Am. J. Potato Res. (2014) 91:414–428
DOI 10.1007/s12230-014-9365-0

Potato Early Dying and Yield Responses to Compost, Green Manures, Seed Meal and Chemical Treatments

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Published online: 25 February 2014

Abstract *Verticillium dahliae* Kleb. is a soilborne fungal pathogen of many crops. In potato, it is the major causal agent of Early Dying. In Manitoba, potato fields planted with cv. Russet Burbank are infested with highly pathogenic *V. dahliae* isolates, which can produce up to 90 % disease severity. The objective of the study was to evaluate selected compost, green manure, and seed-meal treatments, in comparison with the soil fumigant Vapam, for their ability to reduce propagule density of *V. dahliae* in soil and decrease disease, and to enhance potato yield. Select green manure crops (oriental and white mustard, Canada milk vetch, sorghum-sudangrass, rye, alfalfa, oat/pea mixture), organic amendments (composted cattle manure and mustard seed-meal), and Vapam, and crop sequences that contribute to the suppression of *Verticillium*, or the improvement of potato yield were used in a 3-year field study initiated in 2006. Survival in soil of microsclerotia was evaluated as a measure of treatments' success in potentially reducing Early Dying. Compost and seed-meal treatments, compared to an untreated control, reduced incidence to 30 and 40 %, respectively, but only seed-meal reduced *V. dahliae* propagule density. Overall, green manures over 1 or 2-years were ineffective in reducing propagule density or improving potato yield. Vapam was partially effective in reducing the propagule density only at the beginning of the potato season, but it did not reduce disease incidence compared to the control. Compost and seed-meal are promising as alternative control of *V. dahliae*. Only compost reduced disease and increased potato yield, which was associated with improved nutrient availability (phosphorus and sulfate) in soil.

Resumen *Verticillium dahliae* Kleb., es un hongo patógeno del suelo para muchos cultivos. En papa, es el principal agente causal de muerte temprana. En Manitoba, los campos de papa con la variedad Russet Burbank están infestados con aislamientos altamente patogénicos de *V. dahliae*, que pueden producir hasta 90 % de severidad de la enfermedad. El objetivo del estudio fue evaluar composta selecta, abono verde, y harina de semillas, en comparación con el fumigante del suelo Vapam, respecto a su habilidad para reducir la densidad de propágulo de *V. dahliae* en el suelo y la enfermedad posterior, y en el aumento en el rendimiento de papa. En un estudio de campo de tres años iniciado en 2006, se usaron cultivos selectos de abono verde (mostaza oriental y blanca, milkvetch canadiense (*Astragalus Canadensis*, Leguminosae), sorgo-pasto sudan, Centeno, alfalfa, mezcla avena-chícharo), mejoradores orgánicos (estiércol vacuno composteado y harina de semilla de mostaza), y Vapam, con seguimiento al cultivo, en la contribución a la supresión de *Verticillium*, o en el mejoramiento del rendimiento de papa. Se evaluó la sobrevivencia en suelo de los microesclerocios como una medida de éxito de los tratamientos en la

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reducción potencial de la muerte temprana. Los tratamientos con composta y de harina de semillas, comparados con un testigo sin tratar, redujeron la incidencia a 30 y 40 % respectivamente, pero únicamente la harina de semillas redujo la densidad de propágulo de *V. dahliae*. En general, los abonos verdes en uno o dos años, no fueron efectivos en la reducción de la densidad de propágulo o en el mejoramiento en el rendimiento de papa. Vapam fue parcialmente efectivo en la reducción de la densidad de propágulo únicamente al principio del ciclo de cultivo, pero no redujo la incidencia de la enfermedad en comparación con el testigo. La composta y la harina de semillas son prometedoras como control alternativo de *V. dahliae*. Solo la composta redujo la enfermedad y aumentó el rendimiento de papa, que estuvo asociado con el mejoramiento de la disponibilidad de nutrientes (fósforo y sulfato) en el suelo.

Keywords Potato Early Dying · *Verticillium dahliae* · Potato · Green manure · Compost · Seed meal · Microsclerotia · Control

Introduction

Potato Early Dying (PED) is responsible for 30 % to 50 % potato yield reduction in severely affected fields of North America (Davis et al. 2001; Powelson and Rowe 1993). *Verticillium dahliae* Kleb., the main causal agent of PED, is a soilborne fungal pathogen of potato found in many growing regions around the world. Control of PED is extremely difficult due to the formation of microsclerotial resting structures by *V. dahliae*, which can survive in soil for up to 10–20 years (Huisman and Ashworth 1976; Schnathorst 1981). Management of PED depends on a variety of strategies to prevent the germination and/or reduce microsclerotia density in soil. Soil fumigation with metam sodium (Vapam) alone or in combination with other fumigants has been an effective method of controlling PED (Rowe and Powelson 2002; Tsror et al. 2005). However, concern over potential negative effects on human health and/or environmental damage as well as high cost have urged the search for alternative strategies to manage PED (Davis et al. 1996).

Green manuring is a cultural practice that uses plant material incorporated into soil while green or at maturity (Fageria 2007). Green manures as well as organic amendments such as animal manures and composts are frequently used for improving soil quality. They also provide nutrients to crops and may be suppressive to some soilborne pathogens such as *V. dahliae* (Davis et al. 2010a; Davis et al. 1996; Lambert et al. 2005; Ochiai et al. 2008). Incidence of PED has been reduced using pea (*Pisum sativum*), oat (*Avena sativa*), sudan-grass (*Sorghum vulgare*) and corn (*Zea mays)* green manures (Davis et al. 2010b, 1999; Ochiai et al. 2008; Wiggins and Kinkel 2005b), animal manures (Conn and Lazarovits 1999; Tenuta et al. 2002) and compost (LaMondia et al. 1999).

Even though reduction of PED using green manure or organic amendments is often inconsistent and unpredictable, a variety of mechanisms responsible for killing *V. dahliae* microsclerotia have been determined. Tenuta and Lazarovits (2002a) demonstrated that ammonia (NH_3) and nitrous acid (HNO_2) from meat and bone meal killed microsclerotia in soil. Olivier et al. (1999) suggested that incorporation of glucosinolate (GLS) containing plants, such as members of the Brassicaceae, reduce *V. dahliae* propagules due to the toxicity of hydrolysis products such as isothiocyanates (ITCs). Organic amendments such as compost stimulate the activity of microbial communities, such as copiotrophic and oligothrophic bacteria, against *V. dahliae* (Termorshuizen et al. 2006).

However, incorporation of green manures have also resulted in no reduction or even increased *V. dahliae* microsclerotia density in soil (Collins et al. 2005). Studies with Sudan-grass and corn green manures suggest that reduction of disease incidence is not always associated with lowering propagule density of *V. dahliae* (Davis et al. 1999; Davis et al. 1996); it can also be attributed to improved soil fertility (Lambert et al. 2005; Rowe and Powelson 2002) and increased microbial respiration, and increased microbial biomass C and FDA hydrolysis (Ochiai et al. 2008). Canada milk vetch green manure might reduce severity of PED due to the production of fungitoxic compounds or an induction of defense response in potato against *V. dahliae* (Uppal et al. 2008). Oat/peas, fall rye, sorghum-sudangrass and alfalfa green manures and composted beef-cattle manure may also suppress PED by changing soil conditions (N, soil pH, Ca, K, total C and microbial biomass) (Ochiai et al. 2008).

In Manitoba, potato fields planted with the processing potato 'Russet Burbank' are infested with highly pathogenic *V. dahliae* isolates, which can infect up to 90 % of the plant tissue (Uppal et al. 2007) and ultimately reduce potato yield. The effectiveness of green manures and organic amendments for reducing *V. dahliae* microsclerotia and PED has not been examined in Manitoba. Therefore, the objective of this study was to evaluate selected green manure and organic amendments for their ability to reduce *V. dahliae* microsclerotia density in soil, PED development, and to enhance soil fertility and potato yield under Manitoba growing conditions. A chemical treatment of metam sodium was used as a standard control.

Materials and Methods

Crop Sequences and Design

An experiment was conducted at the Canada-Manitoba Crop Diversification Centre (CMCDC) near Carberry, Manitoba, between 2006 and 2008. The soil was a Ramada loam (pH_{water} 6.0, EC 0.46 dSm^{-1} and organic matter 51 g kg^{-1}) naturally infested with *V. dahliae* (7 colony forming units (CFU) g^{-1} soil) (0–15 cm depth; spring, 2006). The field was planted to potato in the season preceding the start of this study in 2005. The experimental design was a randomized complete block with four blocks and 12 treatments. A total of 48 individual plots, 18 m long x 6 m wide, were separated by 2 m between plots and 10 m between each block with fallow soil.

In 2006, plots of ten treatments were planted (23 May 2006) to spring wheat (*Triticum aestivum* L., 'AC Cora') (123 kg ha^{-1}). Plots of two treatments were planted (25 May 2006) to sorghum-sudangrass hybrid (*Sorghum bicolor* L. Moench, 'Super Su 22') (29 kg ha^{-1}) (treatment SS2), and alfalfa (*Medicago sativa* L.) (9 kg ha^{-1}) (treatment ALF), respectively. The spring wheat plots were harvested, plant biomass of the SS2 plots were disc-incorporated to a depth of 15 cm, and ALF plots cut down and mechanically harvested for animal feed on 19 September 2006 (Table 1).

In 2007, alfalfa regrew in the same plots it was planted in 2006, and the SS2 treatment was re-planted with sorghum-sudangrass on the same plots. Plots of one treatment were planted to spring wheat and used as the crop control treatment (control). Plots of six green manure treatments were planted on 29 May 2007 to white mustard (*Sinapis alba* L. 'Ace') (9 kg ha^{-1}) (treatment MUSTwh); oriental mustard (*Brassica juncea* L. 'Cutlas') (5 kg ha^{-1}) (treatment MUSTor); oat (*Avena sativa* L 'Triple Crown')/pea (*Pisum sativum* L. '40-10 Forage Pea' (86 kg ha^{-1}) (treatment O/PEA); Canada milk vetch (*Astragalus canadensis* L.) (11 kg ha^{-1}) (treatment CMV); fall rye (*Secale cereale* L.) (81 kg ha^{-1}) (treatment RYE) and sorghum-sudan-grass (treatment SS1). Composted beef cattle manure (COM) produced at AAFC Brandon, MB), was hand applied to one set of the treatment plots (four plots), before wheat planting, at a rate of 44.5 wet Mg ha^{-1} (45 % moisture, 1.28 % N, 0.5 % P). The remaining set of treatment plots (16 plots) were planted to spring wheat on 30 May 2007. Treatment plots with green manure crops were cut down and mechanically incorporated in soil to a depth of 15–20 cm, between August and September 2007, by either disc or rototiller. The fresh matter (Mg ha^{-1}) of green manures incorporated in 2007 was 34.5 MUSTor, 33.3 MUSTwh, 34.8 O/PEA, 53.8 SS2, 48.8 SS1, 6.5 RYE, 8.3 CMV, and 21.0 ALF. Plots planted to spring wheat were harvested on 15 October 2007. Soil fumigation with Vapam (32.7 % sodium methyldithiocarbamate, United Agriproducts, MB) (VAP) was conducted on 15 October 2007, immediately after harvest of one set of plots with spring-wheat. Before fumigation, plots were cultivated with a tandem disk followed by roto-tilling to loosen the soil to a depth of 15 cm. The fumigant was mixed with water and injected into plot areas, at 5,000 L ha^{-1} (762 L Vapam ha^{-1}) which is what was recommended by the product distributor at the time of the study. The more concentrated product formulation (Vapam HL, 42 % sodium methyldithiocarbamate) was not commercially available in Canada at the time of this study.

Table 1 Crops, green manures, organic amendments and Vapam treatments used in the study

Treatment[a]	2006		2007		2008	
	Crop	Amendment	Crop	Amendment	Crop	Amendment
Control	Spring Wheat	N/A[b]	Spring Wheat	N/A	Potato	N/A
COM	Spring Wheat	N/A	Spring Wheat	May/Compost	Potato	May/Compost
MUSTmeal	Spring Wheat	N/A	Spring Wheat	N/A	Potato	May/Seed-Meal
VAP	Spring Wheat	N/A	Spring Wheat	Oct./Vapam	Potato	N/A
O/PEA	Spring Wheat	N/A	Oat/Peas	August/GM	Potato	N/A
CMV	Spring Wheat	N/A	Can. Milk Vetch	August/GM	Potato	N/A
RYE	Spring Wheat	N/A	Fall rye	August/GM	Potato	N/A
MUSTor	Spring Wheat	N/A	Brown mustard	August/GM	Potato	N/A
MUSTwh	Spring Wheat	N/A	White Mustard	August/GM	Potato	N/A
SS1	Spring Wheat	N/A	Sorghum-Sudan	August/GM	Potato	N/A
SS2	Sorghum-Sudan	Sept./GM[c]	Sorghum-Sudan	August/GM	Potato	N/A
ALF	Alfalfa	Sept./CDH[d]	Alfalfa	August/GM	Potato	N/A

[a] Treatments: Control=wheat-control, COM=compost, $MUST_{meal}$=mustard seed-meal, VAP=Vapam, O/PEA=oat/peas mix, CMV=Canada milk vetch, SS2=2-years sorghum-sudangrass, SS1=1-year sorghum-sudangrass, RYE=fall rye, MUSTor=Oriental mustard, MUSTwh=white mustard, and ALF=alfalfa

[b] *N/A* Non-amendment at that particular time

[c] *GM* Green manure

[d] *CDH* Cut down and mechanically harvested for animal feed

In 2008, to one set of plots previously planted to spring-wheat, mustard seed meal (treatment MUSTmeal) was added as an organic amendment treatment prior planting of potato. The seed meal was in the form of partially de-oiled oriental mustard seed containing a high level of GLS sinigrin (0.85 %), 25 % protein, 8 % moisture and 4–6 % ash content (Mustard Capital Inc., Gravelbourg, SK). Mustard seed meal was broadcasted by hand to the soil surface at a concentration of 0.5 % (w w^{-1}) (field equivalent 9,000 Mg ha^{-1}) and incorporated into soil with a rototiller on 15 May. On 16 May, compost was incorporated at a rate of 44.5 wet Mg ha^{-1} to the same treatment plots it was applied in the previous year (Table 1).

On 21 May 2008, all plots were planted to potato (*Solanum tuberosum* 'Russet Burbank'). Seed rows were 1 m apart, with six rows per plot, and within row spacing of 0.25 m. Prior to planting, each plot received a broadcast application of nutrients based on soil fertility analysis (NO_3^-, Olsen-P, ammonium acetate-K) and recommendation guidelines in Manitoba (Province of Manitoba Soil Fertility Guide). Treatments were grouped according to soil fertility test levels as follows: Group 1-control, VAP, COM, MUSTmeal, RYE, SS2 and ALF treatments received 34 kg N ha^{-1}, 45 kg P_2O_5 ha^{-1} and 78 kg K_2O ha^{-1}; Group 2-O/PEA, MUSTwh, MUSTor and SS1 treatments received 90 kg N ha^{-1}, 45 kg P_2O_5 ha^{-1} and 78 kg K_2O ha^{-1}. Nitrogen, P and K were added as commercial urea, monoammonium phosphate and potassium chloride fertilizers, respectively. Soil was disked to 15 cm following fertilizer application. Potato hilling was done on 24 June 2008 with no additional fertilizer application.

Survival of Buried *Verticillium dahliae* Microsclerotia

An in-situ soil bioassay was used to determine the fungitoxic effect of MUSTmeal, VAP, O/PEA, CMV, SS2, SS1, RYE, MUSTor, MUSTwt and ALF treatments on the survival of *V. dahliae* microsclerotia. Microsclerotia from a highly pathogenic isolate of *V. dahliae* (vd-1396) to potato (Uppal et al. 2007) were produced in the laboratory on semisolid Czapek-Dox medium for 3–4 weeks in the dark at 24 °C (Hawke and Lazarovits 1994). The culture was poured through mesh screens to obtain microsclerotia 75 to 106 μm in diameter. Survival response to the treatments was determined using a microsclerotia in silica sand mixture added to nylon mesh bags (SAATILON® monofilament) (Tenuta and Lazarovits 2002b). The mesh bags were buried in soil to a 10 cm depth immediately following treatment incorporation of green manure, seed meal and Vapam treatments, and retrieved 1 and 3 weeks later. Bags were then dried at room temperature for 12 h and their contents spread onto a pectate–tergitol–agar plate using an Andersen Cascade Impactor (Andersen Instruments Inc., Smyrna, GA). The agar plates were then incubated for 5–7 days in the dark at 24 °C and the germination of microsclerotia determined as the percentage of number of microsclerotia examined that germinated to form colonies.

Verticillium dahliae Propagule Density in Soil

A composite soil sample consisting of 20 soil cores was taken randomly from the hills of each treatment plot with a 2.5 cm diameter soil probe to a depth of 15 cm, at four times: before green manure and spring-wheat crops were planted (May 2007), immediately after spring wheat was harvested and approximately 2 months after incorporation of green manures (September 2007), prior to planting potato (April 2008) and immediately after potato harvest (August 2008). Soil cores from each plot were bulked, placed in polyethylene bags, and transported in an ice chest to the University of Manitoba. The samples were mixed by hand and stored at 4 °C. Samples were stored for no more than 2 weeks before starting determination of *V. dahliae* propagule density in soil.

A 100 g subsample of each soil sample was air-dried for 7 days at room temperature and then 5 g were suspended in 100 mL of sterilized agar-water (1 %), and agitated for 2 min using an orbital shaker at 60 rpm. One milliliter of the suspension was placed onto plates of Sorensen's NP-10 medium (Sorensen et al. 1991) containing chloramphenicol, streptomycin sulphate and chlortetracycline HCl. Each sample was plated ten times. Plates were incubated for 15 days in the dark at 22 °C. They were then rinsed with tap water to remove soil particles from the medium surface. The number of germinated *V. dahliae* microsclerotia was counted using a stereomicroscope, and expressed as *V. dahliae* CFU g^{-1} soil.

Disease Incidence and Severity

PED incidence was determined late in the growing season (25 August 2008) as the proportion of plants examined with symptoms in each plot. The symptoms observed were uneven chlorosis of the lower leaves or flagging unilateral leaf wilting or death (Rowe et al. 1987). For disease severity determination, ten plants were dug from one row on each side of a plot. Disease severity by *V. dahliae* was assessed by taking a stem portion from the upper, middle and lower section of the plant. Stem portions were then vertically split to estimate the percentage of vascular tissue discolored from each section using a predetermined scale of 0 to 5, in which 0=0 %, 1=1–10 %, 2=11–30 %, 3=31–50 %, 4=51–75 %, and 5=76–100 % discoloration (Uppal et al. 2008).

Soil Chemical Analysis

For soil chemical analysis, three cores per plot were taken (from the hills and between plants, if present) on 14 May (before planting), 8 July (middle of the season) and 22 August (after harvest) at two depths: 0–15 cm for sodium bicarbonate extractable-P, ammonium acetate extractable-K, pH and electrical conductivity (by 1:2 soil:water ratio method), and 15–60 cm for the mobile nutrients, nitrate and sulfate. The three soil cores from each plot were bulked, placed in polyethylene bags, and transported on ice to the University of Manitoba, where each bag was mixed by hand and stored at 4 °C. Samples were stored for no more than 24 h before sending to Bodycote Laboratory (Winnipeg, MB.) for analysis.

Potato Yield and Quality

Crop yield was determined by harvesting the two central rows of each plot on 22 August 2008. Experimental plots were mechanically harvested. Potato tubers were placed in open fiber bags, transported to the CMCDC station and stored at 5 °C until sample processing. Samples were then washed and weighed to determine weight loss and washed. Based on tuber weight analysis, the size categories for marketable yield were: non-marketable tubers <85 g, regular 86–170 g, bonus 170.1–340 g and overweight >340 g. Yield was expressed as Mg ha^{-1}. Specific gravity was determined using the weight in air and water method, fry color analysis by using USDA Fry Color Chart, green color, rot and hollow heart by tuber observation, and sugar end analyses by frying and comparing color with a standard chart.

Statistical Analysis

All statistical analyses were performed with the Statistical Analysis Software (SAS Institute, Cary, NC; release 9.1). Prior to analysis, *V. dahliae* propagule density in soil, disease incidence and severity, and marketable yield data sets were checked for normality (PROC Univariate). Survival rate of buried *V. dahliae* microsclerotia was arcsine transformed before analysis, and it is presented as percentage of the control (microsclerotia germination of control=100 %). The data were analyzed by analysis of variance using the PROC MIXED procedure in SAS. Block was considered a random variable. Treatment means were separated using the Bonferronni's procedure if the F-test was significant ($P<0.05$). Contrasts were used to test the significance of differences between groups of treatments. Linear and nonlinear regression analyses were used to examine the relationships of inoculum density and selected soil characteristics on PED disease incidence and potato tuber yield. MUSTmeal was excluded from the regression analyses of measures to tuber yields because plants remained in vegetative growth while other treatments senesced at harvest. *V. dahliae* propagule density in soil, disease incidence and severity, chemical parameters and marketable yield and categories were then subjected to stepwise regression analysis to determine if the relationship between two or more independent variable(s) during the 2-year study can be used to construct a model that predict disease incidence and potato tuber yield. Differences were considered significant at $P<0.05$.

Results

Survival of Buried *Verticillium dahliae* Microsclerotia

The bioassay results showed that germination of buried *V. dahliae* microsclerotia was affected by treatment ($P<0.0001$) and time after treatment application ($P<0.006$). In general, the germination percentage of buried microsclerotia relative to the control was lower at 3 weeks than 1 week post treatment, except for MUSTmeal treatment which increased slightly after 3 weeks (Fig. 1). VAP and MUSTmeal were the most effective treatments reducing germination of microsclerotia ($P<0.0001$). One week following incorporation, MUSTwh, MUSTor and O/PEA reduced germination of microsclerotia by about 40 % compared to the control. In contrast, CMV, SS1, SS2, RYE and ALF treatments did not affect the germination of *V. dahliae* microsclerotia (Fig. 1). Three weeks after application, VAP was the most effective treatment reducing germination to 5 % of the control ($P<0.0001$). In contrast, MUSTmeal treatment was less effective after 3 weeks with the germination of microsclerotia being 24 % of the control (Fig. 1). Germination of *V. dahliae* microsclerotia decreased slightly with green manure treatments ranging from 60 to 90 % of the control. However, germination of microsclerotia in O/PEA, SS2, MUSTor and MUSTwh treatments was not significantly different from the MUSTmeal treatment. Although the germination of microsclerotia observed in SS1, ALF, RYE and CMV treatments was not significantly different from other green manure treatments, it was significantly higher than MUSTmeal ($P<0.05$).

Verticillium dahliae Propagule Density in Soil

Mean soil densities of *V. dahliae* in spring and fall 2007 were 6 and 8 CFU g^{-1} soil, respectively. Propagule density was not affected by incorporation of the green manures in 2007. However, contrast analysis showed lower density in MUSTwh and MUSTor compared to other green manure treatments ($P=0.0296$, Table 2).

Propagule density in soil also differed among treatments in the spring and fall of the potato year of the experiment (2008) ($P=0.0001$). In spring 2008, MUSTwh was the only green manure treatment with a lower density of propagules than the control ($P=0.0002$). MUSTwh, MUSTor and O/PEA treatments maintained low propagule density ranging from 3 to 7 CFU g^{-1} soil. The application of Vapam the previous fall had reduced the density of propagules in soil from 9 to

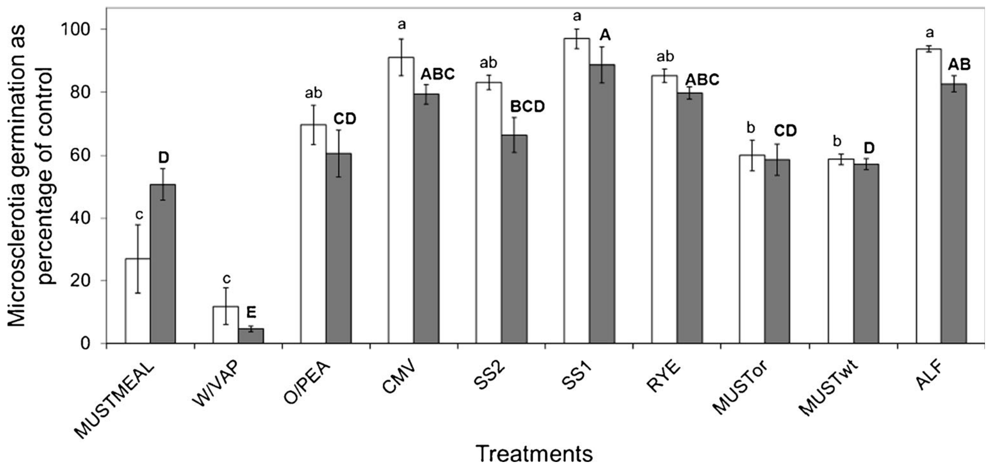

Fig. 1 Bioassay results for the effect of mustard seed meal, Vapam and green manures on germination of buried *V. dahliae* microsclerotia, expressed as a percentage of germination of microsclerotia relative to the control at 1 week (*empty square*) and 3 weeks (*filled square*) after application. $MUST_{meal}$=mustard seed meal, VAP=Vapam, O/PEA=oat/pea mix, CMV=Canada milk vetch, SS2=2-years sorghum-sudangrass, SS1=1-year sorghum-sudangrass, RYE=fall rye, MUSTor=oriental mustard, MUSTwh=white mustard, and ALF=alfalfa. Means within a sampling time followed by the same letter are not statistical different according to the Bonferroni's multiple comparison test (P>0.05). *Error bars* are +1 standard error

3 CFU g^{-1} soil. Contrast analysis showed significant differences for Vapam compared to the control (P<0.0001) and green manure treatments (P=0.0006, Table 2).

After harvest of potato in 2008, the density of propagules in soil increased in all treatments, except for MUSTmeal and COM (Table 2). Propagule density in soil was lower in MUSTmeal and COM plots compared to control plots (P=0.0001). Moreover, MUSTmeal plots had fewer CFU g^{-1} soil at the end of the potato season compared to plots fumigated with VAP (P<0.0001) or green manure treatments (P=0.0004). The application of Vapam did not reduce the density of propagules compared to control and green manure treatments after potato harvest. Contrast analysis also showed that COM treatment had significant lower density of propagules in soil compared to the green manure treatments (P=0.0022, Table 2).

Disease Incidence and Severity

PED incidence data was affected by treatments (P=0.0001) though no individual treatment was lower than the Control (Table 2). MUSTmeal and COM treatments had the numerically lowest incidence of 13 % and 25 %, respectively, whereas SS2 had the highest at 93 %. Contrast analyses showed significant differences for green manures compared to MUSTmeal (P<0.0001) and COM (P=0.0007) treatments. Contrast analyses also showed significantly lower incidence in MUSTmeal than the mustard green manure treatments (P<0.0014).

No treatment reduced disease severity, at the lower, middle and upper section of the potato stems, compared to the control (P>0.05, data not shown). Severity as stem discoloration ranged from 11 to 45 %, 1 to 25 %, and less than 1 % for lower, middle and upper potato stem sections, respectively (data not shown).

Plant and Soil Assessment

In general, organic amendments and green manures promoted significant changes in soil fertility levels and potato yield. MUSTmeal treatment increased NO_3^--N (203 kg N ha^{-1}) in soil by approximately four-fold compared to the control (49 kg N ha^{-1}) (P=0.0001, Table 3). Plant extractable-P concentration was significantly greater in COM (65 kg ha^{-1}) than in green manure treatments (29–38 kg P ha^{-1}, P<0.0001) or any other treatment except for MUSTmeal. MUSTmeal treatment (45 kg N ha^{-1}) also increased extractable-P compared to green manure treatments (P=0.0105). Sulfate concentration was greatest in MUSTmeal and VAP treatments (142 and 139 kg ha^{-1}, respectively) compared to other treatments (34–65 kg S ha^{-1}, P=0.0001, Table 3). The incorporation of green manure and organic amendments into soil had no effect on soil pH (Table 3). No significant difference in EC was observed among green manure, COM and the control treatments (EC ranged from 0.19 to 0.35 dS m^{-1}). However, MUSTmeal treatment showed a significant increase (0.52 dS m^{-1}) compared to the control treatment (P=0.0016, Table 3) and likely a result of accumulation of NO_3^- and sulfate.

The relationship between inoculum density at harvest and PED disease incidence was cubic (P=0.0068, Fig. 2a). At harvest, there was a quadratic relationship between PED incidence and extractable-P (P=0.006, Fig. 2b). Further, multiple stepwise regression analysis revealed relations of high inoculum density (at harvest) and extractable-P on PED incidence.

Table 2 Effect of wheat, green manures, organic amendments and Vapam treatments on soil propagule density of *V. dahliae* (CFU g^{-1} soil) and Potato Early Dying incidence

Treatment[a]	*V. dahliae* (CFU g^{-1} soil)						Incidence (%) 2008 August	
	2007		2008					
	May	Sept.	April		August			
Control	3±1[b]	5±2	13±3	a	27±2	ab	57±11	abc
COM	10±4	6±1	9±3	ab	12±3	cd	25±3	bc
MUSTmeal	10±6	11±2	15±2	a	10±1	d	13±7	c
VAP	4±2	9±5	3±1	b	24±3	abc	70±11	ab
O/PEA	11±5	9±2	7±2	ab	14±4	bcd	47±10	abc
CMV	6±2	12±6	12±4	ab	21±2	abcd	48±2	abc
SS2	4±3	4±1	11±2	ab	25±4	abc	93±7	a
SS1	6±3	11±7	14±3	a	18±2	abcd	62±7	ab
RYE	4±2	12±3	18±4	a	36±7	a	70±10	ab
MUSTor	8±3	7±4	7±2	ab	15±3	bcd	52±16	abc
MUSTwh	3±1	3±2	3±1	b	17±2	abcd	52±6	abc
ALF	7±1	7±3	12±2	ab	20±1	abcd	62±13	ab
$P>F$[c]	ND	ND	0.0002		0.0001		0.0001	
Selected contrasts (significant probability).								
Unamended vs Green manures	ND	ND	0.0382		NS		NS	
Unamended vs VAP	ND	ND	<0.0001		ND		ND	
Vap vs Green manures	ND	ND	0.0006		NS		NS	
COM vs Green manures	ND	ND	ND		0.0022		0.0007	
MUSTmeal vs Green manure	ND	ND	ND		0.0004		<0.0001	
Mustards vs MUSTmeal	ND	ND	ND		0.0332		0.0014	
Mustards vs Non-mustards	ND	0.0296	0.0002		0.0247		NS	

[a] Treatments: Control=wheat-control, COM=compost, $MUST_{meal}$=mustard seed-meal, VAP=Vapam, O/PEA=oat/peas mix, CMV=Canada milk vetch, SS2=2-years sorghum-sudangrass, SS1=1-year sorghum-sudangrass, RYE=fall rye, MUSTor=Oriental mustard, MUSTwh=white mustard, and ALF=alfalfa. (Unamended)=treatments not established, but planted with spring-wheat; mustards=white and oriental mustard; non-mustards=oat/peas, Canada milk vetch, 1- and 2-years Sorghum-sudangrass, fall rye and alfalfa

[b] Values are means±1 standard error. Within columns means followed by the same letter are not statistical different (P>0.05) according to the Bonferroni's multiple comparison test

[c] Treatment significant probability. NS, not significant (P>0.05). ND, not determined

This model explained 66 % of the variability in disease incidence (P=0.0076, Table 5). There was no relation between inoculums density at planting and PED disease incidence (data not shown).

Marketable Yield and Tuber Quality

A significant treatment effect was observed for bonus, overweight and total marketable yield (Table 4), but not for tuber quality (fry colour, specific gravity and sugar ends of tubers) (data not shown). Total marketable yield of potato in plots amended with COM treatment were 26 and 42 % higher than the control and MUSTmeal treatments (P=0.0003), respectively. Contrast analyses revealed an increase in total marketable yield with COM compared to green manure treatments (P<0.0001, Table 4). Bonus tubers, which bring a premium price, increased with COM treatment, from 15 to 71 %, compared to other treatments (Table 4). Contrast analysis indicated that bonus (P=0.0016) and overweight marketable (P=0.0072) tuber classes were higher in COM compared to green manure treatments.

Not including the MUSTmeal treatment, overweight and total tuber yield decreased while bonus yield increased with PED incidence (Fig. 3a). Upon inspection of the regression plots, it is clear that the significant relationships resulted because of three treatment groupings; COM at low, SS2 at high, and other treatments at intermediate PED incidence (Fig. 3a). Regular tuber yield increased with sulfate (P=0.01) but the relationship was driven by the yield and very high concentration with VAP treatment (Fig. 3b) because the relationship was not significant when the treatment was removed from the data set (data not shown). Bonus yield

Table 3 Water extractable nitrate-N (NO_3^--N), sodium bicarbonate extractable-P, ammonium acetate extractable-K, water extractable sulfate (SO_4^{-2}), pH and electrical conductivity (EC) in soil following potato harvest

Treatment[a]	NO_3^--N		P		K	SO_4^{-2}-S		pH	EC	
	kg ha^{-1}								dS m^{-1}	
Control	49±6	b[b]	31±2	b	285±9	28±2	b	6.0±0.1	0.19±0.01	b
COM	73±19	b	65±11	a	455±81	61±9	b	6.7±0.2	0.35±0.05	ab
MUSTmeal	203±33	a	45±9	ab	388±74	142±28	a	5.9±0.2	0.52±0.10	a
VAP	66±17	b	31±3	b	307±8	139±21	a	6.1±0.1	0.27±0.02	ab
O/PEA	66±9	b	36±2	b	304±10	38±3	b	6.0±0.2	0.19±0.02	b
CMV	54±10	b	36±4	b	425±84	42±2	b	6.3±0.1	0.20±0.02	b
SS2	117±17	b	29±2	b	315±18	34±3	b	6.1±0.1	0.22±0.02	b
SS1	58±11	b	31±3	b	330±35	47±6	b	6.5±0.3	0.34±0.09	ab
RYE	88±11	b	29±1	b	351±28	36±3	b	6.3±0.2	0.26±0.03	ab
MUSTor	48±8	b	30±6	b	269±9	54±13	b	6.4±0.2	0.26±0.04	ab
MUSTwh	57±4	b	31±3	b	300±15	65±13	b	6.0±0.1	0.21±0.02	b
ALF	116±22	b	38±5	b	363±63	41±5	b	6.6±0.2	0.30±0.03	ab
$P>F$[c]	0.0001		0.0001		NS	0.0001		NS	0.0016	
Selected contrasts (significant probability)										
VAP vs Green manures	NS		NS			<0.0001			NS	
COM vs Green manures	NS		<0.0001			NS			NS	
MUSTmeal vs Green manure	<0.0001		0.0105			<0.0001			<0.0001	
Mustards vs MUSTmeal	<0.0001		0.0108			<0.0001			<0.0001	
Mustards vs Non-mustards	0.0094		NS			0.0406			NS	

[a] Treatments: Control=wheat-control, COM=compost, $MUST_{meal}$=mustard seed-meal, VAP=Vapam, O/PEA=oat/pea mix, CMV=Canada milk vetch, SS2=2-years sorghum-sudangrass, SS1=1-year sorghum-sudangrass, RYE=fall rye, MUSTor=Oriental mustard, MUSTwh=white mustard, and ALF=alfalfa. Mustards=white and oriental mustard; non-mustards=oat/peas mix, Canada milk vetch, 1- and 2-years Sorghum-sudangrass, fall rye and alfalfa

[b] Values are means±1 standard error. Within columns means followed by the same letter are not significantly different (P>0.05) according to the Bonferroni's multiple comparison test

[c] Treatment significant probability. NS, not significant (P>0.05)

decreased while overweight and total tuber yields increased with extractable-P (Fig. 3c). When the COM treatment with its high extractable-P values were removed from the data set, overweight and total tuber yields still increased with soil concentrations (P<0.05). Tuber quality attributes (green color, rot damage, hollow heart, specific gravity, sugar end, dark end and fry color) were not related with treatments (data not shown).

Potato Early Dying was best predicted (P=0.0076) by the combination of *V. dahliae* propagule density and extractable-P (Table 5, Fig. 4a). The model explained 66 % of the variation in measured PED incidence. Multiple regression analysis showed total yield predicted by PED incidence, sulfate and NO_3^-N (P=0.0002). These three variables explained 92 % of the variability in potato total marketable yield (Table 5). The measured and predicted total marketable yield were well related (r^2=0.93), indicating that the model fit was good (Fig. 4b). Eighty-two percent of the variability in marketable regular tuber category was explained by the combination of sulfate, PED incidence and NO_3^-N, while 86 % of the variability of the marketable bonus category was predicted by PED incidence and sulfate. Finally, the overweight category was best explained by the extractable-P by 61 % (P=0.004) (Table 5).

Discussion

In this study, a variety of green manure crops and organic amendments, such as mustard seed meal and composted beef cattle manure, were assessed for their ability to kill *V. dahliae* microsclerotia, to reduce PED incidence and ultimately, to increase potato yield. VAP and MUSTmeal were the most effective treatments reducing germination of buried microsclerotia within 1 week after incorporation. The efficacy of Vapam in reducing germination of microsclerotia depends on its conversion in the soil to very active and toxic ITC compounds, particularly methyl-isothiocyanate (methyl-ITC), which constitutes 90 % of the conversion products in soil (Leistra et al. 1974). The suppressive activity of the

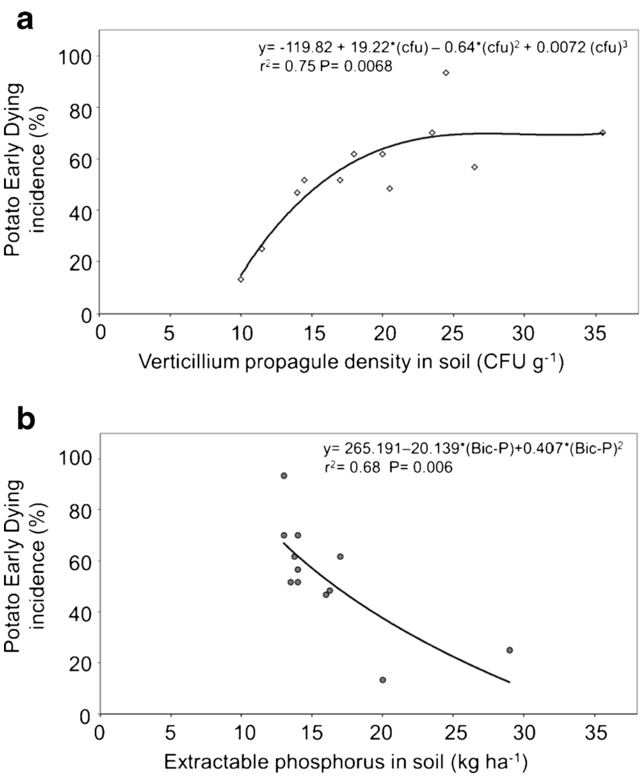

Fig. 2 Relationship between incidence (foliar symptoms) of Potato Early Dying and: **a** propagule density of *Verticillium dahliae* in soil, and **b** extractable phosphorus in soil after potato harvest

MUSTmeal treatment may be associated with the presence of GLS hydrolysis products as well. Partially deoiled mustard seed meal contained sinigrin-GLS, which transformed to 2-propenyl isothiocyanate (2-propenyl-ITC). 2-propenyl-ITC is one of the most biologically active forms of ITCs, and it has been shown to kill soil pathogens like *V. dahliae* (Olivier et al. 1999). Although MUSTmeal treatment reduced the germination of buried microsclerotia 1 week after incorporation, the germination was higher 3 weeks after incorporation. The findings reported here provide evidence that exposure to sub-lethal concentrations of ITC sufficiently weaken microsclerotia that they do not germinate on selective media. Once the ITC concentration dissipated in soil, microsclerotia regained the ability to grow on the plate medium. It is common that some organisms weakened by sub-lethal treatments remain viable but not culturable, which means that some cells will require special stimuli to return to the culturable state (Weichart and Kjelleberg 1996). This condition is often observed in injured organisms, which fail to grow under selective isolation conditions. However, when injured organisms are removed from the sub-lethal conditions, the microorganisms restore their ability to grow in culture (Weichart and Kjelleberg 1996). For example, exposure of microsclerotia to sub-lethal concentrations of liquid swine manure (LSM) or volatile fatty acids (VFA) (Tenuta et al. 2002), NH_3 and HNO_2 (Tenuta and Lazarovits 2002a, 2004) followed by immediate plating onto germination medium resulted in a lower germination of microsclerotia. However, placement of the microsclerotia in soil free of LSM, VFA, NH_3 or HNO_2 for 7 days allowed the organism to recover, resulting in a moderate rebound of germination of microsclerotia. Smolinska et al. (2003) found that *F. oxysporum* isolates exposed to low concentrations of pure 2-propeny-ITC (0.3 μl) for 24 h did not grow on plate medium until the medium was cleared of ITCs. Our result suggests that fungitoxicity of ITCs could be induced by increasing concentration or exposure time of microsclerotia to ITCs.

MUSTwh, MUSTor and O/PEA green manure treatments inhibited germination of buried microsclerotia to 60 % of the control. These green manures show some promise as a means to reduce *V. dahliae* propagule density in soil. Reduction in germination of *V. dahliae* microsclerotia has been observed with several green manures (Lopez-Escudero et al. 2007). Brassicaceae green manure crops have been used to reduce soil populations of *V. dahliae* and incidence of Verticillium wilt in potato (Rowe and Powelson 2002) due to the release of ITCs. The efficacy of Brassicaceae green manures to kill the pathogen depends on the concentration of ITC released from the plant material (shoot or roots tissue or seed meal) (Olivier et al. 1999). The most important factor limiting ITC concentration in soil may not be GLS concentration in the amendment itself, but release of ITC from the amendment to the soil. High release of ITC in soil occurs when cell membranes are broken (Gimsing and Kirkegaard 2009). The level of plant physical disruption necessary to maximize ITC is generally impossible with green manure crops (Matthiessen and Kirkegaard 2006). However, such a limitation does not exist when using seed meal since the seed crushing procedure results in extensive cellular disruption (Brown and Morra 2005). Therefore, the concentration of ITCs released by the mustard seed meal may be 10 times higher than from intact vegetative plant tissue (roots and aerial parts) (Brown and Morra 2005; Kirkegaard et al. 1996). Further, the effectiveness of ITCs release from Brassicaceae green manure crops improves with lower organic matter content of soil (Matthiessen and Kirkegaard 2006). The organic matter content of the loam soil in the current study was 51 g kg^{-1} whereas Brassicaceae green manures are recommended for sand soils of relatively lower organic matter content than the soil used here (Matthiessen and Kirkegaard 2006). In a laboratory study, doubling the fresh matter addition of MUSTor from 34.5 to 69 Mg ha^{-1} equivalent to the same soil used in this current study was not more lethal to *V. dahliae* microscleroatia buried in soil (Molina 2009). Even at that double addition rate of MUSTor, less than half the amount of 2-propenyl-isothiocyanate was produced than seed-meal applied at the rate of this current study. Therefore, we hypothesize that the higher concentration of ITCs released by the MUSTmeal was responsible for greater death of buried *V. dahliae* microsclerotia in soil.

The *Verticillium* propagule density in soil prior to planting was 13 cfu g^{-1} soil and 20 cfu g^{-1} soil at harvest across all

Table 4 Effect of green manure, organic amendments and Vapam treatment on marketable potato yield

Treatment[a]	Marketable yield (Mg ha^{-1})[b]						
	Regular	Bonus		Overweight		Total	
Control	11.0±0.9[c]	16.1±1.1	ab[§]	3.7±1.3	ab	30.8±1.6	b
COM	10.5±0.9	19.5±2.1	a	8.7±1.8	a	38.7±1.3	a
MUSTmeal	8.9±0.4	14.0±1.3	ab	4.3±0.6	ab	27.2±1.8	b
VAP	13.3±1.0	16.6±1.1	ab	3.2±1.0	ab	33.1±1.6	ab
O/PEA	9.5±0.8	16.9±0.8	ab	5.9±1.4	ab	32.3±1.2	ab
CMV	10.9±0.3	15.2±0.9	ab	6.0±1.4	ab	32.0±0.3	ab
SS2	11.7±0.3	11.4±1.2	b	2.9±1.6	b	26.0±1.6	b
SS1	11.1±1.3	14.8±1.2	ab	4.9±0.4	ab	30.8±2.3	b
RYE	11.0±0.9	14.5±1.6	ab	5.6±1.7	ab	31.2±2.6	ab
MUSTor	11.7±1.4	15.5±1.5	ab	3.5±1.0	ab	30.6±1.4	b
MUSTwh	11.7±0.8	15.4±1.1	ab	5.4±0.9	ab	32.6±1.7	ab
ALF	10.1±0.4	15.4±0.9	ab	7.2±0.9	ab	32.6±1.2	ab
$P>F$[d]	NS	0.0321		0.0365		0.0003	
Selected contrasts (significant probability)							
Control vs Green manures		NS[y]		NS		NS	
VAP vs Green manures		NS		NS		NS	
COM vs Green manures		0.0016		0.0072		<0.0001	
MUSTmeal vs Green manure		NS		NS		0.0200	
Mustards vs MUSTmeal		NS		NS		0.0201	
Mustards vs Non-mustards		NS		NS		NS	

[a] Treatments: Control=wheat-control, COM=compost, $MUST_{meal}$=mustard seed-meal, VAP=Vapam, O/PEA=oat/pea mix, CMV=Canada milk vetch, SS2=2-years sorghum-sudangrass, SS1=1-year sorghum-sudangrass, RYE=fall rye, MUSTor=Oriental mustard, MUSTwh=white mustard, and ALF=alfalfa. Mustards=white and oriental mustard; non-mustards=oat/peas mix, Canada milk vetch, 1- and 2-years sorghum-sudangrass, fall rye and alfalfa

[b] Potato tubers graded into weight categories for marketable yield: regular 86–170 g, bonus 170.1–340 g and overweight >340 g

[c] Value are means ± standard error. Within columns means followed by the same letter are not significantly different ($P>0.05$) according to the Bonferroni's multiple comparison test

[d] Treatment significant probability. NS, not significant ($P>0.05$)

treatments. There are no economic threshold guidelines for *Verticillium* in soil for Manitoba or the Prairie Provinces of Canada. Greater than 12 cfu g^{-1} soil is a guideline in Ontario for a high risk of disease and need for control measures (University of Guelph Laboratory Services, Agricultural and Food Laboratory). Disease incidence in the current study ranged from 13 to 93 % indicating sufficient presence of the pathogen to potentially limit yields.

In general, green manure crops did not reduce the germination of microsclerotia or propagule density of *V. dahliae* in soil, compared to the control. Some green manure treatments such as RYE and SS2 increased propagule density as well as PED incidence. The lack of effect of the green manure treatments to reduce propagule density of *V. dahliae* and PED incidence may be due to several factors such as too small number of green manure cycles (Rowe and Powelson 2002) or insufficient physical disruption of plant tissue during plow down that lead to low release of toxic compounds (Morra and Kirkegaard 2002). These factors are relatively important for long-term survival structures of plant pathogens in soil, particularly, microsclerotia of *V. dahliae* (Rowe and Powelson 2002). These results are consistent with those of Davis et al. (1996) who reported that two consecutive years of sudangrass, oat, or rye green manure did not reduce inoculum of *V. dahliae*. Green manure crops can reduce density of propagules in soil and Verticillium wilt incidence in potato (Lopez-Escudero et al. 2007; Ochiai et al. 2008). Three consecutive years of green manure may be required to reduce *V. dahliae* levels in soil depending the type of green manure crop (Davis et al. 1996; Rowe and Powelson 2002). However, 3 years of green manure cropping is usually not feasible for potato production especially with current favourable returns for grain and oilseed rotation crops.

Although buried microsclerotia partially restored their ability to germinate 3 weeks after incorporation of MUSTmeal, germination was reduced by 50 %. Moreover, PED incidence and propagule density of *V. dahliae* were effectively reduced by MUSTmeal. It can be hypothesized that ITCs could reduce

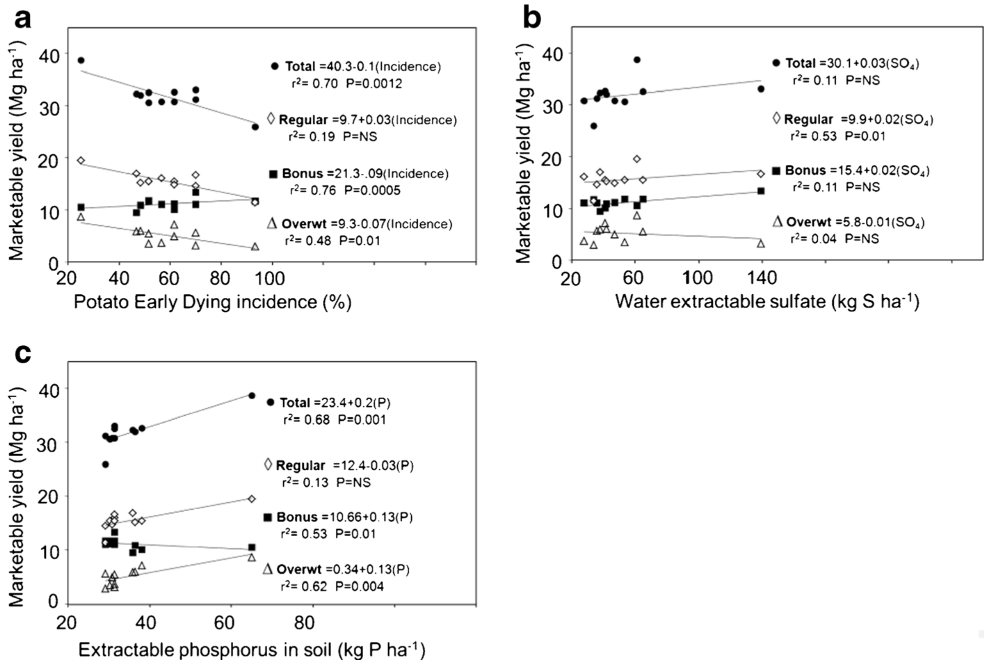

Fig. 3 Relationship between the potato tuber weight categories of marketable yield (Mg ha^{-1}), regular (86–169 g), bonus (170–340 g) and overweight (>340 g), and total yield and: **a** Potato Early Dying incidence (%), **b** Water extractable sulfate (kg S ha^{-1}), and **c** Extractable phosphorus in soil (kg P ha^{-1}). MUSTmeal treatment was not taken in consideration due to the possible negative effects of excess of N incorporated with the mustard seed-meal. Non significant (NS) at $P>0.05$. ($n=11$)

germination of microsclerotia, as well as *V. dahliae* propagule concentration in soil. Besides the toxicity of GLS hydrolysis products (Brown and Morra 1997), the mechanisms by which mustard green manures and seed meals may suppress soil-borne pathogens are varied and often unknown (Wiggins and Kinkel 2005a). MUSTmeal may reduce propagule density indirectly by influencing the indigenous microbial population through compounds released directly from the meal upon application to soil or during decomposition of the meal. For example, Njoroge et al. (2008) detected ITCs in soil amended with mustard materials and observed increased population densities of fluorescent pseudomonads, an antifungal bacteria

Table 5 Multiple-regression models that best predict Potato Early Dying incidence and potato marketable yield

Variable	Response	Variable	R^2	P-value	Coefficient	Intercept
Potato Early Dying (n=12)						
Incidence	Partials	*V. dahliae*[a] (cfu g^{-1})	0.53	0.0071	1.384	
		Extractable-P (Kg ha^{-1})	0.13	0.09	−0.901	
	Model		0.66	0.0076		59.571
Potato marketable Yield (n=11[b])						
Total yield	Partials	Disease incidence (%)	0.70	0.0012	−0.182	
		Sulfate (kg ha^{-1})	0.12	0.046	0.04	
		NO_3^- (kg ha^{-1})	0.10	0.016	0.046	
	Model		0.92	0.0002		36.9
Tuber categories[c]						
Regular	Partials	Sulfate (kg ha^{-1})	0.53	0.01	0.015	
		Disease incidence (%)	0.18	0.05	0.037	
		NO_3^- (kg ha^{-1})	0.10	0.08	0.021	
	Model		0.82			8.99
Bonus	Partials	Disease incidence (%)	0.75	0.0005	−0.098	
		Sulfate (kg ha^{-1})	0.11	0.029	0.021	
	Model		0.86	0.0003		20.152
Overweight	Partials	Extractable-P (kg ha^{-1})	0.61	0.0042	0.1363	
	Model		0.61	0.004		0.348

[a] Inoculum density at harvest

[b] MUSTmeal treatment was not included in this analysis due to possible negative effects on potato growth after excess of N incorporated with the mustard seed meal

[c] Potato tubers graded into weight categories for marketable yield: regular 86–169 g, bonus 170–340 g and overweight >340 g

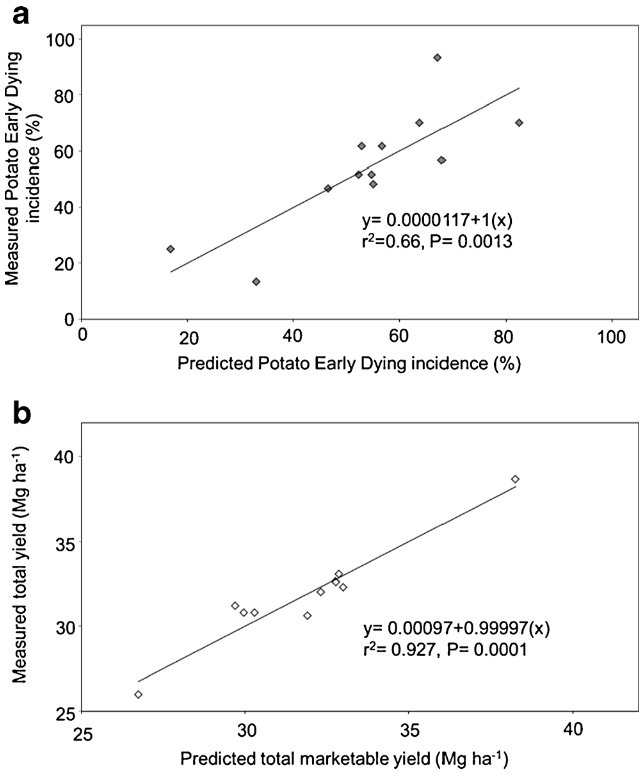

Fig. 4 **a** Relationship between Potato Early Dying incidences predicted by multiple linear models given in Table 5, and measured Potato Early Dying incidence for green manures, composted cattle, seed meal, Vapam, and control treatments (*n*=12). **b** Relationship between total marketable potato yield predicted by multiple linear models given in Table 5 and measured total marketable potato yield for green manure, composted cattle, Vapam, and control treatments. MUSTmeal treatment was not taken in consideration due to the possible negative effects of excess of N incorporated with the mustard seed-meal. (*n*=11)

antagonist against *V. dahliae* (Pegg and Brady 2002). Incorporation of mustard seed meals significantly increased populations of *Streptomyces* spp (Cohen et al. 2005), which were associated with reduction of *V. dahliae* (Krechel et al. 2002). This mechanism of control could explain the consistent reduction of propagule density observed before and after the potato season in plots amended with mustard seed meal.

The application of Vapam reduced propagule density of *V. dahliae* at the beginning of the potato season in 2008. However, severe incidence of PED was observed on potato, as well as an increased number of propagules by immediately after potato harvest of the Vapam treatment. Disease plant residues would not have had time to decompose and release microsclerotia. The biocidal activity of Vapam is provided by methyl isothiocyanate (methyl-ITC) (Gerstl et al. 1977), a very active ITC with high vapour pressure (16.0 mmHg at 20 °C), and thereby elevated potential for volatilization (Zheng et al. 2006). The lack of control in this case could be associated with the lower application rate compared to maximum rates used in potato growing regions in the US, 752 L ha^{-1} this study compared to 935 L ha^{-1}, and the low concentration of active ingredient (32.7 % a.i.) used compared to other commercial formulations of Vapam (37 and 42 % a.i.). Additionally, the lack of efficacy is possibly due to the volatilization loss of methyl-ITC in the upper soil profile, where a large amount of wheat residues may prevent proper sealing of the soil, allowing methyl-ITCs to escape to the atmosphere. Optimum distribution of methyl-ITC in soil for the control of pathogens is rarely achieved with current application practices (Duniway 2002). Soil sealing using irrigation may prevent volatilization of methyl-ITC after application of Vapam. Particularly in cold climates such as Manitoba, producers flush the water out of irrigation equipment after harvest before the onset of freezing temperatures, thus proper soil sealing using irrigation water may not be possible after Vapam application.

Composted materials have been effective for reducing Verticillium wilt of potato in a previous study (Entry et al. 2005). In this study, composted cattle manure reduced incidence of PED and maintained low propagule density of *V. dahliae* in soil. Organic soil amendments might be responsible for changes in soil properties that may disrupt the ability of *V. dahliae* to recognize a potential host, and for the propagules to germinate, grow, or colonize a host (Ochiai et al. 2008). Davis et al. (1990) suggested that reduction of Verticillium wilt may be mediated by improved soil fertility, particularly by optimum available phosphorus in soil. Compost can improve plant health due to the increase of soil nutrient concentration and improvement of soil physicochemical properties (Corti et al. 1998). Composted soil amendments can build a pathogen suppressive soil environment, where biological control is promoted through antagonism, microbial competition, hyperparasitism and antibiosis (Hoitink and Fahy 1986). Kuter et al. (1983) reported reduction of propagule density of *R. solani* in soil amended with composted cattle manure due to enhanced activity of antagonistic microorganism populations. Our results suggest that reduction of PED incidence with compost is not necessarily dependent on reduction of propagule density of *V. dahliae.*

Potato production systems in which organic amendments are applied to soil can improve soil nutrient availability, soil tilth, water holding capacity and plant health (LaMondia et al. 1999; Stark and Porter 2005). In this study, the composted cattle manure (COM) significantly increased marketable potato yield, and neither VAP nor any of the green manure treatments resulted in improvement of marketable yield compared with the control treatment. In addition, only the COM treatment yield (38.7 Mg ha^{-1}) was higher than the control treatment (30.8 Mg ha^{-1}). This increment represents a considerable improvement in potato yield compared to the production average for Manitoba (31.4 Mg ha^{-1}) (Agriculture and Agri-Food Canada 2007).

Soil amendment with composted materials delays decline of photosynthesis in leaves that expand early in the season, increasing the number of leaves and leaf area (Gent et al. 1999) and ultimately, reducing yield losses caused by PED (LaMondia et al. 1999). By this mechanism compost may help potato to defend against the detrimental effect of PED, which is believed to decrease leaf surface area as well as photosynthesis of the plant and ultimately, the supply of assimilates required for later tuber bulking growth (Bowden and Rouse 1991). The factors most closely associated with higher bonus marketable yield were sulfate and disease incidence. Although, extractable-P and extractable-K were not found directly related to the increase of bonus marketable yield, both have been previously reported as the most obvious causes of increased production of larger tubers and total yield, respectively (Moore et al. 2011). Furthermore, the increase in late bulking growth is not just a crop response to better soil nutrient status, it may also be related to the lower disease incidence that was found associated with the soil nutrient increase as well. Optimum nutrient uptake for potato yield is associated with reduced PED incidence as well (Lambert et al. 2005).

Disease suppression by compost was associated with increased microbial activity as well as changes in concentration of N, P, Ca, and soil organic matter of treated soils (Hoitink and Fahy 1986). Increased soil organic matter has been associated with reduction of potato PED incidence in North America (Briar et al. 2009; Davis et al. 2010a). Compost amendments are a source of organic carbon, and when added to the soil should increase soil organic carbon (Magdoff and Weil 2004; Zinati et al. 2001). In this study, the carbon applied with composted beef cattle manure was equivalent to an addition of 1.4 % of total soil mass. Based on a survey of 23 commercial fields, of Russet Burbank potato, increasing soil organic carbon levels was related to decreasing incidence of PED ($r=-0.7$; Tenuta et al., unpublished). On average, soil organic carbon levels >2.8 % had PED incidence levels less than 20 %. Increased organic matter has been reported as one of the major factors in soil associated with the reduction of inoculum density and PED in commercial potato fields in Idaho (Davis et al. 2001). Although, the mechanism involved in the suppression is unclear, the addition of compost may impact the carbon energy available for microorganisms in soil (Weil and Magdoff 2004). Thus, pathogen survival may be reduced by increased microbial activity (Hoitink and Fahy 1986) or presence of biologically active substances from residues and soil microbiota (Lazarovits 2010).

In this study, not all treatments which reduced disease incidence and number of propagules in the soil resulted in increased potato yield. Despite the reduced germination of microsclerotia and reduced density of propagules in soil, the MUSTmeal treatment did not increase potato yield. Incorporation of the mustard seed meal led to significantly higher concentration of N in soil (203 kg N ha^{-1}), but did not significantly increase extractable-P concentration. Mazzola et al. (2007) documented that mustard seed meal usually contains from 5.5 to 6.8 % N. Therefore, mustard meal added at 9,000 kg ha^{-1} added approximately 281 kg N ha^{-1}. In this study, this N content of seed meal was not taken into account to reduce fertilizer application rates. N in seed meal likely led to more vegetative growth and less tuber development. Adequate management of N is critical for optimal potato yield (Sincik et al. 2008), whereas excessive available N results in reduced yields (Lauer 1986; Neeteson and Zwetsloot 1989).

The findings of this study indicate that incorporation of composted beef cattle manure to potato fields planted to the susceptible cv Russet Burbank increases potato yield, decreases PED incidence, and improves nutrient availability. It is hypothesized that improvement of nutrient availability and soil organic matter is possibly involved. Further examination of the mechanisms involved and factors controlling the suppressive activity of compost against PED are required.

One or two years of green manure seem to be ineffective in reducing PED in Manitoba. However, the results of this study suggest that oriental mustard, white mustard and oat/peas mix green manures have potential to reduce germination of *V. dahliae* and soil propagule densities. Therefore, multiple years of green manure may be needed to reduce PED in potato field of Manitoba. Addition of oriental mustard seed meal has the potential to be an important part of PED management program in Manitoba but current high commodity prices may limit use.

The role of mustard seed meal in nutrient management programs needs further investigation for growers who seek a nonchemical alternative to fumigation. However, critical issues remain which must be addressed before mustard seed meal can be considered a viable option. For example, the rates used in this study would be economically unaffordable for potato producers; therefore, it is necessary to investigate whether lower application rates can achieve the desired control of disease.

Acknowledgments We sincerely thank Dr. Maria Antonia Henriquez for reviewing this manuscript. We thank Dr. Tracy Shinners-Carnelley for her comments and inputs on this study. Assistance with several aspects of this study from the staff from The Canada Manitoba Crop Diversification Centre as well as Lorne Adam, Trevor Fraser and Brad Sparling from the University of Manitoba are gratefully appreciated.

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