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SYMPOSIUM

Managing Soilborne Disease of Potatoes Using Ecologically Based Approaches

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Abstract Soil fumigation and planting resistant cultivars remain the primary means for control of soilborne plant diseases. Fumigation however, is being constrained by increased costs, urbanization, and its negative environmental impacts. Resistance genes to soilborne pathogens are not available for many crop species. Using verticillium wilt as model disease we examined the potential of non chemical alternatives, specifically the use of organic amendments and green manures, as disease management tools. Application of organic products reduces disease incidence in controlled settings but its application in commercial use was hampered by inconsistent efficacy. Studies now have demonstrated that by-products of animal and plant production, such as meat and bone meal, feather meal, poultry and swine manure, soy meal, etc. can significantly reduce diseases but that the level of control obtained is product and soil specific. Three mechanisms of action for pathogen reduction were identified: 1) generation of toxic compounds such as ammonia and nitrous acid from high nitrogen-containing materials, 2) presence of volatile fatty acids (e.g. vinegar) and 3) alterations in biological agents that may suppress the activity of plant pathogens. For products that work mostly through the generation of active chemical ingredients, knowing the properties that regulate efficacy allows targeting their use to specific locations and thus, increased activity and consistency. Much less is known about how such materials influence rhizosphere residents involved in regulating plant health. New molecular technologies are being implemented to identify key players in maintaining root health. Through an understanding of the microbial soil ecosystem we should be able to develop disease control strategies that are more economical to growers and are more environmentally benign.

Resumen La fumigación del suelo y el uso de variedades resistentes permanecen como los primeros medios para controlar las enfermedades vegetales originadas en el suelo. No obstante, la fumigación está siendo limitada por el aumento en los costos, la urbanización, y sus impactos ambientales negativos. Los genes de resistencia a los patógenos del suelo no están disponibles para muchas especies de cultivos. Usando el modelo de enfermedad del marchitamiento por verticillium, examinamos el potencial de alternativas no químicas, específicamente el uso de mejoradores orgánicos y abonos verdes, como herramientas para el manejo de enfermedades. La aplicación de productos orgánicos reduce la incidencia de la enfermedad en condiciones controladas, pero su aplicación en uso comercial se impidió por eficacia inconsistente. Ahora los estudios han demostrado que los sub-productos de la producción vegetal y animal, tales como carne y harina de hueso o de plumas, estiércol de bovinos y cerdos, harina de soya, etc., pueden reducir las enfermedades significativamente, pero el nivel de control obtenido es producto específico del suelo. Se identificaron tres mecanismos de acción para la reducción del patógeno: 1) generación de compuestos tóxicos, tales como amonio y ácido nitroso, de materiales con alto contenido de nitrógeno, 2) presencia de ácidos grasos volátiles (por ejemplo, vinagre) y 3) alteración de agentes biológicos que pudieran suprimir la actividad de los fitopatógenos. Para los productos que trabajan mayormente a través de la generación de ingredientes químicos activos, conociendo las propiedades que regulan la eficacia, permite enfocar su uso a ubicaciones

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específicas, y así, aumentar la actividad y la consistencia. Se sabe mucho menos acerca de cómo tales materiales influencian a los residentes de la rizosfera involucrados en la regulación de la sanidad de la planta. Se están implementando nuevas tecnologías moleculares para identificar a los jugadores clave en el mantenimiento de la sanidad de la raíz. A través de un entendimiento del ecosistema microbiológico del suelo, estaremos en capacidad de desarrollar estrategias de control de enfermedades que sean más económicas para los productores y más benignas para el ambiente.

Keywords Soilborne diseases · Organic amendments · Volatile fatty acids · Ammonia · Nitrous acid · Fish emulsion · Green manure · Root microbiome

Introduction

Verticillium wilt, caused by the fungus *Verticillium dahliae* Kleb, remains one of the most important soilborne plant diseases worldwide. Losses to this pathogen likely amount to billions of dollars on a global basis when all susceptible crops are considered. The actual dollar amount lost in production of potatoes is difficult to assess and is probably greatly underestimated as infection typically reduces plant growth rather than causing death outright. *V. dahliae* has become the predominant pathogen of potatoes because its extremely hardy resting structures, called microsclerotia, can survive in soil for over a decade. Furthermore, the fungus can infect and produce inoculum in numerous weed species, making it extremely difficult to eradicate from soil using rotation management. Disease control has focused on reducing the populations of microsclerotia in soil using broad spectrum soil fumigants. Fumigants also kill many of the plant parasitic nematodes known to enhance the early dying syndrome. The high costs and potential negative health and environmental concerns associated with the use of fumigants, combined with increasing urban sprawl, have greatly curtailed their use. Resistance to *Verticillium* spp. has been difficult to identify in potatoes and other crop plants. In tomato the *Ve* resistance gene controlled cop losses to the pathogen for several decades, but new biotypes emerged that overcame resistance and now this crop is again highly susceptible to Verticillium wilt. Grafting tomatoes and eggplants onto Verticillium resistant rootstock has become a common practice but this it not possible for potatoes.

As public concern over the use pesticides increases more efforts are being placed into finding non-chemical control methods for managing soilborne diseases. This article describes three approaches that we've indentified to reduce soilborne disease. The procedures are far from being a replacement for fumigants but their use is increasing as we learn more about how to better utilize them. Recent in depth reviews on the use of organic amendments for the control of *Verticillium* were published by Goicoechea (2009) and for plant pathogenic nematodes by Oka (2010).

Organic Amendments

Disease Reductions in the Field

Almost 60 years ago Wilhelm (1951) tested a spectrum of chemical pesticides and organic amendments for the control of Verticillium wilt of tomato, and found that only blood meal and fishmeal reduced wilt incidence to zero. Why these products were able to control Verticillium wilt was never questioned or explained. Over the last decade our laboratory examined in some detail how products such as blood meal may be controlling plant diseases. We tested many organic by-products derived from animal and plant processing industries, including blood meal, meat and bone meal, feather meal, fish emulsion (Abbasi et al. 2006), soymeal, lignosulfonates from the pulp and paper industry (Lazarovits et al. 2008), condensed distillers solubles from alcohol production (Abbasi et al. 2007), and several types of manures. The products were selected primarily because they were available in large quantities required for field scale applications, they were free of harmful materials such as heavy metals and most important, they had fairly good batch to batch similarity allowing for a degree of reproducibility. Most of the products were considered by-products of the industries that generated them and they were generally inexpensive and all had fertility values that enhanced crop growth. Summaries of the results from many of our studies were previously published (Bailey and Lazarovits 2003; Lazarovits 2001, 2004; Lazarovits et al. 2005).

Many of our initial trials were carried out on growers' fields in Ontario and Prince Edward Island. Products such as poultry manure and soymeal reduced the incidence of Verticillium wilt and potato scab, as well as populations of plant pathogenic nematodes, to near zero (Conn and Lazarovits 1999). In some cases disease reduction persisted for two crop seasons. The disease control efficacy of these treatments however, was often limited to a specific site and/or product. Even when disease control was marginal the added fertility derived from the organic materials usually resulted in increased plant vigor and yield. Field testing of organic products proved to be complicated by many unknowns that included rates required, the effect of soil type, the impact of climatic conditions, factors such as soil moisture and temperature, the nature and source of the amendment, the methods and timing of application, etc. Evaluating even a few of these factors under field conditions, even if it were possible, would have required hundreds of plots to be established. Such studies however, could be done in the laboratory using model systems to simplify evaluations of the impact of these products on pathogen survival.

Mechanisms of Action by which High Nitrogen Amendments Reduce Pathogen Populations

Conversion of ammonium to ammonia Using a microcosm assay we were able to assess the survival of microsclerotia buried in amended soils and predict with reasonable confidence the impact under field conditions (Lazarovits et al. 2005). Furthermore, the assay also allowed the identification of the modes of action for the various amendment types. Amending sandy soils with high levels of organic nitrogen-containing materials, such as poultry manure, soyameal, meat and bone meal etc, resulted in the mortality of microsclerotia within 1 week after incorporation. This proved to be caused by the production of high amounts of the volatile gas ammonia (Tenuta and Lazarovits 2002a, b, 2004). Upon microbial degradation of proteins, NH_4^+ ions are released and accumulation of this ion in soil causes an increase in soil pH. As soil pH increases above pH 8, NH_4^+ starts to be converted into NH_3. The equilibrium (pK_a) between $NH_4^+ \leftrightarrow NH_3$ occurs at pH 9.3 at about 24 C. NH_4^+ is not toxic to microorganisms even at high concentrations, but NH_3 is very toxic (Warren 1962). Tenuta and Lazarovits (2004) showed that with increasing levels of soil organic matter, NH_3 is not formed because the higher populations of microorganisms in such soils rapidly convert NH_4^+ into nitrate (NO_3^-). Thus high nitrogenous amendments are more effective in controlling soilborne plant pathogens in soils with low organic matter content (Tenuta and Lazarovits 2002a, b, 2004). This explains one aspect of the soil specificity of such amendments for disease suppression.

Conversion of nitrite to nitrous acid Under bioassay and field conditions a second phase of pathogen kill was observed to occur 4–6 weeks after of incorporation of meat and bone meal or equivalent nitrogenous product (Tenuta and Lazarovits 2002a). This was shown to be related to the accumulation of nitrite and a significant drop in soil pH to below 5. When NH_4^+ is converted to nitrite (NO_2^-), $4H^+$ ions are released and soil pH can drop precipitously. If the pH drops below 5.0, NO_2^- is converted to HNO_2 (nitrous acid) with the equilibrium (pK_a) between $NO_2^- \leftrightarrow HNO_2$ occurring at pH 3.3. Nitrous acid was found to be 300–500 times more toxic to microsclerotia than NH_3 (Tenuta and Lazarovits 2002a) and was also toxic to many plant pathogens including *Streptomyces scabies*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, as well as to crops and to weed seeds. The formation of HNO_2 is influenced by soil buffering capacity (Tenuta and Lazarovits 2004), as in highly buffered soils nitrification does not lower soil pH sufficiently to form nitrous acid. The toxicity of ammonia and nitrous acid to plant pathogens was predicted by Tsao and Oster (1981) from their work on controlling soilborne pathogens with poultry manure. However, the role of toxic nitrogen molecules is still not considered by most investigators testing nitrogenous products as potential disease management tools.

Mechanism of Action of Materials Containing Volatile Fatty Acids (VFAs)

Most books dealing with potato production indicate that adding animal manures to soil results in increased levels of soilborne diseases such as potato scab. We therefore applied manures to see if we could increase scab severity. We found that even a single application of liquid swine manure (LSM) significantly reduced the severity and incidence of Verticillium wilt and potato scab, but again the effect was site specific, even with the same manure (Conn and Lazarovits 1999). In laboratory assays using soil from a field where disease reductions were optimal we found that microsclerotia were killed within 1-2 days after manure application. Upon analyzing the manure by ion chromatography we found that it contained high concentrations of volatile fatty acids (VFA), with acetic acid representing 60% of the active ingredients, and propionic, butyric, isobutyric, valeric, isovaleric and caproic the remainder (Tenuta et al. 2002). Commercially purchased VFAs adjusted to identical concentrations found in LSM reproduced all the LSM killing activity. Since only the acidic forms of VFA molecules are toxic, disease control only occurs in acidic soils (Conn and Lazarovits 2000). In soils where pH levels are higher than 5.5 VFAs are not toxic to microorganisms. The equilibrium between the toxic and non-toxic counterparts occurs at pH 4.7 (pK_a). Disease suppression with amendments containing VFAs as the active molecules will therefore only occur in acidic soils but not in basic or neutral soils. The concentrations of VFAs in manure can also vary and in dozens of LSM samples tested from different farms only about 50% had sufficient VFAs to kill *V. dahliae* microsclerotia (Conn et al. 2007). Analyzing manure for VFA content can predict disease control potential.

Fish emulsion (FE), the concentrated soup left after menhaden fish (forage fish of the genera *Brevoortia* and *Ethmidium)* is processed in large boilers, was similarly effective in controlling both potato scab and verticillium wilt under laboratory and field conditions (Abbasi et al. 2006). FE applied at rates of 2% (v/v) reduced the viability of *V. dahliae* microsclerotia by 74% 1 day after application and by 98% after 3 days. Initially the evidence pointed to an increase in specific soil microorganisms as the mode of action (Abbasi et al. 2004; Lazarovits et al. 2009) However, subsequent analysis of the fish emulsion showed that all of the toxic VFAs found in liquid swine manure were also present in fish emulsion but at much higher concentrations. FE also contains formic acid which is the most active VFA of all tested against Verticillium (Abbasi et al. 2009). VFA concentrations sufficient to provide effective disease control have been detected in by-products of corn ethanol (Abbasi et al. 2007), in molasses and in fermentation products from the pharmaceutical industry (Lazarovits, unpublished data).

High concentrations of VFAs have been found in young composts (cited in Bailey and Lazarovits 2003) and it is very likely that in some instances the disease suppressiveness and phytotoxic activity are related to VFA content, a factor not yet examined in any detail in studies of disease-suppressive compost. Blok et al. (2000) have successfully reduced soil populations of plant pathogens such as *F. oxysporum*, *Rhizoctonia solani*, and *V. dahliae* by adding large quantities of organic matter to soil. They indicate disease control is achieved by creating anaerobic conditions and quite possibly providing the conditions for VFA formation. Anaerobic decomposition of wheat straw was shown to be phytotoxic due to the production of acetic acid (Lynch 1977, 1978). Browning et al. (2006) demonstrated that butyric acid, a product likely to form in fermenting organic material, inhibited several fungal pathogens including Verticillium and reduced the populations of both *Meloidogyne hapla* and *M. incognita* to almost undetectable levels when applied to strawberries. As VFAs are common components of edible products and have very low toxicity to mammals and no mutagenic potential, they can be used in organic farming systems. VFAs persist in soil and plant tissues for only short times and leave no residues as they are readily metabolized by bacteria and fungi. In our tests, VFAs rarely lasted in soil for more than a few days. In fact, rapid degradation may explain why they do not work at some locations.

Formulation for Site Specific Activity

Adding organic amendments to improve soil structure, a factor often associated with soil "health", is gaining great interest among grower groups. By developing a better understanding of the mechanisms involved in disease reduction we will be able to identify and select materials for optimal benefits to plant health based on soil and other conditions that favor activities derived from their chemical and biological properties. There is now a greater recognition of the value present in products we have been labeling as waste materials, and as a result, attempts are being made to modify their composition for increased disease control. Xiao et al. (2007) used anaerobic digestion of LSM to enrich both VFAs and NH_4^+. Following 4 weeks of incubation the treated manure proved to be superior to untreated manure for reducing egg production by the soybean cyst nematode. Experiments carried out in the laboratory and greenhouse using raw and anaerobically digested LSM showed that VFA-enriched manure was the most effective, the raw manure was intermediate, and the NH_4^+-enriched manure the least effective at inhibiting *H. glycines* hatch and in killing eggs and J2 juveniles (Xiao et al. 2008). Conn and Lazarovits (2007) tested additions of sulfuric acid to soil or to manure prior to application to the field with the intent of temporarily lowering soil pH to increase the concentrations of the acidic forms of the VFAs. An increase in disease control activity of soilborne pathogens was observed; however, the technology comes with an increase in costs. Mahran et al. (2008a, b) compared the effectiveness of LSM to acidified LSM for killing root lesions nematodes (*Pratylenchus* spp.) in potato soils. In a two year trial the treatments were applied annually to two soils in micro-plots and once in a large scale experiment at a potato farm. The treatments included an untreated control, sulfuric acid (1.8 hl ha^{-1}), LSM (58 hl ha^{-1}), and acidified LSM (2.4 hl of H_2SO_4 + 58.5 hl LSM ha^{-1}). The sulfuric acid reduced soil pH to 4.3 and 5.5 in the 2 years of the trial, respectively. Acidified LSM reduced *Pratylenchus* spp. populations in the two microplot soils by over 91% and by 60–93% in the second year. LSM also reduced nematodes by about 60% in the second year. Similar reductions in nematodes were seen under field conditions but only in samples collected soon after application. By harvest, there were no differences among treatments compared to control treatment. Mahran et al. (2009) concluded that liquid hog manure is effective in killing plant-parasitic nematodes while increasing bottom-up food web interactions but not, as with soil fumigants, decimating top-down trophic interactions. Combining fish emulsion with biological control agents increased disease control efficacy of either product alone (El-Tarabily et al. 2003). Combining organic amendments with solarization significantly improved the efficacy of pathogen control compared to either treatment alone (Gamliel and Stapleton 1993). This is very likely because increasing temperatures shift the equilibrium constants for both NH_3 and HNO_2 toward neutrality, thereby increasing the concentrations of the toxic moieties.

Organic products offer a technology for growers who wish to incorporate them as part of a sustainable and holistic crop production system to keep populations of key pathogens below crop loss thresholds. In a survey we conducted many years ago, the only potato grower in Ontario who did not have high levels of Verticillium wilt told us that he applied and incorporated five tons of poultry manure every fall into his soil. It is very possible that such a practice accelerates the degradation of potato tissues and thereby results in a more rapid release of microsclerotia into the soil where they are more prone to be killed by microbial activity. Adding such rates of manure likely also produces sufficient levels of nitrous acid as to kill many of the microsclerotia and possibly the nematodes that play a role in Verticillium disease severity. Unfortunately, large quantities of manure or other organic materials of uniform quality are not available to many growers, and this is a major constraint for adoption of such practices. There is also a substantial cost for transporting these products and for incorporating them into soil. Growers would benefit from having access to equipment that effectively mixes the organic materials into soils as bands so that they can be applied at lower rates.

Green Manures

The incorporation of green biomass into soil, known as green manuring, has been in use in Europe for over 2,000 years (Fig. 1; Pieters 1927). Studies were carried out by Davis et al. (2001) at 100 commercial potato fields in southeastern Idaho to identify what factors in soil were associated with the severity of Verticillium wilt, soil inoculum density of *V. dahliae* and *Colletotrichum coccodes,* colonization of stems, root, and tubers by *V. dahliae and C. coccodes,* and tuber yield, size, and quality. The factors most closely associated with wilt suppression and higher tuber yields were organic matter, organic nitrogen, and increased nutrient availability. Loss of soil integrity, high sodium and reduced nutrient availability were related to increased wilt and lower tuber yields. Thus, organic amendments and green manures may have their greatest impact on production through enhancing the former properties of soil. However, much of the literature focuses on the impact that the carbon energy has on soil microbial activity, pathogen survival, and the release of biologically active substances from residues and microorganisms. Mathre et al. (1999) claimed that the increased productivity observed following a crop rotation sequence including a green manure crop was directly due to changes in microbial populations. They suggested that adding organic amendments brings about soil-sanitization that can be equivalent to that obtained by crop rotation.

Fig. 1 Millett rotations incorporated as green manures into potato fields resulted in 25–50% increases in the yield of the next potato crop

Brassica spp. have been found to be the most successful green manures for disease management in a broad spectrum of crops (Bhat and Subbarao 2001; Lazarovits and Subbarao 2010; Matthiessen and Kirkegaard 2006; Shetty et al. 2000). Koike et al. (1994) showed that broccoli residue plowed into soil reduced Verticillium wilt in the subsequent crops planted. Fresh broccoli residue suppressed *V. dahliae* microsclerotia more than dry broccoli residue over a wide temperature range (Subbarao and Hubbard 1996). Most of the inoculum reduction occurred within 15 days after incorporation suggesting a chemical mode of action. Incorporation of broccoli residue also resulted in consistently taller plants with greater root and shoot biomass and lower levels of infected plants compared to all other treatments studied. For field use, rotations of cauliflower with broccoli proved to be the ideal means of exploiting disease suppression (Subbarao et al. 1999; Xiao et al. 1998). A multiplicity of mechanisms for pathogen reduction by cole crop residues have been suggested, including the toxicity of mustard oils (Subbarao and Hubbard 1996) and the generation of cyanide gas from the glucosinolates in plant tissues (Mayton et al. 1996). Such compounds can also cause severe injury to the main crop and there is often a requirement to delay planting due to potential phytotoxicity. However, pathogen suppression has been observed long after the isothiocyanates had volatilized or were degraded (Lewis and Papavizas 1970) or even when no glucosinolate content was detected (Mazzola et al. 2001). As of yet there have been no reports on the presence of toxic nitrogen intermediates in disease control, but given the large quantities of organic nitrogen introduced, this is quite possible. It is also possible that depletion of oxygen in soil through anaerobiosis occurs, as was found by Blok et al. (2000). The accumulation of acetic and butyric acid was also postulated as being involved under the appropriate circumstances (Momma 2008). Ochiai et al. (2007) found that early dying disease was reduced by incorporating broccoli residues into soils used for potato production but the quantities required were not realistic and the reduced disease did not result in any yield increase.

Davis et al. (1996) showed that Sudan grass or corn green manure incorporated into soils used in a potato rotation reduced wilt and increased yields, whereas canola and legumes increased wilt severity. The increased yields after additions of Sudan grass were associated with increases in populations of Fusarium species considered as potential biological control agents for Verticillium (Davis et al. 2004). They also suggested that some of the control of early dying seen after Sudan grass green manures was due to reductions in populations of plant parasitic nematodes known to act synergistically with *V. dahliae* in causing the early dying syndrome. Ochiai et al. (2008) found that plant residues, regardless of source, when amended at the same rates, resulted in similar levels of suppression of Verticillium wilt, suggesting that it is the quantity and not the chemistry of organic inputs that is the critical factor. They found no evidence for any biofumigation effects in their trials. Wiggins and Kinkel (2005) also observed reduced incidences of Verticillium wilt and common scab and higher potato yields with the green manures they tested that included buckwheat and canola interspersed with rotations of corn and alfalfa.

The evidence of the benefits for green manures for managing soilborne pathogens is continually and rapidly expanding. Even in instances where disease reductions are minimal yield increases are common, possibly because of improved nutrient cycling (Thorup-Kristensen et al. 2001). However, there are also examples where green manures increase disease severity. Based on the literature on the use of organic amendments for controlling Verticillium, Goicoechea (2009) suggested that growers must take into account the type and quantity of the organic amendment they intend to use, as efficacy can vary depending on soil characteristics, climate, the crop, and even the *Verticillium* isolate that may be present. Lazarovits et al. (unpublished results) carried out tests incorporating millet using various strategies over 3 years at two potato field sites. Potato yields increased an average of 20% at one site and 50% at the other, but little reduction in Verticillium wilt incidence was observed. The manner of incorporating the green material impacted yield, although in some cases, the same method did not give the same results at the two sites. The take home message is that we need to launch a major research effort to determine why and how these materials influence plant growth and productivity.

Role of Microbiology in Suppressing Potato Diseases

Increased microorganism populations are almost universally postulated as a mechanism for suppression of Verticillium wilt and other soilborne diseases in studies evaluating the impact of rotations and organic amendments. Evidence has been presented in several studies that the increase in microorganism populations following amendment application leads to disease-suppressive conditions (Cohen et al. 2005; Mazzola 2004; Mazzola et al. 2007; Trankner 1992). This selective enrichment of microbiological ecosystems has tremendous potential if it proves to be transferrable to large scale agriculture. Human medicine has already embraced the concept of probiotic disease management whereby human diseases are prevented by enriching native microflora, thereby keeping pathogens from becoming established either directly or indirectly. In plant disease suppression bacteria are most often identified as the primary candidates responsible for reduced pathogen activity as they most closely interact with the host plant (Sturz et al. 2000). The information available on the bacterial rhizosphere community of potato has been obtained mostly by culture-dependent approaches (Azad et al. 1987; Berg et al 2001; Kloepper et al. 1980; Loper et al. 1985). Krechel et al. (2002) used both cultivation on agar medium and a cultivation-independent approach based on terminal restriction fragment length polymorphism (T-RFLP) of 16S rDNA. Of 440 bacteria screened *in vitro* for antagonism against *V. dahliae* and *Rhizoctonia solani*, the highest proportion of bacteria with antagonistic activity were isolated from the rhizosphere (10%), followed by the endorhiza (9%), phyllosphere (6%), and endosphere (5%). Interestingly, this is a relatively low proportion of antibiotic producers; Lievens et al. (1989) found that of 11,614 bacteria isolated from 7 crop plants about 33% (3910) produced antifungal metabolites. Despite the huge numbers of microorganisms that have been now identified from potato roots with recognized benefits to plant growth, there are few, if any commercial products available to growers for use in production systems. One reason may be that the pesticide industry has not yet bought into developing microorganisms as a plant protection strategy, primarily because of the lack of knowledge on how to use these organisms in a manner that gives consistent control in diverse agroecosystems. Berg et al. (2002) recently commercialized a strain of *Serratia plymuthica* HRO-C48 (Rhizostar), isolated from a strawberry rhizosphere, for use as a biocontrol agent of Verticillium wilt of strawberry (Muller et al. 2004; Scherwinski et al. 2007).

Fluorescent pseudomonads are the most studied bacteria for suppression of plant disease caused by soilborne pathogens (Weller 2007). Kloepper et al. (1980) identified the plant growth promoting benefits of these bacteria on potato 30 years ago but there has been little subsequent work on developing these microorganisms for use in the field because of the variation in efficacy at different locations. Fluorescent pseudomonads are potent biological control agents for Verticillium wilt (Berg et al. 2001; Mercado-Blanco et al. 2004). Strains that produce both biosurfactants and phenazine-1-carboxylic were able to kill microsclerotia (Debode et al. 2007) and to colonize their surfaces, but not their inner matrix. These strains rapidly increased in numbers in the vicinity of Verticillium microsclerotia, suggesting that they may utilize nutrients derived from the microsclerotia for their own growth.

It has been suggested that studies of single soil microorganisms, while useful in specialized cases such as Rhizobia and mycorrhizae, do not yield useful information on the functioning of soil ecosystems, as most soil processes depend upon interactions between suites of organisms (Stockdale and Brookes 2006). Stockdale and Brookes (2006) suggest that it is time to study the "forest rather than an individual tree". Elucidating the soil ecosystem remains one of our last major challenges in agriculture and in ecology. The lack of knowledge about who lives in soil and/or plant roots has hampered efforts to manipulate soil microbiology for the benefit of plant agriculture.

In the last decade, a revolution has occurred in soil microbiology that has been accelerated by the availability of inexpensive high-throughput DNA sequencing (Hall 2007). These new technologies have now been used in numerous studies to track specific microorganisms in selected ecosystems. As yet very few attempts have been made to obtain a holistic and quantitative picture of the entire soil or root microorganism community. Hill et al. (2002) described a technology that they used to successfully identify and enumerate members of complex microbial communities (Hill et al. 2002, 2005a, b, 2006). The technology utilizes a genomic segment of DNA that codes for the chaperonin proteins, which are essential for the folding and assembly of proteins in all bacteria and in the plastids and mitochondria of eukaryotes (Hemmingsen et al. 1988; Saibil and Ranson 2002). In most bacteria, a single copy gene, *cpn*60, encodes this protein and it is an informative target for microbial species identification and for phylogenetics. Hill et al. (2002) described a robust, generic, molecular method for identifying microorganisms based on amplification of a portion of the *cpn*60 gene, the "universal target" (UT), using universal, degenerate PCR primers (Goh et al. 2000). This method was demonstrated to have several advantages over 16S *rRNA*-based methods. The protein-encoding *cpn*60 gene is richer in phylogenetically informative sequence variation than is the structural RNA-encoding 16S *rRNA* gene. While 16S *rRNA* gene sequences are often identical between closely related organisms (Chatellier et al. 1998), *cpn*60 UT sequences usually discriminate different species within a genus, and sometimes serotypes or subspecies within a species (Brousseau et al. 2001). Second, as a single-copy gene, *cpn*60 lacks the potential for some sequencing artefacts that are encountered with the multiple-copy 16S *rRNA* gene. This characteristic is also advantageous for quantitative PCR assays. Last, the 549-567 bp *cpn*60 UT can be sequenced easily with a single reaction, whereas the full length (~1.5 kbp) sequence of the 16S *rRNA* gene is often required to distinguish two given species, if it is possible at all. Thus, high throughput sequencing of large libraries of cloned *cpn*60 gene UT regions is relatively economical and this approach has been developed for identifying and enumerating members of complex microbial communities (Hill et al. 2002, 2005a, b, 2006).

Any sequence-based approach to microbial species identification however, is dependent on the availability of a sufficient collection of reference sequence data. At the moment this is a limitation for studies of soil microorganism communities as the chaperonin sequence database, cpnDB (http://cpndb.cbr.nrc.ca), contains only about 9,000 sequences, including about 1,600 reference sequences from named organisms (prokaryotic and eukaryotic). While it has been used to create phylogenetic context for microbial communities (Hill et al. 2002) and for identifying clinical isolates (Goh et al. 2000) its usefulness for identifying the highly diverse communities of soils and roots has only been superficially evaluated (Lazarovits et al. 2009).

In a recent study carried out in collaboration with Dr. Sean Hemmingsen at Plant Biotechnology Institute in Saskatoon, we set out to examine whether the *cpn*60 technology could be used to determine and compare the microbial profile of soils collected from potato farms in PEI and Ontario. We planted minitubers grown under greenhouse conditions in these soils and grew them for 4 weeks. The objective was to evaluate whether the technology allowed detection of the changes in the microorganism communities of soils collected prior to planting potatoes, compared to those obtained from soils attached to roots and tubers and of the bacteria community that remained on the roots after washing them in running water. The hypothesis was that in general the microorganism communities would be similar in the two soils and that changes would be detected in the community as we approached the root system. Using pyrosequencing of the CPN60 amplicons, about 511,000 sequences were useable for initial identifications of bacterial genera and species based on 70% similarity to known sequences in the data base, the similarity index used as the confidence limit in attaching a phylogenetic status. However, even sequences with at least 70% identity to reference sequences are inadequate for classifying the organisms to genera and species. Sequences with less than 70% identity were not used, although they may be valuable for future analysis.

Based on a very preliminary and as yet superficial analysis, which are at best only predictive of the genus rather than species, the most common organisms tentatively identified to be present in both Ontario and Prince Edwards Island soils were *Solibacter usitatus* and *Bacterium sp. Ellin514*. Relatively little is known about what either of these organisms does in soil (Galperin 2006). Another organism of interest was *Azospirillum brasilense* identified to be common in Ontario soil and PEI soil. This bacterium was previously identified to be present in Ontario soils in the author's laboratory by Mehnaz et al. (2007). The studies revealed alterations in species in the rhizosphere soil with increases in *Sphingopyxis alaskensis* and in the number of *Serratia* species which were relatively rare in bulk soil. The most dramatic changes in microorganism communities occurred on washed roots, where *Pseudomonas* species, *Rhizobium* species and *Serratia* species became dominant. The increases in the prevalence of *Pseudomonas* and *Serratia* species were expected, whereas that of populations of *Rhizobium* was a surprise. The issues of spatial heterogeneity and limitations in sampling were the primary questions in this study, and the results show that we should be focusing on the roots. The results also indicate that the technology underestimates Gram positive bacteria and fungi. Although we do not yet have sufficient genomic information to correctly identify the vast majority of microorganisms that reside in soil this technology offers hope that in the near future we will be able to unravel the microorganism communities that contribute to productive as well as unproductive soils. Studies of the microbial complexes in animals and humans have shown the value of the cpn60 approach. Schellenberg et al. (2009) compared dideoxy sequencing of cloned chaperonin-60 universal target (cpn60 UT) amplicons to pyrosequencing of amplicons derived from vaginal microbial communities. In samples pooled from a number of individuals, the pyrosequencing method produced a data set that included virtually all of the sequences that were found within the clone library and revealed an additional level of taxonomic richness. However, the relative abundances of the sequences were different in the two datasets. The pyrosequencing method generated a reproducible profile of the microbial community structure in replicate amplifications from the same community. In comparing the taxonomic composition of a vaginal microbial community determined by pyrosequencing of 16S rRNA amplicons to that obtained using cpn60 universal primers, the profiles generated from the two molecular targets were highly similar, with slight differences in the proportional representation of the taxa detected. However, the number of operational taxonomic units was significantly higher in the cpn60 data set, suggesting that the protein-encoding gene provides improved species resolution over the 16S rRNA target. These observations demonstrate that pyrosequencing of cpn60 UT amplicons provides a robust, reliable method for deep sequencing of microbial communities.

Sustainable agriculture will require that producers better manage their soil microbial communities in order to minimize losses caused by soilborne pathogens. This will likely involve adding back specific nutrients to soil by way of crop rotations, green manures, composts etc. to encourage the desired organisms. It will probably require years to achieve healthy soils, rather than months or weeks, but once attained, it is expected that we can develop technologies to maintain the desired effect. Through molecular genetic approaches we will be able to fingerprint soils and identify the microorganisms we consider as beneficial residents. Generation of such fingerprints will make it possible to move forward using ecological concepts and knowledge based decision systems for achieving conditions that benefit plant production.

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References

Abbasi, P.A., K.L. Conn, and G. Lazarovits. 2004. Suppression of *Rhizoctonia* and *Pythium* damping-off of radish and cucumber seedlings by addition of fish emulsion to peat mix or soil. *Can J Plant Pathol* 26: 177–187.

Abbasi, P.A., K.L. Conn, and G. Lazarovits. 2006. Effect of fish emulsion used as a preplanting soil amendment on verticillium wilt, scab, and tuber yield of potato. *Can J Plant Pathol* 28: 509–518.

Abbasi, P.A., K.L. Conn, and G. Lazarovits. 2007. Managing soilborne diseases of vegetable crops with a pre-plant soil or substrate amendment of a corn distillation product. *Biocontrol Sci Technol* 17: 331–344.

Abbasi, P.A., G. Lazarovits, and S. Jabaji-Hare. 2009. Detection of high concentrations of organic acids in fish emulsion and their role in pathogen or disease suppression. *Phytopathology* 99: 274–281.

Azad, H.R., J.R. Davis, W.C. Schnathorst, and C.I. Kado. 1987. Influence of verticillium wilt resistant and susceptible potato genotypes on populations of antagonistic rhizosphere and rhizoplane bacteria and free nitrogen fixers. *Appl Microbiol Biotechnol* 26: 99–104.

Bailey, K.L., and G. Lazarovits. 2003. Suppressing soil-borne diseases with residue management and organic amendments. *Soil Tillage Res* 72: 169–180.

Berg, G., A. Fritze, N. Roskot, and K. Smalla. 2001. Evaluation of potential biocontrol rhizobacteria from different host plants of *Verticillium dahliae* Kleb. *J Appl Microbiol* 91: 963–971.

Berg, G., N. Roskot, A. Steidle, L. Eberl, A. Zock, and K. Smalla. 2002. Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different verticillium host plants. *Appl Environ Microbiol* 68: 3328–3338.

Bhat, R.G., and K.V. Subbarao. 2001. Green manure. In *Encyclopedia of plant pathology*, ed. O.C. Maloy and T.D. Murray, 519–520. New York: Wiley.

Blok, W.J., J.G. Lamers, A.J. Termorshuizen, and G.J. Bollen. 2000. Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. *Phytopathology* 90: 253–259.

Brousseau, R., J.E. Hill, G. Préfontaine, S.H. Goh, J. Harel, and S.M. Hemmingsen. 2001. *Streptococcus suis* serotypes characterized by analysis of chaperonin 60 gene sequences. *Appl Environ Microbiol* 67: 4828–4833.

Browning, M., D.B. Wallace, C. Dawson, S.R. Alm, and J.A. Amador. 2006. Potential of butyric acid for control of soil-borne fungal pathogens and nematodes affecting strawberries. *Soil Biol Biochem* 38: 401–404.

Chatellier, S., J. Harel, Y. Zhang, M. Gottschalk, R. Higgins, L.A. Devriese, and R. Brousseau. 1998. Phylogenetic diversity of *Streptococcus suis* strains of various serotypes as revealed by 16S rRNA gene sequence comparison. *Int J Syst Bacteriol* 48: 581–589.

Cohen, M.F., H. Yamasaki, and M. Mazzola. 2005. *Brassica napus* seed meal soil amendment modifies microbial community structure, nitric oxide production and incidence of *Rhizoctonia* root rot. *Soil Biol Biochem* 37: 1215–1227.

Conn, K.L., and G. Lazarovits. 1999. Impact of animal manures on verticillium wilt, potato scab, and soil microbial populations. *Can J Plant Pathol* 21: 81–92.

Conn, K.L., and G. Lazarovits. 2000. Soil factors influencing the efficacy of liquid swine manure added to soil to kill Verticillium dahliae. *Canadian Journal of Plant Pathology—Revue Canadienne de Phytopathologie* 22: 400–406.

Conn, K.L., and G. Lazarovits. 2007. Reduction of potato scab with acidified liquid swine manure soil amendment. *Can J Plant Pathol* 29: 440.

Conn, K.L., M. Tenuta, and G. Lazarovits. 2005. Liquid swine manure can kill verticillium dahliae microsclerotia in soil by volatile fatty acid, nitrous acid, and ammonia toxicity. *Phytopathology* 95: 28–35.

Conn, K.L., E. Topp, and G. Lazarovits. 2007. Factors influencing the concentration of volatile fatty acids, ammonia, and other nutrients in stored liquid pig manure. *J Environ Qual* 36: 440–447.

Davis, J.R., O.C. Huisman, D.O. Everson, and A.T. Schneider. 2001. Verticillium wilt of potato: a model of key factors related to disease severity and tuber yield in Southeastern Idaho. *Am J Potato Res* 78: 291–300.

Davis, J.R., O.C. Huisman, D.T. Westermann, S.L. Hafez, D.O. Everson, L.H. Sorensen, and A.T. Schneider. 1996. Effects of green manures on verticillium wilt of potato. *Phytopathology* 86: 444–453.

Davis, J.R., O.C. Huisman, D.T. Westermann, D.O. Everson, A. Schneider, and L.H. Sorensen. 2004. Some unique benefits with sudangrass for improved U.S. #1 yields and size of Russet Burbank potato. *Am J Potato Res* 81: 403–413.

Debode, J., K.D. Maeyer, M. Perneel, J. Pannecoucque, G.D. Backer, and M. Höfte. 2007. Biosurfactants are involved in the biological control of *Verticillium* microsclerotia by Pseudomonas spp. *J Appl Microbiol* 103: 1184–1196.

El-Tarabily, K.A., A.H. Nassar, G.E.S.J. Hardy, and K. Sivasithamparam. 2003. Fish emulsion as a food base for rhizobacteria promoting growth of radish (*Raphanus sativus* L. var. *sativus*) in a sandy soil. *Plant Soil* 252: 397–411.

Galperin, M.Y. 2006. Sampling of microbial diversity by complete genomes. *Environ Microbiol* 8: 1313–1317.

Gamliel, A., and J.J. Stapleton. 1993. Effect of chicken compost or ammonium phosphate and solarization on pathogen control, rhizosphere microorganisms, and lettuce growth. *Plant Dis* 77: 886–891.

Goh, S.H., R.R. Facklam, M. Chang, J.E. Hill, G.J. Tyrrell, E.C.M. Burns, D. Chan, C. He, T. Rahim, C. Shaw, and S.M. Hemmingsen. 2000. Identification of *Enterococcus* species and phenotypically similar *Lactococcus* and *Vagococcus* species by reverse checkerboard hybridization to chaperonin 60 gene sequences. *J Clin Microbiol* 38: 3953–3959.

Goicoechea, N. 2009. To what extent are soil amendments useful to control verticillium wilt? *Pest Manag Sci* 65: 831–839.

Hall, N. 2007. Advanced sequencing technologies and their wider impact in microbiology. *J Exp Biol* 210: 1518–1525.

Hemmingsen, S.M., C. Woolford, S.M. Van der Vies, K. Tilly, D.T. Dennis, C.P. Georgopoulos, R.W. Hendrix, and R.J. Ellis. 1988. Homologous plant and bacterial proteins chaperone oligomeric protein assembly. *Nature* 333: 330–334.

Hill, J.E., R.P. Seipp, M. Betts, L. Hawkins, A.G. Van Kessel, W.L. Crosby, and S.M. Hemmingsen. 2002. Extensive profiling of a complex microbial community by high-throughput sequencing. *Appl Environ Microbiol* 68: 3055–3066.

Hill, J.E., M. Gottschalk, R. Brousseau, J. Harel, S.M. Hemmingsen, and S.H. Goh. 2005a. Biochemical analysis, cpn60 and 16S rDNA sequence data indicate that *Streptococcus suis* serotypes 32 and 34, isolated from pigs, are *Streptococcus orisratti*. *Vet Microbiol* 107: 63–69.

Hill, J.E., S.M. Hemmingsen, B.G. Goldade, T.J. Dumonceaux, J. Klassen, R.T. Zijlstra, H.G. Swee, and A.G. Van Kessel. 2005b. Comparison of ileum microflora of pigs fed corn-, wheat-, or barley-based diets by chaperonin-60 sequencing and quantitative PCR. *Appl Environ Microbiol* 71: 867–875.

Hill, J.E., J.R. Town, and S.M. Hemmingsen. 2006. Improved template representation in cpn 60 polymerase chain reaction (PCR) product libraries generated from complex templates by application of a specific mixture of PCR primers. *Environ Microbiol* 8: 741–746.

Kloepper, J.W., M.N. Schroth, and T.D. Miller. 1980. Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. *Phytopathology* 70: 1078–1082.

Koike, S.T., K.V. Subbarao, T.R. Gordon, R.M. Davis, and J.C. Hubbard. 1994. Verticillium wilt of cauliflower in California. *Plant Dis* 78: 1116–1121.

Krechel, A., A. Faupel, J. Hallmann, A. Ulrich, and G. Berg. 2002. Potato-associated bacteria and their antagonistic potential towards plant-pathogenic fungi and the plant-parasitic nematode *Meloidogyne incognita* (Kofoid & White) Chitwood. *Can J Microbiol* 48: 772–786.

Lazarovits, G. 2001. Management of soil-borne plant pathogens with organic soil amendments: a disease control strategy salvaged from the past. *Can J Plant Pathol* 23: 1–7.

Lazarovits, G. 2004. Managing soilborne plant diseases through selective soil disinfestation by a knowledge-based application of soil amendments. *Phytoparasitica* 32: 427–432.

Lazarovits, G., K.L. Conn, P.A. Abbasi, and M. Tenuta. 2005. Understanding the mode of action of organic soil amendments provides the way for improved management of soilborne plant pathogens. *Acta Hortic* 698: 215–224.

Lazarovits, G., K.L. Conn, P.A. Abbasi, N. Soltani, W. Kelly, E. McMillan, R.D. Peters, and K.A. Drake. 2008. Reduction of potato tuber diseases with organic soil amendments in two Prince Edward Island fields. *Can J Plant Pathol* 30: 37–45.

Lazarovits, G., P.A. Abbasi, K. Conn, J.E. Hill, and S.M. Hemmingsen. 2009. Fish emulsion and liquid swine manure: model systems for development of organic amendments as fertilizers with disease suppressive properties. In *Biocontrole de doenças de plantas: uso e perspectivas*, ed. W. Bettiol and M.A.B. Morandi, 49–68. Jaguariúna: Embapa Meio Ambiente.

Lazarovits, G., and K. Subbarao. 2010. Challenges in controlling verticillium wilt by the use of nonchemical methods. In *Plant pathology in the 21st Century: Recent developments in management of plant diseases: Contributions to the 9th International*

Congress for Plant Pathology, held in Turin in 2008, ed. U. Gisi, I. Chet, and L. Gullino, 247–264. Dordrecht: Springer.

Lewis, J.A., and G.C. Papavizas. 1970. Evolution of volatile sulfur-containing compounds from decomposition of crucifers in soil. *Soil Biol Biochem* 2: 239–246.

Lievens, K.H., R. van Rijsbergen, F.R. Leyns, B.J. Lambert, P. Tenning, J. Swings, and H.J.P. Joos. 1989. Dominant rhizosphere bacteria as a source for antifungal agents. *Pestic Sci* 27: 141–154.

Loper, J.E., C. Haack, and M.N. Schroth. 1985. Population dynamics of soil pseudomonads in the rhizosphere of potato (*Solanum tuberosum* L.). *Appl Environ Microbiol* 49: 416–422.

Lynch, J.M. 1977. Phytotoxicity of acetic acid produced in the anaerobic decomposition of wheat straw. *J Appl Bacteriol* 42: 81–87.

Lynch, J.M. 1978. Production and phytotoxicity of acetic acid in anaerobic soils containing plant residues. *Soil Biol Biochem* 10: 131–135.

Mahran, A., K.L. Conn, M. Tenuta, G. Lazarovits, and F. Daayf. 2008a. Effectiveness of liquid hog manure and acidification to kill *Pratylenchus spp*. in soil. *J Nematol* 40: 266–275.

Mahran, A., M. Tenuta, M.L. Hanson, and F. Daayf. 2008b. Mortality of *Pratylenchus penetrans* by volatile fatty acids from liquid hog manure. *J Nematol* 40: 119–126.

Mahran, A., M. Tenuta, R.A. Lumactud, and F. Daayf. 2009. Response of a soil nematode community to liquid hog manure and its acidification. *Appl Soil Ecol* 43: 75–82.

Mathre, D.E., R.J. Cook, and N.W. Callan. 1999. From discovery to use: traversing the world of commercializing biocontrol agents for plant disease control. *Plant Dis* 83: 972–983.

Matthiessen, J., and J. Kirkegaard. 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. *Crit Rev Plant Sci* 25: 235–265.

Mayton, H.S., C. Olivier, S.F. Vaughn, and R. Loria. 1996. Correlation of fungicidal activity of *Brassica* species with allyl isothiocyanate production in macerated leaf tissue. *Phytopathology* 86: 267–271.

Mazzola, M. 2004. Assessment and management of soil microbial community structure for disease suppression. *Annu Rev Phytopathol* 42: 35–59.

Mazzola, M., D.M. Granatstein, D.C. Elfving, and K. Mullinix. 2001. Suppression of specific apple root pathogens by *Brassica napus* seed meal amendment regardless of glucosinolate content. *Phytopathology* 91: 673–679.

Mazzola, M., J. Brown, A.D. Izzo, and M.F. Cohen. 2007. Mechanism of action and efficacy of seed meal-induced pathogen suppression differ in a brassicaceae species and time-dependent manner. *Phytopathology* 97: 454–460.

Mehnaz, S., B. Weselowski, and G. Lazarovits. 2007. *Sphingobacterium canadense* sp. nov., an isolate from corn roots. *Syst Appl Microbiol* 30: 519–524.

Mercado-Blanco, J., D. Rodríguez-Jurado, A. Hervás, and R.M. Jiménez-Diaz. 2004. Suppression of verticillium wilt in olive planting stocks by root-associated fluorescent Pseudomonas spp. *Biol Control* 30: 474–486.

Momma, N. 2008. Biological soil disinfestation (BSD) of soilborne pathogens and its possible mechanisms. *Jpn Agr Res Q* 42: 7–12.

Müller, H., S. Kurze, I. Richter, and G. Berg. 2004. Strategies in developing an efficient commercial product for biological control of soil borne fungal pathogens by *Serratia plymuthica* HRO-C48. *Bull OILB/SROP* 27: 199–201.

Ochiai, N., M.L. Powelson, R.P. Dick, and F.J. Crowe. 2007. Effects of green manure type and amendment rate on verticillium wilt severity and yield of Russet Burbank potato. *Plant Dis* 91: 400–406.

Ochiai, N., M.L. Powelson, F.J. Crowe, and R.P. Dick. 2008. Green manure effects on soil quality in relation to suppression of Verticillium wilt of potatoes. *Biol Fertil Soils* 44: 1013–1023.

Oka, Y. 2010. Mechanisms of nematode suppression by organic soil amendments-A review. *Appl. Soil Ecol.* 44: 101–115.

Pieters, A.J. 1927. *Green manuring: principles and practice*. New York: Wiley.

Saibil, H.R., and N.A. Ranson. 2002. The chaperonin folding machine. *Trends Biochem Sci* 27: 627–632.

Schellenberg, J., M.G. Links, J.E. Hill, T.J. Dumonceaux, G.A. Peters, S. Tyler, T.B. Ball, A. Severini, and F.A. Plummer. 2009. Pyrosequencing of the chaperonin-60 universal target as a tool for determining microbial community composition. *Appl Environ Microbiol* 75: 2889–2898.

Scherwinski, K., A. Wolf, and G. Berg. 2007. Assessing the risk of biological control agents on the indigenous microbial communities: *Serratia plymuthica* HRO-C48 and *Streptomyces* sp. HRO-71 as model bacteria. *Biocontrol* 52: 87–112.

Shetty, K.G., K.V. Subbarao, O.C. Huisman, and J.C. Hubbard. 2000. Mechanism of broccoli-mediated verticillium wilt reduction in cauliflower. *Phytopathology* 90: 305–310.

Stockdale, E.A., and P.C. Brookes. 2006. Detection and quantification of the soil microbial biomass—impacts on the management of agricultural soils. *J Agric Sci* 144: 285–302.

Sturz, A.V., B.R. Christie, and J. Nowak. 2000. Bacterial endophytes: potential role in developing sustainable systems of crop production. *Crit Rev Plant Sci* 19: 1–30.

Subbarao, K.V., and J.C. Hubbard. 1996. Interactive effects of broccoli residue and temperature on *Verticillium dahliae* microsclerotia in soil and on wilt in cauliflower. *Phytopathology* 86: 1303–1310.

Subbarao, K.V., J.C. Hubbard, and S.T. Koike. 1999. Evaluation of broccoli residue incorporation into field soil for verticillium wilt control in cauliflower. *Plant Dis* 83: 124–129.

Tenuta, M., and G. Lazarovits. 2002a. Ammonia and nitrous acid from nitrogenous amendments kill the microsclerotia of *Verticillium dahliae*. *Phytopathology* 92: 255–264.

Tenuta, M., and G. Lazarovits. 2002b. Identification of specific soil properties that affect the accumulation and toxicity of ammonia to *Verticillium dahliae*. *Can J Plant Pathol* 24: 219–229.

Tenuta, M., K.L. Conn, and G. Lazarovits. 2002. Volatile fatty acids in liquid swine manure can kill microsclerotia of *Verticillium dahliae*. *Phytopathology* 92: 548–552.

Tenuta, M., and G. Lazarovits. 2004. Soil properties associated with the variable effectiveness of meat and bone meal to kill microsclerotia of *Verticillium dahliae*. *Appl Soil Ecol* 25: 219–236.

Thorup-Kristensen, K., J. Magid, and L.S. Jensen. 2001. Catch crops and green manures as biological tools in nitrogen management in temperate zones. *Adv Agron* 79: 227–302.

Trankner, A. 1992. Use of agricultural and municipal organic wastes to develop suppressiveness to plant pathogens. In *Biological control of plant diseases*, ed. E.C. Tjamos, G.C. Papavizas, and R.J. Cook, 35–42. New York: Plenum.

Tsao, P.H., and J.J. Oster. 1981. Relation of ammonia and nitrous acid to suppression of *Phytophthora* in soils amended with nitrogenous organic substances. *Phytopathology* 71: 53–59.

Warren, K.S. 1962. Ammonia toxicity and pH. *Nature* 195: 47–49.

Weller, D.M. 2007. *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years. *Phytopathology* 97: 250–256.

Wiggins, B.E., and L.L. Kinkel. 2005. Green manures and crop sequences influence potato diseases and pathogen inhibitory activity of indigenous streptomycetes. *Phytopathology* 95: 178–185.

Wilhelm, S. 1951. Effect of various soil amendments on the inoculum potential of the Verticillium wilt fungus. *Phytopathology* 41: 684–690.

Xiao, C.L., K.V. Subbarao, K.F. Schulbach, and S.T. Koike. 1998. Effects of crop rotation and irrigation on *Verticillium dahliae* microsclerotia in soil and wilt in cauliflower. *Phytopathology* 88: 1046–1055.

Xiao, J., J. Zhu, S. Chen, W. Ruan, and C. Miller. 2007. A novel use of anaerobically digested liquid swine manure to potentially control soybean cyst nematode. *J Environ Sci Health, Part B, Pestic Food Contam Agric Wastes* 42: 749–757.

Xiao, J., S. Chen, J. Zhu, and W. Ruan. 2008. Effect of liquid swine manure on hatch and viability of *heterodera glycines*. *J Nematol* 40: 152–160.