disease, yet the impact of such strategies on soil microbial communities is not well understood and inconsistent (143, 152). In general, a better understanding of the keystone bacterial communities found in potato production systemswill aid in the evaluation of the soil amendments used to manage soilborne diseases as well as an increase knowledge of overall soil microbial diversity, especially in fields plagued by intense disease severity (215).

1.3.3 IMPORTANCE OF SOIL MICROORGANISMS

Soil microorganisms are an important aspect of soil fertility because of their involvement in the cycling of nutrients such as carbon and nitrogen (70, 74, 135, 223), which are required for plant growth (82). Soil microorganisms decompose organic matter into suitable forms of chemicals which are then used by plants as food. Furthermore, certain rhizobacteria, mycorrhiza, Protista, and archaea have been shown to exhibit a close association with plants (11, 17, 52, 53, 68, 207) and in potato cropping systems the rhizosphere is dominated by genera within the Proteobacteria, Firmicute, and Actinobacteria phyla (215). Such bacteria have been shown to promote plant growth and antagonize pathogens, through the production of phytohormones for example, thus aiding in plant defense (52, 61, 106, 216). An increasing body of evidence also signifies the importance of the root microbiome which consists of the entire complex of

rhizosphere-associated microbes, their genetic elements and their interactions, in determining plant health (15).

The impact of the root microbiome on plant health is evident most clearly in disease-suppressive soils (109, 118, 168, 194). The microflora of most soils is starved (111) and as a result, there is a battle in the rhizosphere between the microorganisms that compete for plant-derived nutrients (176). Soilborne pathogens tend to grow saprophytically in the rhizosphere (15) to reach their host or must achieve sufficient numbers on their host before they can infect host tissue and effectively escape the rhizosphere. Natural soil, or non-amended soil, has the ability to suppress a pathogen to a certain extent (137) and this phenomenon, known as general disease suppression, is attributed to the total microbial activity (15).

1.4 Geostatistics in Agriculture

1.4.1 A BRIEF HISTORY OF PRECISION AGRICULTURE AND GEOSTATISTICS

Precision agriculture has been implemented by farmers since the early days of agriculture (158). Subsistence farmers worked on small patches of land and divided their landholding into smaller areas to grow crops where the conditions were most suitable (158). The work done at the Rothamsted Research Station in the late 19th century by John Lawes and Joseph Gilbert was, in some aspects, geared toward precision agriculture through their assessments of different combinations and amounts of crop nutrients and crop varieties (122). In the 1920s, Robert Fisher developed a series of statistical tools used as a foundation for most small-plot experiments and his tools along with statistical methods which address problems associated with slopes and systematic differences in soils, such as Latin-square design or the use of replication blocks, have been useful in decreasing the effect of spatial variability and lack of heterogeneity in fields (37).

However, none of these tools are particularly useful for studying spatiel field variability of nutrients, weeds, insects, soilborne pathogens, seeding rates, or other management inputs (37).

The first known recommendation for soil sampling was based on a 12.5 ha field and advised growers to sample at a depth of 15 cm and analyze on a 0.4 ha grid, with additional sample cores to a depth of 30 cm, was brought on by concern of acidic fields in the late 1920s (125). Until the early 1960s the typical soil sample was a composite sample taken from many points throughout the field (140). Although researchers familiar with spatial variability of crop nutrient included cautions to only include relatively uniform, similar soils in a composite sample, sample cores were routinely taken from multiple areas of the field boundary rather than the soils within them (140). These initial soil sampling patterns were based on a philosophy of unbiased sampling and the lack of instruments such as global positioning satellites (GPS) receiving devices. Growers were discouraged from taking samples from unusual areas or in a random manner across the field because of this unbiased sampling approach.

In the early 1960s, Georges Matheron introduced the statistical sub-field discipline of geostatistics (136). The development of geostatistics resulted from the need for a methodology to evaluate the recoverable reserves in mining deposits (72). The approach was based on principles outlined in the thesis work of Danie Krige, an engineer working on gold mining spatial problems in South Africa (114). In geostatistics, priority was given to practicality which is why it is applicable across many disciplines including mining, petroleum, oceanography, hydrogeology, remote sensing, environmental science, and soil science (72). From the time of its conception, geostatistics was used as a means to describe spatial patterns and interpolate the value of an attribute of interest at non-sampled locations (72). Since soil sampling or any sampling within a farm field only identifies the small area from which cores, plants, plant parts, or measurements

were taken, the vast majority of areas within the field are unknown from the observed values (37). Therefore, the values from non-sampled areas of the field must be estimated or interpolated using one of many equations available (100).

1.4.2 CURRENT STATE OF GEOSTATISTICS IN PRECISION AGRICULTURE

Geostatistics and precision agriculture work in concert. Large amounts of georeferenced data at intervals small enough to adequately resolve variations are required for geostatistical analysis and enable reliable variograms to be computed (158). Current mapping techniques are limited by a lack of understanding of the geostatistics necessary for displaying spatial variability of crops and soil and an increased knowledge base in geostatistical methods should improve interpretation of precision agriculture data according to the National Research Council in 1997 (149). Geostatistical methods are used to model spatial patterns using variograms which in turn are used to interpolate values between measured values to produce a predictive map of soil properties (158). In geostatistics, it is crucial to select best-fit functions because this choice affects all subsequent analyses (213). Kriging, a geostatistical method (named for Danie Krige) for interpolation of sparse data for random spatial processes, is currently used by many disciplines, including precision agriculture, in which spatial prediction and mapping are required (158). However, an agronomist's lack of knowledge and training in agricultural geostatistics is an obstacle to the broad-scale adoption of precision agriculture (23).

1.4.3 THEORY OF GEOSTATISTICS

Geostatistics as it is known today has developed from Georges Matheron's coherent foundation of Danie Krige's empirical observations (158). Most properties on, above, or beneath the Earth's surface are continuous, but in general the only economical option is to measure properties at a finite number of places to estimate, or predict, the rest of the points in a spatial

context (213). Furthermore, the spatial variation of those properties is so complex that Matheron developed an alternative approach to the traditional deterministic one for the analysis of those properties (158). The principle reason for geostatistics is to estimate or predict non-sampled values without bias and with minimum error and to deal with properties that vary in ways that are unbiased for the system and at all spatial scales (213). Geostatistics can never provide complete information, but given the data, in can enable researchers to estimate the probabilities that true values exceed specified thresholds and in some situations the conditional probabilities of exceeding thresholds are as important as the estimates themselves (213).

An additional feature of the environment is that at some scale the values of its properties are positively related, or autocorrelated (213). The first law of geography states that everything is related to everything else, but near things are more related than distant things (206). Places closer to one another tend have similar values for a given property, whereas ones that are farther apart, on average, differ more. This is intuitive, but geostatistics expresses this intuitive knowledge quantitatively and then uses the concept for predictive measures (213)

Modern geostatistics assumes that the variable of interest is a random variable. This implies that at each point, $\mathbf{x}$, in space there is a series of values for a property, $Z(\mathbf{x})$, and the one observed, $z(\mathbf{x})$, is drawn at random per some law, from a probability distribution (72, 136, 158). At $\mathbf{x}$, a property $Z(\mathbf{x})$ is a random variable with a mean and a variance. The set of random variables, $Z(\mathbf{x_1})$, $Z(\mathbf{x_2})$,…, is a random process, and the actual value of Z observed is just one of any number of realizations of that process (72, 136, 158). The fact that the values of regionalized variables near to one another tend to be autocorrelated is used to describe the variation of the underlying random process (72, 136, 158). Therefore, spatial covariance can be estimated to describe the relationship between pairs of points [Equation 1.1 (158)]. There can only be one

realization of Z at each point, so the spatial covariance is unsolvable because the means are unknown (72, 136, 158). Ordinarily multiple realizations are required to infer properties, but by using the assumption of ergodicity, just one realization (vector) is sufficient to make reliable assessments about properties (222). By making this assumption of ergodicity, the spatial averages are used in lieu of probabilistic or ensemble averages (222). An issue with this is that the observed data cannot be used to check the validity of the ergodicity assumption (148).

The main purpose of geostatistics is to develop models for the correlation structure of the observed data (51). The concepts of stationarity and isotropy provide theoretical underpinnings for modeling the local source of variability (51). The assumption of stationarity states that certain attributes of the random process are the same everywhere (158). Stationarity assumes that the mean and variance of the spatial process $Z(\mathbf{x})$ do not depend on the exact locations of $\mathbf{x_1}$ and $\mathbf{x_2}$, the mean is assumed constant and the variability only depends on the separation vector $\mathbf{x_1}$-$\mathbf{x_2}$ (51). If the spatial variability for a fixed distance in all directions is the same, then the spatial process is isotropic (51). This applies to any pair of points $\mathbf{x_i}$, $\mathbf{x_j}$ separated by the lag denoted as $\mathbf{h}$ ($\mathbf{h}=\mathbf{x_i}-\mathbf{x_j}$). Since the spatial covariance is a description of the relationship between pairs of points, the covariance is a function of the lag and is denoted by $C(\mathbf{h})$. It is the autocovariance function—auto because it represents the covariance of Z with itself (213). Autocovariance describes the dependence between values of $Z(\mathbf{x})$ with changing lag (158). The autocovariance depends on the scale on which Z is measured; therefore, the autocovariance is often converted to the dimensionless autocorrelation $\rho(\mathbf{h})$ which is done by dividing the autocovariance at lag $\mathbf{h}$ by the covariance at lag $\mathbf{0}$ (158).

In reality, the mean appears to change across a region and the variance will appear to increase indefinitely as the extent of the area increases and because of this there is no value for

the assumed mean and covariance cannot be defined (158, 213). The solution to this is known as the weaker intrinsic hypothesis of geostatistics which states that though the general mean might not be constant, it will be for small lag distances, so the expected differences will be zero (72, 136, 158, 213). Furthermore, by replacing the covariance with the variance of differences as measures of spatial relations, which, like the covariance, depends on lag and not absolute position, the equation becomes a measure of half of the variance of a difference at a lag. This is referred to as the semivariance and denoted as $\gamma(\mathbf{h})$. The semivariance is the variance per point when the points are considered pairs and as a function of **h** it is the semivariogram, or more simply, the variogram (51, 72, 136, 158, 213).

1.4.3.1 The variogram

Unlike the autocovariance and autocorrelation functions, which are measures of similarity, the experimental variogram $\gamma(\mathbf{h})$ measures the average dissimilarity between data separated by vector **h** (72). It is computed as half the squared average difference between the components of every data pair at lag **h** (72). The experimental variogram is obtained by changing **h**. The larger the semivariance at distance **h**, the larger the dissimilarity between the values separated by distance **h**. The typical variogram will increase with distance until reaching a certain point where it will stabilize and the value at which the variogram stabilizes is called the sill (72). The small values of $\gamma(\mathbf{h})$ at short lag distances show that the values of $Z(\mathbf{x})$ are similar, but as the lag distance increases they become increasingly dissimilar on average and a variogram with a monotonic increasing slope indicates that the process is autocorrelated (72, 158). The distance of **h** at which the sill is reached is called the range and an observation separated by distances larger than the range are not correlated to each other (72). Most environmental variables vary in a spatially continuous way therefore $\gamma(\mathbf{h})$ is expected to pass through the origin

at **h=0** (72, 158). However, the variogram will often approach the ordinate at some positive value as **h** approaches **0**. This is often referred to as the nugget effect or the nugget variance (72, 158). Two main causes of nugget effect are variability existing at distances less than the smallest value of **h,** and errors in sampling (72, 100). The accuracy of the variogram calculations is proportional to the number of data points available. In general there are three rules to follow in order to ensure proper variogram estimation: 1) For each computed value of the variogram, the number of pairs should be greater than 30; 2) The region of interest on the fitted variogram is positively sloped section of the graph (from the nugget to the sill) and should be represented by three to four values; and 3) The maximum lag should be limited to one half of the extreme distance in the sampling area (84, 157)

1.4.3.1.1 Variogram modeling

The experimental variogram estimates the underlying variogram, which is a continuous function, as a set of discrete points at particular lag intervals (158, 213). To describe the spatial variation, a model must be fitted to experimental values, the model must be conditionally negative semi-definite (213) so that it will not give rise to negative variances when random variables are combined, and the function must be able to represent the variogram features such as the nugget, sill, and range (158). There are many models which satisfy the conditions listed above but the most commonly fitted models are the exponential, spherical, and Gaussian models (72, 158, 213). Fitting models is often challenging because the accuracy of the semivariances varies and the experimental variogram might fluctuate considerably from point to point (158). Finding a suitable model for the data is fundamental in geostatistics because the model will affect the subsequent analysis. A weighted least squares approach is used because it takes into

account the accuracy of individual semivariances and the residual sum of squares calculation is used to assess the accuracy of the model (158).

1.4.3.2 Interpolation of the data

Interpolation is used to convert a sample observation into a different representation, such as a contour map that can be used to show changes in values across a surface (154). In most interpolation procedures, the predicted values at non-sampled locations can be viewed as a weighted average from the surrounding sampled locations (116). How the weights are determined and the values of those weights depend on the type of interpolation procedure used.

Kriging is often known as a best linear unbiased estimator (BLUE). It is linear because the estimated values are a weighted linear combination of the available data, unbiased because the mean error is 0, and best because it minimizes the variance of the errors (71, 81, 174), Kriging is the geostatistical method of interpolation of sparse data from random spatial processes. Most features of the environment can be measured at any of an infinite number of places, but sampling every point in space is not economical nor feasible (159, 160). Aside from Kriging, several other mathematical methods of interpolation are available including, Thiessen polygons, splines, triangulation, least-squares polynomials, and inverse distance weighting (IDW). Comparisons of the different methods of interpolation have shown that Kriging often performs optimally and that Kriging overcomes many of the shortcomings of the mathematical methods of interpolation by considering the way a property varies in space (121, 158). Additionally, Kriging provides errors for the interpolation, which the mathematical methods cannot. Kriging is a local weighted moving average of the observed values of a random variable within an area in space (32, 158). Ordinary Kriging is the most robust method and is most often used (100).

1.4.4 USING GEOSTATISTICS TO STUDY AGRICULTURAL PESTS

Weeds, insects, nematodes, and soilborne pathogens vary in intensity within a field (59, 171, 180). These pests vary in space from zero up to numbers which can impact yield. With the advent of GPS and variable rate pesticide application, growers have the ability to control pests selectively, where they occur (3). Simultaneously, society has become increasingly concerned with the potential impacts of excess pesticide application to the environment (211). Using geostatistics, agronomists interpolated maps of sample counts and scores of infestations to precisely map levels of infestation across the entirety of a field of interest. For example, weed scientists in Germany have developed an automatic weed detection system using digital image analysis, computer based decision making an GPS controlled path spraying in cereals, maize, winter rape, and sugar beet (64). Despite this research, there has been little practical use of the technology (36, 77). The lack of implementation of this technology, in large part, is due to the perceived risk of applying herbicide doses lower than those recommended by the manufacturers, though data to support this perception is limited (21). The only effective way of mapping for preemptive local control is to count weeds at the seedling stage or count seeds in the soil and use geostatistics to interpolate densities throughout the field (211).

Like weeds, plant-parasitic nematodes tend to be distributed in patches (183). Unlike weeds, the presence of plant-parasitic nematodes becomes obvious only after infection and symptoms are clearly manifested, by diseased plants or poor yield if soil sampling is not done prior to the growing season (211). Effective control of nematodes therefore depends on knowledge of where the nematodes are prior to planting. Fumigation is the most common method of nematode management (210) but is costly and can have environmental impacts (204). Therefore chemical companies have responded by offering packages whereby they estimate

nematode densities by sampling in large blocks within fields and then treating (or not) the field with nematicides on a block by block basis (193). Several studies have been done which show the practicality of using geostatistics to analyze and interpolate parasitic nematode populations in agricultural fields (182, 208, 209, 212, 220). However, much like in weed science, practical and large scale application has been limited by the economics of site-specific application (220).

1.4.5 FUTURE APPLICATIONS OF GEOSTATISTICS IN AGRICULTURE

Research in weed science and nematology has resulted in applications for geostatistics in pest management, but to date, there are few examples of local control of fungi following geostatistical analysis. The biggest stumbling block to the applications of geostatistics into integrated pest management and variable rate pesticide application is not statistical but economic, largely because of the cost of sampling. For potatoes however, management costs are high and these costs may be offset by savings on expensive soil treatment (211). As relationships between soil microbial communities and soilborne pathogens are elucidated, the need for spatial interpolation will become an essential tool to visualize these relationships across entire fields. Preservation or promotion of disease suppressive soils can be a reliable form of sustainable agriculture practice, but determining where these suppressive communities are within the soils in relation to where disease is occurring in the field is the first step.

The harmful effects of broad spectrum soil fumigants on soil microbial communities have led to a push amongst commercial potato growers to reduce fumigant application and develop management tools for combating soilborne pathogens on a sub-field level rather than treating the field as a whole (89, 110, 128, 221). Realization of these methods will include the use of geostatistics to interpolate soilborne pathogen population levels, and quantification of soil microbial communities and diversity for an entire field from limited numbers of sample points.

Consequently, the use of interpolated maps to analyze the relationships between edaphic soil properties, pathogen populations, microbial community quantities and diversity to inform management recommendations is best suited to reducing soilborne disease and preserving soil biological diversity.

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CHAPTER 2: EVALUATION OF CHEMISTRIES AND THEIR TEMPORAL EFFECT ON MANAGEMENT OF VERTICILLIUM WILT IN MICHIGAN POTATO (*SOLANUM TUBEROSUM* L.) PRODUCTION SYSTEMS

Abstract

Verticillium wilt caused by *Verticillium dahliae* Kleb. is a widespread disease of potatoes (*Solanum tuberosum* L.) and is an annual problem for potato growers throughout the US Marketable yield can be reduced by 30-50% in potato fields with high disease severity. The significant cost of pre-plant fumigation, increased restriction of soil fumigants, and demand for high quality tubers has led to a renewed focus on management of Verticillium wilt in Michigan commercial potato production. In 2013 and 2014, trials were conducted to determine the efficacy of commercially available fungicides applied in-furrow at-planting to decrease propagules of *V. dahliae* in the rhizosphere and subsequently of wilt disease in plant stems. Preliminary results from these trials indicated that plots treated with fluopyram had a moderate decrease in wilt symptoms. In 2014 and 2015, the effects of fluopyram at three application times; at-planting, at-emergence, and at-tuber initiation were evaluated for management of Verticillium wilt. The three application times were assessed individually and in combination with other application times to determine an effective application time to reduce Verticillium wilt symptoms. The results from these experiments showed that the at-emergence application of fluopyram or any combination of application times coupled with an at-emergence application led to a reduction in *V. dahliae* propagules in stem sap and subsequent yield increase, but had no effect on *V. dahliae* propagule levels in the rhizosphere. These results may suggest that fluopyram has an effect on *Pratylenchus penetrans*, a root-lesion nematode (RLN), which is known to exacerbate Verticillium wilt

symptoms on potato. In 2015 the effects of seven different at-planting and/or foliar treatment programs for control of Verticillium wilt were evaluated to target RLN levels in the soil. Two treatment programs showed significantly lower levels of Verticillium disease severity (wilt symptoms) at the end of the growing season when compared to the non-treated control, however, no significant difference was observed in tuber yield, *V. dahliae* propagule levels in the soil, or RLN populations in the soil. The observations and data from these trials have directed further research on the effects of soil amendments on RLN populations, *V. dahliae* propagules, and other microorganisms found in the rhizosphere.

2.1 Introduction

Soilborne diseases, such as Verticillium wilt of potato, present significant constraints to potato production in Michigan and throughout the US (53) and *Verticillium dahliae* Kleb. is the predominant species affecting potato production in Michigan (33, 59). *Verticillium dahliae* produces microsclerotia that are well adapted to persist in soil and can remain viable for more than a decade (5). Infection is initiated from microsclerotia that overwinter in soil or infected plant debris (5, 7). Depending on severity, time of occurrence and growing season, potato yields and tuber size may be reduced up to 50 % (7, 53).

The amount of damage to potato plants by *V. dahliae* is directly correlated with the number of microsclerotia in the soil (46). However, temperature, moisture, fertility, organic matter, and host susceptibility are factors that can contribute to disease severity in any given year (3, 8, 39). The only true characteristic symptom of Verticillium wilt, that of unilateral chlorosis and necrosis, is morphologically indistinguishable from senescence symptoms, and is not produced until host maturity, and then often a week or so before normal senescence (23).

Therefore, there are many possibilities for incorrect diagnosis. It is vital to determine the degree of *V. dahliae* colonization in potato tissue if the efficacy of treatments for disease management (e.g., pesticides, cultural practices, cultivar resistance) are to be accurately evaluated (7).

Consistent management of Verticillium wilt is lacking, because the goal of keeping *V. dahliae* inoculum levels below the threshold for disease in the soil is not attainable (32, 46, 53, 54). Soil fumigation using methyl bromide was an effective management strategy for decreasing *V. dahliae* propagules in the soil until it was phased out in the mid 1990s (10, 13, 36, 50). The use of metam sodium as a soil fumigant proved somewhat effective at reducing the number of microsclerotia in the soil, however, this method of management is cost prohibitive and has negative environmental effects (7, 35, 46, 53). With growing concern over the future of soil fumigants as a management strategy for Verticillium wilt, it is necessary to evaluate the efficacy of chemistries with more desirable environmental profiles and economic benefits (38), (17). Previous studies have shown the efficacy of in-furrow, at-planting applications of fungicides for managing soilborne diseases caused by *Rhizoctonia solani*, *Phytophthora erythroseptica*, and *Pythium ultimum* (16, 63). The goal of the research presented in this set of studies was to determine if techniques used in common crop husbandry by growers using similar chemistries could provide adequate protection of potato plants against Verticillium wilt. Though there are currently no fungicides registered for management of Verticillium wilt of potato, there are fungicides registered for management of fungi that are morphologically or phenotypically similar to *V. dahliae* (15).

The efficiency of all non-systemic fungicides is dependent on the correct placement of the fungicide in relation to the pathogen (11). This generalization implies that seedborne diseases are best controlled with seed dressing, soilborne pathogens by soil treatment and airborne

pathogens by application of fungicides to the aerial part of the crop (11). Theoretically, the most effective method for applying fungicide to manage levels of *V. dahliae* would be to apply fungicides directly to the soil. However it has previously been shown that when pathogens, such as *R. solani*, reach high levels of inoculum in the soil, in-furrow application of fungicides may not provide effective control (65). These limitations may be overcome through the use of systemic fungicides capable of translocation via the xylem or phloem (11). As these systems are multidirectional it should be possible to apply systemic chemistries to potato foliage throughout the growing season that will move systemically throughout the plant to promote healthy tuber development and contribute to protection against vascular diseases (11).

Over the course of three growing seasons, multiple chemistries were evaluated for their ability to reduce microsclerotia levels in the soil and reduce levels of *V. dahliae* within potato stems after initial infection to ultimately decrease Verticillium wilt symptoms (39). Initially, eight separate chemistries applied in-furrow at-planting to evaluate product efficacy in decrease microsclerotia in the rhizosphere and, more importantly, minimized vascular colonization. Fluopyram and pyrimethanil packaged together as Luna Tranquility® (Bayer CropScience LP, Research Triangle Park, NC) was identified for further evaluation based on initial experimental field testing. A subsequent trial was established to determine whether application timing influenced the efficacy of treatments in decreasing microsclerotia in the soil or vascular colonization.

Verticillium wilt severity is often exacerbated in the presence of root lesion nematodes, most commonly *Pratylenchus penetrans* (4, 52, 54). Feeding injury by *P. penetrans* does not provide a direct avenue of entry for *V. dahliae* into the root system (4) but as *P. penetrans* feeds it has a physiological effect on the potato root, perhaps increasing the amount of root branching,

and subsequently creating a larger root surface area and greater exposure to *V. dahliae* in the rhizosphere (4, 46). Because of this interaction, a third trial was conducted to determine whether fluopyram labeled as Velum Prime® (Bayer CropScience LP, Research Triangle Park, NC) could decrease *P. penetrans* populations in the soil when applied in-furrow. By decreasing *P. penetrans* levels in the soil, the potato plant would be in theory, exposed to fewer *V. dahliae* propagules. Finding an effective method for *V. dahliae* and *P. penetrans* management that is more economical and less environmentally impactful than soil fumigation would provide a long-term management alternative to potato growers plagued by Verticillium wilt.

2.2 Materials and Methods

2.2.1 IN-FURROW FUNGICIDE TREATMENTS FOR MANAGEMENT OF VERTICILLIUM WILT OF POTATOES

2.2.1.1 Field preparation, planting, and maintenance

In-furrow fungicides were applied at the Michigan State University (MSU) Clarksville Research Center (CRC), Clarksville, MI (Capac loam soil); 42.8733, -85.2604 deg; elevation 273 m and at the MSU Montcalm Research Center (MRC), Entrican, MI (sandy soil); 43.3526, -85.1761 deg; elevation 290 m. Potato seed tubers ("Snowden") were prepared by cutting two days prior to planting. These trials were conducted using potato cultivar "Snowden" due to its susceptibility to Verticillium wilt (2) and its commercial use throughout the state of Michigan and the Midwestern US potato growing region. Tubers were planted on 13 May of 2013 into two-row by 7.62-m plots (~25.4-cm between plants to give a target population of 60 plants at 86.36-cm row spacing) replicated four times in a randomized complete block design. A 1.54-m alley separated the two-row beds. In-furrow at-planting applications were delivered in 9.35 L

water/ha a 17.8-cm band using a single XR11003VS nozzle (Lechler Inc., St Charles, IL) at ~210 KPa. The active ingredients evaluated in this study included: difenoconazole; fludioxonil; azoxystrobin; flutolanil; pyraclostrobin; pyrimethanil + fluopyram; entachloronitro-benzene (PCNB) and oxamyl (Table 2.1). Oxamyl (Vydate C-LV; DuPont USA) was used as a non-fungicidal control to determine whether observed results were based on reduction of *V. dahliae* in the stem, or reduction of nematode populations in the rhizosphere. The non-treated control consisted of applying no in-furrow treatment at planting. Additionally, oxamyl was applied at 11.3 mL/100 row-m via a foliar spray at hilling (21 DAP) and three weeks after hilling (42 DAP) with post application irrigation to manage nematodes within the plot. Foliar applications of oxamyl were applied with a calibrated backpack sprayer (R & D Inc., Opelousas, LA) in 234 L water/ha at ~550 KPa.

Fertilizer was drilled into plots before planting, formulated based on soil tests results. Additional nitrogen (final N ~32.0 kg/ha) was applied to the growing crop with irrigation 45 days after planting (DAP) for a total of ~340 kg N/ha. Weeds were controlled by cultivation, hilling, and with S-metolachlor (Dual II Magnum; Syngenta Crop Protection) at 2.24 L/ha 10 DAP and sethoxydim (Poast; BASF Corporation) at 1.75 L/ha 58 DAP. Insects were controlled with imidacloprid (Admire Pro 2F; Bayer CropScience) at 3.4 mL/100 row-m at-planting, and two applications of Beta-cyfluthrin (Baythroid XL; Bayer CropScience) at 116.9 mL/ha at 60 and 90 DAP, or as needed based on commercial potato pest recommendations. Propamocarb-HCL (Previcur N 6SC; Bayer CropScience) was applied at 0.82 L/ha on a seven-day interval from early canopy closure, for a total of four applications to prevent potato late blight movement into the plots. For the weeks when Previcur was not applied, potato late blight and general foliar diseases were managed with weekly applications of chlorothalonil (Bravo WS; Syngenta Crop

Table 2.1. Products evaluated for effect on Verticillium wilt of potato caused by *Verticillium dahliae* in initial study including product name, active ingredient, mode of action, chemical group name, and FRAC code group

Product Name	Active Ingredient(s)	Mode of Action (MOA)	Group Name	FRAC[a] Code
Inspire	Difenoconazole	Sterol Biosynthesis in Membranes	Demethylation Inhibitors (DMI)	3
Maxim 4FS	Fludioxonil	Signal Transduction	Phenylpyrroles (PP)	12
Quadris	Azoxystrobin	Respiration	Quinone Outside Inhibitor (QoI)	11
Moncut	Flutolanil	Respiration	Succinate Dehydrogenase Inhibitor (SDHI)	7
Headline	Pyraclostrobin	Respiration	Quinone Outside Inhibitor (QoI)	11
Luna Tranquility	Pyrimethanil + Fluopyram	Amino Acid and Protein Synthesis + Respiration	Anilinopyrimidine (AP) + Succinate Dehydrogenase Inhibitor (SDHI)	9 + 7
Blocker	Pentachloronitro-benzene (PCNB)	Lipid Synthesis and Membrane Integrity	Aromatic Hydrocabrons (AC)	14
Vydate	Oxamyl	Acetylcholinesterase (ACHE) Inhibitor	Carbamate	1A (Insecticide)

[a] FRAC = Fungicide Resistance Action Committee.

Protection) at 1.75 L/ha starting at early canopy closure. Vines were killed with diquat dibromide (Reglone 2EC; Syngenta Crop Protection Inc.) applied at 1.2 L/ha on 1 Sep 2013. All plot maintenance applications were applied with a tractor mounted spray boom (R&D Inc. Opelousas, LA) delivering 234 L/ha (550 kPa) and using three XR11003VS nozzles per row. Plots were irrigated to supplement precipitation to about 0.63 cm/ha/4 d period with overhead irrigation. Plots were harvested in late September (~120 DAP) and individual treatments were weighed and graded. Meteorological variables were measured with a Campbell weather station (Campbell Scientific Inc., Logan UT) located at each farm from 1 May to 30 Sep. Weather data were provided by MSU Enviroweather. These trials were repeated in 2014 within the same trial area with the exact treatments, potato cultivar, and maintenance.

2.2.1.2 Soil sampling, medium, and assay procedure for *Verticillium dahliae* CFU quantification

Soil samples were taken from each plot prior to applications of in-furrow treatments at planting. Five samples from each plot row (ten total) were collected with a 25 mm JMC soil corer (Clements Assoc., Newton, IA) to a depth of 100 mm and combined in a one gallon sample bag for total of ~1000 g/ soil per sample. Soil samples were placed in separate labeled plastic sample bags, transported to the laboratory on ice, and stored at 4°C pending further analysis. A second set of soil samples were taken approximately a month prior to harvest (or one week prior to vine kill). These soil samples were collected and stored as described above.

Triplicate 10-g samples from each plot were air-dried for 5-7 d before being suspended in 200 ml of sterile water containing 1% Calgon (detergent) (Reckitt Benckiser Group, Slough, England) and 0.01% Tergitol-NPX (detergent) (Sigma-Aldrich, St. Louis, MO) and blended for 30 s using a countertop blender (Sunbeam Products, Inc., Boca Raton, FL). The soil suspension was then washed through 125- and 37-μm sieves (Endecotts, Inc., Newtown, PA) (19). The

residue that was retained by a 37-µm sieve was collected in 15 ml centrifuge tubes, centrifuged for 5 minutes at 1600 *g*, and the excess water removed via aspiration. The final residue was spread over a total of 5 sodium polypectate (NP-10) agar medium plates [semi-selective for *Verticillium* spp. (26, 42)]. Contents of the medium per liter were 5 g of polygalacturonic acid-sodium salt, 1 g of KNO_3, 1 g of KH_2PO_4, 0.5 g of KCl, 0.5 g of $MgSO_4 \bullet 7H_20$, 0.5 ml of Tergitol NPX, 0.5 g of streptomycin sulfate, 0.25 g of chlorotetracyclin HCl, 0.25 g of chloramphenicol, and 15 g of agar (42). Petri plates containing soil samples were incubated for 14 to 21 d at 21 to 23°C in the dark to allow *Verticillium* propagules to germinate and develop into colonies. Following incubation, the soil residue was removed from the medium surface by washing under a gentle stream of tap water from a faucet. The discrete colonies of *V. dahliae*, which developed below the agar surface, were counted with the aid of a dissecting microscope (Leica Camera AG, Wetzlar, Germany). The average number of colonies per plate was used to calculate an estimate of the population density of *V. dahliae* in the soil collected from each plot.

2.2.1.3 Quantification of vascular colonization of potato by *Verticillium dahliae* assay

To determine the colony forming units (CFU) per ml of plant sap, stem sections approximately 15 cm long were cut from 5 plants per plot, with sterile razor blades, from the soil line approximately 100 DAP. The stem sections where placed into separately labeled plastic bags, transported on ice to the laboratory, and processed within 48 hours of collection. Stem sections were washed in tap water for 60 seconds, soaked in a 0.5% NaOCl solution for 30 seconds and rinsed in sterile water for 30 seconds. Sap was extracted from the basal portion of each stem section by placing them in a hydraulic plant press (Spectrum Technologies, Inc., Aurora, IL) and crushing them into individually labeled centrifuge tubes. Between each stem, the sap press was sterilized with a 0.5% NaOCl solution and rinsed with sterile water. Sap (0.1 mL

aliquots) was plated into 4 separate quadrants of a divided 100 mm quad petri plate (VWR, Radnor, PA). Twenty mL of Czapek-Dox Agar (31) (5 mL/quadrant) was poured into the dish and the dish was gently agitated by hand. A total of 5 plates for each stem (20 0.1 mL aliquots). Plates were incubated at 21-23°C in the dark for 7-14 d. CFU per 0.1 mL of sap were determine by estimating the number of colonies on a plate. Estimates were done by taking the average number of CFU of 10 random microscopic sampling fields then multiplying by the number of times the area of a microscopic sampling field was contained in the total area of the plate (18).

2.2.1.4 Disease severity rating

Disease severity ratings were taken ~90 DAP using a 0-5 scale. The scale was constructed as follows: 0 = No wilt symptoms seen; 1 = Sporadic yellowing and flagging of petioles; 2 = Moderate yellowing and flagging of petioles, some flagged petioles becoming necrotic on lower leaves; 3 = Symptomatic plants beginning to have stems that stand straight up while the rest of plant lays down, upright stems are yellow and petioles are wilted and necrotic; 4 = Majority of plot has upright necrotic stems; and 5 = Entire plot is necrotic, upright stems are brown and petioles completely wilted and necrotic, tubers have stem end vascular browning.

2.2.1.5 Data collection and analysis

Data were subjected to analysis of variance (ANOVA). Means separation was conducted using Fisher's Least Significant Difference (LSD α=0.10). All statistical analysis was performed using JMP Version 10.0 (SAS Inc., Cary, NC).

2.2.2 TEMPORAL EFFECTS OF FLUOPYRAM APPLICATION ON VERTICILLIUM WILT

In-furrow and foliar combinations of the chemical fluopyram were applied at MSU CRC and at MSU MRC. Potato seed tubers were prepared by cutting two days prior to planting. Seed tubers were planted on 15 May, 2014 into two-row by 7.6-m plots (~25.4-cm between plants to

give a target population of 60 plants at 86.4-cm row spacing) replicated four times in a randomized complete block design. A 1.5-m not-planted alley separated the two-row beds. Pyramethanil + fluopyram 4.16SC (Luna Tranquility, Bayer CropScience) was applied at 0.82 L/ha throughout the season at three different times. The first application time was at planting, in-furrow (Time A). A foliar application of fluopyram was applied 21 DAP (Time B) at the same rate as the in-furrow treatment. A final foliar treatment, was applied 42 DAP (Time C) at the same rate. Irrigation was applied to each plot immediately after the application time of treatments B and C to allow the chemical to infiltrate the root system (24). In-furrow at-planting applications were delivered in 9.4 L water/ha in a 17.8-cm band using a single XR11003VS nozzle (Lechler Inc., St Charles, IL) at ~210 KPa. Foliar applications were applied with a calibrated backpack sprayer (R & D Inc., Opelousas, LA) in 234 L water/ha at ~550 KPa. Fertilizer, herbicide, insecticide, fungicide maintenance sprays, and vine desiccant were applied as previously described prior to harvest 120 DAP. Plots were subsequently irrigated to supplement precipitation to about 0.63 cm/ha/4 d period with overhead irrigation. Weather data were collected as described above. This trial was repeated in 2015. Soil sampling, medium, assay procedure for *Verticillium dahliae* CFU quantification from soil, assay to quantify vascular colonization of potato by *Verticillium dahliae*, disease rating, and data collection and analysis were all carried out as described in the sections above.

2.2.3 EVALUATION OF VERTICILLIUM WILT MANAGEMENT PROGRAMS

2.2.3.1 Field preparation, planting, and maintenance

In-furrow and foliar combinations of the chemical fluopyram were applied at MSU MRC. Potato seed tubers (“Snowden’) were prepared by cutting two days prior to planting. Seed tuber pieces were planted on 14 May 2015 into two-row 7.62-m plots (~25.4-cm between plants to

give a target population of 60 plants at 86.4-cm row spacing) replicated four times in a randomized complete block design. A 1.5-m alley separated the two-row beds. A not-treated control was compared with 7 different treatment programs to compare their efficacy in controlling Verticillium wilt [potato early die (PED)]. Application times in the trial were in-furrow at planting (Time A), at 2" emergence (Time B), and 7 days after 2" emergence (Time C). Four products (Table 2.2) and seven treatments (Table 2.3) were evaluated. Irrigation was applied to each plot immediately after the applications of treatment at times B and C to allow the chemical to infiltrate the root system (24). At-planting applications (Time A) were delivered in 9.35 L water/ha in a 17.8-cm band using a single XR11003VS nozzle (Lechler Inc., St Charles, IL) at ~210 KPa. Foliar applications (Times B and C) were applied as described in section 2.2.2. Fertilizer, herbicide, insecticide, fungicide maintenance sprays, and vine desiccant were applied as previously described. Plots were harvest on 21 Sep (130 DAP) and individual treatments were weighed and graded. This trial was repeated in 2016 on-farm at White Pigeon, MI.

2.2.3.2 Soil sampling, *Verticillium dahliae* quantification, disease rating, and data collection

Soil sampling was performed as previously described at three dates; prior to planting, 7 days following time B application (~25 DAP), and 14 days after the time C application (~65 DAP). Medium, assay procedure for *Verticillium dahliae* CFU quantification from soil was done as described above. Disease severity rating, data collection and analysis were all carried out in this trial as described above.

2.2.3.3. Nematode sampling and extraction

Soil and root samples were collected prior to planting, 25 DAP, and 65 DAP and sent to the Michigan State University Plant Diagnostic Clinic to determine populations levels of RLN (*P. penetrans*) prior to and after in-furrow and foliar applications. Samples were collected and

Table 2.2. Products evaluated for the effect of application time on Verticillium wilt of potato caused by *Verticillium dahliae* in study three including product name, active ingredient, mode of action, chemical group name, and FRAC code group

Product Name	Active Ingredient(s)	Mode of Action (MOA)	Group Name	FRAC[a] Code
Luna Tranquility	Pyrimethanil + Fluopyram	Amino Acid and Protein Synthesis + Respiration	Anilinopyrimidine (AP) + Succinate Dehydrogenase Inhibitor (SDHI)	9 + 7
Velum Prime	Fluopyram	Respiration	Succinate Dehydrogenase Inhibitor (SDHI)	7
Serenade Soil	QST 713 Strain of *Bacillus subtilis*	Lipid Synthesis and Membrane Integrity	Microbial (*Bacillus* spp.)	44
Vydate	Oxamyl	Acetylcholinesterase (ACHE) Inhibitor	Carbamate	1A (Insecticide)

[a] FRAC = Fungicide Resistance Action Committee.

Table 2.3. The seven treatment programs use in the evaluation of Verticillium wilt management programs trial and the times of each application

Treatment	Time of Application[a]
Not-treated Control	
Pyrimethanil + fluopyram	B
Pyrimethanil + fluopyram	C
Pyrimethanil + fluopyram	B & C
Fluopyram Pyrimethanil + fluopyram	A B & C
Bacillus subtilis Pyrimethanil + fluopyram	A B & C
Oxamyl Pyrimethanil + fluopyram	A B & C
Oxamyl	B & C

[a] In-furrow at planting (time A), 2" Emergence (time B), 7 Days after 2" Emergence (time C).

stored per protocols established by the Michigan State University Plant Diagnostic Clinic.

2.2.3.4 Vascular discoloration evaluation and trial repetition

Following harvest, 25 tubers from each plot (100 total) were evaluated for stem end vascular discoloration. The tubers were sliced 0.5 cm from the stem end to reveal the vascular ring. In severe cases of Verticillium wilt, stem end discoloration will be present in tubers (9) (Fig. 2.1). The total number of tubers from each plot were collected and analyzed to determine an average % for each treatment. This trial was repeated at a new location in White Pigeon, MI in 2016 on land which had a 25-year history of potatoes grown in a two-year rotation with seed corn. The trial was moved in hopes of finding levels of pre-plant nematode populations which could cause disease (46).

2.3 Results

2.3.1 IN-FURROW FUNGICIDE TREATMENTS FOR MANAGEMENT OF VERTICILLIUM WILT OF POTATOES

In the preliminary field trial at MRC in 2013, weather data was conducive for disease (Table 2.4). No treatments were significantly different in % emergence or average number of stems per plant (α=0.10) (data not shown) at either location in any year. The pre-season, prior to planting soil sampling revealed significantly higher levels of *Verticillium dahliae* CFU/g soil among treatment plots where difenoconazole was applied compared to plots that were designated for the not-treated control (NTC), fludioxonil, flutolanil, pyraclostrobin, pyrimethanil + fluopyram (pyr + fluo), PCNB, and oxamyl (Table 2.5). The late season sampling (~100 DAP) found that oxamyl had significantly lower levels of *V. dahliae* CFU/g (2.20) compared to difenoconazole (21.1), fludioxonil (35.5), pyraclostrobin (32.0), pyr + fluo (19.9), and PCNB

Figure 2.1. In potato plants that display severe symptoms of Verticillium wilt, tuber stem-end browning and discoloration can be seen

Table 2.4. Mean daily air temperature, mean relative humidity, mean daily soil temperature, and mean monthly precipitation data for Montcalm Research Center (Entrican, MI) and Clarksville Research Center (Clarksville, MI) in 2013

	Mean Daily Air Temp. (°C) [Days >30°C]		Mean Daily Relative Humidity (%)		Mean Daily Soil Temp. (°C at 10-cm Depth)		Mean Monthly Precipitation (cm)	
Location	MRC[a]	CRC[b]	MRC	CRC	MRC	CRC	MRC	CRC
May	16.7 [0]	18.6 [0]	71.4	64.8	18.2	21.3	9.8	7.9
June	18.9 [0]	20.8 [4]	40.7	71.4	22.6	24.3	5.7	8.4
July	20.8 [3]	19.5 [0]	72.6	72.1	26.7	19.8	3.4	8.1
August	19.6 [0]	15.4 [0]	72.0	72.7	19.9	17.8	12.6	4.5
September	15.8 [0]	16.7 [0]	74.1	74.7	24.7	17.5	3.4	4.3

[a] Montcalm Research Center (Entrican, MI).
[b] Clarksville Research Center (Clarksville, MI).

Table 2.5. Effects of in-furrow pesticide treatments on propagules of *Verticillium dahliae* in the soil prior to and after treatment application of fungicide or insecticide (one month prior to harvest), propagules of *V. dahliae* recovered from stem sap of potato, wilt disease rating, and total yield of potato at Montcalm Research Center, Entrican, MI in 2013 and 2014

	CFU[a]/g Soil Pre-Season Sample[b]		CFU/g Soil Late-Season Sample[c]		Stem CFU/0.1 mL Sap		Wilt Disease Rating[d]		Total Yield (t/ha)	
Treatment	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
NTC[e]	1.43 b[g]	8.50	12.5 ab	9.50	30.9 a-d	90.8 a	4.63 a	4.63 a	15.4 b	22.0 c
Difenoconazole	4.93 a	6.50	21.1 a	7.80	36.1 abc	48.8 cd	3.75 bc	4.63 a	21.1 a	23.1 bc
Fludioxonil	2.35 b	6.50	35.5 a	9.30	63.4 ab	79.3 ab	3.75 bc	4.75 a	23.2 a	22.6 bc
Azoxystrobin	2.85 ab	5.80	14.9 ab	8.30	110 a	19.8 e	3.25 c	4.00 b	21.5 a	27.7 ab
Flutolanil	1.53 b	6.80	11.7 ab	9.30	30.0 a-d	51.0 cd	3.88 bc	4.13 b	19.2 ab	21.6 c
Pyraclostrobin	1.52 b	6.00	32.0 a	9.50	21.1 bcd	51.8 cd	4.13 ab	4.00 b	21.5 a	23.6 bc
Pyr + fluo[f]	2.39 b	6.00	19.9 a	8.00	5.74 d	29.3 de	2.25 d	2.13 d	21.2 a	23.6 bc
PCNB	2.11 b	5.30	19.3 a	7.80	40.2 abc	64.5 bc	3.50 c	2.75 c	22.8 a	23.1 bc
Oxamyl	1.62 b	7.30	2.20 b	7.80	8.90 cd	52.5 c	3.75 bc	4.00 b	19.0 ab	29.8 a

[a] CFU = colony forming units of *Verticillium dahliae*.
[b] Sampled prior to planting;
[c] Sampled one month prior to harvest (~100 DAP).
[d] Verticillium Wilt Scale: 0=No Verticillium wilt seen; 1=Small amounts of yellow and flagging of petioles; 2=Moderate amounts of yellowing and flagging of petioles, some of the flagged petioles becoming necrotic; 3=Symptomatic plants are start to have stems stand straight up while the rest of the plant is laying down, the upright stems are yellow and petioles are wilted and necrotic;

Table 2.5. (cont'd)
4=Majority of the plot has upright necrotic stems and 5=Entire plot is necrotic, upright stems are brown and petioles are wilted and necrotic, tuber may have brown speckling throughout the stem-end.
[e] NTC = Not-treated control.
[f] Pyr + fluo = pyramethanil + fluopyram.
[g] Means followed by same letter are not significantly different (α=0.10, Fisher's LSD).

(19.3) (Table 2.5). Analysis of CFU in stem sap (~100 DAP) showed that no in-furrow treatment had significantly lower *V. dahliae* CFU/0.1 mL sap than the NTC, however, significant differences were seen among treatments (Table 2.5). Azoxystrobin had significantly more *V. dahliae* CFU/0.1 mL recovered from stem sap (110) than pyraclostrobin (21.1), pyr + fluo (5.74), and oxamyl (8.90). Additionally, pyr + fluo had significantly lower *V. dahliae* CFU/0.1 mL than difenoconazole (36.1), fludioxonil (63.4), and PCNB (40.2). Oxamyl treatments had significantly lower CFU in the stem sap than fludioxonil. Disease severity on a 0-5 scale (described above) revealed that all treatments, aside from pyraclostrobin, were significantly lower than the NTC (4.63) (Table 2.5). Disease severity (2.25) in pyr + fluo treatments was significantly lower than difenoconazole (3.75), fludioxonil (3.75), azoxystrobin (3.25), flutolanil (3.88), pyraclostrobin (4.13), PCNB (3.50), and oxamyl (3.75). All treatments had significantly higher total yield in t/ha, aside from flutolanil (19.2) and oxamyl (19.0), than the NTC (15.4 t/ha). Total yield ranged from 15.4 to 23.2 t/ha (Table 2.5).

The other preliminary trial established at CRC in 2013 again had weather conducive for disease (Table 2.4). The pre-season, prior to planting soil sampling revealed significantly lower CFU/g in plots treated with fludioxonil, azoxystrobin, pyr + fluo, and PCNB compared to the NTC (Table 2.6). The late season sampling (~100 DAP) ranged from 3.20 to 5.30 CFU/g soil with no significant differences among any treatments. Analysis of CFU in stem sap (~100 DAP) ranged from 14.7 to 42.6 CFU/0.1 mL sap with no significant differences among treatments. Disease severity on a 0-5 scale revealed that pyr + fluo (1.50) was significantly lower than the NTC (4.50), difenoconazole (4.83), fludioxonil (4.50), azoxystrobin (4.38), flutolanil (4.38), pyraclostrobin (4.13), and oxamyl (4.38) (Table 2.6). PCNB (3.63) was not significantly different from the NTC or pyr + fluo. Analysis of yield in t/ha revealed that difenoconazole

Table 2.6. Effects of in-furrow pesticide treatments on propagules of *Verticillium dahliae* in the soil prior to and after treatment with fungicide or insecticide (one month prior to harvest), propagules of *V. dahliae* recovered from stem sap of potato, wilt disease rating, and total yield of potato at Clarksville Research Center, Clarksville, MI in 2013 and 2014

	CFU[a]/g Soil Pre-Season Sample[b]		CFU/g Soil Late-Season Sample[c]		Stem CFU/0.1 mL Sap		Wilt Disease Rating[d]		Total Yield (t/ha)	
Treatment	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
NTC[e]	9.20 a[g]	3.50 cd	3.75	30.0 ab	38.3	78.8 a	4.50 a	4.75 a	19.0 b	21.1
Difenoconazole	4.30 abc	5.50 a	3.70	32.8 a	33.6	16.8 e	4.38 a	3.88 cde	26.6 a	23.4
Fludioxonil	3.40 bcd	4.50 abc	4.45	26.5 abc	23.8	21.0 d	4.50 a	4.25 bc	23.3 ab	23.6
Azoxystrobin	1.40 cd	5.00 ab	3.20	17.0 c	32.8	8.30 f	4.38 a	4.38 ab	27.5 a	22.9
Flutolanil	4.60 ab	3.00 de	4.40	23.5 abc	15.7	24.3 cd	4.38 a	4.25 bc	26.0 ab	24.6
Pyraclostrobin	3.70 a-d	2.30 e	4.95	33.5 a	30.7	28.8 b	4.13 a	4.13 bcd	25.4 ab	26.5
Pyr + fluo[f]	3.20 bcd	3.00 de	4.60	24.0 abc	14.7	13.3 e	1.50 b	1.88 f	25.9 ab	29.7
PCNB	1.10 d	4.00 bcd	5.10	34.0 a	15.5	23.3 d	3.63 ab	3.75 de	23.6 ab	25.8
Oxamyl	5.20 ab	3.80 cd	5.30	20.5 bc	42.6	27.8 bc	4.38 a	3.63 e	26.4 a	25.2

[a] CFU = colony forming units of *Verticillium dahliae.*
[b] Sampled prior to planting.
[c] Sampled one month prior to harvest (~100 DAP).
[d] Verticillium Wilt Scale: 0=No Verticillium wilt seen; 1=Small amounts of yellow and flagging of petioles; 2=Moderate amounts of yellowing and flagging of petioles, some of the flagged petioles becoming necrotic; 3=Symptomatic plants are start to have stems stand straight up while the rest of the plant is laying down, the upright stems are yellow and petioles are wilted and necrotic;

Table 2.6. (cont'd)
4=Majority of the plot has upright necrotic stems and 5=Entire plot is necrotic, upright stems are brown and petioles are wilted and necrotic, tuber may have brown speckling throughout the stem-end.
[e] NTC = Not-treated control.
[f] Pyr + fluo = pyramethanil + fluopyram.
[g] Means followed by same letter are not significantly different (α=0.10, Fisher's LSD).

(26.6), azoxystrobin (27.5), and oxamyl (26.4) had significantly higher total yield compared to the NTC (19.0). Fludioxonil (23.3), flutolanil (26.0), pyraclostrobin (25.4), pyr + fluo (25.9), and PCNB (23.6) were not significantly different from the NTC, difenoconazole, azoxystrobin, or oxamyl (Table 2.6). Total yield ranged from 19.0 to 27.5 t/ha.

At MRC in 2014 weather conditions were conducive for disease (Table 2.7). The pre-season, prior to planting soil sampling ranged from 5.8 to 8.5 CFU/g soil with no significant differences observed. The late season sampling (~100 DAP) ranged from 7.8 to 9.5 CFU/g soil with no significant differences among any treatments (Table 2.5). Analysis of CFU in stem sap (~100 DAP) found that difenoconazole, azoxystrobin, flutolinil, pyraclostrobin, pyr + fluo, PCNB, and oxamyl had significantly lower *V. dahliae* CFU/0.1 mL recovered from stem sap (48.8, 19.8, 51.0, 51.8, 29.3, 64.5 and 52.5 respectively), compared to the NTC (90.8) (Table 2.5). Azoxystrobin and pyr + fluo had significantly lower CFU/0.1 mL sap compared to fludioxonil (79.3), PCNB, and oxamyl (Table 2.5). Disease severity on a 0-5 scale revealed that azoxystrobin (4.00), flutolanil (4.13), pyraclostrobin (4.00), pyr + fluo (2.13), PCNB (3.50), and oxamyl (3.75) were significantly lower compared to the three remaining treatments. Pyr + fluo disease severity was significantly lower than azoxystrobin, flutolanil, pyraclostrobin, PCNB, and oxamyl. Disease severity in plots with PCNB in-furrow were significantly lower than in plots treated with azoxystrobin, flutolanil, pyraclostrobin, and oxamyl. azoxystrobin and oxamyl had significantly higher total yield in t/ha (27.7 and 29.8 respectively) compared to the NTC (22.0) and flutolanil (21.6). Moreover, oxamyl had significantly higher total yield in t/ha compared to difenoconazole (23.1), fludioxonil (22.6), pyraclostrobin (23.6), pyr + fluo (23.6), and PCNB (23.1) (Table 2.5). Total yield ranged from 21.6 to 29.8 t/ha.

The trial was repeated at CRC in 2014 and weather was conducive for disease (Table

Table 2.7. Mean daily air temperature, mean relative humidity, mean daily soil temperature, and mean monthly precipitation data for Montcalm Research Center (Entrican, MI) and Clarksville Research Center (Clarksville, MI) in 2014

	Mean Daily Air Temp. (°C) [Days >30°C]		Mean Daily Relative Humidity (%)		Mean Daily Soil Temp. (°C at 10-cm Depth)		Mean Monthly Precipitation (cm)	
Location	MRC[a]	CRC[b]	MRC	CRC	MRC	CRC	MRC	CRC
May	15.5 [0]	15.6 [0]	70.1	67.2	16.5	15.7	5.4	10.7
June	15.9 [0]	18.8 [0]	71.5	74.4	20.9	21.9	8.3	10.4
July	19.6 [0]	19.7 [1]	74.5	72.3	25.6	22.8	9.1	8.4
August	17.2 [0]	18.9 [0]	72.0	71.5	24.3	19.8	4.3	8.1
September	15.1 [0]	15.9 [0]	74.1	75.7	19.8	16.7	6.0	6.9

[a] Montcalm Research Center (Entrican, MI).
[b] Clarksville Research Center (Clarksville, MI).

2.7). The pre-season, prior to planting soil sampling ranged from 2.30 to 5.50 CFU/g soil with significant differences among treatments (Table 2.6). The late season sampling (~100 DAP) found that only plots treated with azoxystrobin had significantly lower CFU/g soil (17.0) than the NTC (30.0) (Table 2.6). Analysis of CFU in stem sap (~100 DAP) revealed that all fungicide and insecticide treatments had significantly lower CFU per 0.1 mL sap compared with the NTC (78.8). Stem sap CFU/0.1 mL ranged from 8.30 to 78.8 (Table 2.6). Disease severity on a 0-5 scale showed that all treatments, aside from azoxystrobin (4.38), had significantly lower disease severity (wilt symptoms) compared to the NTC (4.75). Pyr + fluo (1.88) had significantly lower disease severity than all other treatments (Table 2.6). Total yield ranged from 21.1 to 29.7 t/ha and no significant differences were seen among treatments.

2.3.2 TEMPORAL EFFECTS OF FLUOPYRAM APPLICATION ON VERTICILLIUM WILT

As a follow-up to the preliminary in-furrow fungicide treatments for management of Verticillium wilt of potatoes trials conducted at MRC and CRC in 2013, a subsequent trial was established at two locations (MRC and CRC) in 2014 to assess the temporal effects of fluopyram in reducing incidence of Verticillium wilt. No significant differences were seen between treatments in emergence and plant stand at either location in either year (data not shown) (α=0.10). The preliminary trial at MRC in 2014 had weather which was conducive for disease (Table 2.7). The pre-season prior to planting soil sampling revealed that only the treatment with a B & C application time had significantly less CFU/g soil compared to the NTC (Table 2.8). The late-season sampling (~100 DAP) found that no treatment had significantly lower CFU/g soil compared to the NTC (26.0) but application time B alone (31.8) and application times A, B & C combined (31.3) had significantly higher CFU/g soil compared to the NTC (Table 2.8). Analysis of CFU in stem sap (~100 DAP) revealed that at application time A alone, time C

Table 2.8. Effects of three different application times of fluopyram both individually and in combination with all application times on propagules of *Verticillium dahliae* in the soil prior to after fungicide treatment (one month prior to harvest), propagules of *V. dahliae* recovered from potato stem sap, wilt disease rating, and total yield of potato at Montcalm Research Center, Entrican, MI in 2014

	CFU[b]/g Soil Pre-Season Sample[c]	CFU/g Soil Late-Season Sample[d]	Stem CFU/0.1 mL Sap	Wilt Disease Rating[e]	Total Yield (t/ha)
Time of Application[a]	2014	2014	2014	2014	2014
NTC[f]	12.0 a[g]	26.0 bc	15.2 c	4.38 a	22.5 f
A	10.3 ab	28.8 ab	21.2 b	4.42 a	21.6 f
B	10.5 ab	31.8 a	13.9 c	2.98 b	28.9 cd
C	8.30 ab	21.5 c	34.0 a	4.75 a	28.0 d
A & B	11.5 a	24.0 bc	14.0 c	1.88 c	26.0 e
A & C	8.00 ab	26.8 ab	33.6 a	4.75 a	29.8 c
B & C	6.30 b	24.5 bc	7.50 d	1.76 c	34.2 b
A, B & C	9.50 ab	31.3 a	5.10 e	0.92 d	41.1 a

[a] In-furrow at planting (Time A), 21 DAP (Time B), 42 DAP (Time C).
[b] CFU=colony forming units of *Verticillium dahliae*.
[c] Sampled prior to planting.
[d] Sampled one month prior to harvest (~100 DAP).
[e] Verticillium Wilt Scale: 0=No Verticillium wilt seen; 1=Small amounts of yellow and flagging of petioles; 2=Moderate amounts of yellowing and flagging of petioles, some of the flagged petioles becoming necrotic; 3=Symptomatic plants are start to have stems stand straight up while the rest of the plant is laying down, the upright stems are yellow and petioles are wilted and necrotic; 4=Majority of the plot has upright necrotic stems and 5=Entire plot is necrotic, upright stems are brown and petioles are wilted and necrotic, tuber may have brown speckling throughout the stem-end.

Table 2.8. (cont'd)

[f] NTC=Not-treated control.

[g] Means followed by same letter do not significantly differ (α=0.10, Fisher's LSD).

alone, and at application times A & C combined had significantly higher numbers of stem sap CFU/0.1 mL sap (21.2, 34.0, and 33.6 respectively) compared to the NTC (15.2). Application time B alone (13.9) and times A & B combined (14.0) were not significantly different from the NTC. Times B & C combined (7.50) and times A, B & C combined had significantly lower CFU/0.1 mL recovered from stem sap compared to the NTC and at application times A, B & C combined were significantly lower than application times B & C (Table 2.8). Disease severity on a 0-5 scale showed that application time A alone (4.42), time C alone (4.75), and application times A & C combined (4.75) was not significantly lower than the NTC (4.38). Disease severity in treatments with application time B alone (2.98), times A & B combined (1.88), times B & C combined (1.76), and times A, B & C combined (0.92) was significantly lower than the NTC. Application times A & B combined and times B & C combined had significantly less disease severity compared to time B alone, but were not significantly different from one other. Application times A, B & C combined had significantly less disease severity than all treatments (Table 2.8). All treatments aside from time A alone had significantly higher total yield in t/ha (21.6) compared to the NTC (22.5) (Table 2.8). Significant differences were seen among treatments in total yield, but application times A, B & C combined (41.1) had significantly higher total yield in t/ha than all other treatments. Outside of application times A, B & C combined, application times B & C combined (34.2 t/ha) had significantly higher yields than all other treatments (Table 2.8).

The same trial was established at CRC in 2014. Weather was conducive for disease (Table 2.7). The pre-season prior to planting soil sampling ranged from 6.50 to 11.0 CFU/g soil with no significant differences (Table 2.9). The late-season sampling (~100 DAP) had a range from 22.3 to 28.3 CFU/g soil with no significant differences. Analysis of *V. dahliae* CFU/0.1 mL

Table 2.9. Effects of three different application times of fluopyram both individually and in combination with all application times on propagules of *Verticillium dahliae* in the soil prior to after fungicide treatment (one month prior to harvest), propagules of *V. dahliae* recovered from potato stem sap, wilt disease rating, and total yield of potato at Clarksville Research Center, Clarksville, MI in 2014 and 2015

	CFU[b]/g Soil Pre-Season Sample[c]		CFU/g Soil Late-Season Sample[d]		Stem CFU/0.1 mL Sap		Wilt Disease Rating[e]		Total Yield (t/ha)	
Time of Application[a]	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
NTC[f]	10.8	3.00 ab[g]	26.3	30.0 ab	22.7 b	4.10	4.83 a	4.75 a	20.3 c	21.1
A	9.30	2.30 b	28.3	32.8 a	13.0 c	2.40	3.75 c	3.88 cde	21.2 c	23.4
B	8.30	4.80 ab	24.5	26.5 abc	3.70 d	1.20	2.67 d	4.25 bc	37.2 a	23.6
C	8.00	5.30 ab	26.8	17.0 c	24.7 a	3.20	4.83 a	4.38 ab	19.3 c	22.9
A & B	6.50	2.30 b	26.5	23.5 abc	2.30 e	3.70	1.75 e	4.25 bc	25.4 b	24.6
A & C	7.30	5.00 ab	25.8	33.5 a	21.5 b	1.60	4.25 b	4.13 bcd	21.7 c	26.5
B & C	11.0	5.50 ab	23.0	24.0 abc	13.0 c	2.10	3.75 c	1.88 f	38.5 a	29.7
A, B & C	10.3	6.00 a	22.3	34.0 a	2.00 e	3.50	0.88 e	3.75 de	40.7 a	25.8

[a] In-furrow at planting (Time A), 21 DAP (Time B), 42 DAP (Time C).
[b] CFU=colony forming units of *Verticillium dahliae*.
[c] Sampled prior to planting.
[d] Sampled one month prior to harvest (~100 DAP).
[e] Verticillium Wilt Scale: 0=No Verticillium wilt seen; 1=Small amounts of yellow and flagging of petioles; 2=Moderate amounts of yellowing and flagging of petioles, some of the flagged petioles becoming necrotic; 3=Symptomatic plants are start to have stems stand straight up while the rest of the plant is laying down, the upright stems are yellow and petioles are wilted and necrotic;

Table 2.9. (cont'd)

4=Majority of the plot has upright necrotic stems and 5=Entire plot is necrotic, upright stems are brown and petioles are wilted and necrotic, tuber may have brown speckling throughout the stem-end.

[f] NTC=Not-treated control.

[g] Means followed by same letter do not significantly differ (α=0.10, Fisher's LSD).

sap in stem (~100 DAP) showed that application time C alone had significantly more CFU/0.1 mL recovered from plant sap (24.7) compared to the NTC (22.7). Application times A & C combined did not have significantly more *V. dahliae* CFU/0.1 mL sap (21.5) compared to the NTC (Table 2.9). Application time A alone (13.0), time B alone (3.70), times A & B combined (2.30), times B & C combined (13.0), and times A, B & C combined (2.00) all had significantly lower CFU/0.1 mL recovered from stem sap than the NTC. Application time B alone, times A & B combined, and times A, B & C combined had a significantly lower amount of CFU/0.1 mL recovered from stem sap compared to application time A alone and application times B & C combined (Table 2.9). Analysis of *V. dahliae* CFU/0.1 mL recovered from stem sap showed application times A & B combined and times A, B & C combined were significantly lower than application time B alone, but were not significantly different from each other. All treatments had significantly lower disease severity on a 0-5 scale compared to the NTC (4.83) except for time C alone (4.83). Application times A & B combined (1.75) and times A, B & C combined (0.88) had significantly lower disease severity than all other treatments, but were not significantly different from each other (Table 2.9). Application time B alone (2.67) had significantly lower disease severity compared to application time A alone (3.75) and time C alone (4.83) (Table 2.9). Analysis of yield in t/ha showed that application time A alone (21.2), time C alone (19.3), and times A & C combined (21.7) were not significantly different from the NTC (20.3). Time B alone (37.2), times A & B combined (25.4), times B & C combined (38.5), and times A, B & C combined had significantly higher total yield than the NTC. Application time B alone, times B & C combined, and times A, B & C combined had significantly higher total yield than times A & B combined but were not significantly different for each other (Table 2.9).

At CRC in 2015 weather was conducive for disease (Table 2.10). The pre-season soil

Table 2.10. Mean daily air temperature, mean relative humidity, mean daily soil temperature, and mean monthly precipitation data for Montcalm Research Center (Entrican, MI) and Clarksville Research Center (Clarksville, MI) in 2015

	Mean Daily Air Temp. (°C) [Days >30°C]		Mean Daily Relative Humidity (%)		Mean Daily Soil Temp. (°C at 10-cm Depth)		Mean Monthly Precipitation (cm)	
Location	MRC[a]	CRC[b]	MRC	CRC	MRC	CRC	MRC	CRC
May	15.8 [0]	16.6 [0]	71.6	69.4	15.6	15.6	7.5	10.2
June	18.2 [0]	21.2 [0]	73.7	73.1	20.7	20.1	12.2	7.7
July	20.1 [0]	22.1 [0]	73.0	70.5	23.2	23.1	4.4	0.7
August	19.7 [0]	17.8 [0]	77.0	72.7	21.5	22.0	6.1	7.8
September	18.2 [0]	15.8 [0]	76.6	73.6	19.2	20.9	9.9	8.9

[a] Montcalm Research Center (Entrican, MI).
[b] Clarksville Research Center (Clarksville, MI).

sampling prior to planting ranged from 2.30 to 6.0 CFU/g soil with no treatment significantly different from the NTC (Table 2.9). Analysis of *V. dahliae* CFU/g soil at the late-season sampling (~100 DAP) revealed that only time C alone (17.0) had significantly lower CFU/g soil compared with the NTC (30.0). All other treatments were not significantly different from the NTC. Average CFU/0.1 mL recovered from stem sap ranged from 1.2 to 4.1 CFU/0.1 mL with no significant differences (Table 2.9). Average wilt disease rating on a 0-5 scale showed that all treatments aside from application time C alone (4.38) had significantly lower disease compared to the NTC (4.75) (Table 2.9). Average total yield ranged from 21.1 to 29.7 t/ha with no significant differences.

2.3.3 EVALUATION OF VERTICILLIUM WILT MANAGEMENT PROGRAMS

The preliminary results of the temporal effect trial as well as the two years of results from the in-furrow treatments trial led to a trial utilizing different pesticides and using them at separate times throughout the growing season. The initial trial was established at MRC in 2015. Weather data was conducive for disease (Table 2.10). No treatment differed significantly from the NTC in % emergence. Evaluation of CFU/g soil sampled 7 days after the application of treatment B (~25 DAP) ranged from 15.3 to 31.8 CFU/g soil with no significant differences (Table 2.11). No treatment differed significantly from the NTC in *Verticillium dahliae* CFU/g soil at the sampling time of 14 days after time C applications (~65 DAP). Root-lesion nematodes (RLN) were not found in the plots at the sampling times . Analysis of disease severity (wilt symptoms) on a 0-5 scale revealed that pyr + fluo at application time B alone (3.30), pyr + fluo at application times B & C combined (3.30), fluopyram at time A followed by pyr + fluo at times B & C combined (3.30), and oxamyl at application times B & C combined (2.80) had significantly lower disease severity compared to the NTC (Table 2.11). Analysis of yield in t/ha revealed that pyr + fluo at

Table 2.11. Effects of in-furrow, at planting, and foliar fungicide and insecticide treatments on emergence percent, *Verticillium dahliae* colony forming units (CFU) in soil, wilt ratings, total yield of potato, and vascular discoloration of potato tubers at Montcalm Research Center, Entrican, MI in 2015

Treatment	Time of Application[a]	Emergence %	CFU[b]/g Soil Early-Season Sample[c]	CFU/g Soil Mid-Season Sample[d]	Wilt Disease Rating[e]	Total Yield (t/ha)	%VD[f] in Tubers
Not-treated Control		99.6	24.0 ab[g]	24.3 ab	4.00 a	38.6 ab	63.0 abc
Pyr + fluo	B	94.0	18.8 ab	26.5 ab	3.30 bc	40.2 ab	70.0 a
Pyr + fluo	C	98.7	15.5 b	20.5 b	3.50 ab	33.3 c	33.0 d
Pyr + fluo	B & C	98.5	15.3 b	30.3 ab	3.30 bc	38.3 ab	65.0 ab
Fluopyram Pyr + fluo	A B & C	98.6	20.3 ab	40.0 a	3.30 bc	37.8 ab	43.0 bcd
Bacillus subtilis Pyr + fluo	A B & C	96.9	19.5 ab	36.3 ab	3.80 ab	36.2 bc	40.0 cd
Oxamyl Pyr + fluo	A B & C	95.2	31.8 a	26.3 ab	3.50 ab	33.1 c	43.0 bcd
Oxamyl	B & C	98.6	22.8 ab	25.0 ab	2.80 c	41.4 a	55.0 a-d

[a] In-furrow at planting (Timing A), 2" Emergence (Timing B), 7 Days after 2" Emergence (Timing C).
[b] CFU=colony forming units of *Verticillium dahliae*.
[c] Sampled 7 days after B application.
[d] Sampled 14 days after C application.
[e] Verticillium wilt scale: 0=No Verticillium wilt seen; 1=Small amounts of yellow and flagging of petioles; 2=Moderate amounts of yellowing and flagging of petioles, some of the flagged petioles becoming necrotic; 3=Symptomatic plants are start to have stems

Tabled 2.11. (cont'd)
stand straight up while the rest of the plant is laying down, the upright stems are yellow and petioles are wilted and necrotic; 4=Majority of the plot has upright necrotic stems and 5=Entire plot is necrotic, upright stems are brown and petioles are wilted and necrotic, tuber may have brown speckling throughout the stem-end.
[f] VD=Vascular discoloration of the stem end; percentage calculated from 100 tubers (25 per plot).
[g] Means followed by same letter do not significantly differ (α=0.10, Fisher's LSD).

application time C alone, *Bacillus subtilis* at application time A followed by pyr + fluo at application times B and C combined, and oxamyl at application times B and C combined had showed that pyr + fluo at application time C alone had significantly lower percentage of tubers with vascular discoloration compared to the NTC (Table 2.11).

The follow-up trial was established in White Pigeon, MI in 2016. Weather was conducive for disease (Table 2.12). No treatment had significantly more emergence (%) compared with the NTC (Table 2.13). However, pyr + fluo at application time B alone, pyr + fluo at application time C alone, pyr + fluo at application times B & C, and oxamyl at application time A followed by pyr + fluo at application times B & C had significantly lower emergence (%) compared to the NTC. Evaluation of soil sampled 7 days after the application of treatment B (~25 DAP) revealed that no treatment reduced the number of CFU/g soil compared to the NTC. Conversely, oxamyl at application times A & B had significantly higher CFU/g soil compared to the NTC. *Verticillium dahliae* CFU/g soil at the sampling time of 14 days after time C applications (~65 DAP) ranged from 3.0-8.8 CFU/g soil with no significant differences. Additionally, RLN were not found in the plots at any sampling times. Analysis of disease severity (wilt symptoms) on a 0-5 scale revealed that pyr + fluo at application time C alone (2.3), and *Bacillus subtilis* at application time A followed by pyr + fluo at application times B & C (2.4) had significantly lower disease severity compared to the NTC (3.0) (Table 2.13). Assessment of yield in t/ha from each treatment revealed no difference in yield compared to the NTC (52.0), but pyr + fluo at application time C alone (39.5) and *Bacillus subtilis* at application time A followed by pyr + fluo at application times B& C (39.7 t/ha) had significantly lower yields compared to the NTC. None of the treatments were different compared to the NTC in vascular discoloration.

Table 2.12. Mean daily air temperature, mean relative humidity, mean daily soil temperature, and mean monthly precipitation data for White Pigeon, MI in 2016

	Mean Daily Air Temp. (°C) [Days >30°C]	Mean Daily Relative Humidity (%)	Mean Daily Soil Temp. (°C at 10-cm Depth)	Mean Monthly Precipitation (cm)
April	7.9 [0]	64.9	8.4	6.7
May	16.1 [0]	61.2	13.8	7.4
June	20.9 [2]	64.7	16.9	12.4
July	22.9 [6]	68.1	23.6	4.4
August	23.4 [6]	70.0	24.5	19.8

Table 2.13. Effects of in-furrow, at planting, and foliar fungicide and insecticide treatments on emergence percent, *Verticillium dahliae* colony forming units (CFU) in soil, wilt ratings, total yield of potato, and vascular discoloration of potato tubers at White Pigeon, MI in 2016

Treatment	Time of Application[a]	Emergence %	CFU[b]/g Soil Early-Season Sample[c]	CFU/g Soil Mid-Season Sample[d]	Wilt Disease Rating[e]	Total Yield (t/ha)	%VD[f] in Tubers
Not-treated Control		91.9 a[g]	3.5 b	5.0	3.0 a	52.0 a	57.5 ab
Pyr + fluo	B	79.4 bc	3.5 b	5.0	3.1 a	42.5 ab	37.5 a
Pyr + fluo	C	78.8 c	4.8 ab	3.3	2.3 c	39.5 b	32.5 a
Pyr + fluo	B & C	78.8 c	3.5 b	7.3	2.9 ab	42.9 ab	37.5 a
Fluopyram Pyr + fluo	A B & C	94.4 a	6.8 ab	8.8	2.9 ab	48.3 ab	67.5 b
Bacillus subtilis Pyr + fluo	A B & C	88.1 ab	3.5 b	3.0	2.4 bc	39.7 b	45.0 ab
Oxamyl Pyr + fluo	A B & C	80.0 bc	6.0 ab	8.5	2.6 abc	49.9 ab	45.0 ab
Oxamyl	B & C	85.6 abc	7.3 a	6.3	2.6 abc	48.9 ab	47.5 ab

[a] In-furrow at planting (Timing A), 2" Emergence (Timing B), 7 Days after 2" Emergence (Timing C).
[b] CFU=colony forming units of *Verticillium dahliae*.
[c] Sampled 7 days after B application.
[d] Sampled 14 days after C application.
[e] Verticillium wilt scale: 0=No Verticillium wilt seen; 1=Small amounts of yellow and flagging of petioles; 2=Moderate amounts of yellowing and flagging of petioles, some of the flagged petioles becoming necrotic; 3=Symptomatic plants are start to have stems

Table 2.13. (cont'd)
stand straight up while the rest of the plant is laying down, the upright stems are yellow and petioles are wilted and necrotic; 4=Majority of the plot has upright necrotic stems and 5=Entire plot is necrotic, upright stems are brown and petioles are wilted and necrotic, tuber may have brown speckling throughout the stem-end.
[f] VD=Vascular discoloration of the stem end; percentage calculated from 100 tubers (25 per plot).
[g] Means followed by same letter do not significantly differ (α=0.10, Fisher's LSD).

2.4 Discussion

The locations of the experimental field trials (MRC, CRC, and White Pigeon) were chosen because they contain soil type typical of the soil used throughout Michigan to grow potatoes commercially and the history of potato trials established at these locations provide for adequate levels of *Verticillium dahliae* propagules via natural infestation. Environmental conditions were optimal for potato growth (18-20°C) and soil temperatures were ideal for growth of *Verticillium dahliae* (23-27°C) (25, 47, 55). The exact soil environment for optimal *Verticillium dahliae* growth is not completely elucidated, but temperature is a very important factor in germination (55). Additionally, soil microbial diversity, interactions with other microorganisms, and soil edaphic characteristics all influence Verticillium wilt severity, but their specific roles require further research (54). This lack of understanding can be overcome by planting in locations where potatoes have been grown for many years along with the use of potato cultivar with susceptibility to Verticillium wilt (2).

The preliminary in-furrow pesticide trials established at MRC and CRC in 2013 were used to determine which, if any, current, registered fungicides for potato might be used in-furrow as a replacement for soil fumigation or supplement to reduce amounts of soil fumigation used extensively in management programs for Verticillium wilt. Pre-season sampling and analysis revealed low levels of CFU/g soil but near the threshold for infection (5 CFU/g soil) (39, 46)Steere, 2016 #347}. The late-season soil sample revealed that no treatment was better than the NTC in the reduction of CFU in the rhizosphere of the plant. One possible explanation is that none of the chemistries are able to degrade the highly melazined resting structure of *Verticillium dahliae* (62). Although no treatment was significantly different than the NTC when looking at CFU recovered from stem sap, plots treated with pyr + fluo in-furrow had the lowest numbers of

CFU/0.1 mL stem sap. Additionally, at both locations in 2013, pyr + fluo showed lower disease severity (wilt symptoms) compared to the NTC. Two possible explanations for these findings are: 1) pyr + fluo persists in the soil after in-furrow application long enough to break-down the more susceptible germ tubes (64) put forth by *Verticillium dahliae* to penetrate young root hairs when the mother tuber begins germination (6) and 2) pyr + fluo is taken up by young roots and provides systemic protection to the root cortex of the potato plant while it is most vulnerable to infection (12). Total yield results were inconsistent likely due to the persistence of other soilborne and foliar potato diseases, insects, or nematodes (1, 34, 40, 51).

The results of the follow-up trials at MRC and CRC in 2014 were consistent at both locations in 2013. Similar results were seen in soil CFU, which supports the hypothesis that the chemistries used in these trials are unable to disrupt the microsclerotia of *V. dahliae*. In 2014, pyr + fluo had lower CFU in stem sap than the NTC at both locations. This may provide evidence that pyr + fluo has some efficacy in reducing the effects of Verticillium wilt. Fluopyram, one of the active ingredients in pyr + fluo, has been previously reported as having efficacy against other soilborne pathogens (27, 45, 49), so its relative effectiveness against *V. dahliae*, though not completely understood, is not inconceivable.

From the initial trials in 2013 and the follow up trials in 2014, it was evident that the efficacy of pyr + fluo as a management tool for Verticillium wilt required further investigation. Previous studies have found that timing of application and vicinity of application to the causal organism is important for maximum effectiveness of a fungicide (12)Evans, 1971 #332}. *Verticillium dahliae* inhabits the soil and is able to persist in the soil for many years without a host (21, 23, 55), so it is logical to apply fungicides to the soil in order to maximize effectiveness (22, 44, 55, 59, 60), especially fluopyram which has limited movement in the xylem (12). The

time during the potato growing season that is most critical for infection by *V. dahliae* is not well understood (29). For infection, roots need to be in close proximity to the pathogen as the germinating hyphae are never more than 2 mm from the infection site (57). As the roots and root hairs of the young plants grow through the abrasive soil and in the presence of a plant-eating microfauna, they may suffer extensive damage to root hairs and the piliferous layer in an otherwise healthy root system (44). Microsites of dead cells resulting from adverse physical of physiological conditions thus can offer a non-living infection court for *V. dahliae* (43). Furthermore, it has been shown that *Verticillium* spp. are capable of directly penetrating intact root hairs (56) as well as young roots on the root cap and in the zone of elongation (14). This would indicate that the early growth and development of young potato roots is when the host is most susceptible to infection (0-60 DAP).

In an effort to test this assumption of 0-60 DAP being the critical time for infection by *V. dahliae*, the temporal effects of pyr + fluo trials were established at two locations in 2014 (MRC and CRC). The goal of these trials was to determine the effect of application time of pyr + fluo on Verticillium wilt. As expected, no application time or combination of application times were better than the NTC at reducing *V. dahliae* CFU in the soil. This is further evidence that pyr + fluo has no effect on the melanized microsclerotia found in the soil. Differences were evident in levels of CFU recovered from stem sap. Of the three application times when applied alone, applications at time B alone (~21 DAP) had lower stem CFU/0.1 mL than the time A alone or time C alone at both locations. At CRC in 2014 application at time B alone had lower stem sap CFU than the NTC, but no different from the NTC at MRC. Furthermore, any combination of application times which included time B at CRC in 2014 had significantly lower stem sap CFU compared to the NTC and at MRC all combinations which included application time B had lower

stem sap CFU compared to the NTC except for the combination of times A & B, which was not significantly different from the NTC. Disease severity followed a similar trend at both locations. Plots which had applications of pyr + fluo applied at time B alone and combinations which included time B consistently had lower disease severity compared with the NTC at both locations in 2014. These results may indicate that the time-period when the potato plant is emerging (21 DAP) is critical in the etiology of Verticillium wilt. Moreover, plots that did not receive an application of pyr + fluo at emergence (time A alone, time C alone, times A & C combined) were either no different from the NTC in recovered stem sap CFU, or had higher levels of recovery of stem sap CFU compared to the NTC. For example, an application at time C alone showed much higher recovery of stem sap CFU at both locations which may mean that application at 42 DAP is too late for effective management of Verticillium wilt. Possible explanations for this require further exploration, but may be attributed to plant defenses blocking certain parts of the vascular system (37, 61) after infection by *V. dahliae*, thus not allowing the fungicide access the infected areas in the stem. Total yield at both location was significantly higher in plots including an application of pyr + fluo at time B when compared to the NTC. While these differences in yield should not be contributed to pyr + fluo application time alone, it is interesting to note the correlation between stem sap CFU, wilt disease rating, and total yield. A follow up trial, which was conducted only at CRC in 2015 showed similar pre-season and late-season soil CFU. Unfortunately, this trial fell victim to heavy rains throughout the month of June and many plots were washed out. Most likely due to these environmental conditions, no significant differences were seen between treatments and the NTC in recovery of stem sap CFU or yield. In plots that survived, all application times aside from time C alone showed lower disease severity (wilt symptom rating) compared to the NTC.

The results obtained from these first two trials indicate three things: 1) No tested treatment had an effect on *V. dahliae* CFU in the soil; 2) pyr + fluo may have some efficacy in managing Verticillium wilt compared to other pesticides applied in a similar manner and 3) an application of pyr + fluo at emergence (21 DAP) was more efficient in reducing CFU within the stem, and disease severity than an in-furrow application alone or an application at 42 DAP of the same product. The results of these trials indicate that pyr + fluo may have viability as an alternative or supplement to traditional methods of managing Verticillium wilt (i.e. soil fumigation). The first two trials however, failed to determine whether these treatments had any effect on *P. penetrans* which exacerbates the disease caused by *V. dahliae* (4, 46, 52, 54). The disease caused by co-infection of nematodes and *V. dahliae* appears similar to that caused by the fungus alone, which has led researchers to attribute the interaction to enhanced pathogenesis of *V. dahliae* when the nematode is present (47, 48). Therefore, a third trial was established which encompassed the results of the first two trial but along with sampling for *V. dahliae* CFU, plots were sampled for treatment effect on populations of root-lesion nematodes. Previous studies have shown positive results when using fluopyram in-furrow as a nematicide (12, 20). For this trial pyr + fluo in the form of Luna Tranquility was evaluated as a foliar application at times B and C, but three separate in-furrow treatments were evaluated at application time A; fluopyram in the form of Velum Prime; a strain of *Bacillus subtilis* which has been reported to have nematicidal properties (58); and oxamyl in the form of Vydate, a well-known nematicide (28, 41). An additional treatment of oxamyl at times B & C combined, without an application at time A, was included as a comparison to pyr + fluo at times B & C, without an application at time A, to assess the efficacy of pyr + fluo as a foliar application nematicide. As reported before, analysis of *V. dahliae* CFU/g soil revealed no differences between treatments and the NTC at any of the

three sampling times (pre-season, early-season, or late-season). Analysis of wilt disease symptoms showed that pyr + fluo at time B alone, times B & C combined, fluopyram at time A followed by pyr + fluo at times B & C combined, and oxamyl at times B & C combined had significantly lower wilt disease symptoms compared to the NTC but no treatment had significantly higher yield than the NTC. The additional cost of nematode sampling did not allow for stem sap CFU/0.1 mL analysis to be done. No nematodes were recovered from soil or roots in any plot at any sample. Possible explanations for this include sampling error and/or the absence of nematode populations. In order to determine the role that fluopyram plays in the management of Verticillium wilt, future experimental field trials are needed to determine how fluopyram interacts with root-lesion nematodes in potato cropping symptoms, and laboratory investigation into the biochemical interaction of fluopyram with *V. dahliae* must be conducted.

2.5 Conclusions

Verticillium wilt is an important disease that can have devastating effects on potato tuber yield. Effective and environmentally conscience management of Verticillium wilt remains elusive, partially due to the resilience or the microsclerotia in the soil and lack of chemical control measures (30). To date, the results gathered in these trials have shown that pyr + fluo has consistently shown an ability to decrease levels of CFU in stems and disease severity (wilt symptoms) but whether this is related to reduction in nematode populations, protection of roots from *V. dahliae* propagules in the soil, or other factors remains to be elucidated. While promising, these results support prior work suggesting that management of Verticillium wilt requires an integrated approach that combines the use of host resistance, cultural control methods, and improved chemical control methods. Further research is needed to identify factors

contributing to Verticillium wilt of potato and identifying new management strategies that are effective, economical, environmentally sustainable, and are likely to include a genetic approach (2).

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CHAPTER 3: A GEOSTATISTICAL APPROACH TO VISUALIZE THE DIVERSITY OF SOIL INHABITING BACTERIA AND EDAPHIC QUALITIES IN POTATO (*SOLANUM TUBEROSUM)* PRODUCTION SYSTEMS

Steere, L., Rosenzweig, N., Gerondale, B., & Kirk, W. W. (2016). A Geostatistical Approach to Visualize the Diversity of Soil Inhabiting Bacteria and Edaphic Qualities in Potato (Solanum tuberosum) Production Systems. *American Journal of Potato Research 93*(5), 518-532.

Abstract

A study was conducted in Michigan (MI) to assess spatial patterns of soil biological and physiochemical factors related to yield in potato production. The project developed an approach to integrate techniques including: high-throughput DNA sequencing; geographic information systems (GIS); geostatistics; traditional soil analyses; and yield data. Twenty soil samples were taken and global positioning satellite (GPS) marked in the fall/spring of 2012-13 from a grower's field scheduled to be in potato production, and total genomic DNA was extracted. Parallel sequencing targeting the 16S rRNA gene was used to assess bacterial diversity. The total number of taxa identified by sequence analysis was 21, 81, 140, 300 and 814 at the level of phyla, class, order, family and genus, respectively. Sequencing results and information gathered on yield at each point was used to generate multi-layer GIS-based maps.

3.1 Introduction

Microorganisms endemic to the soil are essential to global biogeochemical cycles and have an influence on nutrient cycling and mineral solubilization, which are important to soil health and crop productivity (7, 17, 55, 64, 66, 69). The microorganisms living in the soil have direct impact on plant fitness as pathogens (31, 72), beneficial mutualists (48), and indirectly

effect overall crop health as decomposers (82, 83), or through antagonistic activity against plant pathogens (18, 25, 64, 65, 87). Moreover, some beneficial bacteria have the capacity to produce plant hormones (3) and induce systemic disease resistance responses in plants (78).

Additionally, soil macro- and micronutrients have been implicated in modifying disease severity (34-36, 49). Traditionally decisions on the amount, source and timing of fertilizer by potato growers is based on optimization of financial return for yield and tuber quality while minimizing harvest, storage, environment, and marketing risks (46) on a farm-wide basis. Thus, there is little consideration given to the totality of spatial variability of abiotic factors, biotic factors and available plant nutrients within a grower's field.

The negative impact of activity by microorganisms as soilborne pathogens is an annual concern for commercial potato production worldwide (31). Approximately 90% of major diseases that impact crops are caused by soilborne pathogens (50). These diseases have accounted for nearly half of all U.S. crop losses (roughly $4 billion annually), (50, 90) and represent the largest source of limitation to yield potential (after water insufficiency), especially in no-till agricultural systems (77). In agricultural production, the negative interactions of plants with soilborne pathogens are a major consideration for crop rotation (52). In particular, commercial potato production has long been plagued by a number of persistent soilborne pathogens (56).

Potatoes may be used fresh-cooked, baked or fried, or processed for starch products (57). For these purposes, potato tubers need to meet various quality standards, such as: size, specific gravity, and appearance, and many other qualities as described fully by Johnson (41) and Navarre and Pavek (58). Potato is one of the most intensively managed crops (61), and cultivation using vegetative tubers as seed make the crop vulnerable to several recurrent and

persistent soilborne diseases (19, 23, 80, 89). These include potato common scab (PCS) caused by *Streptomyces* spp. and potato early die (PED) caused by the *Verticillium dahliae/Pratylenchus* spp. complex (72). Although other soilborne pathogens, such as *Fusarium* spp., *Rhizoctonia solani*, and *Colletotrichum coccodes*, are persistent in Michigan soils, PCS and PED in particular are consistently identified as chronic and yield limiting problems for potato production systems (44, 56).

Soilborne diseases in potato production are currently managed using a combination of chemical fungicides, bio-fungicides, fumigation, crop rotation, soil amendments, and/or other cultural practices. Currently available chemistries for soil fumigation are not consistently effective against soilborne diseases (8, 9), in contrast to their efficacy against nematodes and some insects, and are cost prohibitive especially at labeled rates of application (54). These fumigants create concerns over adverse effects to the environment (28, 29). The application of fumigants for management of soilborne pathogens typically results in a reduction of pathogen populations in the soil (16, 26, 62). However, the non-specific nature of fumigants also reduces general soil microbial community, including that of antagonists and disrupts the natural microbial balance of the soil (47, 53).

Potato production in Michigan is valued at around $207 million (2015) and ranks seventh nationally (http://www.nass.usda.gov). Recently potato growers identified an increase in soilborne disease complexes (e.g. PCS and PED) and declining yields across the state (http://potatodiseases.org/). This has raised concerns over the ability of the potato industry in Michigan to serve the high-demand chipping markets, as approximately 70% of Michigan potatoes are used in chip production (33). Accordingly, growers in

MI estimate the amount of acreage affected by potato soilborne diseases increased from 11 to > 50% over the last decade.

The diversity and relative abundance of important microbes that contribute to naturally occurring disease control, as well as soil and plant health, may provide the necessary resources to develop novel tools for sustainable, integrated soilborne disease management strategies. Numerous studies have shown species-specific effects of plants on the composition and relative abundance of microbial populations in the rhizosphere of agricultural crops and of cultivated native plant species (17, 21, 27, 70, 75). Using a DNA based culture-independent approach, a recent study of the phylogenetic composition of bacterial communities in the rhizosphere of three potato cultivars grown at two distant field sites, found that sequence abundance was the highest for Proteobacteria (46%), followed by Firmicutes (18%), Actinobacteria (11%), Bacteroidetes (7%) and Acidobacteria (3%) (85). The relative abundance of the bacterial families *Streptomycetaceae*, *Micromonosporaceae*, and *Pseudomonadaceae* was dependent on potato cultivar in this study (85).

Next-generation sequencing (NGS) platforms allow for millions of DNA strands to be sequenced in parallel (5), leading to substantially higher throughput of DNA sequencing and minimizing the need for older fragment cloning methods, and have recently become more accessible, and affordable to researchers (93). This has enabled wider analyses of microbial communities in the soil (37, 51). High-throughput NGS platforms are capable of producing approximately 10^6 sequence reads of 400 base pairs (bp) in a single run, and provide greater depth than traditional DNA sequencing technologies and greater detection of rare species (37). Next-generation sequencing using the multiplex coded-primer (tag) approach enables high-throughput processing of multiple soil samples (1, 38, 42). This approach coupled with DNA

amplification using conserved primers of phylogenetically informative regions of the 16S rRNA generates sufficient taxonomic information necessary for bacterial community diversity analysis (92). In theory thousands or millions of sequences are processed simultaneously and in parallel, and NGS platforms can generate hundreds of megabases to gigabases of nucleotide sequence outputs in a single instrument run, depending on platform (79). NGS platforms result in lower cost of DNA sequencing/bp compared to traditional sequencing allowing for more affordable detection of soil microbial communities.

GIS technology has proven to be a successful tool in precision agriculture (95). GIS and GPS technology helps growers to identify disease loci or "hot-spots" within a field (84) and make management decisions to target those specific locations. This means fields no longer need to be managed as a whole, but that problem areas can be managed as separate entities (32). GIS has enabled growers to improve productivity, apply fertilizers and pesticides at variable rates, and precisely guide equipment across fields (2). Since the 1980's when precision agriculture first increased in use, tools such as GIS and GPS have increased growers understanding of the variation that exists within their fields. In the case of the research presented herein, ArcGIS (Environmental Systems Research Institute (ESRI) Inc, Redlands, CA) has allowed advanced analysis of spatial data and GIS mapping of soil, which can enable growers to visualize the relationships between measured variables such as soil bacterial diversity as well as soil physical and chemical properties.

The goal of this study was to begin to develop trans-disciplinary tools to integrate DNA technologies, GIS and computational biology to assess soil conditions, soil bacterial biodiversity and crop productivity. These tools could potentially be utilized by potato growers, and across cropping systems. This technology has the potential to improve productivity, by informing

prescribed sub-field soil disease management decisions to reduce chemical inputs, improve soil quality and aid in sustainable, high-quality agricultural crop production.

3.2 Materials and Methods

3.2.1 SOIL SAMPLING, STUDY AREA AND DATA COLLECTION

The field site for this study was in Saint Joseph County, in a large potato production area in southwestern Michigan. The field (41°52'18.4435", -085°22'57.3227") was roughly 30 ha with 36% Oshtemo sandy loam and 64% Spinks loamy sand (Fig. 3.1). The field was on a two-year rotation, alternating between seed corn (*Zea mays*) and potatoes (cv. Atlantic). Following corn harvest in the fall of 2012, a grid-sampling scheme (Fig. 3.1) was established to obtain samples proportionally throughout the entire field and 20 soil cores were collected with a 25 mm JMC soil corer (Clements Assoc., Newton, IA) to a depth of 100 mm around a central point in each grid (400 total soil cores). The position of each point was recorded using a handheld GPS device (Trimble Juno 3D, Trimble Navigation Limited, Sunnyvale, CA). Soil samples were placed in separate labeled plastic bags, transported to the laboratory on ice, and stored at 4°C pending further analysis. Soil samples were sent to the Michigan State University Soil and Plant Nutrient Laboratory for analyses of soil quality and physical properties such as organic matter, potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P) and pH (http://www.spnl.msu.edu/_pdf/Soil_Test_Report.pdf).

Potatoes were planted in the spring of 2013 according to industry standards (41). Prior to commercial harvest in early September of the field, a sample yield dig of 3 m-rows was performed on 30 Aug 2013 by hand within a 3 m radius of each original sample point, ensuring no blank spaces occurred in the 3 m row section. Tubers from the 3 m section were sorted based

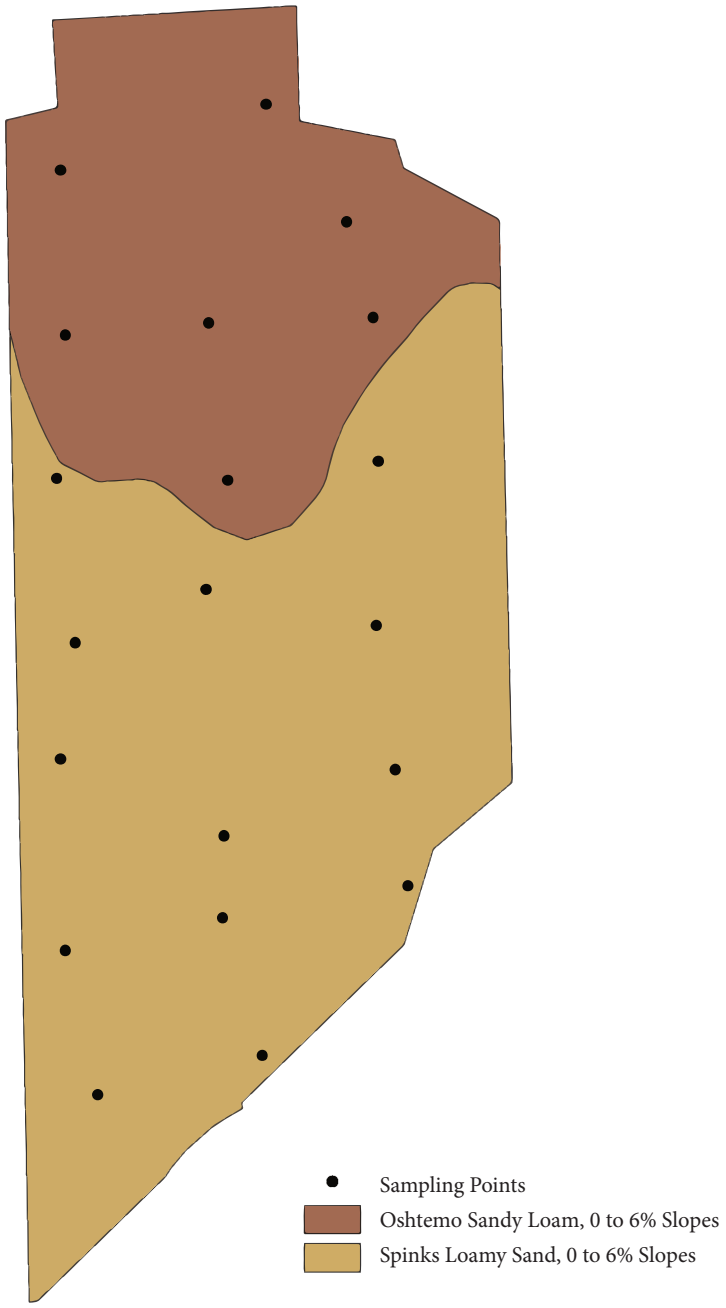


Figure 3.1. Map of a field used in potato production in Saint Joseph County, MI (41°52'18.4435", -085°22'57.3227") including soil types and points sampled in the field for this study. Soil types obtained through the Soil Survey Geographic database (SSURGO) made available from the Natural Resources Conservation Service (http://www.nrcs.usda.gov) and sampling points (n=20) of study area

on size profile described previously (22) and weighed using an digital electronic scale (Pure Fishing Inc., Columbia, SC) in the field. Weights were recorded then converted into tons per hectare (t/ha) using Agricultural Research Manager (ARM) 9 Software (Gylling Data Management, Inc., Brookings, SD). Soil physical data and molecular analyses data (described below) were entered relative to their spatial reference data and plotted and analyzed using ArcGIS 10.2 (ESRI Inc., Redlands, CA).

Using the data collected from each sample point, predictive maps were made to provide a visual representation of the correlation of variables throughout an entire field to better understand the interactions between soil physical characteristics, soil microbial diversity and yield. ArcGIS was used to analyze and visually display soil sampling and DNA sequencing results. Data for each field were entered to a GIS database and analyzed using the Spatial Analyst and Geostatistical toolset in ArcGIS.

3.2.2 DNA EXTRACTION, AMPLICON LIBRARY CONSTRUCTION, SEQUENCING AND COMPUTATIONAL ANALYSIS

Total genomic DNA was extracted from 0.5 g of soil from each composite soil sample using a kit for soil (FastDNA SPIN, MP Biomedicals LLC, Solon, OH) per the manufacturer's instructions. DNA samples were stored at -20°C until used. Total genomic DNA from the soil sample was used as a template for polymerase chain reaction (PCR) amplification of the bacterial 16S rRNA gene (86, 91). Preparation of amplicons and library construction was performed according to the previously described protocol (45). Amplicon libraries were submitted to the Michigan State University Research and Technology Support Facility (MSU RTSF) for next-generation sequencing on the MiSeq Illumina platform (San Diego, CA). The resulting sequence data were analyzed using the previously described analysis pipeline (45) with the mother

v.1.33.0 software package (68) and processed using the protocol from http://www.mothur.org/wiki/MiSeq_SOP accessed on November 4, 2015. An additional phylotype assignment was determined by analysis of processed sequence data using the ribosomal database project (RDP) 16S rRNA gene training set (version 9) (14, 15). Subsequently to initial sequence analysis preprocessing the dataset was normalized by selecting a sub-sample (9,957) at random from each DNA from sample. Finally, the inverse of the Simpson Index was calculated to determine the overall bacterial diversity from each of the sub-samples. The reciprocal of Simpson's index (1/Diversity) was chosen to characterize the microbial communities in our soil samples because they have good to moderate discriminating ability and are used widely in ecological studies (97). The use of 1/diversity instead of the original formulation of Simpson's index ensure that an increase in the reciprocal index reflects an increase in diversity (97).

3.2.3 STATISTICAL ANALYSIS

Pearson correlation coefficient analysis using a row-wise comparison method was used to compare the relationships among measured soil biotic and abiotic variables. Additionally, principle component analysis was used to determine the positive and negative correlation among the measured soil variables. All statistical analyses were performed using JMP v. 10 (SAS Institute, Inc. Cary, NC).

3.2.4 GIS ANALYSIS

Point data were collected in the field using Trimble GPS units and were uploaded into ArcGIS for further analyses. Data from soil fertility and DNA analysis were imported to ArcGIS as data tables and then linked to the point data information from the field to create a database. The database included soil physical properties, DNA sequences, and positional attributes.

Geostatistical analyses were conducted on measured variables using the Spatial and Geostatistical Analyst toolsets in ArcGIS. Geostatistics were used to predict and interpolate the values of spatially distributed data. Spatial interpolation is based on Tobler's First Law of Geography, that data is often spatially dependent or autocorrelated, or a value of a variable at one location will be similar to values of nearby variables (47, 63, 76). The basis of this research was to examine whether there was a distance-based relationship between variables, such as yield, DNA sequence information and soil physical characteristics and to create predictive maps to display these relationships. Two techniques to predict values of variables at not-sampled locations; a) deterministic (43) and b) probabilistic (39), were compared for the variable "yield" and presented in the study field. As soil property variables are continuous across fields, researchers use interpolation from sample data to reconstruct the complete surface and to gain an understanding of how variables differ across space (59). To further examine the process of creating predictive maps and to provide an example of the capabilities of GIS in sub-field soil management, one field and a single variable, yield measured as t/ha are described in this chapter.

3.2.5 SPATIAL CONTINUITY AND VARIABILITY

To create accurate predictive maps, it is important to assess the spatial continuity and variability of sample points. The similarity or dissimilarity between data separated by a certain distance can be quantified by several measures (30). The measures used in this study were, a) covariance, b) correlogram and c) semivariogram. Covariance and correlogram are used to measure the similarity between non co-located data (63)**.** The covariance between data values separated by a vector *h* is as follows (30):

$$C(h) = \frac{1}{N(h)} \sum_{\alpha=1}^{N(h)} (z_i(\alpha) z_i(\alpha + h)) - m_{-h} m_{+h} \qquad (3.1)$$

where $N(h)$ is the number of pairs separated by distance h, $z_i(\alpha)$ is the value from the original data point (tail), $z_i(\alpha + h)$ is the value of the data point separated by distance h from the $z_i(\alpha)$ point (head point), m_{-h} is the mean of the tail values, and m_{+h} is the mean of the head values. Covariance is a measure of how much two variables change together (Fig. 3.2). A correlogram is a unit-free measure of similarity between data and the standardized form of the covariance (30). It is calculated as follows:

$$\rho(h) = \frac{C(h)}{\sigma_- \sigma_+} \tag{3.2}$$

where $\rho(h)$ is the correlation coefficient and σ_- and σ_+ are the standard deviations of the tail and head values respectively. The correlogram describes the relationship between the distance h and the correlation coefficients between the pairs of points. The covariance and correlogram equations were used to show how similar data are at certain distances. Both measures were used to determine how the similarity between points changed with an increase in distance.

Unlike the previous functions, which measured similarity, the semivariogram measured the average dissimilarity between data. As such dissimilarity increases as points get further apart (Fig. 3.3) (30). It is computed as follows:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{\alpha=1}^{N(h)} (z_i(\alpha) - z_i(\alpha + h))^2 \tag{3.3}$$

The semivariogram is computed as half the average squared difference between the data values separated by h. The semivariogram was made to fit a mathematical model that best described the spatial variation of the data. The semivariogram is a continuous function that must be fitted to experimental values to deduce values for any possible distance h required by interpolation algorithms, and also to smooth out sample fluctuations (30).

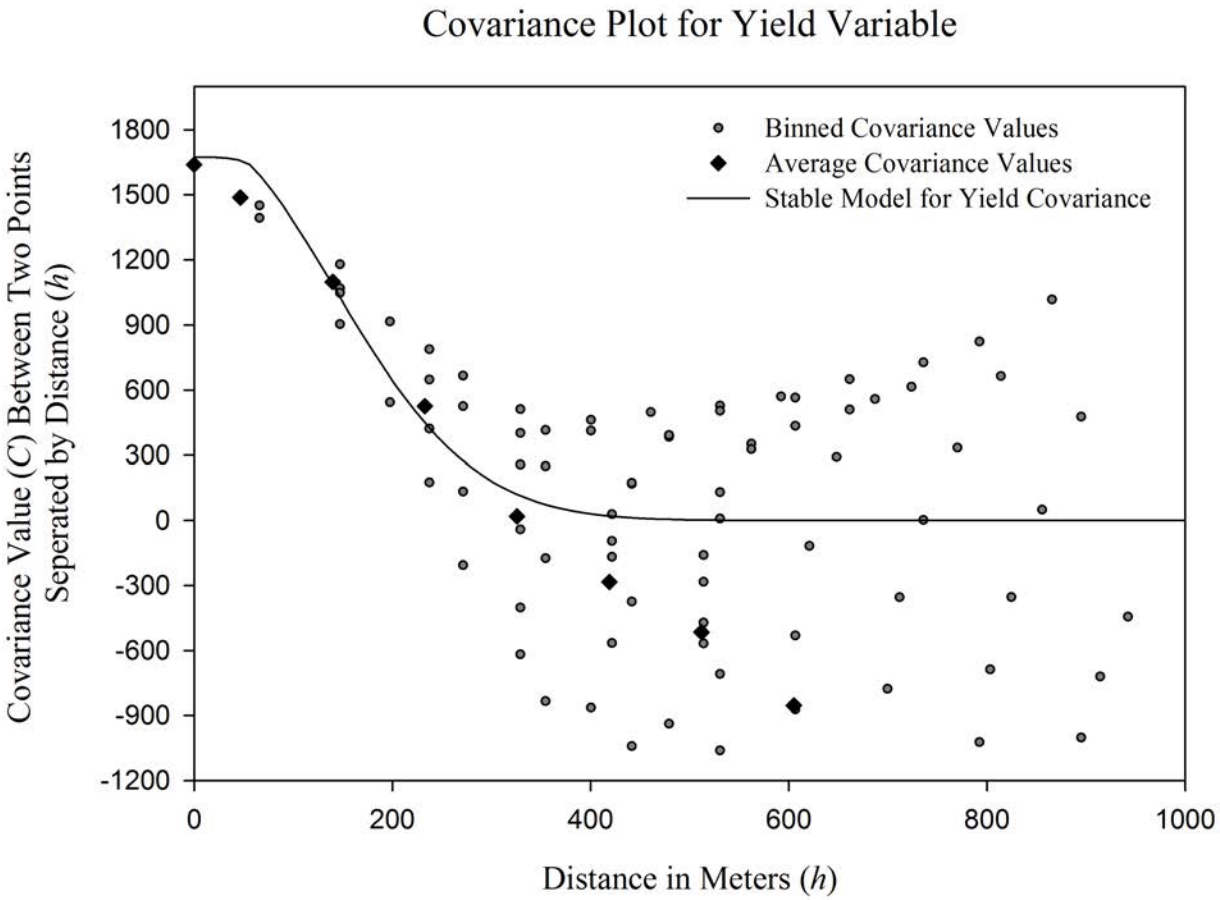


Figure 3.2. The covariance function of the variable for yield for potato ("Atlantic") in a single field shows the relationship between the covariance value (*C*; Y-axis) and the distance between the paired points (*h*; X-axis). Represents how similar two points are at (*h*) distance. As distance increases, similarity decreases. The similarity value for each pair of yield values are represent by ⚬, the average covariance value for any distance *h* is represented by ◆, and the line represents the stable statistical model fit to the data set

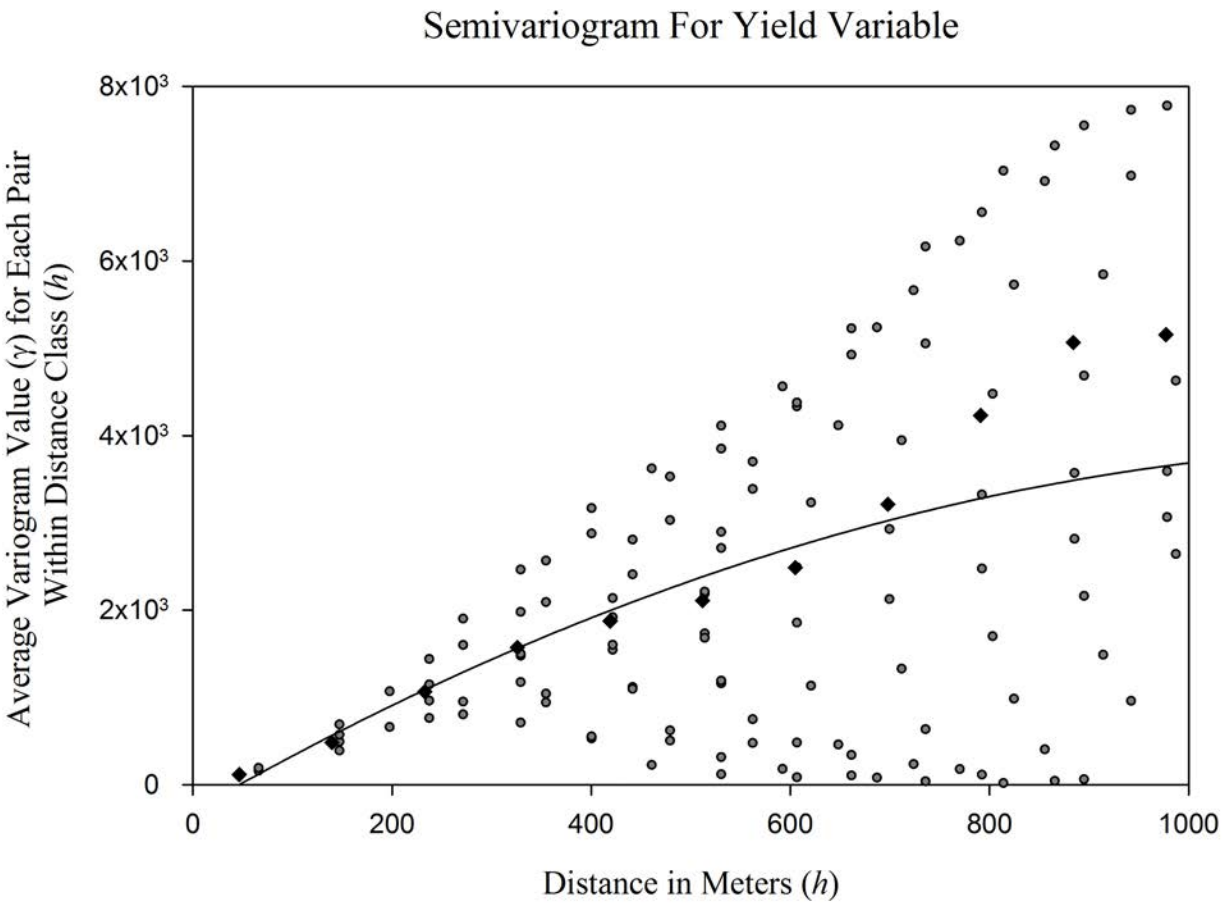


Figure 3.3. Semivariogram for yield of potato ("Atlantic") in a single field shows the dissimilarity of two values as distance increases. Distance (*h*; X-axis) represents the distance classes for all possible distances between points. Average variograms are calculated for each pair within the class (γ; Y-axis). The semi-variogram is generated by plotting half of the average squared distance between data values separated by distance (*h*). This represents the dissimilarity between data points separated by distance (*h*). As distance (*h*) increases, dissimilarity (γ) increases. Each pair of yield values are represented by ⚬, the average semivariogram value for any distance *h* is represented by ◆, and the line represents the stable statistical model fit to the data set

Three different variogram models, spherical, exponential, and Gaussian, were tested on the potato yield semivariogram to evaluate which predictive model provided the best fit. The spherical model exhibited linear behavior near the origin and was often best for spatial distribution with patches of larger or smaller values of similar size. The model is calculated as follows where c_0 is the nugget value, c is the partial sill, and a is the range:

$$\gamma(h) = c_0 + c\begin{cases} 1.5\frac{h}{a} - 0.5\left(\frac{h}{a}\right)^3 \; if \; h \leq a \\ 1 \; if \; h > a \end{cases} \tag{3.4}$$

The exponential model, like the spherical model, exhibited linear behavior near the origin, but at shorter distances the exponential model increased more steeply than the spherical model. However, as distance increased the rate of increase became less than that of a spherical model. The model is computed as follows where c_0 is the nugget value, c is the partial sill, and a is the range:

$$\gamma(h) = c_0 + c(1 - \exp\left(-\frac{3h}{a}\right)) \tag{3.5}$$

The Gaussian model exhibits parabolic behavior near the origin and was often used to model variables with high spatial continuity, such as elevation. The Gaussian model reaches the sill asymptotically, meaning that a practical range rather than the actual range is achieved. A practical range is defined as the distance at which the model value is at 95% of the sill (30). The Gaussian model is computed as follows where c_0 is the nugget value, c is the partial sill, and a is the range:

$$\gamma(h) = c_0 + c(1 - \exp\left(-\frac{3h^2}{a^2}\right)) \tag{3.6}$$

3.2.6 GEOSTATISTICAL PARAMETERS

The parameters of the model: nugget semivariance, range and sill or total semivariance were determined. Nugget semivariance is the variance at zero distance, the sill is the constant value of the variogram when the variables influence each other and the range is the distance at which the value of one variable becomes spatially independent of another. Two indices of spatial dependence were employed. One was the Q value [calculated as (sill-nugget)/sill], which indicates the spatial structure at the sampling scale. The Q value varies between 0 and 1. When it is close to 0, no spatial structure is detected at the sampling and support scale used. As the Q value approaches 1, more of the spatial variation can be explained by the semivariogram model at the analysis scale used. The other index is the range, which indicates the limit of spatial dependence.

3.2.7 CROSS-VALIDATION

Cross-validation removes each data location one at a time and predicts the associated data value (20) The variance of the errors is calculated and compered with the average Kriging variance for all estimations. If the prediction errors are unbiased, the mean prediction errors (ME) should be near zero:

$$ME = \frac{1}{N}\sum_{i=1}^{N}[\hat{Z}(x_i) - Z(x_i)] \tag{3.7}$$

where $\hat{Z}(x_i)$ is the predicted value at the cross-validation point, $Z(x_i)$ is the measured value at point x_i, and N is the number of data sets measured. However, this value depends on the scale of the data so it is beneficial to standardize these values. The mean standardized error (MSE) equation is as follows:

$$MSE = \frac{1}{N}\sum_{i=1}^{N}\frac{\hat{Z}(x_i) - Z(x_i)}{\hat{\sigma}(x_i)} \tag{3.8}$$

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

The square root of the mean error (not shown) squared is the root mean squared error (RMSE) for the model. This indicates how closely the model predicts the measured values but the scale of the data can affect these results. The equation for RMSE is as follows:

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}[\hat{Z}(x_i) - Z(x_i)]^2} \tag{3.9}$$

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

The root mean square standardized error (RMSSE) shows the model's accuracy in assessing variability. The root mean sum of the squared error cross-validation statistic is computed as follows:

$$RMSSE = \sqrt{\frac{1}{N}[\sum_{i=1}^{N}\frac{\hat{Z}(x_i) - Z(x_i)}{\hat{\sigma}(x_i)}]^2} \tag{3.10}$$

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

Finally, the "slope" is the value of the slope of the regression function for that model. The regression function plots the predicted values against the measured values. In addition, the stable

variogram model was included in the table because it was a best-fit model of the data set. The stable model is built into the ArcGIS geostatistical analysis but is proprietary and the equation for the stable variogram model is not available to the user. Comparison of the three models discussed previously (spherical, exponential, and Gaussian) using these parameters did not give a clear picture as to what model was best for yield values.

The smaller the RMSE, the closer the prediction was to the actual measured values. The closer the RMSSE is to 1, the more accurate the prediction of variability for that model. If the RMSSE is greater than one, the model has underestimated the variability in the predictions. If the RMSSE is less than one, the model has overestimated the variability in the predictions. The closer the slope is to 1 or -1, the closer the prediction was to the measured values for that model.

3.2.8 INTERPOLATING DATA

Once a model was found that best fit the semivariogram, two interpolation methods, inverse distance weighting (IDW) (30) and ordinary Kriging (30), were tested against one another to see which provided a better interpolation of the data set, to estimate values of variables between known data points. In GIS, interpolation is used to convert a sample of observations into a different representation, such as a contour map that can be used to shows changes in values across a surface (59). After the more accurate interpolation method was established, a map of "yield" was created. The "yield" map could be compared to maps of several variables created using similar interpolation techniques to visually identify relationships between variables.

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

$$Z^*(x_0) = \sum_{i=1}^{n} \lambda_i Z(x_i) \tag{3.11}$$

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

The first interpolation method used on the data set was IDW. The larger the distance a sample point was from the not-sampled location, the less weight that sample point had on the interpolation process. IDW assumes that points located closer to each other were more related than those farther apart. A mathematical formula was used to calculate values across a surface and assigned a higher weight to closer locations when calculating a local mean (59). The IDW method uses the general interpolation equation (equation 3.11) and the weights are calculated as follows:

$$\lambda_i = \frac{(1/d_i)^p}{\sum_{i=1}^{n}(1/d_i)^p} \tag{3.12}$$

where d_i is the distance between the non-sampled point that is being predicted and the known sample point. The larger the distance d_i a sample point $Z(x_i)$ is from the non-sampled point $Z^*(x_0)$, the less weight that sample point will have on the interpolation. Dividing it by the sum of all the weights standardizes each weight. The exponent p is used to provide flexibility to the weights. The greater the value of *p,* the greater the value of the weights at closer distances will be.

The second interpolation method, Kriging, assigned weights to points based on distance, predicted values and variance, and the overall spatial arrangement of data. Kriging uses the underlying spatial structure of the known data points to assign weights (59). Kriging is associated with the term Best Linear Unbiased Estimator (BLUE); it is linear because the

estimated values are weighted linear combinations of the available data, unbiased because the mean error is 0, and best because it minimizes the variance of the errors. The general kriging equations is as follows:

$$Z^*(x) - m(x) = \sum_{i=1}^{n} w(x_i)[Z(x_i) - m(x_i)] \quad (3.13)$$

where $w(x_i)$ is the weight assigned to the observation *i* at location x_i, The weights are assigned to the actual observations $z(x_i)$, but we interpret these observations as realizations of a random function $Z(x_i)$. The $m(x)$ and $m(x_i)$ are the expected values of the random functions at locations *x* and (x_i). The total number of the observed data used to estimate the value at non-sampled location *x* is equal to *n*. The error at each non-sampled location is also a random variable obtained as the difference between estimated and true values:

$$R(x) = Z^*(x) - Z(x) \quad (3.14)$$

where *Z(x)* is the true value. For the random function *Z*(x) at location *x*:

$$Z(x) = R(x) + m(x) \quad (3.15)$$

where *R(x)* is the error components and *m(x)* is the mean or trend component. In the case of ordinary kriging, the mean component is assumed to be constant, but the value is unknown. Minimizing the variance of the errors is what distinguishes Kriging from IDW. Kriging is best used when an estimate of the error of interpolation is needed. There are several different types of Kriging techniques, but the most common one and the one used in this research, was ordinary Kriging.

The interpolation techniques described above were used to approximate the distribution of data at not-sampled locations and to understand how soil properties, such as yield, were spatially correlated across the field or surface. After the data were evaluated with both methods,

the two methods were compared to determine which method was more accurate. There are no universal approaches known that determined the best interpolation method for every variable. Rather, methodology was dependent on the type of data being interpolated, the number of control points, and the particular problem being addressed (59). Although "yield" was described for one field in detail (described above) as an example for this paper, this interpolation process was applied to several measured variables (Figs. 3.4a and 3.4b) and for multiple commercial potato field locations (data not shown).

3.3 Results

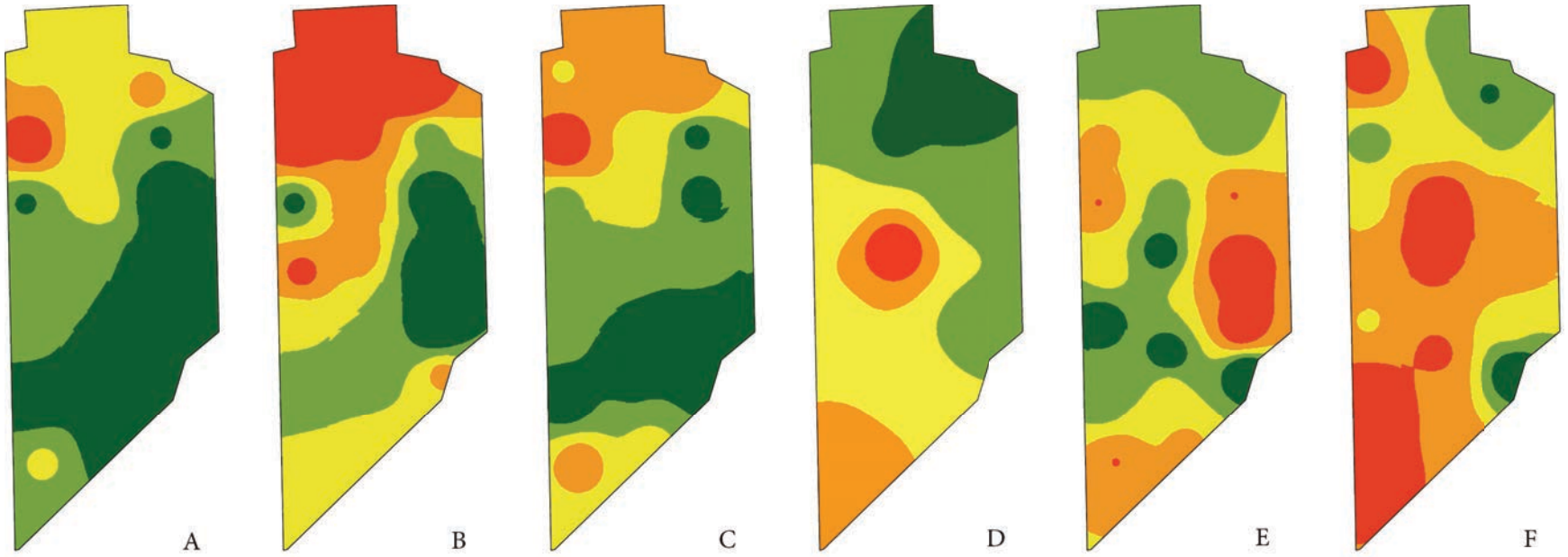
3.3.1 NEXT-GENERATION SEQUENCING OF THE 16S RRNA GENE

The total number of taxa identified by DNA sequences identified to the level of phyla, class, order, family and genus was 28, 81, 140, 300 and 814 respectively (Table 3.1). Multi-layer GIS-based maps were generated from sequencing results and information gathered on tuber yield from each point at the end of the growing season (Figs. 3.4a and 3.4b). The results provided baseline information on the ecology of bacteria across a potato production field related to soil physical properties, soilborne disease severity and total yield. Based on numbers of DNA sequences the relative abundance of bacterial taxa was determined for each sample, and field-wide. DNA sequence assignment was dominated by 9 of the 21 bacterial phyla recovered, comprising nearly 96% of the total bacterial community assemblage (Table 3.1). These phyla were present in every soil sample from the potato production field, and consisted of major bacterial groups including Proteobacteria (27%), Acidobacteria (15%), Actinobacteria (12%), Verrucomicrobia (4%), Firmicutes (3%) with unclassified bacteria representing 21% of the total bacterial phyla (Figs. 3.4a and 3.4b).

Table 3.1. Summary of 16S DNA sequencing effort from a 30 ha potato/corn rotation field located in southwestern Michigan (41°52'18.4435", -085°22'57.3227") in 2013

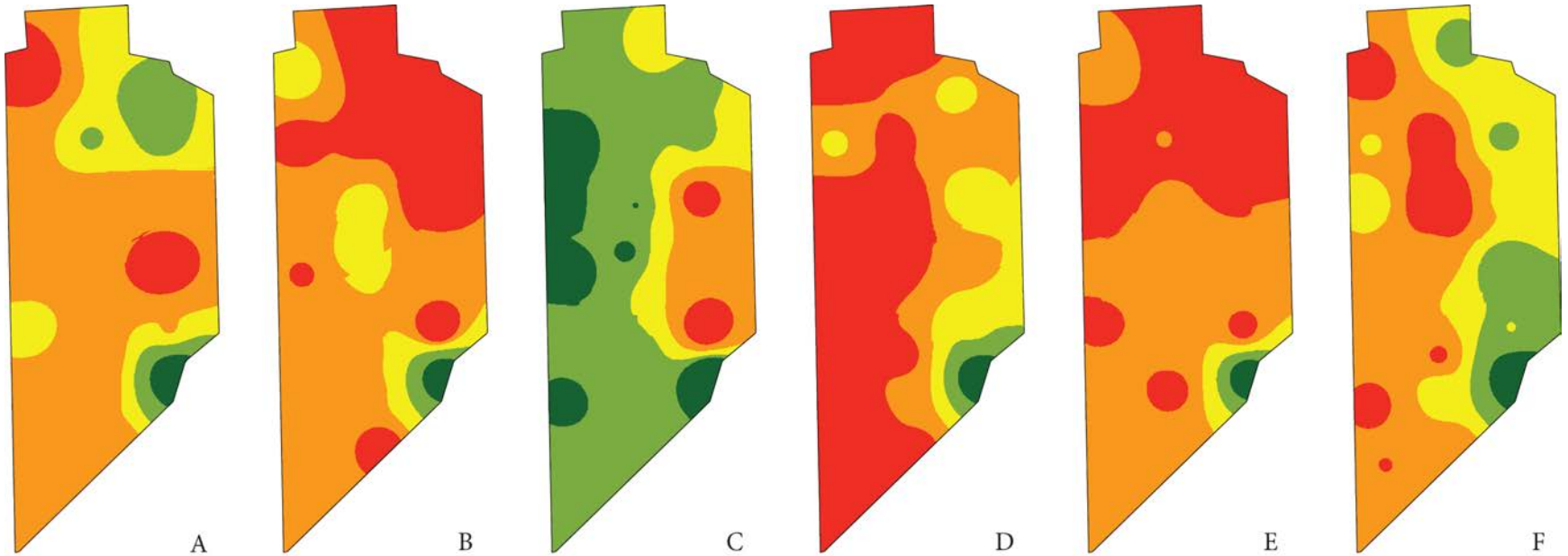
Total no. of soil samples	No. of samples sequenced	Total no. of sequences	Average no. of sequences/soil sample	Classification[a]				
				Phyla	Classes	Orders	Families	Genera
20	20	674,920	33,746	21	81	140	300	814

[a] Taxonomic classification level was determined based on the Ribosomal Database Project



Color Key	Organic Matter (A)	Potassium (B)	Magnesium (C)	Yield (D)	pH (E)	Inverse Simpson Index (F)
	1 - 1.15 (38.9%)	77.0 - 110.4 (8.6%)	49.0 - 63.0 (26.6%)	104.6 - 117.3 (14.6%)	6.1 - 6.28 (5.9%)	0.04 - 127.5 (0.8%)
	1.15 - 1.3 (26.6%)	110.4 - 128.8 (27.2%)	63.0 - 73.7 (38.8%)	117.3 - 130.1 (35.2%)	6.29 - 6.37 (37.3%)	127.5 - 199.3 (4.4%)
	1.3 - 1.5 (11.7%)	128.8 - 144.7 (32.5%)	73.7 - 84.7 (28.0%)	130.1 - 142.8 (31.3%)	6.38 - 6.47 (26.2%)	199.3 - 248.3 (35.5%)
	1.5 - 1.7 (20.0%)	144.7 - 165.1 (25.0%)	84.7 - 100.0 (5.3%)	142.8 - 155.7 (16.0%)	6.48 - 6.6 (23.7%)	248.3 - 302.2 (50.0%)
	1.7 - 2.0 (2.8%)	165.1 - 204.0 (6.8%)	100.0 - 123.0 (1.4%)	155.7 - 168.4 (3.0%)	6.61 - 6.8 (7.0%)	302.2 - 416.5 (9.3%)

Figure 3.4a. GIS maps of soil properties measured for a potato field in Saint Joseph County, MI (41°52'18.4435", -085°22'57.3227") in 2013, A: Organic matter in ppm; B: Potassium in ppm; C: Magnesium in ppm; D: Total yield (t/ha); E: pH; F: Inverse Simpson Diversity Index of microbial communities based on 16S rRNA next-generation sequencing data



Color Key	Acidobacteria (A)	Actinobacteria (B)	Firmicutes (C)	Unclassified (D)	Proteobacteria (E)	Verrucomicrobia(F)
	0 - 760 (0.7%)	0 - 488 (0.6%)	0 - 244 (1.1%)	0 - 850 (0.7%)	0 - 896 (0.9%)	0 - 210 (1.8%)
	761 - 1,215 (1.1%)	489 - 907 (0.7%)	245 - 315 (72.6%)	851 - 1,486 (0.9%)	897 - 1,712 (1.3%)	211 - 329 (13.7%)
	1,216 - 1,418 (16.9%)	908 - 1,136 (1.3%)	316 - 434 (12.8%)	1,487 - 1,838 (2.5%)	1,713 - 2,288 (2.8%)	330 - 379 (28.2%)
	1,419 - 1,697 (74.0%)	1,137 - 1,245 (26.7%)	435 - 588 (12.1%)	1,839 - 2,082 (33.2%)	2,289 - 2,676 (54.7%)	380 - 434 (44.2%)
	1,698 - 2,1523 (7.3%)	1,246 - 1,464 (70.8%)	589 - 820 (1.5%)	2,083 - 2,493 (62.8%)	2,677 - 3,412 (40.4%)	435 - 582 (12.2%)

Figure 3.4b. GIS maps of spatial distribution of soil microbial communities based on next-generation 16S rRNA sequencing data for a potato field in Saint Joseph County, MI (41°52'18.4435", -085°22'57.3227") in 2013, A: Acidobacteria (sequence abundance), B: Actinobacteria (sequence abundance); C: Firmicutes (sequence abundance); D: Unclassified (sequence abundance); E: Proteobacteria (sequence abundance); F: Verrucomicrobia (sequence abundance). Taxonomic classification level was determined based on the Ribosomal Database Project

3.3.2 STATISTICAL ANALYSIS

Based on the Pearson coefficient analysis the correlation of 15 pair-wise comparisons were significant at α=0.01 (Table 3.2). These included: OM with K and Mg, K with Mg, pH and Firmicute abundance, yield with inverse Simpson index and unclassified bacteria abundance, pH with Firmicute abundance, Acidobacteria abundance with Proteobacteria abundance, Actinobacteria abundance with unclassified bacteria abundance, Firmicute abundance with unclassified bacteria abundance and finally unclassified bacteria abundance with Proteobacteria abundance (Table 3.2). Further, the Pearson coefficient analysis of the correlation of 6 variables were significant at α=0.05 (Table 3.2). These included: OM with Firmicute abundance, pH with unclassified bacteria abundance, Acidobacteria abundance with Actinobacteria abundance, Actinobacteria abundance with Firmicute and Verrucomicrobia abundance and unclassified bacteria abundance with Verrucomicrobia abundance (Table 3.2).

The relationships of abiotic and biotic soil parameters were compared within the field using principle component analysis. Inverse Simpson diversity index was significantly positively correlated with Acidobacteria abundance, yield, unclassified bacteria abundance and Verrucomicrobia abundance (Fig. 3.5). Organic matter was significantly positively correlated with K and Mg (Fig. 3.5). Actinobacteria abundance was significantly positively correlated with Proteobacteria abundance (Fig. 3.5). Firmicutes abundance was significantly positively correlated with pH (Fig. 3.5). Proteobacteria abundance was significantly negatively correlated with inverse Simpson diversity index, Acidobacteria abundance and yield (Fig. 3.5). Actinobacteria was significantly negatively correlated with unclassified bacteria abundance and Verrucomicrobia abundance (Fig. 3.5). Firmicute abundance and pH was significantly negatively correlated with pH, OM, K and Mg (Fig. 3.5). In principle component analyses, abiotic and

Table 3.2. Correlation matrix of abiotic and biotic soil parameters from soil collected in 2012-13 from a 30 ha potato/corn rotation field in southwestern Michigan (41°52'18.4435", -085°22'57.3227")

	OM	K	Mg	Yield	pH	ISI[a]	Aci[a]	Act[a]	Fir[a]	Unc[a]	Pro[a]	Ver[a]
OM	1.000											
K	0.898*	1.000										
Mg	0.932*	0.833*	1.000									
Yield	-0.164	-0.040	-0.131	1.000								
pH	-0.528	-0.714*	-0.375	-0.271	1.000							
ISI[a]	-0.128	-0.036	-0.112	0.789*	-0.149	1.000						
Aci[a]	-0.200	-0.141	-0.266	0.303	-0.019	0.775*	1.000					
Act[a]	-0.486	-0.584	-0.411	-0.527	0.656	-0.524*	-0.393**	1.000				
Fir[a]	-0.572**	-0.676*	-0.572	-0.393	0.705*	-0.187	0.089	0.835**	1.000			
Unc[a]	0.361	0.400	0.370	0.751*	-0.433**	0.625*	0.226	-0.844*	-0.805*	1.000		
Pro[a]	0.001	0.021	0.004	-0.610	0.018	-0.853*	-0.675*	0.434	0.154	-0.638*	1.000	
Ver[a]	0.547	0.563	0.483	0.190	-0.392	0.427*	0.271	-0.419**	-0.406	0.537**	-0.675	1.000

[a] ISI: Inverse Simpson Diversity Index; Aci: Acidobacteria; Act: Actinobacteria; Fir: Firmicutes; Unc: Unclassified; Pro: Proteobacteria; Verrucomicrobia: Ver.

* Correlation significant at the level of α=0.01.

** Correlation significant at the level of α=0.05.

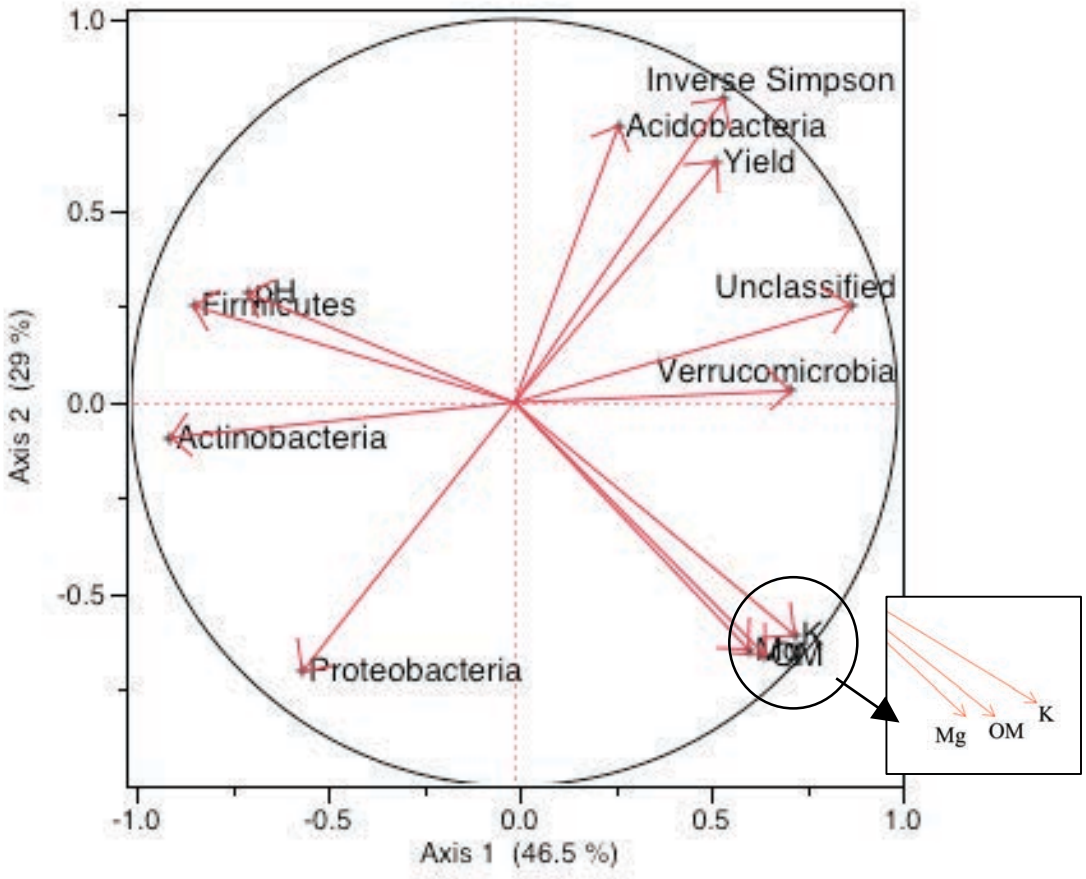


Figure 3.5. Principle component analysis summarizing relationships between abiotic and biotic soil parameters in a 30 ha field located in southwestern MI (41°52'18.4435", -085°22'57.3227"), planted to potatoes in 2013

biotic soil parameters were separated by axis 1 representing 46.5% of variability and by axis 2 representing 29% of variability (Fig. 3.5).

3.3.3 FITTING A VARIOGRAM MODEL AND INTERPOLATION OF DATA TO CREATE MAPS

Multi-layer GIS-based maps were generated from sequencing results and information gathered on tuber yield from each point at the end of the growing season (Figs. 3.4a and 3.4b). Variables was analyzed using geostatistical methods. The results provided baseline information on the ecology of bacteria across a potato production field related to soil physical properties, soil disease severity and total yield.

3.3.3.1 Geostatistical parameters of abiotic and biotic soil properties and yield

The calculated parameters from the variograms (nugget variance, partial sill, range and Q value) (Tables 3.3 and 3.4) of the soil abiotic properties showed spatial dependence ranging from 328.579 to 854.005 m, the soil biotic properties ranged from 281.282 m to 535.317 m, and yield range was 607.356 to 1116.930 m. The Q values of the four abiotic chemical parameters measured (organic matter, potassium, magnesium, and pH) were between 0.425 and 0.697 indicating that most of the variance could be explained by the semivariogram model. The Q value of yield was 1.0, indicating that the model chosen explains the spatial variation well. The Q value of the soil biotic properties ranged from 0.602 to 1.0 indicating that most of the variance could be explained by the semivariogram models chosen. Each property was characterized by a null nugget variance indicated the absence of spatial structure of this parameter at a finer scale than that investigated with our sampling design. Properties which showed nugget variances of any value greater than 0 indicate a significant residual variance at distance zero.

Table 3.3. Cross validation parameters semivariogram properties for organic matter, potassium, magnesium, yield, pH and inverse Simpson index (measure of total bacterial diversity) from a 30 ha potato/corn rotation field in Saint Joseph County, MI (41°52'18.4435", -085°22'57.3227")

Variable	Model	ME[a]	MSE[b]	RMSE[c]	RMSSE[d]	Nugget (λ)[f]	Range (m)[g]	Partial Sill (λ)[h]	Q[i]
Organic Matter	Spherical	0.006	0.006	0.282	1.447	0.030	854.005	0.087	0.742
	Exponential	0.003	0.002	0.268	1.223	0.016	854.005	0.095	0.856
	Gaussian	0.027	0.072	0.341	1.871	0.044	831.134	0.088	0.670
	Stable	-0.001	-0.007	0.254	1.055	0.044	831.134	0.088	0.670
Potassium	Spherical	-0.183	-0.008	35.227	1.100	734.285	558.786	1049.482	0.588
	Exponential	-0.562	-0.015	33.950	0.991	574.457	738.673	1322.744	0.697
	Gaussian	0.471	-0.019	41.396	1.931	930.325	522.111	900.429	0.492
	Stable	-0.885	-0.021	34.161	0.956	930.325	522.111	900.429	0.492
Magnesium	Spherical	-0.249	-0.021	19.672	1.209	165.406	854.005	371.078	0.692
	Exponential	-0.301	-0.018	18.882	1.213	114.872	854.005	386.606	0.771
	Gaussian	1.126	0.027	23.004	1.782	214.598	854.005	408.938	0.656
	Stable	-0.612	-0.027	17.938	1.015	214.598	854.005	408.938	0.656
Yield	Spherical	2.239	0.036	44.824	1.024	0.000	1116.930	3354.337	1.000
	Exponential	1.776	0.025	43.728	0.880	0.000	1116.930	2583.919	1.000
	Gaussian	-0.336	-0.040	52.425	2.020	58.896	607.356	2647.033	0.978
	Stable	0.849	0.011	46.036	0.888	0.000	1116.930	3976.063	1.000
pH	Spherical	0.001	-0.005	0.194	1.051	0.023	359.291	0.024	0.506
	Exponential	-0.005	-0.021	0.211	1.029	0.017	451.049	0.033	0.668
	Gaussian	0.015	0.062	0.192	1.439	0.027	328.579	0.020	0.425
	Stable	0.000	0.002	0.206	1.008	0.027	328.579	0.020	0.425
Inverse Simpson Index	Spherical	-0.059	0.011	66.022	0.903	0.000	281.282	7192.746	1.000
	Exponential	2.311	0.034	72.941	0.917	0.000	297.516	7241.321	1.000
	Gaussian	-1.048	0.017	68.851	1.298	1894.944	281.282	5611.248	0.748
	Stable	-0.312	0.008	65.775	0.919	0.000	281.282	7556.912	1.000

Table 3.3. (cont'd)

[a] Mean error is the averaged difference between the measured and predicted values. This value is dependent on the scale of the data. The closer this value is the zero the closer the prediction is to the measured values.

[b] Mean standardized error is the average of the standardized errors. The closer this value is to zero the closer the prediction is to the measured values.

[c] Root mean squared error, the root value of the mean squared error. This value is dependent on the scale of the data. The smaller the value is, the closer the prediction is to the measured values

[d] Root mean squared standardized errors should be close to one if the prediction standard errors are valid. If the root mean standardized error is greater than one, an underestimation of the variability occurred. If the root mean squared standardized error is less than one, an overestimation of the variability occurred.

[e] Slope of the regression equation comparing predicted values to actual values using a given model. The closer the slope is to one, closer the prediction is to the measured values.

[f] In theory, the semivariogram value at the origin (0 lag) should be zero. If it is significantly different from zero for lags very close to zero, then this semivariogram value is referred to as the nugget. The nugget represents variability at distances smaller than the typical sample spacing, including measurement error.

[g] The lag distance at which the semivariogram model first flattens out (or reaches its sill) is known as the range. Sample locations separated by distances closer than the range are spatially autocorrelated, whereas locations farther apart than the range are not.

[h] The semivariance value at which the variogram levels off. This scale of this value is dependent on the context of the data.

[I] Q value [(sill – nugget)/sill] indicates the spatial structure at the sampling scale. The Q value varies between 0 and 1. When it is close to 0, no spatial structure is detected at the sampling and support scale used. As the Q value approaches 1, more of the spatial variation can be explained by the semivariogram model at the analysis scale used.

Table 3.4. Cross validation parameters and semivariogram properties of Kriging prediction methods for sequence abundance of Acidobacteria, Actinobacteria, Unclassified, Proteobacteria, and Verrucomicrobia from a 30 ha potato/corn rotation field in Saint Joseph County, MI (41°52'18.4435", -085°22'57.3227")

Variable	Model	ME[a]	MSE[b]	RMSE[c]	RMSSE[d]	Slope[e]	Nugget (λ)[f]	Range (m)[g]	Partial Sill (λ)[h]	Q[i]
Acidobacteria	Spherical	-9.475	-0.021	282.095	1.067	-0.746	0.000	346.572	107980.219	1.000
	Exponential	-6.248	-0.009	279.570	0.983	-0.840	0.000	470.200	116249.485	1.000
	Gaussian	-11.551	-0.027	293.759	1.386	-0.596	12666.532	292.803	96615.149	0.884
	Stable	-12.324	-0.029	284.881	1.145	-0.666	0.000	319.836	111090.907	1.000
Actinobacteria	Spherical	-7.842	-0.047	157.281	1.290	-1.200	2564.055	295.011	17304.081	0.871
	Exponential	-6.516	-0.039	139.412	1.064	-1.020	0.000	348.320	20859.065	1.000
	Gaussian	-9.523	-0.083	181.052	2.083	-1.315	8242.752	295.011	11946.511	0.592
	Stable	-6.920	-0.042	144.761	1.117	-1.034	0.000	295.011	20501.914	1.000
Firmicutes	Spherical	-9.046	-0.031	123.637	0.936	-0.660	0.000	496.895	44671.472	1.000
	Exponential	-7.334	-0.023	129.670	0.807	-0.745	0.000	535.317	42612.022	1.000
	Gaussian	-9.269	-0.065	187.251	2.738	-0.721	46.903	397.397	46902.808	0.999
	Stable	-20.019	-0.161	225.821	5.692	-0.830	46.903	397.397	46902.808	0.999
Unclassified	Spherical	-11.900	-0.027	292.553	1.218	-0.800	0.000	303.265	90277.442	1.000
	Exponential	-7.493	-0.018	270.575	1.052	-0.841	0.000	450.383	99501.988	1.000
	Gaussian	-19.247	-0.019	353.713	2.411	-0.809	22123.059	295.011	70507.775	0.761
	Stable	-8.423	-0.013	288.510	1.212	-0.797	0.000	295.011	92821.990	1.000
Proteobacteria	Spherical	28.139	0.059	302.962	1.219	-0.835	46420.718	319.392	36236.713	0.438
	Exponential	14.663	0.028	295.201	1.070	-0.938	23931.152	281.282	59834.949	0.714
	Gaussian	53.519	0.147	364.966	2.069	-0.767	56343.934	294.341	26485.010	0.320
	Stable	8.543	0.014	301.027	1.057	-1.000	19607.799	281.282	64164.273	0.766
Verrucomicrobia	Spherical	1.060	0.023	74.155	1.096	-0.845	2979.506	281.282	3851.372	0.564
	Exponential	2.977	0.039	73.920	0.947	-0.938	2723.871	281.282	4127.705	0.602
	Gaussian	-1.549	-0.018	89.455	2.062	-0.724	4933.975	281.282	1815.625	0.269
	Stable	4.085	0.050	75.509	0.934	-0.980	3789.587	281.282	3035.773	0.445

Table 3.4. (cont'd)

[a] Mean error is the averaged difference between the measured and predicted values. This value is dependent on the scale of the data. The closer this value is the zero the closer the prediction is to the measured values.

[b] Mean standardized error is the average of the standardized errors. The closer this value is to zero the closer the prediction is to the measured values

[c] Root mean squared error, the root value of the mean squared error. This value is dependent on the scale of the data. The smaller the value is, the closer the prediction is to the measured values

[d] Root mean squared standardized errors should be close to one if the prediction standard errors are valid. If the root mean standardized error is greater than one, an underestimation of the variability occurred. If the root mean squared standardized error is less than one, an overestimation of the variability occurred.

[e] Slope of the regression equation comparing predicted values to actual values using a given model. The closer the slope is to one, closer the prediction is to the measured values.

[f] In theory, the semivariogram value at the origin (0 lag) should be zero. If it is significantly different from zero for lags very close to zero, then this semivariogram value is referred to as the nugget. The nugget represents variability at distances smaller than the typical sample spacing, including measurement error.

[g] The lag distance at which the semivariogram model first flattens out (or reaches its sill) is known as the range. Sample locations separated by distances closer than the range are spatially autocorrelated, whereas locations farther apart than the range are not.

[h] The sill is semivariance value at which the variogram levels off. The partial sill = (sill-nugget) which accounts for the variance of the nugget. This scale of this value is dependent on the context of the data.

[I] Q value [(sill – nugget)/sill] indicates the spatial structure at the sampling scale. The Q value varies between 0 and 1. When it is close to 0, no spatial structure is detected at the sampling and support scale used. As the Q value approaches 1, more of the spatial variation can be explained by the semivariogram model at the analysis scale used.

3.3.3.2 Geostatistical analysis of potato yield

The behavior of the semivariogram for yield showed that as distance increased between data points, dissimilarity increased (Fig. 3.3). This was further evidence that yield values had a strong spatial autocorrelation and were suitable for interpolation**.** Each variogram model fitted to the data set was evaluated using several cross-validation techniques (Table 3.3). This procedure produces a list of actual and theoretical errors. From the list, the actual errors are averaged. If the estimation is unbiased the average should be zero. The ratio between these two quantities is expected to be one, if the estimation procedure has been carried out correctly (13). Mean standardized error (MSE) is the average of the standardized errors and this value should be close to 0 (Table 3.3).

The exponential model had the MSE closest to zero and the smallest RMSE, the spherical model had the RMSSE closest to 1, and the Gaussian model had the ME closest to 0 and the slope closest to -1. The Gaussian model had the slope closest to -1 but had the MSE furthest from 0, the largest RMSE value and the RMSSE that was furthest from 1 of the three models. In this case, having the ability to create a stable model in ArcGIS is advantageous since the spherical, exponential, and Gaussian models do not provide a statistically rigorous fit. The stable model for the yield data set had an MSE of 0.011 and the slope closest to -1. The yield semivariogram was then fitted with the stable model, as it was the best model to use in the interpolation process (Fig. 3.3).

Once the data were tested for spatial continuity and a best-fit model was selected to fit the semivariogram, the data from the sample points were interpolated to predict the values at non-sampled locations. The two interpolation methods compared in this study were inverse distance weighting and ordinary Kriging. Mean standardized error (MSE) and RMSSE are not available

for IDW so the three cross-validation measures used in the comparison between IDW and ordinary Kriging were ME, RMSE and slope. For the example of the yield variable the ME and RMSE for ordinary Kriging stable model was closer to 0, with a value of 0.849 and 46.036 respectively, compared to the IDW model ME and RMSE values of 2.989 and 46.354 respectively (Table 3.5). Two maps were created for the variable yield (Fig. 3.6). The slope (Table 3.5) for ordinary Kriging was closer to -1 than the slope for IDW (-0.926 and -0.916, respectively). Comparison of these parameters showed that ordinary Kriging more correctly predicted the values at not-sampled locations.

3.3.3.3 Geostatistical analysis of soil physiochemical factors

The spatial variability of soil physicochemical parameters was also evaluated as described above (Table 3.3). For potassium (K), magnesium (Mg) and pH variables the best interpolation method was the ordinary Kriging with MEs of -0.562, -0.612, and 0.000 respectively; RMSEs of 33.950, 17.938, and 0.206 respectively; and slopes of -0.652, -0.811, and -1.028 respectively compared to the IDW MEs of -0.792, -1.375, and -0.007 respectively; RMSEs of 35.943, 18.324, and 0.213 respectively; and slopes of -0.771, -0.855, and -1.045, respectively (Table 3.5). For organic matter (OM) the best interpolation method was the IDW interpolation method with and ME of -0.006 compared to -0.001 for ordinary Kriging, RMSE of 0.248 compared to 0.254 for ordinary Kriging, and a slope of -0.771 compared to -0.725 for ordinary Kriging (Table 3.5).

The interpolated maps for soil physicochemical properties showed a range of spatial variability (Fig. 3.4a). For organic matter, based on the IDW interpolation map the highest percentage of area was 38.9% (11.7 ha) fell within the range of 1-1.15% OM (Fig. 3.4a). For the spatial map for variable Mg, which used the ordinary Kriging stable model interpolation, the

Table 3.5. Cross validation parameters of Kriging prediction methods for Inverse Distance Weighting and ordinary Kriging prediction methods for variables from a 30 ha potato/corn rotation field in Saint Joseph County, MI (41°52'18.4435", -085°22'57.3227")

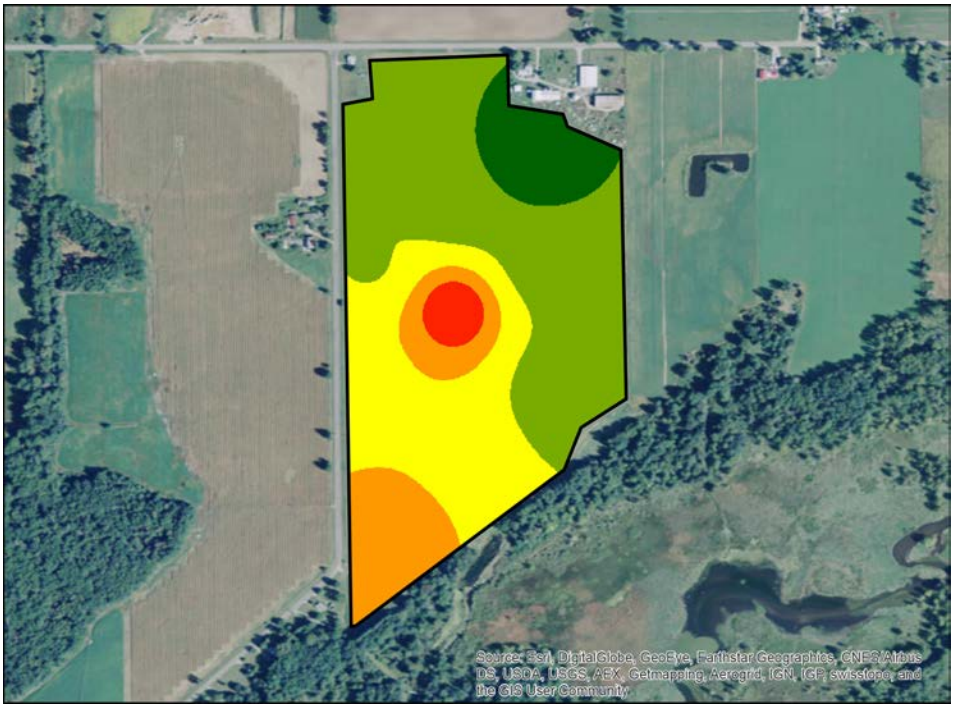
Variable	Interpolation Technique (Model)	ME[a]	RMSE[b]	Slope[c]
Organic Matter	Inverse Distance Weighting	-0.006	0.248	-0.771
	Ordinary Kriging (Stable)	-0.001	0.254	-0.725
Potassium	Inverse Distance Weighting	-0.792	35.943	-0.771
	Ordinary Kriging (Exponential)	-0.562	33.950	-0.652
Magnesium	Inverse Distance Weighting	-1.375	18.324	-0.855
	Ordinary Kriging (Stable)	-0.612	17.938	-0.811
Yield	Inverse Distance Weighting	2.989	46.354	-0.916
	Ordinary Kriging (Stable)	0.849	46.036	-0.926
pH	Inverse Distance Weighting	-0.007	0.213	-1.045
	Ordinary Kriging (Stable)	0.000	0.206	-1.028
Inverse Simpson Index	Inverse Distance Weighting	2.965	70.118	-0.704
	Ordinary Kriging (Stable)	-0.312	65.775	-0.503
Acidobacteria	Inverse Distance Weighting	-10.281	290.008	-0.908
	Ordinary Kriging (Exponential)	-6.248	279.570	-0.840
Actinobacteria	Inverse Distance Weighting	-14.889	135.105	-1.015
	Ordinary Kriging (Exponential)	-6.516	139.412	-1.020
Firmicutes	Inverse Distance Weighting	-16.231	132.417	-0.714
	Ordinary Kriging (Exponential)	-7.334	123.637	-0.660
Unclassified	Inverse Distance Weighting	3.785	262.128	-0.858
	Ordinary Kriging (Exponential)	-7.493	270.575	-0.841
Proteobacteria	Inverse Distance Weighting	22.549	286.099	-0.916
	Ordinary Kriging (Stable)	8.543	301.027	-1.000
Verrucomicrobia	Inverse Distance Weighting	4.066	74.186	-0.957
	Ordinary Kriging (Exponential)	1.060	73.920	-0.938

[a] Mean error is the averaged difference between the measured and predicted values. This value is dependent on the scale of the data. The closer this value is the zero the closer the prediction is to the measured values.

[b] Root mean squared error, the root value of the mean squared error. This value is dependent on the scale of the data. The smaller the value is, the closer the prediction is to the measured values

[c] Slope of the regression equation comparing predicted values to actual values using a given model. The closer the slope is to one, closer the prediction is to the measured values.

A



B

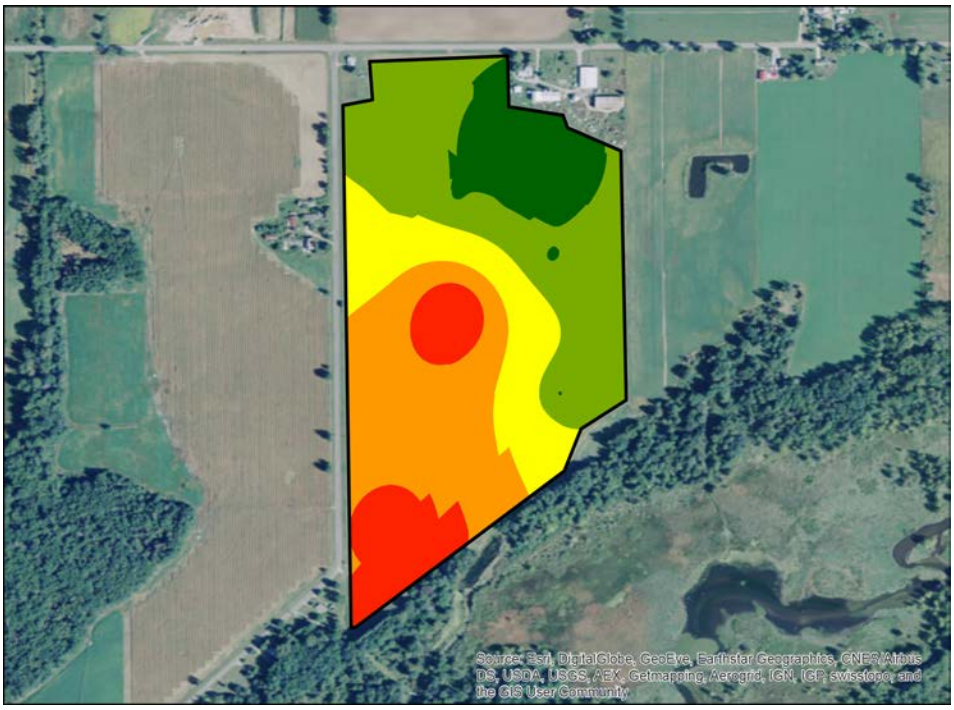


Figure 3.6. Two contour maps generated from the sample points (n=20) where potato yield was measured to predict yield values at any non-sampled point in the field using the Inverse Distance Weighting (IDW) method of interpolation (A) and the ordinary Kriging (B) method of interpolation from a 30 ha potato/corn rotation field in Saint Joseph County, MI (41°52'18.4435", -085°22'57.3227") ranging from 105 t/ha (dark green) to 168 t/ha (red)

highest percentage of area was 37.3% (11.2 ha) in a range of 63.0-73.7 ppm (Fig. 3.4a). Similarly, using ordinary Kriging stable model interpolation for variable pH the highest percentage of area was 37.3% (11.2 ha) in the range of 6.29-6.37 (Fig. 3.4a). Unique among the soil physicochemical properties, the spatial map of K was based on the ordinary Kriging exponential model with the highest percentage of area being 32.5% (9.8 ha) within the range of 128.8-144.7 ppm (Fig. 3.4a).

3.3.3.4 Geostatistical analysis of soil bacterial diversity

The spatial variability of soil bacterial diversity was also evaluated as described above (Table 3.4). For total bacterial diversity based on the inverse Simpson index, the best interpolation method was the ordinary Kriging stable model with an ME of -0.312, RMSE of 65.775, and a slope of -0.503 compared to the IDW ME of 2.965, RMSE of 70.118, and slope of -0.704 (Table 3.5). For sequence abundance of Acidobacteria, Firmicutes, and Verrucomicrobia the best interpolation method was ordinary Kriging using an exponential model with MEs of -6.248, -7.334, 1.060 respectively; RMSEs of 279.570, 123.637, and 73.920 respectively; and slopes of -0.840, -0.660, and -0.938 respectively compared to IDW MEs of -10.281, -16.231, and 4.066 respectively; RMSEs of 290.008, 132.417, and 74.186 respectively; and slopes of -0.908, -0.714, and -0.957 respectively (Table 3.5). The best interpolation model for sequence abundance of Proteobacteria was the ordinary Kriging interpolation using a stable model with an ME of 8.543, RMSE of 301.027, and a slope of -1.000 compared to the IDW ME of 22.549, RMSE of 286.099, and slope of -0.916 (Table 3.5). Finally, the best interpolation model for sequence abundance of Actinobacteria and unclassified bacteria showed that IDW more correctly predicted the values at not-sampled locations with MEs of -14.889 and 3.785, respectively; RMSEs of 135.105 and 262.128 respectively; and slopes of -1.015 and -0.858, respectively

compared to ordinary Kriging MEs of -6.516 and -7.493, respectively; RMSEs of 139.412 and 270.575, respectively; and slopes of -1.020 and -0.841, respectively (Table 3.5).

The interpolated maps for soil bacterial diversity showed a range of spatial variability (Figs. 3.4a and 3.4b). For total bacterial diversity based on the inverse Simpson index based on the ordinary Kriging stable model map the highest percentage of area was 50.0% (15 ha) with indices in the range of 248.3 to 302.2 (Fig. 3.4a). The spatial map for the variables Actinobacteria and unclassified, which used IDW interpolation, the highest percentage of area was 70.8% (21.2 ha) in the range of 1,246 to 1,464 sequences and 62.8% (18.8 ha) in the range of 2,083 to 2,493 sequences, respectively (Fig. 3.4b). For the spatial map for the variable Proteobacteria, which used the ordinary Kriging stable model interpolation, the highest percentage of area was 54.7% (16.4 ha) in the range of 2,289 to 2,676 sequences (Fig. 3.4b). Using the Kriging exponential model for the variables Acidobacteria, Firmicutes and Verrucomicrobia bacteria the highest percentage of area was 74.0% (22.2 ha), 72.6% (21.8 ha) and 44.2% (13.3 ha) respectively, corresponding to total number of sequence in ranges of 1,419-1,697, 245-315, and 380-434, respectively (Fig. 3.4b).

3.4 Discussion

The goals of this research were to use the procedures and methods developed during this study to build a framework on which to construct tools for understanding microbial interactions within soil as well as visualizing soil properties, both biological and edaphic, across individual fields as additional components of an integrated disease management system. Currently the tools used in this study to develop predictive mapping are computationally intensive and costly to perform manually. Therefore, the utilization of a program to automate the interpolation process

and map-making pipeline and continue to evaluate different sampling schemes for fields will be essential to ensure adaptability for a variety of measured variables.

The long-term goals of this project include: 1. Use the baseline data presented here to develop a trans-disciplinary tool combining the latest DNA technologies, GIS and computational biology at the sub-field management scale so that growers can easily monitor soil conditions, soil biodiversity and pathogen levels. This technology has the potential to improve productivity, reduce chemical inputs (71), and improve soil quality (40) for reduced disease pressure and sustainable high-quality crop production and 2. To expand DNA based detection and mapping to target specific bacterial, fungal and oomycete soilborne pathogens of potato. The incorporation of predictive and conditional probability maps would be a component of integrated soilborne disease management in commercial potato production. The ultimate goal is to build a framework for the development of sub-field management tools that may be used in precision production systems to provide a disease risk-advisory for potato growers, allowing for the delineation and management of homogenous classes within the field rather than managing a field as a whole (73).

To better understand the soil ecology related to potato production systems, this study used a trans-disciplinary approach (e.g. DNA sequencing, GIS and geostatistics) to develop spatial maps as a foundation to develop tools and recommendations to inform crop production management. The use of the Illumina sequencing platform enabled the detection of a wide diversity of bacteria associated with a potato production field. Due to the complexity and depth of the bacteria identified, this study focused on the relative abundance of a sub-sample of the most abundant taxa at the phylum level. This included the phyla Actinobacteria, Acidobacteria, Verrucomicrobia, Proteobacteria and Firmicutes, which are common to most soil types at high

levels of relative abundance similar to previous work (92, 94), including potato production systems (4, 65, 85). Similar to previous studies (10, 65) the most abundant sequences corresponded to unclassified bacteria, due to database bias of well-sampled organisms whose genomes are annotated (65).

The spatial variability was similar among the phyla Actinobacteria, Acidobacteria, Verrucomicrobia, Proteobacteria and unclassified bacteria, where sequence abundance was coincident in the same areas of the field. Moreover, total diversity based on the inverse Simpson index had a similar spatial distribution as the sequence abundance of Actinobacteria, Acidobacteria, Verrucomicrobia, Proteobacteria and unclassified bacteria. Conversely in the case of the phyla Firmicutes the spatial distribution of low numbers of sequence abundance were similar visually to parts of the field where Actinobacteria, Acidobacteria, Verrucomicrobia, Proteobacteria, unclassified bacteria and total diversity was high. In this particular field, areas with high total bacterial diversity were similar to areas in the field with the greatest total yield and low soil organic matter, which has been previously reported in cucumber (*Cucumis sativus* L.) (6). Recent studies using the Illumina-based platforms focused on quantifying the beta-diversity between communities using database-dependent methods (11, 12, 88). While important for comparing communities, the use of measuring beta-diversity is limited to comparisons where there are clear differences between communities, and it does little to inform one of the details of the subtle differences between communities (45) and the composition of communities in space. Therefore, the use of spatial maps for comparing soil microbial communities is useful to visualize the differences of their distributions in crop production fields.

The phylogenetic composition of bacterial communities in the area influenced by the roots (rhizosphere) of three potato cultivars grown at two separate field sites found that sequence

abundance was the highest for Proteobacteria (46%), followed by Firmicutes (18%), Actinobacteria (11%), and Acidobacteria (3%) (85). Other work has found species-specific effects of plants on the composition and relative abundance of microbial populations in the rhizosphere of crops and of cultivated native plant species (17, 21, 27, 70, 75). Therefore, the rhizosphere has proven to be a valuable resource to exploit for studies on bacterial diversity to develop host specific tools to enable sustainable agricultural crop production systems (96). The phyla Actinobacteria and Proteobacteria also found in relatively high numbers from bulk soil, based on sequence abundance in this study. Within the Actinobacteria and Proteobacteria the bacterial families *Streptomycetaceae* and *Pseudomonadaceae* respectively showed the strongest response at the potato cultivar level using a DNA based culture-independent approach (85). Members of both the *Streptomycetaceae* and *Pseudomonadaceae* are important plant pathogens and others have been exploited for their antimicrobial activities as biological controls (81). Commercially available biocontrol rhizobacteria include strains of *Streptomyces griseoviridis* K61 (Mycostop;AgBio development), *Pseudomonas fluorescens, Pseudomonas putida* and *Pseudomonas chlororaphis* (Cedomon; BioAgri) (60, 67).

The spatial variability of OM, K and Mg were similar, with low levels of all three parameters similar in the same areas of the field. In the case of pH the spatial distribution was more variable, and in the current study, the areas of the field with high pH were areas with low OM, K and Mg. Areas with low OM, K and Mg were in similar areas of the field with high numbers of sequence abundance of Actinobacteria, Acidobacteria, Verrucomicrobia, Proteobacteria and unclassified bacteria, and lower sequence abundance of Firmicutes. Studies evaluating simple physicochemical and microbiological soil parameters using geostatistics found similar trends in that soil pH, microbial biomass, and organic nitrogen differed from the structure

of microbial communities (24), but direct correlations of microbial communities and yield are yet to be elucidated.

Mineral nutrients to some degree can affect soilborne potato diseases via chemical physical, physiological or structural components involved in plant defense mechanisms (46) along with other important effects (74). Moreover nutrient availability in the soil can increase antagonistic microbial activity, thus affecting soilborne pathogens directly or indirectly (46). Unfortunately, disease minimization may or may not be consistent with optimal fertilization for yield, quality and probability (46). Optimization for management of one disease may not be the same for another, and the exact mechanisms involved are often complex and poorly understood (46). Therefore, sub-field fertility management recommendations for crop health, disease suppression coupled with increased yield, quality and economic considerations for site-specific conditions are more desirable than broadcast applications. The availability of precision agriculture technology with increased computing capacity and GIS can inform and direct these site-specific recommendations for both fertility and soilborne disease management programs for commercial potato production.

3.5 Conclusion

From an agronomic perspective, having the ability to take a relatively small amount of data points and use those points to get predicted values for an entire field could prove invaluable. Although only one field was described in depth, other potato production fields were mapped using a similar interpolation analysis pipeline. The hope was to visually resolve distinctive trends and similarities between variables across a field (e.g. high yielding areas and high concentrations of organic matter). Visual correlation did reveal similar trends among variables across the same

potato field. Therefore, further statistical and analytical techniques, coupled with more intensive and alternative sampling will be needed to validate the usefulness and accuracy of predictive mapping. Although assessing the spatial continuity of the data and then fitting that data to the correct model may enable accurate predictions with very little error is essential for further comparative studies. The current study was a baseline study where only a few variables were assessed. Additionally, the spatial information from this study is specific to the field in which the study was conducted. Though the data in this field is useful for the grower who farms this field on a yearly basis, the methods discussed above may be useful for other researchers conducting similar studies in other locations. Further investigation of the spatial correlation and interpolation process of different sets of variables is essential. Future research will incorporate the use of predictive mapping into overall assessment of soil health and soil microbial interactions and community diversity. In a related study the use of indicator Kriging, which maps the probability of an area being above a set threshold as a disease-risk assessment tool, was evaluated for implementation into commercial integrated pest management (71). Indicator Kriging may be important to reduce production costs and chemical inputs used in the management of commercial potato.

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CHAPTER 4: USING CONDITIONAL PROBABILITY AND A NONLINEAR KRIGING TECHNIQUE TO PREDICT VERTICILLIUM WILT CAUSED BY *VERTICILLIUM DAHLIAE*

Steere, L., Rosenzweig, N., and Kirk, W. 2016. Using conditional probability and a nonlinear Kriging technique to predict potato early die caused by *Verticillium dahliae*. Pages 142-151. in: Geographical Information Systems Theory, Applications and Management C. Grueau, and J. G. Rocha, eds. Springer International Publishing, Cham, CH.

Abstract

Verticillium dahliae is a plant pathogenic fungus that can be devastating to commercial potato production. Potato growers in the state of Michigan have experienced yield declines and decreased marketability. These yield declines may be the result of the persistence of *V. dahliae* in soil. Research was conducted using geostatistics and geographic information systems (GIS) to assess the spatial variability of *V. dahliae* in the soil. The use of a nonlinear Kriging method allowed the for the prediction of where infection may occur based on published established thresholds. Nonlinear Kriging is a useful tool for creating conditional probability maps based on a threshold, which can be built into the equation. *Verticillium dahliae* has an inoculum threshold needed to cause infection in a potato plant. Using this threshold, maps can be created based on a probability of any point in space being greater than the threshold. The methods used in this paper show how geostatistics can be used for fumigation disease management recommendations for commercial growers.

4.1 Introduction

Potatoes (*Solanum tuberosum* L.) are mainly consumed as fresh, chipping, frozen, or starch products and require tubers that meet a high-quality standard in either cosmetic appearance or

structural integrity from producers. Potato is one of the most intensively managed crops (47), and cultivation using vegetative tubers as seed makes the crop vulnerable to several recurrent and persistent soilborne diseases (12, 15, 29, 59, 65, 69). Nearly 90% of major diseases that impact crops (including potato) are caused by soilborne pathogens (72). Soilborne diseases in potato production are currently managed using combinations of chemical fungicides, biological fungicides, fumigation, crop rotation, soil amendments, and other cultural practices (23). Soil fumigation is not consistently effective against soilborne diseases (8, 9), compared to its effectiveness on reducing populations of nematodes and some insects (37, 66). Furthermore, fumigation is cost prohibitive especially at labelled rates and creates environmental concerns (20).

In 2012, a team comprised of potato growers and university researchers was formed to develop a research agenda to address the issue of declining yields and decreased tuber quality in some potato production areas in Michigan dedicated (59). The goals of the research were 1. to better understand the spatial variability of soilborne pathogen inoculum levels in potato fields; 2. to better understand the soil biology and quantify soilborne disease inoculum and 3. to predict where in the field an infection may occur based on pathogen levels determined by conditional probability. These goals are the culmination of a survey in which growers throughout the state of Michigan indicated, in their opinion, the major factors contributing to yield losses in potato production.

Verticillium wilt, caused by the soilborne fungus *Verticillium dahliae*, is a pathogen that is particularly significant and in severe cases, can lead to yield reductions of up to 50% (52, 60). *Verticillium dahliae* has a wide host range including bell pepper (*Capsicum annuum*) (5), eggplant (*Salanum melongena* L.) (6), mint (*Mentha* spp.) (45), potato (48, 52, 53), and tomato (*Solanum lycopersicum* L.) (4). Potato plants are infected directly via penetration of root hairs by the fungus

(7, 46, 55). Once the fungus has penetrated the root cortex it enters the xylem where it quickly plugs the vascular system leading to premature senescence (48, 52, 53, 58) (Fig 1.1). Verticillium wilt is an annual production concern for commercial potato growers and impacts plant health and subsequently, crop yield. *Verticillium dahliae* is in the Ascomycota phylum and is a well-documented pathogen of potato plants (13, 14, 28, 39, 40, 43, 46, 48, 53, 58). The use of conditional probability may help determine where infection by *V. dahliae* might occur based on inoculum levels at sampled locations.

This research used geographic information systems (GIS) and geostatistics to create predictive maps of entire fields from known sample points. GIS technology has proven to be a successful tool in precision agriculture (1). GIS and global positioning satellite (GPS) technology helps growers to identify problem areas across a field and make management decisions to target those specific locations (67). This means fields no longer need to be managed as a whole, but that problem areas can be managed as separate entities (26). GIS has enabled growers to improve productivity, apply fertilizers and pesticides at variable rates, and precisely guide equipment across fields (1). Since the 1980's when precision agriculture first increased in use, tools such as GIS and GPS have increased growers understanding of the complex relationships that exist across their fields.

The use of linear Kriging methods in soil science has been well documented (33, 35, 41, 73). This project evaluated a nonlinear Kriging model to interpolate data for *V. dahliae* inoculum levels in the soil. Nonlinear Kriging techniques have advantages over linear Kriging techniques due to their ability to account for uncertainty (36) and therefore are often used to predict the conditional probability for categorical data at non-sampled locations (17, 21).

Estimating a value of the variable of interest has been investigated thoroughly (17); however, estimating an indicator has been given little attention. This research investigated conditional probability (indicator) maps of Verticillium wilt potential using the nonlinear geostatistical model called indicator Kriging. To generate conditional probability maps, a variable of interest must be converted into a binary variable (0 or 1) by choosing a threshold (17). If values are above the set threshold, they become 1 and if they are below the threshold, they become 0. Therefore, the interpolation is between 0 and 1, and the estimates can be interpreted as the probability of a variable being 1 (or 100% probability) (57). If a threshold is used to create the indicator variable, the resulting interpolated map shows the probabilities of exceeding or falling below the threshold. Indicator Kriging is a nonlinear Kriging technique that is flexible and can be modified to fit specific management or research goals by modifying the critical threshold criteria (56). Conditional probability maps generated using indicator Kriging can be used to visualize the probability of any point in space (within the field of interest) being greater than a set threshold. When a known threshold value is available for certain pathogens and insects, a conditional probability map can be a valuable agronomic crop management tool.

Timely implementation of management practices for Verticillium wilt requires that some estimate of potential effects of disease on yield be available before the crop is planted. Traditionally this has been done by considering the disease and crop history of a given planting site and basing management strategies on past experience (48). To allow growers to approach management options from a more informed basis, various yield-loss risk-assessment systems have been developed to serve as decision aids (18, 19, 30). Growers can make management decisions based on soil sampling and whether that sampling reveals pathogen populations that exceed previously published action threshold values (18, 43, 44, 68). Although there is some variability

in published threshold values, they fall in the range of 5-30 CFU/g air-dried soil. These thresholds are used as decision aids in determining the need for soil fumigation. By using the low end of a pre-determined threshold value (5 CFU/g soil) as a cut-off in an indicator Kriging model, this project will give growers a more realistic visualization of where in their fields they can expect to find Verticillium wilt and plan management strategies accordingly.

4.2 Materials and Methods

4.2.1 STUDY AREAS AND COLLECTION OF SOIL

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

4.2.2 QUANTIFICATION OF *VERTICILLIUM DAHLIAE* COLONY FORMING UNITS

To estimate *V. dahliae* colony forming units (CFU), 10 g of soil from each sample point (3 replicates) was prepared using the wet sieving method (27, 42). Residue left in the 37μm sieve (Endecotts Inc., Newtown, PA) was centrifuged for 5 minutes at 1600 *g*, and excess water was

removed via aspiration. The final residue was plated onto an NP-10 medium (32) which served as a selective nitrogen source and promoted the development of CFU of *V. dahliae* while inhibiting the growth of other soilborne fungi and bacteria. Contents of the medium per liter were 5 g of polygalacturonic acid-sodium salt, 1 g of KNO_3, 1 g of KH_2PO_4, 0.5 g of KCl, 0.5 g of $MgSO_4 \bullet 7H_20$, 0.5 ml of Tergitol NPX, 0.5 g of streptomycin sulfate, 0.25 g of chlorotetracyclin HCl, 0.25 g of chloramphenicol, and 15 g of agar (45). Isolates were stored at 22°C for 14-21 days and observed at 4x magnification under a dissecting microscope (Leica Microsystems Inc., Buffalo Grove, IL) and number of microsclerotia (CFU) were recorded. Each sample point was replicated 3 times to confirm the accuracy of the initial CFU enumeration.

4.2.3 GEOSTATISTICAL ANALYSIS AND INTERPOLATIONS

Data from CFU enumeration were imported to ArcGIS as data tables then linked to the point data information from the field to create a database from which predictive maps could be developed. Geostatistics were used to predict and interpolate the values of spatially distributed data. Spatial interpolation is based on Tobler's First Law of Geography, that data is often spatially dependent or autocorrelated, or a value of a variable at one location will be similar to values of nearby variables (51, 63).

4.2.3.1 General interpolation

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

$$Z^*(x_0) = \sum_{i=1}^{n} \lambda_i Z(x_i) \qquad (4.1)$$

where $Z^*(x_0)$ is the non-sampled location that is being predicted, $Z(x_i)$ are the values at n sampled locations and λ_i are the weights assigned to each sampled data point (22). The difference between interpolation methods is dependent on how λ_i is calculated and what their respective values are.

4.2.3.2 Indicator Kriging interpolation method

The indicator Kriging model assumes an unknown, constant mean. The technique has been well documented (31, 57) and the general form can be computed as follows (17):

$$I(s) = \mu + \varepsilon(s) \tag{4.2}$$

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

$$I(x, z_k) = \begin{cases} 1, if\ z(x) \geq z_k \\ 0, otherwise \end{cases} \tag{4.3}$$

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

$$I^*(x_o; z_k) = \sum_{i=1}^{n} \lambda_i I(x_i; z_k) \tag{4.4}$$

and the indicator Kriging, given $\Sigma\lambda=1$, is:

$$\sum_{j=1}^{n} \lambda_j \gamma_I(x_j - x_i) = \gamma_I(x_o - x_i) - \mu \tag{4.5}$$

where λ_j is the weight coefficient, γ_I is the semivariance of the indicator Kriging codes at the respective lag distance, and μ is the Lagrange multiplier (34),

4.2.3.3 Model evaluation

The models used in this study were evaluated based on two criteria: the accuracy and the successfulness of the model in estimating the variability. The accuracy of the indicator Kriging model was evaluated by using the root mean square error (RMSE) cross-validation calculated as (49):

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}[\hat{Z}(x_i) - Z(x_i)]^2} \quad (4.6)$$

where $\hat{Z}(x_i)$ is the predicted value at the cross-validation point, $Z(x_i)$ is the measured value at point x_i and N is the number of data sets measured. The smaller the RMSE, the closer the prediction was to the measured values. The successfulness of the model in assessing the variability was evaluated by using the root mean squared standardized error (RMSSE) cross-validation statistic calculated as (49):

$$RMSSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left[\frac{\hat{Z}(x_i) - Z(x_i)}{\sigma^2(x_i)}\right]^2} \quad (4.7)$$

where $\hat{Z}(x_i)$ is the predicted value at the cross-validation point, $Z(x_i)$ is the measured value at point x_i, N is the number of data sets measured, and $\sigma^2(x_i)$ is the variance at cross-validation point x_i. If the RMSSE is close to 1, the variability of the prediction is correctly assessed.

4.3 Results and Discussion

Cross-validation statistical analysis was performed on data for the three fields with a low-, high- and variable-risk for Verticillium wilt based on the spatial distribution of CFU (Table 4.1). These cross-validation statistics are used to determine how well the indicator Kriging equation interpolated the *V. dahliae* CFU numbers for each of the three fields. The closer the RMSE is to zero (0.11-0.50), the closer the prediction is to the measured values (50). All three fields had RMSE values relatively close to zero meaning that the model derived from the data points in each of the respected fields accurately predicted the probability of any point in space, within the field being greater than the threshold of 5 CFU/g of soil.

Table 4.1. Cross-validation parameter root mean squared error (RMSE) and root mean squared standardized error (RMSSE) are used to assess the accuracy of the models predictions and the model's successfulness in assessing variability

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

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

The RMSSE shows the model's successfulness in assessing variability. The closer the RMSEE is to 1, the more successful the modes was in predicting variability (50). The calculations using the indicator Kriging equations (above) for each of the three fields of interest showed high levels of accuracy in predicting and assessing variability. Each of the three equations performed well in accuracy of predictions of probability of an established threshold (CFU > 5 CFU/g of soil) at points that were not sampled.

Conditional probability maps were generated for the three individual fields (Figs. 4.1-4.3). These maps spatially represented the probability of Verticillium wilt incidence based on a 5 CFU/10 g of soil threshold. A conditional probability map was generated of the low-risk field (Fig. 4.1). Based on the 20 original *V. dahliae* CFU values and a threshold value of 5 CFU/g of soil, the indicator Kriging model developed for this field predicts a low incidence of Verticillium. The small portion of the field colored red had a probability from 0.95 to 1 of Verticillium wilt. Much of the field, colored in blue had a probability between 0 and 0.1 for Verticillium wilt. This in contrast to the conditional probability map for the high-risk field (Fig. 4.2). Most of this field had a probability between 0.95 and 1 for Verticillium wilt. This is in contrast from the low-risk field. Finally, a conditional probability map was generated of the variable-risk field (Fig. 4.3). The result is a map where the probability of being above the established Verticillium wilt threshold varied throughout the field.

The visualized differences among these three maps shows how the use of conditional probability can be used to predict the spatial distribution of soilborne pathogens which may cause plant diseases, and provide an informational tool for commercial potato growers. To help reduce inoculum levels of *V. dahliae* and other soilborne pathogens, growers will often elect to use soil fumigants. For many years, soil fumigants such as methyl bromide were used, with great

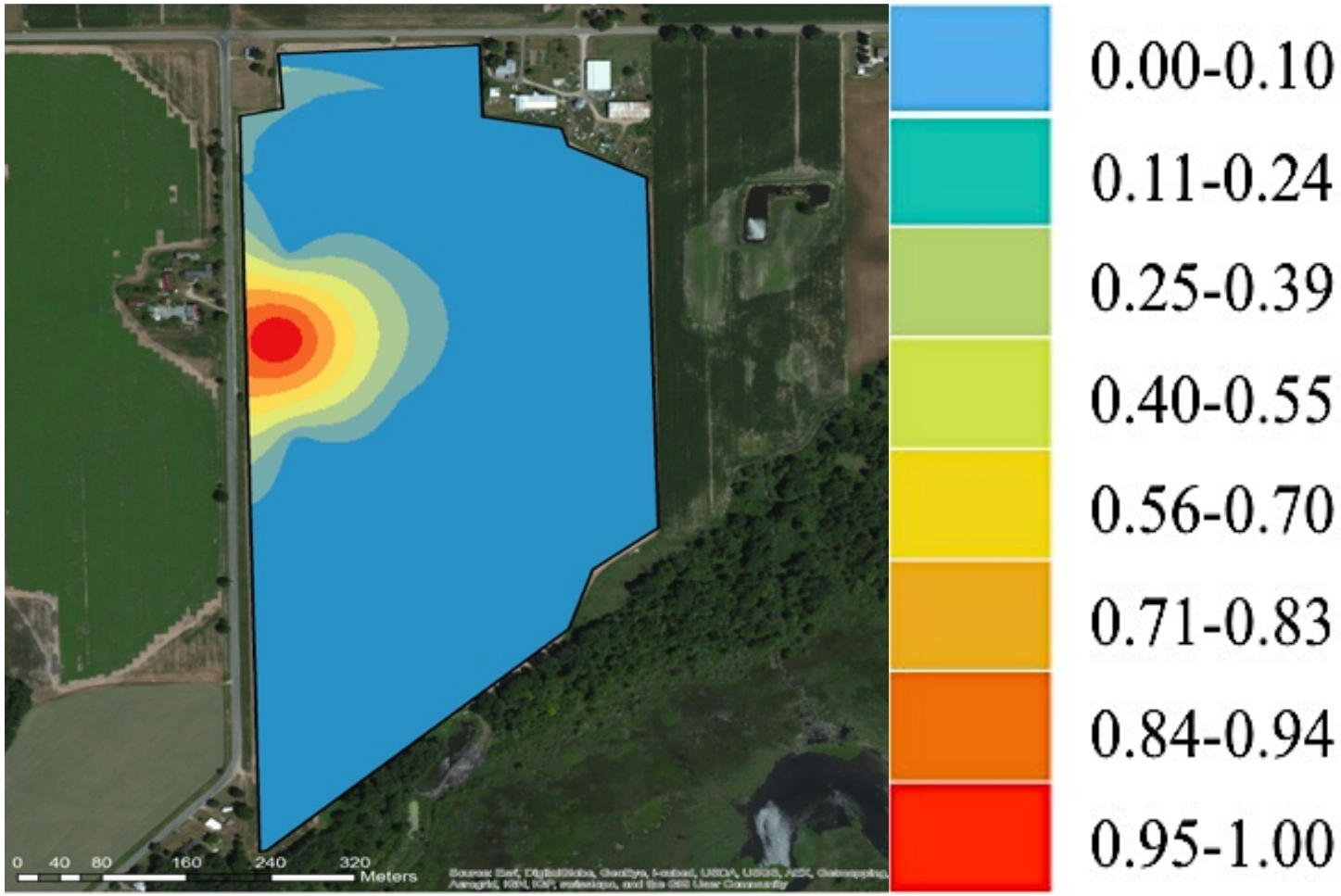

Figure 4.1. Conditional probability maps developed for low-risk field using the indicator Kriging method of interpolation with the threshold set at 5 CFU/g of soil. The conditional probability map represents the risk for the development of Verticillium wilt based on the probability of that area in space having greater than 5 CFU/g of soil with the color red representing a high probability and the color blue representing a low probability based on predicted values of *Verticillium dahliae* CFU at that location in the field

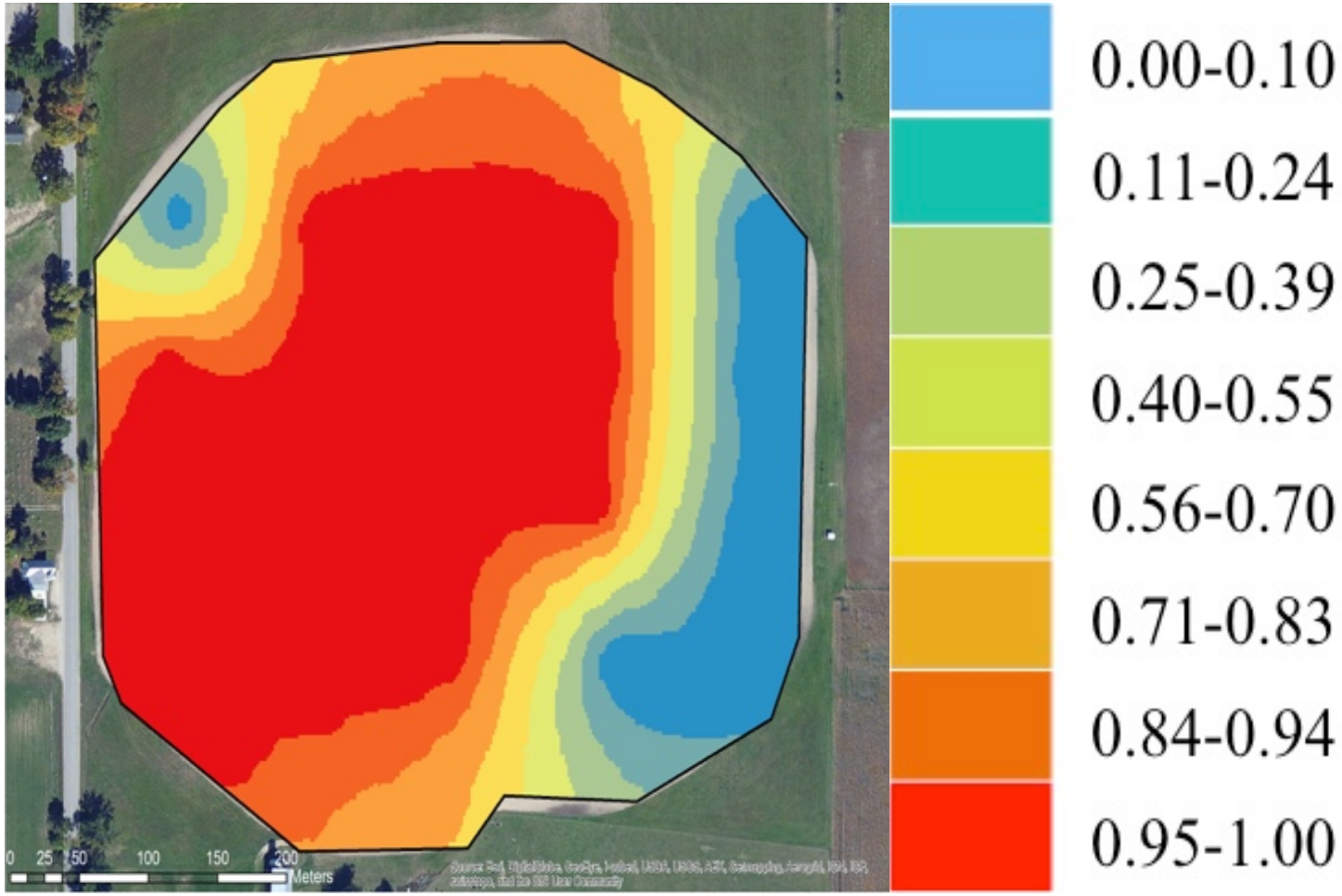

Figure 4.2. Conditional probability maps developed for high-risk field using the indicator Kriging method of interpolation with the threshold set at 5 CFU/g of soil. The conditional probability field represents the risk for the development of Verticillium wilt based on the probability of that area in space having greater than 5 CFU/g of soil with the color red representing a high probability and the color blue representing a low probability based on predicted values of *Verticillium dahliae* CFU at that location in the field

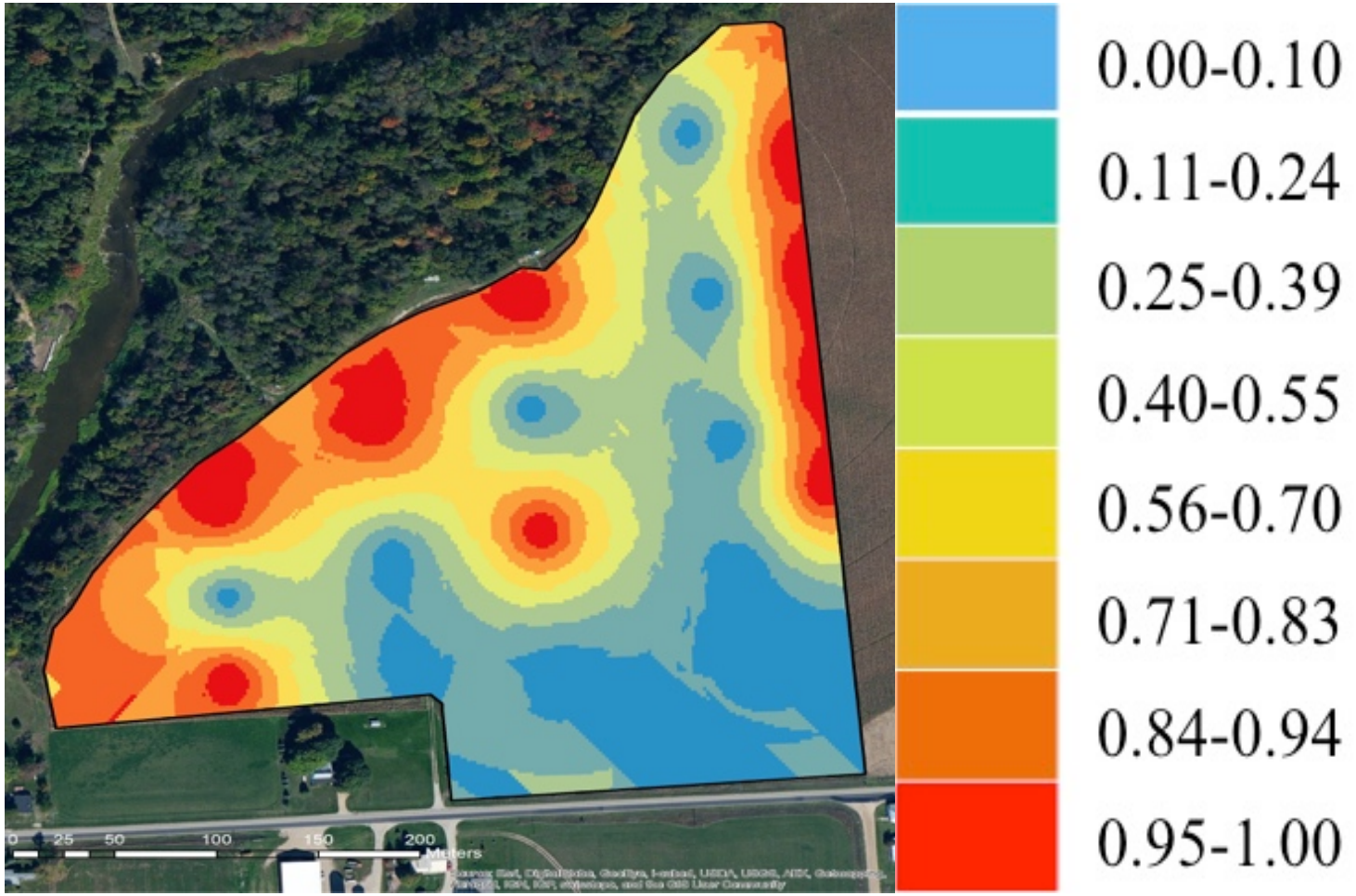

Figure 4.3. Conditional probability maps developed for variable-risk field using the indicator Kriging method of interpolation with the threshold set at 5 CFU/g of soil. The conditional probability map represents the risk for the development of Verticillium based on the probability of that area in space having greater than 5 CFU/g of soil with the color red representing a high probability and the color blue representing a low probability based on predicted values of *Verticillium dahliae* CFU at that location in the field

effectiveness, in eliminating or mitigating the impact of soilborne plant pathogens such as *V. dahliae* (16, 70, 71). More recently the use of methyl bromide has been prohibited due to its negative effect on the environment (61). New soil fumigants such as metam sodium and chloropicrin have taken the place of methyl bromide (38) but as researchers begin to better understand the role of beneficial soil microorganism related to plant health (25, 62) the use of any broad-spectrum fumigant is being re-evaluated in a new context. While these soil fumigants may control soilborne pathogens (16, 23), they may be reducing the beneficial soil microorganism populations that assist in plant growth and natural defense against plant pathogenic bacteria and fungi (10, 64). Thus, it is important to reduce the dependency of fumigation application as a routine management practice to decrease diseases caused by soilborne pathogens.

The accessibility of conditional probability maps could become a useful informational tool for growers implementing integrated disease management. Rather than making crop management decisions for a farming operation's acreage in its entirety, a grower would be able to assess each field individually, or even at the sub-field level to determine problem fields or areas of the field that would benefit from soil fumigation or some other soil amendment for management of Verticillium wilt. If the grower maintained a low-risk field (Fig. 4.1), they could use conditional probability as a management tool to determine a lack of need for fumigation in that field based on the Verticillium wilt risk. Conversely, if the grower assesses the conditional probability for Verticillium wilt and the results indicate a high-risk for Verticillium wilt above the established threshold (Fig. 4.2), the grower may elect to treat with applications of soil fumigants. Lastly, if a grower is managing a variable-risk field for Verticillium wilt (Fig. 4.3), this would allow the grower to make decisions based on a sub-field management approach and only apply treatment to the portions of the field that present a greater probability of Verticillium wilt. By moving away

from generalized, large-scale management practices and into single field and sub-field management strategies with the incorporation of geostatistics and GIS, growers have the potential to greatly decrease input cost and negative environmental effects (61) brought on by regular treatment regimes of soil fumigants and other inputs.

4.4 Conclusions

Over the last 50 years, commercial potato growers have applied large amounts of soil fumigants to combat soilborne pathogens. New research has indicated that these soil fumigants are disrupting the soil ecology and often removing beneficial microbes from the soil (11, 38). Conditional probability maps may allow growers to make more informed decisions on when and where soil fumigation or other treatments are necessary. By decreasing the amount of amendments made to the soil it is possible that more beneficial microorganisms will survive (64) and over time a balanced soil environment may be attained (24). Though this research studied only one pathogen in one cropping system, the methods and tools used have the potential to be incorporated into any cropping system that is plagued by soilborne pests. Most soilborne pathogens that affect commercial crops have been researched for decades (3, 54). Information on pathogen thresholds needed to cause infection is available for most soilborne pests. Conditional probability mapping can be inserted into most integrated pest management plans for crops that have soilborne pests.

The long-term goals of this project include using the baseline data presented here to develop a trans-disciplinary tool combining DNA technologies, GIS and computational biology at the sub-field management scale so that growers can easily monitor soil conditions, soil biodiversity, and pathogen levels. This technology has the potential to improve productivity, reduce chemical inputs, and improve soil quality for less disease pressure and sustainable high-

quality crop production. The research team is currently developing a high throughput DNA sequencing protocol to more rapidly quantify *V. dahliae* CFU in hopes of getting information back to growers in a timely manner. By eliminating the 21-day growth period for quantifying CFU (2), a grower would be able to make management decisions earlier in the season. The goal is to build a framework for the development of sub-field crop management tools that may be used in precision production systems to provide a disease risk-advisory for commercial potato growers.

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CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

Verticillium wilt caused by the fungus *Verticillium dahliae* is an annual production concern for potato growers in Michigan and throughout the US. Levels of *V. dahliae* colony forming units (CFU) in the soil have a direct correlation with level of observed disease severity in above ground plant parts, so managing CFU in the soil is the common practice in commercial potato production. Historically, growers have relied on strict soil fumigation regimes to decrease *V. dahliae* propagules in the soil, but these soil fumigation programs can be cost prohibitive. Furthermore, the negative impact that these fumigants can have on the environment, coupled with growing concern over their impact on beneficial microorganisms in the soil has led to an increased focus on the development of alternative management strategies for Verticillium wilt. The results of experimental field trials from 2013-2016 provided baseline information on the effects of several commercially available fungicides and their ability to manage Verticillium wilt of potato. Initial comparison of seven fungicides found that pyramethanil + fluopyram (pyr + fluo) (Luna Tranquility, Bayer CropScience) when applied in-furrow at planting significantly decreased disease severity and CFU in the stem were reduced but had no effect on CFU units in the soil. Similarly, applying pyr + fluo at emergence may decrease disease severity and CFU in the stem.

Whether these results are due to inhibition of *V. dahliae* in the stem by pyr + fluo or a reduction of root-lesion nematodes (RLN) in the soil are yet to be elucidated. Though the cause of the reduction in CFU in the stem and disease severity is not yet known, it is apparent that pyr + fluo is useful in managing Verticillium wilt. Determining the effect of pyr + fluo on microsclerotial germination *in vitro* will be essential in helping researchers to determine how best to use this chemistry for management of Verticillium wilt. Future research should attempt to understand the temporal effect that pyr + fluo is having on RLN and whether this interaction is contributing to a

decrease in Verticillium wilt symptoms when pyr + fluo is applied to potato in-furrow and at emergence.

For potato growers to reduce soil inputs for Verticillium wilt, a better understanding of the soil microbiology in potato cropping systems is needed, particularly how it changes in space. Initial efforts to do this used a trans-disciplinary approach involving DNA sequencing, traditional soil sampling, geographic information systems (GIS) and geostatistics. Together, these techniques were used to develop spatial maps as foundational tools and to inform crop management recommendations. The use of next-generation sequencing platforms enabled the detection of a wide diversity of bacteria associated with potato production. This data along with soilborne pathogen levels and other edaphic soil properties was used to develop a spatial interpolation system which allowed for visual representation of variables throughout the entire field of interest. From an agronomic management perspective, the ability to sample a relatively small number of points to get predicted values for an entire field could prove invaluable. Although only one field was described in depth in this study, the process described has been used to interpolate many fields in potato production throughout the state of Michigan. The hope of this study was to visually resolve distinctive trends and similarities between variables across the same field (e.g. high yielding areas and high concentrations of organic matter). Assessing the spatial continuity of a data set and fitting that data to the correct model enables accurate prediction with very little error however further statistical and analytical techniques, coupled with new sampling techniques will be needed to validate the usefulness and accuracy of predictive mapping.

Though the data in this study from this field is site specific, the methods discussed in the study may be useful for other researchers conducting similar studies in other locations. It is important to understand that this research did not set out to make conclusions about the effects of

microbial diversity on yield and disease but to develop a tool and improve methods for managing soilborne diseases in potato cropping systems. Future research must incorporate other variables which contribute to the overall soil microbial community that were not assessed in this study such as fungi, archaea, Protista and other higher level organisms. As additional information is revealed and alternative methods for the study of these organisms become available, they can easily be imported into the spatial analysis system that has been developed during this research. To validate these methods, large scale field trials will need to be conducted using spatial interpolation to develop spatially customizable management decisions.

The large scale trans-disciplinary study provided a wealth of data that was labor intensive and time consuming to analyze. Until computing capacity and empirical knowledge of the relationships between soil microbial communities and soilborne plant pathogens are increased, the data set will have many confounding relationships. Alternatively, the methods and information from the previous study could be used to reassess the necessity of current management strategies for diseases caused by soilborne pathogens, such as Verticillium wilt. Over the past half century, commercial potato production has relied on scheduled broadcast application of soil fumigants to combat these soilborne pests. The development and subsequent use of conditional probability maps based on critical levels of *V. dahliae* will allow growers to make more informed decisions on when and where soil fumigation is needed.

This approach to management has the potential to reduce inputs and make more informed decisions, but will require constant refinement to ensure long-term adaptability. Most importantly, potato cultivars currently used in large scale commercial potato production need to be assessed for thresholds of soilborne pathogens at which disease will occur, as current thresholds have been established for cultivars no longer present in commercial production. Additionally, interpolation

and geostatistical analysis needs to be done on the multivariate level for more accurate assessment. The *V. dahliae* threshold for disease can be reduced by up to 50% when RLN reach a certain threshold so it is important to develop techniques that take both pathogens into consideration when predicting where Verticillium wilt may occur. Finally, the quantification assay for *V. dahliae* CFU needs to shift towards molecular diagnostics. The turn-around time for quantifying CFU is from 21 to 28 days and limits the ability to make input management recommendations, because the crop may already have been planted in this time-frame. Though the development of a rapid and accurate real-time quantitative polymerase chain reaction (qPCR) technique, inoculum levels can be determined in a 1-2 day period. By decreasing the amount of time required for inoculum level determination, growers using conditional probability maps can make management decisions less than one week after sampling.