Use of Green Manure Crops to Reduce Soil-Borne Pests and Diseases of Potato Crops in Alberta
Project 2006-F052R (2006-2010)

FINAL REPORT

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Executive Summary

At the request of the Research Committee of the Potato Growers of Alberta, a project was initiated in 2006 to study the potential for using green manure crops in potato production systems to reduce the incidence and severity of potato early dying complex and Verticillium wilt. Initially, green manure crops were selected that had been reported to suppress Verticillium wilt in other potato production areas. Additional collaborative work was planned to evaluate the effect of the same green manure crops on other soil-borne diseases of potatoes (black scurf and scab) on research sites in Saskatchewan. Commercial fields with a potato history were selected in Alberta to study potato early dying to ensure sufficient disease pressure for the trial. Although some Brassicaceous crops (mustard, rapeseed, oilseed radish, etc.) have been successfully used as green manure crops to suppress potato diseases, all such crops were dropped from the trial in Alberta after the first field season in response to concerns about the potential for contamination of hybrid canola crops produced in potato rotations.

Green manure crops were initiated in late spring or early summer depending on the year and the availability of commercial co-operator fields. When soil moisture was adequate or the cooperating grower was willing to irrigate, good stands of green manure crops were established. Inadequate soil moisture for establishment of the green manure crop affected one site in 2006 and one in 2007. Clean fields or appropriate weed control options are required to aid in establishing green manure crops and producing sufficient green manure biomass. Weed pressure at several locations negatively affected the establishment or growth of some green manure crops. Pest control (grasshoppers, aphids and flea beetles) became an issue at some locations – especially as the crops surrounding the green manure plots matured. At most of the field sites, the green manure crops produced good biomass and that was incorporated prior to fall frosts. Hail affected the biomass production at two locations in 2008. In Saskatchewan, an oat-Austrian winter pea-vetch mixture and oriental mustard provided good biomass. In Alberta, Sorghum Sudan grass, Canadian Forage Pearl Millet, and the oat-pea-vetch mixture typically produced the most biomass. Only one year of data was collected using Oriental mustard and oilseed radish in Alberta and establishment of these crops was hampered by soil moisture issues and inadequate weed and pest control. In Saskatchewan, mustard meal was used as an amendment in place of the Brassica green manure crops in 2008.

Verticillium inoculum was measured using soil dilution assays and molecular techniques. The soil dilution assays were specific for *Verticillium dahliae* while the molecular assays picked up several Verticillium spp. In spite of selecting commercial fields for the trial, *V. dahliae* populations were not detected in all fields at the beginning of the trial. As expected, the presence of a potato crop increased the inoculum level relative to fallow areas of the field. Where *V. dahliae* was present prior to planting the green manure crops, a reduction in *V. dahliae* inoculum was measured with most green manure crops. *V dahliae* populations typically increased during the potato crop year regardless of the level of inoculum following the green manure crop year. No soil samples were collected in the year following potato production.

Although the trial was established specifically to address Verticillium wilt in potatoes, potato early dying complex can be aggravated by plant diseases other than Verticillium. In potato stem assays, the presence of Verticillium was sometimes verified, while at other sites, *Fusarium* and *Colletotrichum* were frequently identified from stem assays in moist chambers. To verify these findings, soil samples collected from some of the commercial fields were used to set up bioassays in a greenhouse using eggplants, known to be very sensitive to Verticillium wilt. Some Verticillium wilt was observed in the eggplant bioassays, especially from the two fields with higher disease pressure. Black dot (*Colletotrichum coccodes*) also affected the eggplants and could be cultured from root and stem tissue

of eggplants grown on soil from several sites. *Fusarium* spp. were also identified on eggplants grown in soil collected from green manured potato fields It is not clear whether the *Fusarium* spp. recovered are pathogenic to potatoes or perhaps antagonistic to Verticillium as has been observed in other green manure studies. *Rhizoctonia* was not very prevalent in the eggplant bioassays.

Many papers identify a link between potato early dying complex and root lesion nematode populations. Root lesion nematodes have also been shown to act synergistically with *Verticillium dahliae* to increase the incidence of wilt (Rotenberg et al. 2004). The initial proposal included nematode analyses but was dropped in the revised application to reduce trial costs. Soil samples collected to assay for Verticillium inoculum were shared with nematologists from Agriculture and Agri-Food Canada to determine the population of root lesion nematodes. The nematode analyses included in this report were provided *gratis* to complement our work on green manures. Root lesion nematodes were identified in soil samples from the commercial fields. *Pratylenchus neglectus*, rather than *Pratylenchus penetrans*, was the species present. In 2007, root lesion nematodes were found in most samples. Some crops appeared to reduce the population of root lesions nematodes in potato fields. The interaction between *P. penetrans* and *V. dahliae* has been studied, but is not thoroughly understood. Even less is known about interactions between *P. neglectus* and Verticillium or other pathogens associated with potato early dying. Additional work in this area may prove beneficial to our understanding and control of potato early dying complex.

Potatoes were grown by commercial co-operators on all but one site following the green manure year. Russet Burbank was grown on all but one site in Alberta. Fresh market varieties were used in Saskatchewan because of their greater disease susceptibility and relevance to the local industry. Regardless of the biomass produced or the effect of the green manure crop on *V. dahliae* inoculum, no yield improvements (total or marketable) were observed as a result of the incorporation of the green manure crop in Alberta. At a few sites, better yield was observed from the areas of the field not planted to green manure, but this may have been a fallow effect or a result of greater residual fertility in these areas of the field. As in Alberta, the use of green manure crops did not increase the yield in the potato crop the following year in any of the trials conducted in Saskatchewan.

In the Saskatchewan trials, a reduction in the incidence of black scurf (*Rhizoctonia solani*) on Russet Burbank was observed following Oriental mustard in 2007. Common scab and black scurf were reduced in plots where red root pigweed dominated the green manure crop. In 2009, both powdery scab and black scurf appeared to be aggravated by mustard meal amendment prior to potatoes.

As a result of this preliminary study using green manure crops in potato production systems in western Canada, we learned that potato early dying is likely a result of the interaction between several potato pathogens, but is not always caused by *Verticillium dahliae*. These pathogens may include other *Verticillium* spp., *Colletotrichum coccodes*, *Alternaria alternata*, and *Fusarium* spp. Several of the green manure crops studied can be effectively grown and incorporated in western Canada within a short growing season. These crops could be utilized as an under-seeded crop or planted following an early harvest of silage or field peas. Management of the green manure crops will influence the effectiveness of these crops to reduce disease, or increase yield. Agronomic work and pest control work may be required to establish green manure crops with sufficient biomass. We know that root lesion nematodes are present in potato production systems in western Canada, but little is known about the prevalent species, *Pratylenchus neglectus*, or its effect on potato yield. If these root lesion nematodes play a role in potato early dying in western Canada, more work is required to determine which potato pathogens are synergistically affected by the root lesion nematode populations. No consistent reduction in disease was noted as a result of incorporating green manure crops in

Saskatchewan field infested with scab and black scurf. As noted by Cherr et al. (2006), green-manure based systems may provide alternatives to current approaches to crop production; however, the use of green manures may not be economically justified without the provision of multiple services such as nutrient supply, pest and weed control, and improvement of soil characteristics for crop production.

Project Overview

Historically, the primary approach for maintaining soil fertility in intensive cropping systems around the world, green manure use in modern agricultural systems has been nearly replaced by synthetic fertilizer, weed, and pest control inputs after the post-World War II development of the agrochemical industry (Cherr et al. 2006). Work in other potato producing areas (Idaho, Minnesota, Quebec, Maine, Washington, Oregon and Ontario) indicates that green manure crops may be effective for control of nematodes and Verticillium wilt. Some work has been conducted in Atlantic Canada to show that green manures may also be effective at controlling Rhizoctonia (black scurf). A green manure crop or cover crop differs from a rotational crop in that it is not harvested, but is instead worked into the soil prior to maturity (Finnigan 2001). Benefits of green manure include: improved soil condition, increased organic matter, improved water penetration, reduction of some diseases, reduced nematode population, and increased availability of nutrients. Green manure crops can be established in spring, summer or fall, and incorporated in the fall or in the spring prior to potato planting. In some studies, multiple years of green manure were required to have significant impact, but even one season of green manure prior to potatoes has been reported as beneficial. Work in SK indicated that approximately 8 weeks were required to establish an effective biomass of many green manure crops. Consultations with potato industry contacts in AB indicated that fall incorporation would likely fit best with current industry practices.

Crop rotation is recommended in all potato growing regions to reduce pests and maintain healthy soil, but economic constraints encourage shortened rotations and the inclusion of high-value crops in the rotation rather than crops that benefit the environmental health of the cropping system. Short rotations, such as one or two years planted to non-host crops between potato crops did not provide consistent reductions in the incidence of Verticillium wilt in subsequent potato crops (Easton et al. 1992).

Root lesion nematodes (*Pratylenchus* spp.) are of concern to potato growers because they can reduce yield and make plants more susceptible to fungal and bacterial diseases (Hafez and Sundararaj 2001). There is also a positive correlation between root lesion nematodes and the incidence of Verticillium wilt (early dying) caused by the fungal pathogen *Verticillium dahliae* (Morgan et al 2002). A 3-year analysis of commercial Russet Burbank fields in Idaho revealed that infections on potato roots by *V. dahliae* accounted for over 51% of the field variability related to potato yield losses (Davis 2001). Severe early dying symptoms in potato crops can result from interactions between *V. dahliae* and root lesion nematodes (Davis et al. 2001). Early dying and Verticillium wilt have been identified in southern AB as increasingly important factors affecting the competitiveness of our processing industry.

AB potato growers face a short, but intensive growing season. Early dying and Verticillium wilt were identified in southern AB as increasingly important factors affecting the competitiveness of our processing industry. Research is required to evaluate several green manure crops to determine which green manure crops will grow in AB. We also proposed to evaluate these crops to determine which, if any, reduce Verticillium wilt infections and improve tuber yield of Russet Burbank potato.

Further, we had an opportunity to determine the effect of some of the same green manure crops on scab and black scurf from infested plots in a number of field trials conducted at the University of Saskatchewan SK.

Objectives of the Project

- **i.** To determine whether the use of green manure crops is effective in Alberta for reducing soil-borne potato pests and diseases;
- **ii.** To determine which green manure crop is most effective at reducing specific potato pests and diseases;
- **iii.** To determine the impact of using green manure crops on yield and quality in subsequent potato crops, and
- **iv.** To provide economically viable alternatives to soil fumigation.

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Literature Review / Background

In fields where potatoes have been part of the production cycle for several years, a pattern of pre-mature vine death and declining yields often develops (Powelson et al. 1993). This syndrome, called potato early dying, limits production in many areas (Powelson et al. 1993). Potato early dying disease (PED) is complex with the root lesion nematode, *Pratylenchus penetrans*, and the wilt causing fungus, *Verticillium dahliae*, usually implicated as the causal agents (Warner 2009). Both organisms are pathogens of potato, but when present together they often interact to produce more significant yield losses than they would cause individually (Warner 2009). Co-infection of potatoes with *V. dahliae* and *P. penetrans* can result in early onset of disease symptoms and thus lower yields (Rowe and Powelson 2002). In North America, yield reduction in moderately diseased fields can easily be 10 to 15%, and in severely diseased fields it can be as high as 30 to 50% (Rowe and Powelson 2002). Tsror (Lahkim) and Haznovsky (2001) indicate that potato early dying syndrome in most potato growing regions has been associated with complexes of soil-borne parasites including *V. dahliae, Pratylenchus* spp., *Colletotrichum coccodes*, *Rhizoctonia solani* and *Erwinia carotovora* spp. Each year in southern Alberta, some potato fields have been observed to mature early, resulting in a loss of size and yield. Concerns about potato early dying prompted the Potato Growers of Alberta to collect plant samples in 2004 to determine whether *Verticillium* spp. were linked to the premature death of these crops. Dr. Bud Platt, Agriculture and Agri-Food Canada, tested the samples and reported that Verticillium was present in many of the samples.

Potatoes are subject to many diseases caused by soil-borne pathogens. Black scurf, caused by *Rhizoctonia solani*, and scab, both common and powdery scab, are important diseases of potato crops on the Canadian Prairies. Chemical control options are limited for many soil-borne pathogens. Two pathogens of particular significance are the soil-borne fungi, *Verticillium dahliae* and *Verticillium albo-atrum* (Rowe and Powelson, 2002). Verticillium wilt, caused by the fungus *V. dahliae* Kleb, remains one of the most important soilborne plant diseases worldwide (Lazarovits 2010). Because of their wide host range, the two species of Verticillium can maintain themselves at low populations on the roots of many symptomless crop and weed species, such as wheat and sunflower (Powelson et al. 1993). In the arid and semi-arid regions of the world, Verticillium wilt, caused by *V. dahliae* Kleb., is a common limiting factor of potato production (Davis et al. 2001). The actual dollar amount lost in production is difficult to assess and is probably greatly underestimated as infection typically reduces plant growth rather than causing death outright (Lazarovits 2010). Once established in a field, the fungus can persist in the soil for many years as microsclerotia (*V. dahliae*) or melanized hyphae (*V. albo-atrum*), either free or embedded in plant debris (Powelson et al. 1993, Ochiai et al. 2007).

The potato root is also host to the root-lesion nematode (RLN) *Pratylenchus penetrans*. Root lesion nematodes (*Pratylenchus* spp.) are of concern to potato growers because they can reduce yield and make plants more susceptible to fungal and bacterial diseases (Hafez and Sundararaj 2001). There is also a positive correlation between root lesion nematodes and the incidence of Verticillium wilt (early dying) caused by the fungal pathogen *Verticillium dahliae* (Morgan et al 2002). Soil samples tested by Growers Supply Limited revealed significant populations of root-lesion nematodes in southern Alberta potato fields, although *Pratylenchus neglectus* was the dominant species, not *P. penetrans*. While root-lesion nematodes contribute to the early dying complex, it is uncertain whether nematode control by rotation with non-host or nematode-antagonistic crops would effectively manage potato early dying (LaMondia 2006).

The two fungal pathogens alone, or in conjunction with *P. penetrans* cause a disease called potato early dying (Powelson et al. 1993, LaMondia 2006). Severe early dying symptoms in potato crops can result from interactions between *V. dahliae* and root lesion nematodes (Davis et al. 2001). Potato early dying is a vascular wilt disease characterized by a general decline of plants 4 to 6 weeks earlier than normal senescence. Although specific diagnostic symptoms are not associated with the disease, foliage show various degrees of chlorosis and necrosis, sometimes associated with wilting or dying of individual stems (Rowe and Powelson, 2002). In the early stages, individual vines may die and remain conspicuously erect in contrast to healthy plants (Rowe and Powelson, 2002). A light brown vascular discoloration in basal stem tissues is usually present in symptomatic plants. This symptom, however, is not diagnostic since vascular discoloration can result from stress factors unrelated to PED (Rowe and Powelson, 2002). In severe cases, plants across an entire field will die over a period of several weeks. A 3-year analysis of commercial Russet Burbank fields in Idaho revealed that infections on potato roots by *V. dahliae* accounted for over 51% of the field variability related to potato yield losses (Davis 2001).

In many potato producing areas of North America, soil fumigation and planting resistant cultivars remain the primary means for control of soilborne plant diseases and pests (Lazarovits 2010). Fumigation, where registered products are available, is constrained by increased costs, urbanization and its negative environmental impacts (Lazarovits 2010). The use of soil fumigants in Canada has not been widely adopted for the control of Verticillium wilt. Fumigants are non-specific, expensive and toxic to applicators and the environment. In 2005, the Research Committee of the Potato Growers of Alberta issued a call for proposals to study the effects of green manure crops on early dying of potatoes.

Crop rotation is recommended in all potato growing regions to reduce pests and maintain healthy soil. Crop rotation reduces pest problems by changing the environmental conditions in the field (McGuire 2003). In general, rotating crops that have different planting dates, different growth habits or different susceptibility to pests prevents any one pest from becoming a problem (McGuire 2003). Economic considerations favour shortened rotations and the inclusion of high-value crops in the rotation rather than crops which inherently benefit the environmental health of the cropping system.

Work in other potato producing areas (Idaho, Minnesota, Quebec, Maine, Washington, Oregon and Ontario) indicated that green manure crops may be effectively used for control of nematodes and Verticillium wilt. A green manure is a crop used primarily as a soil amendment and a nutrient source for subsequent crops (Cherr et al. 2006). A green manure crop or cover crop differs from a rotational crop in that it is not harvested, but is instead worked into the soil prior to maturity (Finnigan 2001). Benefits of green manure include: improved soil condition, increased organic matter, improved water penetration, reduction of some diseases, reduced nematode population, and increased availability of nutrients (Davis et al. 2010). The incorporation of green biomass into soils, known as green manuring, has been in use in agriculture for over 2000 years (Lazarovits 2010). Green manure approaches to crop production may improve economic viability, while reducing the environmental impacts of agriculture (Cherr et al 2006). Such approaches are complex, however, because they depend on interactions between the green manure, the environment, and management (Cherr et al 2006). Green manure crops can be established in spring, summer or fall, and incorporated in the fall or in the spring prior to potato planting.

The mechanism by which green manures may influence disease are varied and often unknown (Wiggins and Kinkel 2005). Green manures may influence pathogens directly through the breakdown of glucosinolates or by releasing fungitoxic compounds such as isothiocyanates (Wiggins and Kinkel

2005). Green manures may also affect soilborne pathogens indirectly by influencing indigenous microbial populations (Wiggins and Kinkel 2005). These changes in the microbial community may affect pathogen populations through competition, parasitism, predation or antagonism (Wiggins and Kinkel 2005).

In some studies, multiple years of green manure were required to have significant impact (Easton et al. 1992), but even one season of green manure prior to potatoes has been reported as beneficial (Davis et al. 2010). Preliminary work in SK indicated that approximately 8 weeks were required to establish an effective biomass of many green manure crops. Consultations with potato industry contacts in AB indicated that a mid-summer planting of green manure would allow producers to harvest a paying crop prior to establishing green manure crops. Fall incorporation would likely fit best with current industry practices. Davis et al (1996) reported evidence that the suppression of Verticillium wilt of potato can be achieved with green manure treatments. Compared with 3 years of a weed-free fallow, three successive green manure crops of **Sudan grass** decreased disease severity by 81% and increased marketable yield by 35%, a response equivalent to soil fumigation (Davis et al. 1996). For green manuring to be a viable approach for management of PED, a shorter time frame is required. McGuire (2003) reported that potato yields following **white mustard** (*Sinapis alba* Martigena) or **oriental mustard** (*Brassica juncea* Cutlass) green manures was not statistically different from yields of potatoes on soils fumigated with metam sodium. Some green manure crops, such as **Canadian Forage Pearl Millet Hybrid 101** (*Pennisetum glaucum* L.) have been reported to suppress root lesion nematodes in the subsequent potato crop (Ball-Coelho et al. 2003). Other crops grown as green manures, such as Austrian winter pea, canola, oats, and rye also have suppressed disease and enhanced tuber yields. Some work has been conducted in Atlantic Canada to show that green manures may also be effective at controlling Rhizoctonia (black scurf).

Duval (2003) suggested that green manures established in the year preceding a potato crop would improve nutrient cycling and soil tilth and could have an impact on certain crop pests. Beans showed an inhibiting effect against nematodes and *Rhizoctonia* (black scurf) in a subsequent potato crop while barley caused an increase in scab levels on subsequent potato crops (Duval 2003). Mustard was considered a suitable green manure crop in a potato rotation as it has shown a suppressive effect against potato scab and black scurf (Duval 2003). The biofumigation potential of Brassicaceous crops has been evaluated in Australia (Sarwar et al. 1998). *In vitro* testing of the biofumigant products from *Brassica* crops showed that *Rhizoctonia* is sensitive to the isothiocyanates produced by *Brassica* species, however it was concluded that more work was needed to determine which *Brassica* species produce substances most toxic to specific pathogens (Sarwar et al. 1998).
Over the past 15 years, numerous soil ammendments have been evaluated for their potential to suppress Verticillum wilt, including animal manures (Conn and Lazarovits 1999), composted materials (LaMonide et al 1999), industrial by-products (Lazarovits 2010) and green manures (Davis et al 1996).

Lazarovits (2010) summarized in a review that field testing of organic products and soil amendments 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, would require hundreds of plots to be established, even if it were possible (Lazarovits 2010).

Alberta Trials

Green Manure Crop Establishment

In 2006, green manure crops were planted in two commercial southern Alberta fields on June 27 (Field A) and July 13 (Field B) (Table 1). Planting was delayed initially to allow vetch seed to be delivered. Planting in Field B was further delayed as a result of spring rains and a low spot in the field. The trial sites were located under centre pivot irrigation in wheat fields. Cooperating growers left approximately 1 acre vacant for green manure plot establishment. Field A had only recently been used for potato rotations, while Field B had a long history of potatoes in the rotation. A randomized block design was used with four replicates of each the six treatments that were planted in 8 x 7 m plots, with 3m pathways between each plot. Green manure crops from the Brassicacea family were not planted in Field A because the producer grows hybrid seed canola in rotation with potato and contamination would be unacceptable. Two alternate green manure crops, California Bluebell and annual ryegrass, were substituted for oilseed radish and Oriental mustard as shown in Table 1).

Just prior to seeding, soil was sampled from each trial area. Four samples were collected at random per replicate at sample depths of 0-15, 15-30, 30-60 and 60-90 cm from each of the four quadrants of the plot (total of 16 samples) and the four quadrant samples were combined to give four composite samples. These samples were analysed for NO_3-N, PO_4-P, K and SO_4 by an ARD laboratory as baseline soil fertility samples for each field.

Table 1: Green manure crops planted in Alberta plots in 2006.

Field	***Crop***	***Species & Cultivar***	***Rate***
Field A	California Bluebell	*Phacelia tanacetifolia*	6 lbs/ac
	Annual ryegrass	*Lolium multiflorum*; Proven HPS Italian Ryegrass	10 lbs/ac
	Sorghum Sudan Grass	*Sorghum x drummondii*; Grazex	20 lbs/ac
	Wooly Pod Vetch	*Vicia villosa* spp. *dasycarpa*	10 lbs/ac
	Canadian Forage Pearl Millet	*Pennisetum glaucum* L.; CFPM Hybrid 101	10 lbs/ac
	Hard Red Spring Wheat	*Triticum aestivum*	120 lbs/ac
Field B	Oilseed Radish	*Raphanus sativus* var. *Oleiferus*	10 lbs/ac
	Oriental Mustard	*Brassica juncea* ; Cutlass	10 lbs/ac
	Sorghum Sudan Grass	*Sorghum x drummondii*; Grazex; Grazex	20 lbs/ac
	Wooly Pod Vetch	*Vicia villosa* spp. *dasycarpa*	10 lbs/ac
	Canadian Forage Pearl Millet	*Pennisetum glaucum* L.; CFPM Hybrid 101	10 lbs/ac
	Hard Red Spring Wheat	*Triticum aestivum*	120 lbs/ac

The seed was broadcast in a barrel spinner using seeding rates double those used in a conventional row seeder (Table 1). The seed bed was moist, and irrigation was applied to the green manure crops and the surrounding wheat crop as required to maintain conditions conducive to wheat harvest. In Field A, these conditions favoured plant establishment in the green manure plots. In Field B, some plots were too dry and others were very wet. Plot establishment in each replicate varied depending on location in the field. Plots at both sites were hand-weeded in 2006 to reduce weed-pressure on the green manure crops.

In 2007, green manure crops were planted in two commercial southern Alberta fields on July 11(Field D) and July 19 (Field C) (Table 2). Finding co-operators for the 2007-2008 year was challenging. Annual ryegrass and California bluebell were used in place of the oilseed radish and oriental mustard in both fields due to the high density of hybrid canola production in southern Alberta. An oat-pea-vetch mixture replaced the woolly pod vetch in 2007. The trial sites were located under centre pivot irrigation in commercial cereal fields. In Field C, the trial area was sprayed out with glyphosate and worked prior to planting the green manure crops. We removed standing crop and planted in stubble in Field D.

Planting green manure crops 2007.

Just prior to seeding, soil was sampled from each trial area. Four samples were collected at random per replicate at sample depths of 0-15, 15-30, 30-60 and 60-90 cm from each of the four quadrants of the plot (total of 16 samples) and the four quadrant samples were combined to give four composite samples. These samples were analysed for NO_3-N, PO_4-P, K and SO_4 by an ARD laboratory as baseline soil fertility samples for each field.

Table 2: Green manure crops planted in Alberta plots in 2007.

Field	***Crop***	***Species & Cultivar***	***Rate***
Field C	California Bluebell	*Phacelia tanacetifolia*	6 lbs/ac
	Annual ryegrass	*Lolium multiflorum*; Proven HPS Italian Ryegrass	10 lbs/ac
	Sorghum Sudan Grass	*Sorghum x drummondii*; Grazex	20 lbs/ac
	Oat-Pea-Vetch*	*Avena sativa; Pisum sativum* spp. *arvense; Vicia villosa* spp. *dasycarpa*	169 lbs/ac
	Canadian Forage Pearl Millet	*Pennisetum glaucum* L.; CFPM Hybrid 101	16 lbs/ac
	Hard Red Spring Wheat	*Triticum aestivum*	120 lbs/ac
Field D	California Bluebell	*Phacelia tanacetifolia*	6 lbs/ac
	Annual ryegrass	*Lolium multiflorum*; Proven HPS Italian Ryegrass	10 lbs/ac
	Sorghum Sudan Grass	*Sorghum x drummondii*; Grazex	20 lbs/ac
	Oat-Pea-Vetch*	*Avena sativa; Pisum sativum* spp. *arvense; Vicia villosa* spp. *dasycarpa*	169 lbs/ac
	Canadian Forage Pearl Millet	*Pennisetum glaucum* L.; CFPM Hybrid 101	16 lbs/ac
	Hard Red Spring Wheat	*Triticum aestivum*	120 lbs/ac

* Wooly Pod Vetch (30 lbs/ab) ; Austrian Winter Pea (89 lbs/ac) ; Oats (50 lbs/ac) plus inoculum

Monocot crops in plots at both sites were sprayed with Refine (12 g/ac) and MCPA (228 mL/ac) in 2007 for weed control, while others were hand-weeded. Phacelia was sprayed out in Field C because the weed pressure was very high and the crop did not catch well. Also, one replicate of the Oat-pea-vetch treatment in Field C was mis-planted and was deleted from the trial.

In 2008, green manure crops were planted in two commercial southern Alberta fields on June 18 (Fields E and F) and at CDCS in Brooks June 19 (Field G) (Table 3). Annual ryegrass and Teff were used in place of the oilseed radish and oriental mustard in all three fields due to the high density of hybrid canola production in southern Alberta. The commercial trial sites were located under centre pivot irrigation in commercial cereal fields. The cereal crop was sprayed out with glyphosate and plots were cultivated prior to planting the green manure crops.

Just prior to seeding, soil was sampled from each trial area. Four samples were collected at random per replicate at sample depths of 0-15, 15-30, 30-60 and 60-90 cm from each of the four quadrants of the plot (total of 16 samples) and the four quadrant samples were combined to give four composite samples. These samples were analysed for NO_3-N, PO_4-P, K and SO_4 by an ARD laboratory as baseline soil fertility samples for each field.

Table 3: Green manure crops planted in Alberta plots in 2008.

Field	***Crop***	***Species & Cultivar***	***Rate***
Field E	Teff	*Eragrostis tef*	8 lbs/ac
	Annual ryegrass	*Lolium multiflorum*; Proven HPS Italian Ryegrass	10 lbs/ac
	Sorghum Sudan Grass	*Sorghum x drummondii*; Grazex	20 lbs/ac
	Oat-Pea-Vetch*	*Avena sativa; Pisum sativum* spp. *arvense; Vicia villosa* spp. *dasycarpa*	169 lbs/ac
	Canadian Forage Pearl Millet	*Pennisetum glaucum* L.; CFPM Hybrid 101	16 lbs/ac
	Hard Red Spring Wheat	*Triticum aestivum*	120 lbs/ac
Field F	Teff	*Eragrostis tef*	8 lbs/ac
	Annual ryegrass	*Lolium multiflorum*; Proven HPS Italian Ryegrass	10 lbs/ac
	Sorghum Sudan Grass	*Sorghum x drummondii*; Grazex	20 lbs/ac
	Oat-Pea-Vetch*	*Avena sativa; Pisum sativum* spp. *arvense; Vicia villosa* spp. *dasycarpa*	169 lbs/ac
	Canadian Forage Pearl Millet	*Pennisetum glaucum* L.; CFPM Hybrid 101	16 lbs/ac
	Hard Red Spring Wheat	*Triticum aestivum*	120 lbs/ac
Field G	Teff**	*Eragrostis tef*	8 lbs/ac
	Annual ryegrass	*Lolium multiflorum*; Proven HPS Italian Ryegrass	10 lbs/ac
	Sorghum Sudan Grass	*Sorghum x drummondii*; Grazex	20 lbs/ac
	Oat-Pea-Vetch*	*Avena sativa; Pisum sativum* spp. *arvense; Vicia villosa* spp. *dasycarpa*	169 lbs/ac
	Canadian Forage Pearl Millet	*Pennisetum glaucum* L.; CFPM Hybrid 101	16 lbs/ac
	Hard Red Spring Wheat	*Triticum aestivum*	120 lbs/ac

* Wooly Pod Vetch (30 lbs/ab) ; Austrian Winter Pea (89 lbs/ac) ; Oats (50 lbs/ac) plus inoculum

** Teff establishment was poor at CDCS and Hairy Nightshade (*Solanum sarrachoides*) was abundant. Nightshade was allowed to grow in place of teff as a negative control at this site.

Monocot crops in plots at both sites in 2008 were sprayed with Refine (12 g/ac) and MCPA (228 mL/ac) for weed control, while others were hand-weeded.

Above-ground biomass samples were collected from all green manure plots prior to incorporation. Biomass samples were collected from Field A plots August 30, 2006 and from Field B plots September 6, 2006. Biomass samples were collected from Field D plots September 19, 2007 and from Field C September 26, 2007. Biomass samples were collected from the plots in Fields E and F September 8, 2008 and in Field G September 11, 2008. Two 0.5 x 0.5 m areas were sampled from each plot. The fresh weight of all above-ground biomass was measured in the field. To determine dry-weight, a sub-sample of up to 300g was collected, placed in a paper bag in a drying room, and dried until a constant weight was obtained.

The green manure crops in Field A were mowed and incorporated with a three-point hitch disc September 1, 2006. The green manure crops in Field B were mowed and incorporated with a three-point hitch rototiller September 6 and 7, 2006. The green manure crops in Field C were incorporated with a three-point hitch rototiller October 2, 2007. The green manure crops were incorporated in Field D with a three-point hitch rototiller October 4, 2007. The green manure crops were incorporated with a three-point hitch rototiller in late September. Field C had a touch of frost and two crops (CPFM, Sorghum Sudan grass) were killed prior to incorporation.

Photographs of the plots were taken throughout the season and prior to incorporation. Examples of some of the crops are shown in Figure 1.

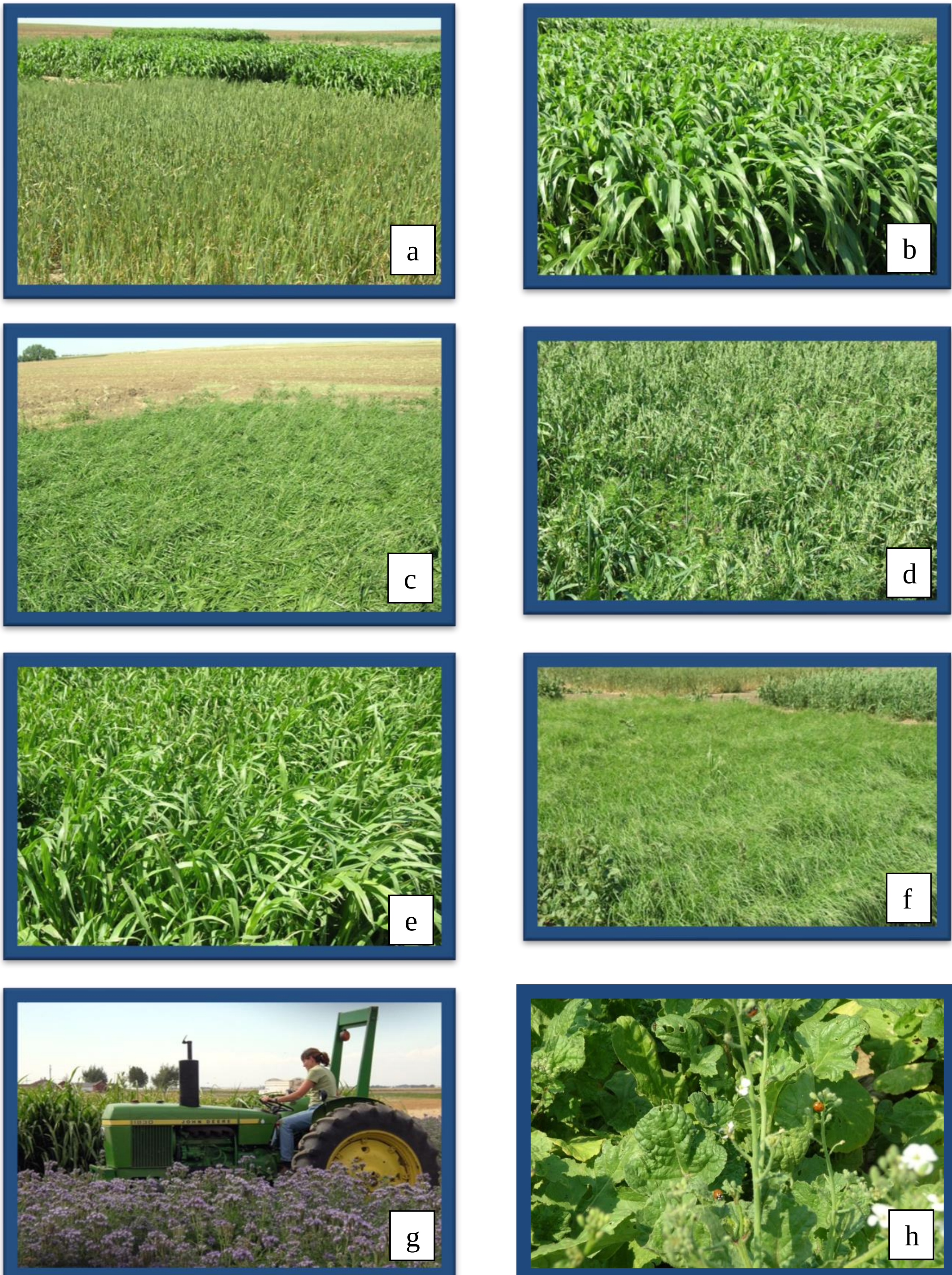


Figure 1: Green manure crops grown in potato rotations in southern Alberta: a) hard red spring wheat; b) sorghum Sudan grass; c) annual ryegrass; d) oat-pea-vetch; e) Canadian Forage Pearl Millet; f) teff; g) California bluebell; h) oilseed radish.

Green Manure Biomass

Fresh and dry weight data are presented (Table 4). Biomass production varied with each field location and by year, likely as a result of the time of year the crop was planted, the fertility at each site and the irrigation applied throughout the season. Fresh weight of each green manure crop is affected by the moisture status at the time of sampling. Dry matter produced by each green manure crop may more accurately represent the quantity of biomass produced. In Field A, Sorghum Sudan grass produced the most biomass on a dry weight basis, followed by CFPM, then Phacelia. Woolly vetch, annual ryegrass and wheat produced the least biomass in Field A. In Field B, there were no statistical differences in dry matter produced between CFPM, Sudan grass, woolly vetch, oriental mustard, and oilseed radish. Wheat again produced the least biomass, but not statistically less than woolly vetch. Much less biomass was produced in Field B than in Field A. There was variation in soil moisture between replicates and insect and weed pressure in Field B that were not observed in Field A. In Field C, there were no statistical differences in dry weight of biomass produced by the 5 green manure crops sampled. In Field D, similar quantities of biomass was recovered from all green manure crops except wheat which did not survive and annual ryegrass which produced less biomass than the other 4 crops. Fields C and D were planted in July of a hot, dry summer (2007). Irrigation was applied as required by the crop surrounding the green manure plots, but was insufficient or not timely for the green manure crop. A hail storm in August in 2008 affected the green manure crops in both Field E and Field F. The green manure crops recovered and the producer continued to irrigate the plots although the surrounding crop was written off by insurance adjusters. In Field E, Sorghum Sudan grass and CPFM produced the greatest dry weight of biomass although not significantly greater than wheat and the oat/pea/vetch mixture. Annual ryegrass produced the least biomass and teff was not significantly different from ryegrass, oat/pea/vetch or wheat. In Field F, CFPM produced a significantly greater dry weight of biomass than the other crops which were not statistically different from one another. Field G was located at a research centre and was irrigated independently of any other crop. At this location, Sorghum Sudan grass produced the greatest biomass by dry weight, followed by CFPM, the oat/pea/vetch mixture, wheat, nightshade and annual ryegrass. Nightshade overtook the teff planted as green manure and was left to determine the effect of this solanaceous weed on soil inoculum and potato yield the following year.

Ochiai et al (2007) reported that 12 Mg/ha (1,200 g/m^2) of dry biomass was required to reduce wilt severity. While not all green manure crops employed at all locations reached this threshold, CFPM 101, Sorghum Sudan Grass, the Oat Pea Vetch mixture and Teff could be expected to reach this target under good agronomic conditions fields A, E, F and G). The Brassica species, Oriental Mustard and Oilseed Radish, were only included at one site in one year of the trial and this did not give a good indication of the quantity of biomass typically expected from these crops. Annual ryegrass, Phacelia, Woolly Pod Vetch alone and wheat often did not provide sufficient biomass to have a predictable impact on wilt symptoms in subsequent crops.

Table 4: Fresh weight (g/m^2) and dry weight (g/m^2) of green manure crop biomass by field and crop. Data followed by the same letter in each row were not statistically different ($p \geq 0.05$).

Field	Wheat	CFPM 101	Sudan-grass	Vetch/OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Night-shade
Fresh Weight										
A	1317 c	6925 a	6865 a	3780 b	2770 b	5435 a				
B	1143 d	5210 a	3380 bc	1168 d				2010 cd	3828 ab	
C	2875 ab	2595 b	2445 b	2885 ab	3270 a					
D		708 bc	944 b	1455 a	517 c	1827 a				
E	4090 c	12470 a	11970 ab	9220 b	5800 c		5550 c			
F	2750 b	16950 a	4950 b	5170 b	6400 b		3550 b			
G	8110 c	14690 ab	15910 a	12130 abc	8950 c					9653 c
Dry Weight										
A	479 d	1094 b	1405 a	541 d	393 d	766 c				
B	175 b	711 a	524 a	417 ab				481 ab	626 a	
C	575 a	566 a	514 a	542 a	583 a					
D		280 a	342 a	340 a	105 b	308 a				
E	1743 ab	2329 a	2413 a	1779 ab	871 c		1516 bc			
F	1164 b	3493 a	1197 b	1306 b	980 b		1002 b			
G	3038 bc	4466 ab	5012 a	3051 bc	1564 c					2226 c

Soil Fertility

Soil Samples to Determine Available Nitrogen

After green manure crops were incorporated in 2006, soil samples were collected from Fields A and B October 12, 2006. A sub-sample of each sample was incubated moist (80% field capacity) at 25°C for 4 weeks to maximize N mineralization prior to analysis for N, NH_4-N and NO_3-N. Total N was determined using a combustion analyzer. NO_3-N and NH_4-N were determined from 10 g of soil using an equilibrium extraction with a 2.0 M KCl solution, followed by air-segmented continuous flow analysis (CFA).

After green manure crops were incorporated in 2007, soil samples were collected from Field D October 15 and from Field C October 17. Each green manure crop was sampled to a depth of 15 cm with 12 soil cores per plot. Sub-samples of 25 to 50 mg were analysed for total N, NH_4-N and NO_3-N. Total N was determined using a combustion analyzer. NO_3-N and NH_4-N were determined from 10 g of soil using an equilibrium extraction with a 2.0 M KCl solution, followed by air-segmented continuous flow analysis (CFA). Samples in 2007 were also analyzed for PO_4-P using a Modified Kelowna method. Potassium (K) concentration was determined from this same extract using a Flame Emission Photometric method. SO_4 was determined from soil using a 2:1 equilibrium extraction with a 0.01 M $CaCl_2$ solution.

After green manure crops had been incorporated in 2008, Fields E and F were sampled October 8 and Field G was sampled October 17. Sampling and lab analyses were as described in 2007.

Results of Soil Samples Prior to Planting Green Manure Crops

In order to assess overall background nutrient levels and the potential spatial variability at both sites, soil samples were collected in July 2007, just prior to seeding the green manure crops. Analytical results are given in Table 5 and summarized below.

Table 5. Summary of the soil sample analyses from plots in July 2007 prior to planting green manure crops.

	2007 Pre-Green Manure Soil Samples†							
	NO_3-N (kg/ha)		PO_4-P (kg/ha)		K (kg/ha)		SO_4 (kg/ha)	
Field C								
Depth (cm)								
0-15	29.5	a	88.5	a	12.8	a	7.75	b
15-30	16.3	b	56.0	b	6.25	b	15.0	b
30-60	16.8	b	36.5	b	13.8	a	52.3	b
60-90	16.8	b	8.75	c	11.8	a	148	a
Replicates								
1	27.3	a	44.8	a	10.3	a	73.8	a
2	16.5	a	38.5	a	11.5	a	41.3	a
3	16.3	a	44.8	a	11.3	a	48.3	a
4	19.3	a	61.5	a	11.5	a	59.3	a
Field D								
Depth (cm)								
0-15	7.00	a	58.8	a	9.50	a	10.0	a
15-30	3.75	b	57.8	a	7.00	b	7.75	a
30-60	6.50	a	31.8	b	10.3	a	99.5	a
60-90	9.25	a	15.0	c	9.75	a	210	a
Replicates								
1	8.75	a	50.5	a	9.25	a	22.0	a
2	7.75	ab	40.5	ab	9.25	a	13.8	a
3	5.25	b	32.5	b	9.25	a	131	a
4	4.75	b	39.8	ab	8.75	a	161	a
Both Sites								
Field C	19.8	a	47.4	a	11.1	a	55.6	a
Field D	6.63	b	40.8	a	9.13	b	81.9	a

† Numbers in the same undivided column, which are followed by the same letter are not significantly different at the 5% level.

At both sites, NO_3-N was least within the 15-30 cm depth range (Table 5). Overall, Field C had greater background NO_3-N levels than Field D. In Field D, there was some initial spatial variability in NO_3-N, with Rep 1 (east side of plot) being significantly greater than Reps 3 and 4 (west side of plot).

At both sites, PO_4-P was greatest at the soil surface (Table 5) and phosphate is not very mobile in soils. Overall, Field C had greater initial levels of PO_4-P, but the difference was not statistically significant. In Field D, soil PO_4-P was significantly greater in Rep 1 (east) than Rep 3), with Reps 2 and 4 being intermediate.

At both sites, K was least in the 15-30 cm depth range (Table 5). Overall K was spatially uniform. K was significantly greater in Field C than in Field D.

At both sites, SO_4 was greatest at the deepest depth range sampled (60-90 cm) (Table 5). Irrigation water naturally contains SO_4 due to the limestone minerals present in the mountain sources of southern Alberta's irrigation water. On average, 30 cm of irrigation water will add approximately 34 kg/ha of SO_4-S to the soil. Unused amounts of sulphur are very mobile and, therefore, prone to leaching through the soil profile. The large SO_4 differences between depth ranges were not statistically significant in Field D, because of the huge amount of variability in the 30-60 and 60-90 cm depth ranges. The amounts of SO_4 were greater in Reps 3 and 4 (west) due to the greater SO_4 concentrations in the 30-60 and 60-90 cm depth ranges. SO_4 was somewhat greater at Rep 1 (west) due to the greater concentration found in the 60-90 cm depth range. Overall SO_4 was greater in Field D than in Field C, mainly due to amounts in the 30-60 and 60-90 cm depth ranges. Overall, SO_4 at both sites was very similar in the surface 0-30 cm.

In 2008, soil samples were collected just prior to seeding the green manure crops in order to assess overall background nutrient levels and the potential spatial variability at all three sites. Analytical results are given in Table 6 and summarized below.

Table 6. Summary of the soil sample analyses from samples collected in July 2008.

	2008 Pre-Green Manure Soil Samples†							
	NO_3-N (kg/ha)		**PO_4-P (kg/ha)**		**K (kg/ha)**		**SO_4 (kg/ha)**	
Field E								
Depth (cm)								
0-15	80.0	b	126.3	a	34.3	a	180.5	b
15-30	116.5	b	86.8	b	19.3	b	449.3	b
30-60	219.0	a	30.8	c	12.5	b	1166.0	a
60-90	182.8	a	10.5	c	13.0	b	1025.8	a
Field F								
Depth (cm)								
0-15	22.0	b	105.0	a	26.0	a	9.0	a
15-30	33.0	b	41.5	b	11.5	b	83.8	a
30-60	143.8	a	22.3	b	13.8	b	568.8	a
60-90	114.5	a	8.5	b	14.5	b	796.3	a
Field G								
Depth (cm)								
0-15	15.3	a	73.8	a	27.3	a	5.0	b
15-30	49.3	a	45.8	b	16.3	b	43.8	b
30-60	174.8	a	24.5	c	10.8	b	830.5	a
60-90	171.3	a	37.3	bc	11.5	b	657.3	a
All Sites								
Field E	149.6	a	63.6	a	19.8	a	705.4	a
Field F	78.3	b	44.3	a	16.4	a	364.4	a
Field G	102.6	ab	45.3	a	16.4	a	384.1	a

† Numbers in the same undivided column, which are followed by the same letter, are not significantly different at the 5% level.

At all three sites, NO_3-N was lowest within the 0-15 cm depth range, followed by the 15-30 cm depth range (Table 6). NO_3-N was greatest within the 30-60 cm depth range, at all three sites. Overall, Field E had significantly greater background NO_3-N levels than Field F, in the 0-90 cm depth range. The 0-90 cm NO_3-N in Field G was intermediate and not significantly different from either of the other two sites.

At all three sites, NO_3-N was least within the 0-15 cm depth range, followed by the 15-30 cm depth range (Table 6). NO_3-N was greatest within the 30-60 cm depth range, at all three sites. Overall, Field E had significantly greater background NO_3-N levels than Field F, in the 0-90 cm depth range. The 0-90 cm NO_3-N in Field G was intermediate and not significantly different from either of the other two sites.

At all three sites, PO_4-P was greatest at the soil surface (0-15 cm depth) and decreased with increasing soil depth. Overall, Field E had greater initial levels of PO_4-P than the other two sites, in the 0-90 cm depth range, but the difference was not statistically significant.

At all three sites, K was significantly greater in the 0-15 cm depth range than all other depths sampled. In Field F, K was least in the 15-30 cm depth range and greatest in the 0-15 cm depth range (Table 6). In Field E and Field G, K was greatest in the 0-15 cm depth range and least in the 30-60 and 60-90 cm depth ranges. K was greater in Field E than at the other two sites, but the difference was not statistically significant.

In Field F, SO_4 was greatest at the deepest depth range sampled (60-90 cm) (Table 6); however, differences among the soil depths were not significant because of two extremely large values in Replicate 1, for 30-60 and 60-90 cm. In Fields E and G, SO_4 was greatest in the 30-60 cm depth range, followed by the 60-90 cm depth range. Soil SO_4 was significantly less in the two surface depth ranges (0-15 and 15-30 cm). Overall SO_4 was greater in Field E than at the other two sites, but the difference was not statistically significant. When the depth increments were analyzed separately (data not shown) soil SO_4 in Field E was significantly greater than at the other two sites for both the 0-15 and 15-30 cm depth ranges.

Results of Soil Samples After Incorporation of Green Manure Crops

In 2006, total N, which includes plant available (inorganic) N and unavailable (organic) N, was statistically the same for all green manure crops (for averages of both sites and averages within sites) (Table 7). This is as expected because N removed from the soil by the crop would have been returned to the soil during incorporation of the plant material. The only exception would be vetch, which should have theoretically added nitrogen to the soil through fixation of atmospheric N. However, no discernible benefit was reflected in the measurement of Total N. From the crops that overlapped between sites (wheat, Sorghum Sudan grass, CFPM and vetch), it was apparent that the average total soil nitrogen was greater in Field A (2661 kg/ha) than in Field B (2461 kg/ha), this difference was also statistically significant and was likely a result of different approaches to N fertilization in the surrounding wheat crop.

Table 7: Total and available N (kg/ha) in soil samples taken from green manure plots following incorporation in 2006. Numbers in the same column, which are followed by the same letter are not significantly different at the 5% level.

	Pre-Incubation											
	Total N (kg/ha)						**Available N (kg/ha)**					
Name	Field A		Field B		Overall		Field A		Field B		Overall	
Wheat	2626	a	2358	a	2492	a	33.1	ab	27.7	ab	30.4	bc
Sorghum Sudan Grass	2570	a	2470	a	2520	a	18.7	b	20.5	b	19.6	c
CFPM 101	2778	a	2526	a	2652	a	37.9	ab	35.7	ab	36.8	ab
Woolly Pod Vetch	2671	a	2492	a	2582	a	37.4	ab	44.2	a	40.8	ab
Oriental Mustard	-	-	2436	a	2436	a	-	-	29.5	ab	29.5	bc
Oilseed Radish	-	-	2447	a	2447	a	-	-	43.4	a	43.4	ab
Phacelia	2514	a	-	-	2514	a	49.5	a	-	-	49.5	a
Ryegrass	2576	a	-	-	2576	a	41.2	ab	-	-	41.2	ab

There was a greater degree of variability for available nitrogen (a sum of nitrate nitrogen and ammonium nitrogen) than for total nitrogen (Table 7). At the end of the 2006 growing season, shortly after incorporation of the green manure stand, Phacelia had the greatest amount of available N. Radish, ryegrass), woolly vetch and CFPM had the next greatest amount of available N and were not statistically different from one another or from Phacelia. The check treatment (wheat), and mustard had available N tests that were significantly less than Phacelia. Sorgum Sudan grass gave the least overall available N, significantly less than all other crops, and had a large amount of above ground biomass (Table 4). This indicates that, shortly after incorporation, much of the soil N that was immobilized by the crop remained unavailable to plants (organic form). This occurred despite thorough chopping of plant material, followed by a period of almost 40 days for potential N mineralization. Of the four green manure crop that were common to both sites (wheat, Sorghum Sudan grass, CFPM and vetch) there was no trend or significant differences between growers. Wheat and CFPM had greater available N in Field A and Sorghum Sudan grass and vetch had greater available N in Field B.

Analytical results from the 2007 green manure sites (Table 8) were taken from soil that was collected on October 15 and 17, 2007. Total N includes plant available (inorganic) N and unavailable (organic) N (Table 2). Total N was statistically the same for all crops in Field D and for the average of both sites. This is as expected because any N removed from the soil by the crop would have been returned to the soil during incorporation of the plant material. The only exception would be the oat-pea-vetch mix, which should have theoretically added nitrogen to the soil through fixation of atmospheric N. In Field D site, the oat-pea-vetch mix had the second-greatest total N. Surprising results were observed in Field C, where wheat had the greatest total N, which was significantly different from the total N in CFPM and the oat-pea-vetch mix. Total N in Field D was significantly greater than in Field C.

Table 8. Summary of the soil sample analyses from samples collected in October 2007.

	2007 Post-Green Manure Soil Samples†											
	Total N (kg/ha)						**Available N (kg/ha)**					
Name	Field C		Field D		Overall		Field C		Field D		Overall	
Wheat	2909	a	2887	a	2898	a	25.1	a	120.4	a	72.8	a
Sorgum Sudan	2611	ab	2974	a	2792	a	20.9	a	87.8	a	54.4	a
Grass	2295	b	3178	a	2736	a	25.1	a	92.2	a	58.6	a
CFPM 101	2215	b	3140	a	2743	a	20.2	a	103.9	a	68.0	a
Oat-Pea-Vetch	-	-	3033	a	3033	a	-	a	100.2	a	100.2	a
Phacelia	2380	ab	3045	a	2712	a	20.9	a	83.5	a	52.2	a
Ryegrass												
Both Sites												
Field C	2496			b			22.5			b		
Field D	3043			a			98.0			a		

† Numbers in the same column, which are followed by the same letter are not significantly different at the 5% level.

Available nitrogen is a sum of nitrate nitrogen and ammonium nitrogen. There was no statistical significance among green manure crops at either of the sample sites in 2007 (Table 8). Available N in Field D was significantly greater than in Field C. In Field D, wheat had the greatest overall available N remaining after incorporation of the green manure crop. Like available N, NO_3-N in Field D (88.9 kg/ha) was also significantly greater than in Field C (14.5 kg/ha) (results not shown). Between July and October, in Field C, NO_3-N decreased from 29.5 to 14.5 kg/ha, in the 0-15 cm depth range. In that period of time, N-mineralization would have contributed to the amount of soil NO_3-N, however uptake by the green manure crop would have immobilized N, resulting in the net decrease in NO_3-N. Between July and October 2007, in Field D, average NO_3-N increased from 7.00 to 88.9 kg/ha, in the 0-15 cm depth range. These results are surprising and suggest that the site may have received mineral N fertilizer in the intervening time, possibly through the pivot.

Analytical results from the 2008 green manure sites (Table 9) were taken from soil that was collected on October 8 and 17, 2008. Total N includes plant available (inorganic) N and unavailable (organic) N (Table 9). Total N was statistically the same for all crops at all three sites. This is as expected because any N removed from the soil by the crop would have been returned to the soil during incorporation of the plant material. The only exception would be the oat-pea-vetch mix), which could have theoretically added nitrogen to the soil through fixation of atmospheric N. The greatest total N values, in Field E, were found in the oat-pea-vetch mix), teff and ryegrass. At In Field F, the greatest total N was found for wheat and the oat-pea-vetch mix). The average total N in Field G was the same for each green manure crop (2688 kg/ha). This result occurred despite differences among the individual replicates for each crop, which ranged from 2240 to 3136 kg/ha. Field F had the greatest average total soil N in the 0-15 cm depth range, which was significantly greater than the average total N at the other two sites.

Table 9. Summary of the soil sample analyses from samples collected in October 2008.

	2008 Post-Green Manure Soil Samples†											
	Total N (kg/ha)						**Available N (kg/ha)**					
Name	Field E		Field F		Field G		Field E		Field F		Field G	
Wheat	2632	a	3080	a	2688	a	19.7	b	36.3	a	23.1	b
Sorgum Sudan	2576	a	3024	a	2688	a	22.3	b	32.4	a	19.7	b
Grass	2464	a	3248	a	2688	a	41.5	a	21.8	a	30.7	b
CFPM 101	2632	a	3864	a	2688	a	28.9	ab	39.8	a	24.6	b
Oat-Pea-Vetch	2464	a	3864	a	2688	a	25.4	b	32.9	a	27.6	b
Teff	2408	a	3752	a	2688	a	35.8	ab	52.9	a	47.0	a
Ryegrass												
All Sites												
Field E			2529	b					28.9	a		
Field F			3472	a					36.0	a		
Field G			2688	b					28.8	a		

† Numbers in the same column, which are followed by the same letter are not significantly different at the 5% level.

Available nitrogen is a sum of nitrate nitrogen and ammonium nitrogen. There was no statistical significance among green manure crops in Field F (Table 9). The greatest available N in Field F, was for ryegrass). Available N was greatest for CFPM in Field E. In Field G, ryegrass) had the greatest overall available N remaining after incorporation of the green manure crop. There was no statistical significance among the three sites for average available N.

Like available N, October NO_3-N in the 0-15 cm depth range was greatest in Field F (32.2 kg/ha), compared to Field E (25.0 kg/ha) and Field G (25.3 kg/ha). Between July and October 2008, in Field F, average NO_3-N increased from 22.2 to 32.2 kg/ha, in the 0-15 cm depth range. Between July and October 2008, in Field G, NO_3-N increased from 15.1 to 25.4 kg/ha, in the 0-15 cm depth range. In that period of time, N-mineralization could have contributed to the amount of soil NO_3-N; however, the results are surprising because it would have been expected that the growing green manure crop would have immobilized N. Between July and October 2008, in Field E, NO_3-N decreased from 80.1

to 25.0 kg/ha, in the 0-15 cm depth range. It is expected that the green manure crop would have immobilized N, resulting in the net decrease in NO_3-N.

Results of Soil Sample Incubations to Determine Mineralizable Nitrogen

The purpose of the warm/moist incubation in 2006 was to maximize mineralization of N held in organic matter within residue from the green manure crop. The available N, from this analysis, was a measure of readily mineralizable N, which includes the available N already present prior to incubation plus any N that was mineralized during incubation minus any N that might have been immobilized during the incubation period. Mould was noted on the surface of samples during the incubation process, which suggests that some immobilization may have occurred.

The total N before and after incubation should remain the same, unless nitrogen fixation, leaching or volatilization occurred during the incubation period. Leaching was prevented because containers were sealed at the bottom. Nitrogen fixation is unlikely, as no plants were growing the small soil samples used, so there were no hosts available for *Rhizobium* nodules.

Based on the recommendations developed in 2006, sub-samples were not incubated in 2007 or 2008 for mineralizable N.

Table 10: Total and available N (kg/ha) in soil samples taken in 2006 from green manure plots following incorporation and incubation to achieve maximum mineralization.

	Post-Incubation†											
	Total N (kg/ha)						**Available N (kg/ha)**					
Name	Field A		Field B		Overall		Field A		Field B		Overall	
Wheat	2722	a	2402	a	2562	a	54.3	ab	58.9	ab	56.6	b
Sorghum Sudan Grass	2537	a	2503	a	2520	a	34.0	b	44.0	b	39.0	c
CFPM 101	2811	a	2666	a	2738	a	61.0	ab	66.5	a	63.7	ab
Woolly Pod Vetch	2531	a	2582	a	2556	a	61.8	ab	78.6	a	70.2	ab
Oriental Mustard	-	-	2475	a	2475	a	-	-	66.4	a	66.4	ab
Oilseed Radish	-	-	2464	a	2464	a	-	-	76.3	a	76.3	ab
Phacelia	2498	a	-	-	2498	a	84.7	a	-	-	84.7	a
Ryegrass	2542	a	-	-	2542	a	75.7	a	-	-	75.7	ab

† Numbers in the same column, which are followed by the same letter are not significantly different at the 5% level.

Total N, after the incubation period, was statistically the same for all green manure crops (for averages of both sites and averages within sites) (Table 10). Total N values remained very similar after incubation to values determined before incubation. From the crops that overlapped between sites (wheat, Sorghum Sudan grass, CFPM and vetch), it was apparent that the average total soil nitrogen was somewhat greater in Field A (2650 kg/ha) than Field B (2538 kg/ha), this difference was not statistically significant.

The available soil N, after the incubation period, was greater than prior to incubation. This indicates that mineralization of organic N occurred during the 28 days of warm/moist conditions, as would be expected. It also indicates that the amount of mineralization exceeded any potential losses of N that may have occurred. Individual ranking of the crops, as indicated by the letters representing significant difference within columns, were very similar before and after incubation. As with the soil analysis prior to incubation, the post-incubation available N for Phacelia was greatest. After incubation, radish), ryegrass), vetch), mustard and CFPM had the next greatest amount of available N and were not statistically different from one another or from Phacelia. Wheat had post-incubation available N tests that were significantly less than Phacelia. Sorgum Sudan grass gave the least overall available N, which was significantly less than all other crops both before and after incubation of the soil samples. This suggests that much of the soil N immobilized by the crop remained unavailable, even after 28 days of warm/moist conditions. In the context of this incubation experiment, the mineralization rates of Sorghum Sudan grass residues would be the slowest of the eight green manure crops tested to supply early season N to a subsequent crop of potatoes. A different (in-field) measure of mineralization is needed to determine if this conclusion holds true under field conditions. Of the four crops that were common to both sites (wheat, Sorghum Sudan grass, CFPM and vetch) there were no trend or significant differences between fields. Wheat and CFPM had greater available N in Field A and Sorghum Sudan grass and woolly vetch had greater available N in Field B.

<u>Differences Between Post- Incubation and Pre-Incubation N, as a Measure of Net Mineralization</u>

The available N after the warm/moist incubation, was a measure of readily mineralizable N, which includes available N present prior to incubation plus N mineralized during incubation minus N immobilized during the incubation period. The difference, for any given sample, between the available N prior to and after incubation is an estimate for net mineralization for that period and under the imposed conditions (28 days at 25°C and 80% of field capacity).

In order to determine whether or not changes in soil N were meaningful, they were calculated as a percent of the pre-incubation N content.

Differences between pre-incubation and post-incubation total N showed no significant trend and were near zero (Table 11). Averages did not exceed ±6% of the pre-incubation total N, and therefore will not be discussed.

Table 11: Difference in total and available N (% of pre-incubation concentration) in soil samples taken from incorporated green manure plots before and after and incubation to achieve maximum mineralization.

	Difference (Post minus Pre-Incubation) †											
	Total N (% of Pre-Incubation)						**Avail. N (% of Pre-Incubation)**					
Name	Field A		Field B		Overall		Field A		Field B		Overall	
Wheat	4.0	a	2.3	a	3.1	a	76	a	112	a	94	a
Sorgum Sudan Grass	-1.4	a	1.4	a	-0.022	a	88	a	125	a	106	a
CFPM 101	1.8	a	5.8	a	3.8	a	61	a	89	a	75	a
Wooly Pod Vetch	-5.1	a	3.7	a	-.73	a	68	a	87	a	77	a
Oriental Mustard	-	-	1.1	a	1.1	a	-	-	127	a	127	a
Oilseed Radish	-	-	0.53	a	0.53	a	-	-	85	a	85	a
Phacelia	-0.29	a	-	-	-0.29	a	80	a	-	-	80	a
Ryegrass	-1.5	a	-	-	-1.5	a	128	a	-	-	128	a

Changes in available N were substantially greater than for total N, as expected. All values were positive, indicating that a net mineralization occurred, i.e. that mineralization exceeded any potential losses due to fixation or volatilization. Although there were no statistically significant differences among green manure crops, some notable trends were observed. Ryegrass showed the greatest increase in available N (128% of the pre-incubation available N). This crop, however, also had the greatest degree of variability of all the crops. The values for individual replicates were 76%, 348%, 34% and 55%, respectively. Therefore the high value may be an outlier and the true mean for this crop is considerably less. Sorghum Sudan grass and mustard also showed a slightly greater amount of N mineralized during the incubation period. Of the four crops that overlap at the two sites (wheat, Sorghum Sudan grass, CFPM and vetch), the individual ranking for each site was similar, Sorghum Sudan grass > wheat >vetch≈ CFPM in Field A and Sorghum Sudan grass > wheat > CFPM ≈vetch in Field B. Of these four crops common to both sites, the average in Field B (103%) was greater than the average for Field A (73%), however the post-incubation available N was still less in Field B (see previous section).

Recommendations for Future Sampling and Analysis

It may be preferable, in order to save costs on future soil testing and analysis, that soil samples following the green manure crop be taken just before planting of the subsequent potato crop, in order to correctly gauge the available N. The laboratory incubation process for estimating mineralizable N may give a good estimate for warm season N mineralization rates but will not be useful in estimating the amount of N that will be readily available under field conditions, where seasonal and diurnal fluctuations in temperatures and variable moisture conditions occur.

If sampling then does occur after planting, it should be decided whether cores should be taken from the hill, furrow, mid-slope or a combination.

The sampling regime used in 2006 (12 core samples per plot, 0-15 cm depth, 2 cm diameter) provided for a sufficient sample size and was ample to account for in-plot spatial variability in soil N.

Verticillium Inoculum

Green Manure Crops Root Analysis in 2006
Materials and Methods

On August 24, 2006, an initial disease evaluation of the green manure crops in both Fields A and B was conducted by digging out and bagging 5 entire plants, including all roots, from each subplot. All bags were then refrigerated at 5°C until they were processed. On Sept 22 each set of plants was individually washed under running tap water to remove the soil and crop debris, and foliage was discarded. Lower stems and roots were visually and/or microscopically examined for evidence of root rot, galls, stunting and discoloration. For each of these parameters, this disease severity (DS) rating scale was used: 0 = no root rot (or galls or stunting or discoloration) present; 1 was a slight amount of the symptom; 2 was a moderate level; and finally 3 meant that severe levels were present. Then, one feeder root/plant/subplot) ca. 2 cm long, was placed onto a glass microscope slide with a drop of lactophenol + acid fuchsin and examined microscopically for *Verticillium* spp. microsclerotia and mycelium. Results were recorded as either positive or negative for this pathogen, so no statistical analysis was performed.

Also, a composite sample of feeder roots of various sizes from each of the five root systems /subplot was sterilized in 1% sodium hypochlorite (NaOCl) for 30 sec., rinsed in sterile water, and then randomly cut into at least 20 pieces (5 mm). Five root pieces were aseptically placed onto each of four plates of agar medium/subplot as follows: two plates of CzaPEK Solution Agar amended with streptomycin (CZA-S) and acidified potato dextrose agar (PDA-A). These plates were incubated at RT for ca. 2-weeks, before microscopically examining them specifically for *Verticillium* spp. and also noting other fungal genera growing on them. As some of the cultures still had either no growth or fungal sporulation, they were rechecked at a later date after cold storage.

Results and Discussion

Data for all ratings were summarized and analyzed using the ARM 7 statistical software program (Gylling Data Management, Brookings, SD) with the DI and DS experimental means results presented as either raw or detransformed data. Duncan's Multiple Range Test (($P \leq 0.05$) was used.

Root rot and root swellings were more common in the Field B as opposed to Field A. Cereal plants in Field B were stunted as the result of a virus infection. Fungal isolations were performed on root samples to check for the presence of *Verticillium spp.*, and the plates were examined in mid-October. There was no growth of this pathogen on the agar plates after 2-3 weeks of incubation. Two pathogens that were present on the roots of some plants at both sites were *Fusarium spp.* and *Alternaria spp*.

Table 12 – Fields A and B
For Field A, crops in this location were definitely much healthier than Field B upon root evaluations. Although the root rot and gall formation results were insignificant, only Sudan grass and vetch had root rot, with very low values of < 7.5% DI and <0.1 DS. Galls only formed on the vetch crop (22.9 % DI but just 0.08 DS), Sudan grass, and wheat – both with very negligible levels. Root stunting was more prevalent, but only Sudan grass and ryegrass had significantly lower DI values of 16.3% and 25% respectively while the remaining crops were in a much higher range, from 70 % (millet) to 100% (wheat). The lowest stunting DS results (0.27 and 0.31) were found with the same crops as in the DI ratings but included millet too, in the same group (1.19 DS). Again, wheat had the highest DS rating at 2.79 out of 3. For root discoloration, the DI ratings were insignificant, although ryegrass had the lowest value at only 5% and the highest was Sudan grass at 68.8%. However for the DS rating,

ryegrass was extremely low at 0.05 but was in the same statistical grouping as wheat (0.27), millet (0.25), and vetch (0.29). Sudan grass and *Phacelia* had the most root discoloration (0.8 and 1.16).

Table 12 shows the microscopic root examination from the two fields, with the DS and DI means of the four replications /crop. For Field B, all data were statistically significant ($P \leq 0.05$) and overall, mustard had the least root rot, gall formation (results of 0 DS and DI), root stunting and discoloration. For root rot, roots from the other crops had DI levels of 100%, whereas mustard was nearly half of that at 55%. Likewise, these roots had a DS severity of 0.58 (scale from 0-3 points) and Sudan grass had the next lowest level of 1.10 followed by vetch at 1.26 but these two crops were statistically higher than mustard. Wheat had the most root rot with a DS of 1.84 but was statistically similar to millet and radish. Root galls formed on 86.7 % of the vetch roots but there were none in mustard and radish. However for the DS results, wheat, mustard and radish were all ≤ 0.1 while again, vetch was the highest at 1.23 but was statistically similar to millet at 0.48. After examining each crop for stunted roots, mustard, millet and Sudan grass had significantly lower symptoms for both DI /DS (results ranged from 10% /0.01 for mustard up to 25% /0.23 for Sudan grass). Wheat had the highest rating (100% /2.36) but this was statistically similar to radish (70% /0.97). Root discoloration usually means that disease may be present, and only the mustard roots (DI = 35% and DS = 0.32) were significantly less discoloured.

Table 13 - Fields A and B

Table 13 shows the % of roots /crop that were infected with *Verticillium spp*. upon microscopic examination from Fields A and B. For Field A, Sudan grass roots were 100% infected with *Verticillium* but the other crops had DI levels of 0%. From this data, Sudan grass roots appeared to be highly susceptible to infection by *Verticillium spp.* For Field B, wheat was the most infested crop, with 30% of the roots showing either *Verticillium* hyphae, spores or both. This was followed by Sudan grass with 15% and then mustard and vetch crops, both showing disease incidence levels (DI) of 5%. Only radish and millet were uninfected with this pathogen.

When other root pieces were plated to CZA-S and PDA-A culture media and microscopically examined for *Verticillium spp*. (hyphae, spores or microsclerotia), in Field A showed that only roots from millet for Replicates 1 and 3 grew *V. dahliae* on a CZA-S and PDA-A plate respectively. Other fungal isolates were *Alternaria*, *Fusarium spp.*, *Cladosporium Epicoccum, Trichoderma*, *Ulocladium, Acremonium, Penicillium, Mucor* and *Rhizopus.* Fusarium grew on nearly all of the culture plates from this field. Davis et al. (2004) reported that incorporation of sudan grass and sorghum-sudangrass hybrids was closely associated with significant increases in populations of *Fusarium equiseti, F. oxysporum*, and *F. solani*. In Field B, only wheat roots from Replication 1 grew *V. dahliae*. Other fungi genera growing on plates were, *Alternaria*, *Fusarium spp., Epicoccum, Trichoderma, Ulocladium, Acremonium, Penicillium, Mucor* and *Rhizopus.* Data from these results are not on a table.

From the results obtained in Tables 12 and 13 for the green manure crops in the two fields overall, Field B had the lowest disease levels in the mustard crop whereas ryegrass was the lowest in Field A. However, these two crops were each only grown in one field.

A complicating factor in disease management through crop rotation is that roots of some non-hosts including cereals (barley, buckwheat, field corn, oats, Sudan grass, and wheat) legumes (alfalfa, Austrian winter pea, clover, and milkvetch), and brassica crops (canola, radish, and turnip) support low populations of *V. dahliae* (Rowe and Powleson 2002).

Table 12: Root evaluations performed from Fields A and B during the green manure crop year (2006).

Field	**Crop**	**DI (%)[2,5,7]**	**DS (0-3)[3,6,7]**	**DI (%)[2,5,7]**	**DS (0-3)[3,6,7]**	**DI (%)[2,4,7]**	**DS (0-3)[3,5,7]**	**DI (%)[2,4,7]**	**DS (0-3)[3,5,7]**
A	**Wheat**	0.0 a	0.00 a	2.3 a	0.01 a	100.0 a	2.79 a	30.0 a	0.27 bc
	Sudan Grass	2.3 a	0.01 a	2.7 a	0.02 a	16.3 b	0.27 c	68.8 a	0.80 ab
	Millet	0.0 a	0.00 a	0.0 a	0.00 a	70.0 a	1.19 bc	25.0 a	0.25 bc
	Vetch	7.2 a	0.10 a	22.9 a	0.08 a	85.0 a	1.77 ab	30.0 a	0.29 bc
	Phacelia	0.0 a	0.00 a	0.0 a	0.00 a	80.0 a	1.93 ab	66.7 a	1.16 a
	Ryegrass	0.0 a	0.00 a	0.0 a	0.00 a	25.0 b	0.31 c	5.0 a	0.05 c
Field	**Crop**	**Root rot[1]**		**Gall formation[1]**		**Stunting of roots[1]**		**Root discoloration[1]**	
		DI (%)[2,4,7]	**DS (0-3)[3,5,7]**	**DI (%)[2,4,7]**	**DS (0-3)[3,6,7]**	**DI (%)[2,4,7]**	**DS (0-3)[3,6,7]**	**DI (%)[2,4,7]**	**DS (0-3)[3,5,7]**
B	**Wheat**	100.0 a	1.84 a	30.0 bc	0.01 cd	100.0 a	2.36 a	93.8 a	1.62 a
	Sudan Grass	100.0 a	1.10 b	35.0 bc	0.33 bc	25.0 cd	0.23 cd	100.0 a	1.19 a
	Millet	100.0 a	1.44 ab	65.0 ab	0.48 ab	15.0 d	0.07 d	85.0 a	1.39 a
	Vetch	100.0 a	1.26 b	86.7 a	1.23 a	53.3 bc	0.55 bc	73.3 ab	0.86 ab
	Mustard	55.0 b	0.58 c	0.0 c	0.00 d	10.0 d	0.01d	35.0 b	0.32 b
	Radish	100.0 a	1.49 ab	5.0 c	0.01 cd	70.0 ab	0.97 b	70.0 ab	0.93 ab

[1]Results are the means of four replications.
[2]Root symptom disease incidence means (DI) are based on the percentage of roots evaluated per crop that had either root rot, gall formation, stunting of the roots and discoloration.
[3]Root symptom disease severity (DS) means are based on a 0-3 point scale, where 0 = no root rot (or galls or stunting or discoloration) present, 1 = slight symptom level, 2 = moderate symptom level and finally 4 = severe symptoms.
[4]Raw data were used for analysis.
[5]Square root-transformed data were used for analysis with detransformed means presented.
[6]Arcsine-transformed data were used for analysis with detransformed means presented.
[7]Numbers within the same undivided column followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Table 13. Microscopic examination of the roots from each green manure crop.

Field A	Positive *Verticillium spp.* on roots (% /crop) [1,2]	Field B	Positive *Verticillium spp.* on roots (% /crop)[1,2]
Wheat	0	**Wheat**	30
Sudan Grass	100	**Sudan Grass**	15
Millet	0	**Millet**	0
Vetch	0	**Vetch**	5
Phacelia	0	**Mustard**	5
Ryegrass	0	**Radish**	0

[1]Results are the means of four replications.
[2]Root symptom disease incidence means (DI) are based on the percentage of roots evaluated per crop that had *Verticillium spp*. growing on them, including hyphae and/or spores.

Verticillium Inoculum in Soil
Materials and Methods

Soil sampling - 2006 – Fields A and B (Green manure crops)
Soil samples were collected from Fields A and B August 24-25, 2006. A composite sample was obtained using a spade. A large spade-full of soil was taken from 10 to 25 cm below soil level at five sites within each subplot to make a composite sample. Soil samples were taken from fallow areas between plots as a control. Samples were stored at 5°C until they were processed in the fall. In October, following green manure crop incorporation into the soil, post-incorporation soil samples were collected in the same manner.

During the winter months, the technologists thoroughly mixed the soil in each composite sample, taking care to break up any large lumps, remove any rocks and avoid cross-contamination between all soil samples by disinfecting the mixing trays with 85% denatured ethanol. From each sample, two 500 mL sub-samples were taken. One set of sub-samples was shipped to Dr. Guy Bélair, AAFC, Saint-Jean-sur-Richelieu, PQ, for root lesion nematode analysis. The other set of sub-samples was sent April 2007 to Dr. Larry Kawchuk, AAFC, Lethbridge, for *Verticillium* analysis using molecular methods. The remainder of the composite soil sample was retained for soil dilution plating. Also, these soil samples were used in 2008 for a bioassay using a *Verticillium*-susceptible cultivar of eggplant, carried out in a CDCS greenhouse and this methodology is described in a separate section. Soil dilution plating, the eggplant bioassay and PCR molecular analysis are compared in the main project report for their speed, accuracy and practicality, as assays for *Verticillium* species in potato soils. In April 2007, four samples from each field were sent to Dr. Bélair for a preliminary evaluation to decide on the feasibility of sending the remaining samples. He decided that after the samples had been in refrigerated storage for over half a year, the nematodes likely wouldn't have survived anyway so these samples weren't sent to him. Consequently then, soil samples for nematode analysis were always sent to the analytical laboratories shortly after obtaining them!

Soil sampling – 2007 - Fields A and B (Potatoes) and Fields C and D (Green manure crops)
Staff collected soil samples, using the same methodology as outlined above, following potato harvest (Fields A and B) and crop incorporation (Field C and D) in the fall, 2007. Baseline soil samples were also collected and composited from five areas outside each replicate in Fields C and D. In 2007 again, 500 mL sub-samples were submitted to Dr. Kawchuk and Dr. Guy Belair. The remaining soil was refrigerated (5˚C) for soil dilution plating.

Soil Sampling – 2008 - Field C (Potatoes) and Fields E, F and G (Green manure crops)
In 2008, soil samples were collected from plots areas in Field C, and from areas that had been fallow in 2007 (Outside Plot Soil). Soil samples were not collected from Field D in 2008 as potatoes were not planted following the green manure crops. Composite samples and baseline soil samples (see 2007 above) were obtained from Fields E, F and G. A smaller soil volume of >1 kg was obtained from each subplot by using a soil auger to reach a depth of ≥ 20cm in five areas within it and 10 scoops of soil (using a garden trowel) were obtained from all of them as a composite sample. A 500 mL sub-sample from each sample was shipped to Dr. Kawchuk for verticillium testing and another 250 g sub-sample was shipped to Dr. Thomas Forge for nematode testing in Agassiz, British Columbia. The remaining samples were retained at 5˚C for dilution plating and eggplant bioassays.

Soil Sampling – 2009 - Fields E, F and G (Potato crop year)
In 2009, following potato harvest, soil samples were collected as before from the centre of each subplot in Fields E, F and G. Samples were collected from between green manure plots in Fields E and F only, (Outside Plot). In Field G, potatoes were only planted into pot areas so no soil was collected from areas between plots. A 500 mL sub-sample from each sample was shipped to Dr. Kawchuk for verticillium testing and another 250 g sub-sample was shipped to Dr. Thomas Forge for nematode testing. The remaining samples were retained at 5˚C for dilution plating and eggplant bioassays.

Dilution Plating

The method of Mpofu and Hall (2003) was modified to estimate Verticillium populations in field soil samples. Two selective agar media, Soil Pectate Tergitol (SPT; Dr. Kenneth Conn, personal communication) and Sorensen's NP-10 medium (SNP-10; Dr, Fuoad Daayf, personal communication), were evaluated for recovery and identification of Verticillium spp.

Although both Verticillium dahliae and Verticillium albo-atrum were recovered from autoclaved soil samples spiked with prepared lab suspensions of each species, V. albo-atrum was indistinguishable from other light coloured fungi when field soil dilutions were evaluated. V. dahliae colonies produced sclerotia that were distinctive.

In 2008, dilution plating of the 2006-07 soil samples was used to enumerate only V dahliae on SNP-10 media, with dilutions ranging from 10^1 - 10^3dilutions /soil sample and three replicate plates /dilution. Results were calculated as colony-forming units, expressed as cfu/g of soil. Few colonies were obtained from the 10^1 and 10^2 dilutions; and for all other soil samples evaluated, only the 10^1 dilutions were required. Soil dilution plates were allowed to grow for approximately 3 weeks in the dark before colonies were counted. In 2009, oven-dried soil weights (ODW), rather than field soil weights were used to calculate the cfu/g to account for any differences in the moisture content of field soil.

Results and Discussion

Green Manure Crop Year – Fields A and B only
In the first year of the trial, samples were collected from plot areas while green manure was growing. There wasn't any verticillium inoculum in any of the soil samples collected from Field A, while green manure crops were growing. The *V. dahliae* inoculum in Field B ranged from 18.75 to 31.42 cfu/g of soil and were the highest values observed throughout the project. There were no statistical differences

between plot areas in Field A or Field B prior to green manure incorporation. In all other years of the trial, areas sampled outside of the green manure plots were used as checks.

After Green Manure Crop Incorporation

No verticillium inoculum was recovered from soil samples collected following the incorporation of the green manure crop in Field A, Field C and Field G. No *V. dahliae* inoculum was recovered from the areas outside of the green manure plots either, indicating that these fields were not high risk fields. The inoculum levels in Field B following the incorporation of wheat, CFPM, Sudangrass and mustard and were statistically lower than following incorporation of oilseed radish. Less inoculum was recovered from soil samples after incorporation of wheat, CFPM, Sorghum Sudangrass and the oat/pea/vetch mixture than while the green manure crops were growing. In Field D, the inoculum level in *Phacelia* plots was significantly higher those from other green manure crops. *Phacelia,* which had been introduced as a source of biomass in place of *Brasscia* relatives, was not used in subsequent years of the trial. The inoculum levels measured in Field E were extremely low and not significantly different from one another, ranging from 0 for "no green manure, wheat and teff up to 0.17 cfu/g of soil for ryegrass. Data from Field F showed significantly higher verticillium inoculum after Sorghum Sudangrass than the after incorporation of the other green manure crops.

After Potato Crop Year

Following potato production in Field A, verticillium inoculum levels were low but detectable, with the highest values of 1.67 and 1.68 cfu/g (Sudangrass and *Phacelia*) and the lowest for wheat, millet and ryegrass (all 0). Soil inoculum levels in the Sudangrass, *Phacelia* and Woolly Vetch plots were greater following potato than following the incorporation of the green manure crops. Soil inoculum levels in Field B were not statistically different from one another ranging from 3.42 cfu/g of soil (Sudangrass) to 7.08 (mustard). Surprisingly, these values were lower following the potato crop than following the green manure incorporation.

The soil inoculum in Field C increased following potato production from the previous year, regardless of which green manure crop was used. Although not statistically different from the inoculum in the green manured plots, soil inoculum was highest in the area where no green manure had been incorporated. Significant differences were observed between plots in Fields E and F. In Field E, inoculum values following potatoes were higher overall than following green manure incorporation. Interestingly, the plots with the highest inoculum prior to potatoes, also had the highest level of inoculum following potatoes. Soil from OPV and ryegrass (1.25 and 1.50 cfu/g of soil) had higher verticillium inoculum than teff or areas that had not been green manured. In Field F, the highest verticillium inoculum levels were observed from Sorghum Sudangrass, ryegrass and "no green manure", and the lowest inoculum levels were observed in teff plots. In Field G, verticillium inolculum was recovered from CFPM and Sudangrass soils following a potato crop, whereas this pathogen wasn't present in any of the green manured plots the previous year.

Table 14: Verticillium inoculum (cfu/g of soil) in fields in southern Alberta before and after incorporation of green manures. Data followed by the same letter in each row were not statistically different ($p \geq 0.05$).

Field	No Green Manure	Wheat	CFPM 101	Sorghum Sudangrass	Vetch/ OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Nightshade
Green manure crop year											
A	0	0	0	0	0	0	0				
B		30.75 a	31.42 a	30.83 a	30.00 a				18.75 a	19.83 a	
C	0	0	0	0	0	0	0				
D	0	0	0	0	0	0	0				
After green manure incorporation											
A	0	0	0	0	0	0	0				
B		14.33 b	16.17 b	15.42 b	20.0 ab				17.00 b	25.58 a	
C	0	0	0	0	0	0	n/a				
D	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.33 a				
E[2]	0.00 a	0.00 a	0.08 a	0.08 a	0.08 a	0.17 a		0.00 a			
F[2]	2.58 b	2.17 b	2.08 b	5.92 a	2.00 b	3.75 b		2.00 b			
G	0	0	0	0	0	0					0
After potato crop year											
A		0.00 a	0.00 a	1.67 a	0.83 a	0.00 a	1.68 a				
B		4.83 a	5.25 a	3.42 a	6.25 a				7.08 a	6.83 a	
C	1.00 a	0.56 a	0.33 a	0.75 a	0.56 a	0.25 a	n/a				
D[1]	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.33 a				
E[2]	0.17 c	0.75 abc	0.67 abc	0.50 abc	1.25 ab	1.50 a		0.25 c			
F[2]	5.92 a	3.75 ab	3.33 ab	5.08 ab	3.17 ab	5.00 ab		1.92 b			
G	0.00 a	0.00 a	0.33 a	0.08 a	0.00 a	0.00 a					0.00 a

[1]Field not planted to potatoes following green manure crop year.
[2]Hail storms in summer (2008 and 2009) affected green manure crops and potato crop in the following year.

Eggplant Bioassay

Materials and Methods

Eggplant, *Solanum melongena*,is a member of the nightshade family, and is very closely related to the potato plant (*Solanum tuberosum*). Some cultivars of eggplant are highly susceptible to verticillium wilt. A bioassay trial was conducted in a double poly hoop house, in 2008. The purpose of this bioassay experiment was to further verify *V. dahliae* inoculum in field soil samples collected from commercial potato fields. A highly susceptible eggplant variety, Black Belle II, was chosen after preliminary testing. Black Belle II eggplant seedlings were transplanted into soil samples collected from Fields A, B and C and D in 2006 and 2007, including samples taken from outside the subplots (described as “no green manure” in this report and negative controls (landscaping soil), totalling 203 soils for testing. Three to four 15 mm (6”) pots were planted, depending on the quantity of soil remaining after soil dilution plating, *Verticillium* and nematode testing.

The eggplants were watered and maintained in the hoop house from August to October, 2008, until verticillium wilt foliar symptoms developed (leaf wilting, bright yellow chlorosis along leaf veins, stunting of the plants, wilting, or death). When symptoms developed, each plant was rated for disease severity (DS) on a scale of 0-4 points: where 0 = no symptoms; 1 = single leaf wilting or half of a leaf was chlorotic; 2 = multiple leaves yellowing /wilting, leaf roll; 3 = whole plant wilts with extensive yellowing, possibly was stunted and finally; 4 = plant death. One diseased plant, (rating from 1-4 points), if any, was chosen from each replicate for root/stems fungal isolations.

The stems from each plant selected were cut off at the soil level with the foliage discarded. The root/lower stems were gently washed. The stems were surface-sterilized with 95% ETOH and allowed to air-dry. Stems were sectioned into six pieces and placed onto one half each of a plate of potato dextrose agar amended with penicillin and tetracycline (PDA-PT) and CzaPEK Solution Agar amended with streptomycin (CZA-S). Roots were washed free of adhering soil, sectioned into three pieces and directly plated onto the remaining halves of the same plates. These were incubated for at least 14 days in the dark at room temperature, and refrigerated until evaluation. The roots and stem pieces were microscopically examined for the presence of *Fusarium spp., V. dahliae, Colletotrichum coccodes (*black dot) and *R. solani* (rhizoctonia) fungal growth. Disease incidence (DI) levels for each pathogen were calculated as the percentage of stems with each of these pathogens present.

Another bioassay trial was set up in 2010, using soil samples collected from Fields E, F and G in 2009. Eggplant seedlings (variety Black Belle II) were transplanted into two replicate pots for each green manured plot in each field. During this 2010 bioassay, there was a heavy aphid infestation in the hoop house, and Safer Brand Insecticidal Soap and Pirimor were used to control the infestation. Some seedlings were replaced once the aphids were under control. Many of the plants prematurely developed severe black dot and blight (*Alternaria spp*. and *Ulocladium*) and this confounded the plant disease evaluations, as these two pathogens also cause necrosis and leaf wilting. Plants were evaluated as in 2008, and fungal isolations were also performed.

Results and Discussion

Green Manure Crop Year – Fields A and B only

Tables 15 and 16: 2006 was the only year that soil samples were obtained prior to green manure soil incorporation from all of the subplots, including areas between the replications (no green manure column in Tables 1 – 10) and the negative control soil (DS = 0.0). Field A was a very healthy field when viewed in 2006 and as expected, when eggplants were planted into these soils, they were nearly wilt-free as opposed to the plants grown in the Field B soils. In Tables 1 and 2 for the first field, all of the DS (0-4 points) and DI% levels were 0.00 with the exceptions of vetch and ryegrass, both at only 0.06 /2,7% (DS/DI) but all had statistically insignificant data ($p \geq 0.05$). There were significant differences ($p \leq 0.05$) between the eggplant DS wilt levels however, for Field B, where the negative control was significant lower than "no green manure", Sudangrass, vetch and radish (DS results for these were from 0.93 for radish to 1.24 for "no green manure). The remaining crops were statistically similar to the negative control, with relatively low DS results ranging from 0.52 to 0.66. When the percentage of the wilted eggplants were compared for this field (DI %), the crops with significantly more disease than the negative control were "no green manure", Sudangrass, vetch, mustard and radish (results ranged from50% to 68.8%). Only wheat and CFPM (millet) were similar but had DI values of 31.3% and 37.5% respectively.

Table 15: Verticillium wilt disease severity (DS)[4] in Black Belle II eggplants that were planted into field soils from southern Alberta before and after incorporation of green manures and following the subsequent potato crop. Data followed by the same letter in each row were not statistically different ($p \geq 0.05$).

GM Crop	No Green Manure	Wheat	CFPM 101	Sorghum Sudangrass	Vetch/ OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Nightshade	Negative Control
Field												
Green manure crop year												
A[6]	0.00 a	0.00 a	0.00 a	0.00 a	0.06 a	0.06 a	0.00 a					0.00 a
B[6]	1.24 a	0.52 ab	0.66 ab	1.00 a	1.20 a				0.64 ab	0.93 a		0.00 b
After green manure incorporation												
A[6]	0.00 a	0.00 a	0.00 a	0.00 a	0.06 a	0.06 a	0.00 a					0.00 a
B[5]	1.13 a	1.13 a	1.69 a	1.81 a	1.50 a				1.44 a	1.81 a		0.00 b
C[5]	0.88 b	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a						0.00 a
D[8]	0.00	0.50	0.00	0.00	0.00	0.00	0.00					0.00
After potato crop year												
A[8]		0.00	0.00	0.00	0.00				0.00	0.00		0.00
B[5]		1.25 a	1.50 a	1.00 a	1.67 a				1.00 a	1.00 a		0.00 a
C[3]	---	---	---	---	---	----						
D[1]	---	---	---	---	---	---	---					---
E[2,5]	1.38 a	1.75 a	0.88 a	0.88 a	0.88 a	1.38 a		0.50 a				1.50 a
F[2,6]	0.56 a	0.11 a	0.11 a	0.50 a	0.20 a	0.65 a		1.56 a				1.47 a
G[5]	----[9]	0.63 a	0.50 a	0.63 a	0.50 a	0.00 a					1.75 a	1.50 a

[1]Field not planted to potatoes following green manure crop year.
[2]Hail storms in summer (2008 and 2009) affected green manure crops and potato crop in the following year.
[3]Insufficient soil quantities for eggplant bioassays were collected in 2008.
[4] Plant symptom disease severity (DS) means are based on a scale of 0-4 points: where 0 = no symptoms; 1 = single leaf wilting or half of leaf chlorotic; 2 = multiple leaves yellowing /wilting, leaf roll; 3 = whole plant wilts with extensive yellowing; possibly stunted and finally; 4 = plant death.
[5]Raw data were used for analysis.
[6]Square root-transformed data were used for analysis with detransformed means presented.
[7]Arcsine-transformed data were used for analysis with detransformed means presented.
[8]No statistical analysis was performed for this row because either these results were all 0s or there was only one diseased eggplant in all.
[9]There were no potatoes that were planted outside this experimental plot to obtain soil from.

Table 16: Verticillium wilt disease incidence (DI%)[4] in Black Belle II eggplants that were planted into field soils from southern Alberta before and after incorporation of green manures and year with potato crops. Data followed by the same letter in each row were not statistically different ($p \geq 0.05$).

GM Crop	No Green Manure	Wheat	CFPM 101	Sorghum Sudangrass	Vetch/ OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Nightshade	Negative Control
Field												
Green manure crop year												
A[6]	0.0 a	0.0 a	0.0 a	0.0 a	2.7 a	2.7 a	0.0 a					0.0 a
B[5]	68.8 a	31.3 ab	37.5 ab	66.7 a	66.7 a				50.0 a	50.0 a		0.0 b
After green manure incorporation												
A	0.0 a	0.0 a	0.0 a	0.0 a	2.7 a	2.7 a	0.0 a					0.0 a
B[5]	56.3 b	56.3 b	87.5 a	93.8 a	100.0 a				87.5 a	100.0 a		0.0 c
C[8]	---	---	---	---	---	----						---
D[1]	---	---	---	---	---	---	---					---
E[2,3]	---	---	---	---	---	---		---				
F[2,3]	---	---	---	---	---	---		---				
G[3]	---	---	---	---	---	---					---	
After potato crop year												
A[9]		0.0	0.0	0.0	0.0	0.0	0.0					0.0
B[8]		---	---	---	---				---	---		---
C[3]	---	---	---	---	---	---						
D[1]	---	---	---	---	---	---	---					---
E[2,5]	75.0 a	62.5 a	37.5 a	37.5 a	50.0 a	50.0 a		37.5 a				---[8]
F[2,5]	50.0 a	12.5 a	12.5 a	25.0 a	12.5 a	37.5 a		50.0 a				---[8]
G[5]	----[10]	25.0 a	12.5 a	25.0 a	12.5 a	0.0 a					50.0 a	---[8]

[1]Field not planted to potatoes following green manure crop year.
[2]Hail storms in summer (2008 and 2009) affected green manure crops and potato crop in the following year.
[3]No samples were collected prior to incorporation of green manure in 2007 and 2008 or insufficient soil quantities for eggplant bioassays were collected in 2008.
[4] Plant disease incidence means (DI) are based on the percentage of roots evaluated per crop that had verticillium wilt symptoms.
[5]Raw data were used for analysis.
[6]Square root-transformed data were used for analysis with detransformed means presented.
[7]Arcsine-transformed data were used for analysis with detransformed means presented.
[8]As there was only one plant/replication/subplot for either all or one of the crops, DI% were not performed.
[9]No statistical analysis was performed for this row either because these results were all 0s or only one plant was infected.
[10]There were no potato crops that were planted outside this experimental plot to obtain soil from.

Tables 17 – 24: For Field A, after enumerating the eggplant root /stem culture plate colonies that were positive for black dot, fusarium, *V. dahliae* and rhizoctonia (DI%), only black dot and fusarium grew on them and just from the vetch and ryegrass subplots (Tables 17-20) with all data insignificant. Black dot DI% values on these roots and stems were both 6.2%, with higher fusarium DI levels seen on the same plant parts at 20.6 and 8.7% respectively. For ryegrass, similarly low black dot results were obtained with root/stem DI levels at 4.8% and 6.2% with fusarium results at 12.5% and 6.2%. There didn't appear to be *V. dahliae* or rhizoctonia inoculum in any of the soils.

However for the Field B culture plates, statistically significant data were obtained for eggplant roots infested with black dot and fusarium. The negative control (DI = 0%) had a significantly much less black dot DI than the crops, which had very high disease levels ranging from 41.1 % for "no green manure" to 68.9% for vetch. The same pattern held for the stem results, except that millet was lowest for the crops at 35.7% and this time, the "no green manure" replication means were the highest (65.4%). Fusarium also grew at even higher levels on roots and stems from all of the soil samples (60.1% -83% on roots and 77.6% -95.7% on stems), except for the negative control (0% DI). Conversely very low, statistically insignificant *V. dahlia* levels were observed on the eggplant roots, with only the "no green manure" subplots having this pathogen at 1.95% DI but as expected, the stems had higher levels, ranging from 2.0% (radish) to 23.4% (Sudangrass). *R. solani* only grew in the stem culture plates from eggplants in Sudangrass soil (DI of 4.2%).

From these results, fusarium and black dot were the major pathogens that caused the eggplants to wilt in soils from both fields while specifically, those grown in the Sudangrass and vetch crops soil samples had higher DI% levels, but this trend wasn't statistically significant in all cases. In addition to causing tuber blemish symptoms in potato, *C. coccodes* also causes symptoms on stems and foliage, resulting in crop losses in some countries, and is implicated as a factor in the potato early dying disease complex (Lees and Hilton 2003).

Table 17: Black dot disease incidence (DI%)[4] means on eggplant root culture isolates from plants growing in the field soils from southern Alberta, before and after green manures incorporation and potato crops. Data followed by the same letter in each row were not statistically different ($p \geq 05$).

Field	No Green Manure	Wheat	CFPM 101	Sorghum Sudangrass	Vetch/ OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Nightshade	Negative Control
Green manure crop year												
A[6]	0.0 a	0.0 a	0.0 a	0.0 a	6.2 a	4.8 a	0.0 a					0.0 a
B[6]	41.1 a	58.0 a	58.0 a	64.8 a	68.9 a				65.4 a	66.1 a		0.00 b
After green manure incorporation												
A[6]	0.0 a	0.0 a	0.0 a	0.0 a	3.4 a	7.5 a	0.0 a					0.0 a
B[5]	75.0 a	66.7 a	62.5 a	95.8 a	66.7 a				83.3 a	87.5 a		0.0 b
C[5]	37.5 a	0.0 b	0.0 b	0.0 b	0.0 a	0.0 b						0.0 b
D[8]	0.0	4.2	0.0	0.0	0.0	0.0	0.0					0.0
E[2,3]	---	---	---	---	---	---		---				
F[2,3]	---	---	---	---	---	---		---				
G[3]	---	---	---	---	---	---					---	
After potato crop year												
A[8]		0.0	0.0	0.0	0.0	0.0	0.0					0.0
B[5]		12.5 a	37.5 a	16.7 a	72.2 a				33.3 a	16.7 a		0.0 a
C[3]	---	---	---	---	---	---						
D[1]	---	---	---	---	---	---	---					---
E[2,5]	58.3 a	79.2 a	50.0 a	45.8 a	66.7 a	62.5 a		50.0 a				79.2 a
F[2,5]	75.0 a	16.7 a	25.0 a	16.7 a	25.0 a	62.5 a		58.3 a				79.2 a
G[5]	----[9]	20.8 a	25.0 a	25.0 a	20.8 a	0.0 a					58.3 a	37.5 a

[1]Field not planted to potatoes following green manure crop year.
[2]Hail storms in summer (2008 and 2009) affected green manure crops and potato crop in the following year.
[3]No samples were collected prior to incorporation of green manure in 2007 and 2008 or insufficient soil quantities for eggplant bioassays were collected in 2008.
[4] Plant disease incidence means (DI) are based on the percentage of roots evaluated per crop growing on PDA-A and CZA-S agar plates with black dot.
[5]Raw data were used for analysis.
[6]Square root-transformed data were used for analysis with detransformed means presented.
[7]Arcsine-transformed data were used for analysis with detransformed means presented.
[8]No statistical analysis was performed for this row either because these results were all 0s or only one plant was infected.
[9]There were no potato crops that were planted outside this experimental plot to obtain soil from.

Table 18: Black dot disease incidence (DI%)[4] means on eggplant stem culture isolates from plants growing in the field soils from southern Alberta, before /after green manures incorporation and potato crops. Data followed by the same letter in each row were not statistically different ($p \geq 05$).

Field	No Green Manure	Wheat	CFPM 101	Sorghum Sudangrass	Vetch/ OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Nightshade	Negative Control
Green manure crop year												
A[6]	0.0 a	0.0 a	0.0 a	0.0 a	6.2 a	6.2 a						0.0 a
B[6]	65.4 a	55.1 a	35.7 a	42.9 a	64.5 a				39.1 a	62.3 a		0.0 b
After green manure incorporation												
A[6]	0.0 a	0.0 a	0.0 a	0.0 a	2.0 a.	6.2 a	0.0 a					0.0 a
B[7]	44.4 a	69.1 a	67.8 a	95.6 a	75.0 a				73.5 a	98.9 a		0.0 b
C[5]	41.7 a	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b						0.0 b
D[8]	0.0	4.2	0.0	0.0	0.0	0.0	0.0					0.0
E[2,3]	---	---	---	---	---	---		---				
F[2,3]	---	---	---	---	---	---		---				
G[3]	---	---	---	---	---	---					---	
After potato crop year												
A[8]		0.0	0.0	0.0	0.0				0.0	0.0		0.0
B[6]		0.0 b	37.5 ab	16.7 b	66.7 a				20.8 b	8.3 b		0.0 b
C[3]	---	---	---	---	---	---						
D[1]	---	---	---	---	---	---	---					---
E[2,5]	29.2 a	79.2 a	37.5 a	29.2 a	50.0 a	45.8 a		33.3 a				29.2 a
F[2,5]	58.3 a	0.0 a	12.5 a	0.0 a	20.8 a	29.2 a		25.0 a				29.2 a
G [5]	----[9]	25.0 a	16.7 a	20.8 a	16.7 a	0.0 a					16.7 a	37.5 a

[1]Field not planted to potatoes following green manure crop year.
[2]Hail storms in summer (2008 and 2009) affected green manure crops and potato crop in the following year.
[3]No samples were collected prior to incorporation of green manure in 2007 and 2008 or insufficient soil quantities for eggplant bioassays were collected in 2008.
[4] Plant disease incidence means (DI) are based on the percentage of stems evaluated per crop growing on PDA-A and CZA-S agar plates with black dot.
[5]Raw data were used for analysis.
[6]Square root-transformed data were used for analysis with detransformed means presented.
[7]Arcsine-transformed data were used for analysis with detransformed means presented.
[8]No statistical analysis was performed for this row either because these results were all 0s or only one plant was infected.
[9]There were no potato crops that were planted outside this experimental plot to obtain soil from.

After Green Manure Incorporation during Green Manure Crop Year: Fields A -D

Tables 15 and 16: For Field A, the eggplant wilt DS and DI% results were identical for soils obtained during the green manure crop growing season and were extremely low, so incorporation apparently made no difference. However, for Field B, higher results were obtained after incorporation, as opposed to before. For comparing the DS levels to each other, the negative control with DS=0 (scale 0-4), was statistically lower than the green manure crops, but now the "no green manure" and wheat means were at 1.13, with the remaining crops ranging from 1.44 (mustard) to 1.81 (Sudangrass and radish). On Table 2, again the negative control (0% DI) was the lowest, followed by "no green manure" and wheat (both 56.3%) in another Duncan's grouping but the eggplants in the millet and mustard were even more diseased (87.5%), then Sudangrass (93.8%) and finally, those in vetch and radish soils were the most wilted (100% DI%). With Field C, only the eggplants grown in the "no green manure" soils were wilted (DS of 0.88). Those from Field D were not wilted at all, and therefore, weren't cultured. Insufficient soil sample amounts were collected from Fields E, F and G for the bioassay.

Tables 17 - 24: When the culture plates were rated for Fields A and C, Field A had similarly low, statistically insignificant, black dot and fusarium DS and DI% levels calculated as" before incorporation", with these pathogens only growing on eggplant roots and stems from the vetch and ryegrass soils. For Field B however, all four of the fungi (black dot, fusarium, *V. dahliae* and *R. solani*) grew on the roots and stems (DI %), but the first two were most prevalent. Statistically significant data were obtained for black dot on roots /stems and for both fusarium and *V. dahliae* on stems only. Specifically for black dot, this fungus grew at high DI levels on the roots, from 62.5% (millet) up to 95.8% (Sudangrass), but the negative control at 0% was significantly lower. The stems had the same Duncan's groupings but this time, radish was 98.9% DI. For fusarium on roots, all of the DI% values, except for the negative control, were very high, ranging from 54.2 % (wheat) to 100% (mustard). On the stems, those from the green manure crops had significantly higher DI% levels than the negative control, with wheat (60.4 %) grouped the same as "no green manure" and millet (both 93.4%) with the remaining soils even more infested. *V. dahliae* was observed at very low, statistically insignificant levels on roots from the wheat and millet soils. However on the stems, the lowest DI% results were on the negative control, wheat (16.7%), then millet, Sudangrass and radish (ca. 35%), "no green manure" and mustard (mid-range) and finally, vetch (77.8%). *R. solani* only grew on eggplant roots from millet, Sudangrass and radish soils (2.0 - 8.4% DI) but infested stems from vetch and mustard soils (2.9 and 2.0 DI%).

Field C culture evaluations showed statistically significant, moderately high DI% levels in the "no green manure" roots /stems (black dot was 37.5% /41.7% with fusarium at 58.3% /70.8%) with the remaining crop values for the at 0%. *V.dahliae* grew just on the stems for this same crop (25% DI); significantly higher than the others. *R. solani* also was tabulated at insignificantly low levels on these same roots (4.17% DI). For this field, the green manure crops likely suppressed these pathogens, so appeared to be promising.

The Field D soil samples must have been nearly pathogen-free as very low if any, of the fungi grew on the roots and stems at insignificant DI% levels (i.e. black dot /fusarium levels were just 4.2% for the wheat soil and the rest were 0). *V. dahliae* was observed on the eggplant stems from the wheat soil at 12.5% DI and *R. solani* wasn't present at all. Although the eggplants grown in these soil samples were extremely healthy, unfortunately the farmer didn't plant this field to potatoes the following year, so no further data was obtained.

For all of the above fields, this data strongly suggests that black dot and fusarium are actually the principle pathogens causing early dying and wilt – not *V. dahliae.*

<u>After Potato Crop Year – Fields A, B, E, F and G</u>

Tables 15 and16: After the eggplant seedlings were transplanted into the <u>Field A</u> soil (2009 bioassay experiment), they all remained very healthy and therefore, were not cultured. Thus, the pathogen culture DI% results in ***Tables 21 - 24*** for this field are all 0, with no statistical analysis performed. Perhaps, these green manure crops suppressed pathogen growth, but there weren't any "no green manure" soil samples taken, as a comparison.

<u>Field B</u>, that was heavily disease-infested the previous year, also didn't have any "no green manure" soil samples collected but there was still the negative control soil, which grew very healthy, disease-free eggplants. Only DS values were calculated, as there was only just plant/subplot. This data were insignificant, with results from 1.0 (Sudangrass, mustard and radish) to 1.67 (vetch) and most values were only slightly decreased from the green manure crop year. <u>For Field C</u>, insufficient soil quantities were also collected so there wasn't enough for this bioassay and <u>Field D</u> was no longer available.

In the <u>Field E</u> 2010 bioassay, the negative control (eggplants grown in local landscaping soil) actually had wilted plants, with a DS of 1.50 (0-4 point scale), using raw data statistical analysis. This was nearly as high as for the most diseased eggplants in wheat soil (1.75) but teff was lowest (DS of 0.50) and the remaining plants had mid-range DS values. Eggplants grown in the crop soils ranged from 37.5% DI (Sudangrass and teff) to 62.5 % (wheat) and finally, 75% ("no green manure"), although this was just a trend with no statistical significance. Teff showed the most disease (DS of 1.56 /DI of 50%) in <u>Field F</u>, with trends only, suggesting that the lowest DS /DI ratings were for the wheat /millet soils (both 0.11 DS, 12.5% DI) followed by vetch (0.2 DS /12.5% DI).

With <u>Field G</u>, however, nightshade was grown rather than teff, so there is an additional column on all of the tables for this crop. As there wasn't a potato crop around this plot, it didn't have the "no green manure" treatment. Eggplants grown in the nightshade soil actually had a wilt DS rating of 1.75, which was ca. 3X higher than the remaining soil samples, ranging from 0 (ryegrass) to 0.63 (wheat and Sudangrass) but data were insignificant. Also, 50% of the plants growing in nightshade soil were wilted, whereas ryegrass had no wilting and the remaining crops were 12.5 – 25% DI.

Tables 17 – 24: For the culture plates ratings (black dot, fusarium, *V. dahliae* and rhizoctonia), a general observation was that the first two pathogens were very prevalent, agreeing with previous findings from the green manure crop year. There weren't any <u>Field A</u> diseased plants for dissection, because the results were all 0%. <u>Field B</u> showed lower DI% trends for both black dot and fusarium on the eggplant roots during the potato crop rotation, as compared to the green manure crop incorporation year, except for those in the vetch soil with slightly higher black dot levels. Although this data were statistically insignificant, it is still noteworthy that the black dot DI levels for the other soil samples were decreased, by at least 65% from before. Fusarium root disease levels were also greatly reduced on the eggplants in the mustard and radish soils, suggesting that green manure crop incorporation *may* be effective for disease prevention in potato crops. With the stem cultures, the black dot DI% data were statistically significant, with the eggplants in the vetch soil having more disease (66.7% - potato year vs. 75% green manure incorporation year) than plants in the other soil samples. Also, these same crop soils (excluding vetch) showed very dramatic DI decreases since the previous year, especially for wheat (0%), Sudangrass (16.7 %,) mustard (20.8%) and radish (8.3%). The *V. dahliae,* fusarium and *R. solani* eggplant stem DI% data were insignificant but the fusarium DI levels (except vetch), were at least 40% lower during the potato crop year. This data was then very interesting and promising, due to this contrast between the two years.

<u>Fields E, F and G</u>, as noted above, didn't have soil samples collected from them during the green manure incorporation year to compare with. All eggplant black dot DI data were insignificant for <u>Field E</u> and as the negative control values were 79.2 % (roots) and 29.2% (stems), the crop soil sample results were moderately high as well. The fusarium DI data was very high but also insignificant, with root DI % from 50% (millet and teff), 95.8% ("no green manure") and 100% (wheat). Stem DI % was 29.2% (negative control), followed by 41.7% (millet) and up to 100% ("no green manure" /wheat). However, the *V. dahliae* stem and root DI values were all statistically significant, as the negative control (roots) was unfortunately at 79.2% DI and the roots in wheat soil were 20.8% diseased with the remainder at 0%. Eggplant stems from the "no green manure", Sudangrass, vetch and teff soils were also 0%, with millet and ryegrass in the same grouping (2.0% /3.4%) but wheat and the negative control were significantly increased (21% /23.1%). *R. solani* was only present on roots from the "no green manure" soils (8.3% DI) and vetch, ryegrass and teff (4.2%), which was significantly less than the negative control (79.2%). For the stems, the negative control had a DI value of 29.2%, with significantly more diseased stems than "no green manure", Sudangrass and ryegrass (8.3%) with the rest at 0%.

After <u>Field F</u> black dot and fusarium DI data were analyzed, the eggplant stems and roots from all of the soil samples grew these pathogens but with insignificant results. As a trend though, the roots had more black dot than the stems, with the lowest DI demonstrated in the wheat and Sudangrass crops (16.7%), followed by millet and vetch (25%), then teff (58.3%), "no green manure" soil (75%) and finally, the negative control (79.2 DI%). The eggplant stems from wheat and Sudangrass soils had no black dot, the negative control was 29.2% with the "no green manure" soil (58.3% DI) highest of all. The fusarium DI root data showed another trend, with 70.8% to 80% of them diseased from the following soils: "no green manure", ryegrass, teff and the negative control. The stems had similar values for these three crops and the negative control was now 29.2% DI, with the remaining crops having ca. 25% DI for both roots and stems. *V. dahliae* data were statistically significant for these ratings too, showing no growth on any of roots, except for the negative control (79.2%). Similarly, the only stems that grew this pathogen were from the "no green manure", ryegrass and teff crops (extremely low DI values of 4.2%), with the negative control significantly much higher at 29.2%. *R. solani* grew on roots from the ryegrass and teff soils (4.2% /16.7%), which was significantly lower than the negative control (79.2% DI). The eggplant stems, from the "no green manure" and teff soils, showed DI levels of 16.5% and 14.8% respectively, which were statistically similar to the negative control (23.1% DI). Those from the ryegrass soil were significantly less diseased (2.0%) and were in the same Duncan's grouping as the remaining stems (0% DI).

After the <u>Field G</u> data evaluation, statistically insignificant data were tabulated for root /stem black dot; however, the highest DI value was on roots grown in nightshade soil (58.3%) followed by the negative control (37.5%) with the other crops at ca. 25%, except for ryegrass (0%). For the eggplant stems, the negative control was the same but the other crop soil DI values ranged from 0% (ryegrass) to 25% (wheat). Fusarium root and stem DI values were similar, although the stems had statistically significant data, where all eggplants, except for those grown in nightshade soil (70.8%), were less diseased than the negative control at 95.8% DI. Ryegrass soil (0% DI) was in the same Duncan's grouping as vetch (16.7%), wheat, millet and Sudangrass (all 25%). Neither *V. dahliae* nor *R. solani* grew on any of the eggplant roots but *V. dahliae* was observed at significant levels on the negative control stems (8.3% DI), with the remaining crops at 0% DI.

For this final potato crop year, the negative control perhaps was stored longer and became more diseased unfortunately, so it wasn't suitable to use for this study but this wasn't apparent until these evaluations were performed and results were statistically significant because of this factor. The hail storms of 2008 and 2009 also confounded some of the data for Fields E and F, so that results were likely higher for these fields due to plant damage. For the eggplant roots and stems, grown in the field soils as part of this bioassay experiment, black dot and fusarium caused the most disease on them, showing the high economic impact these pathogens may have also on potato crops.

Table 19: *Fusarium spp.* disease incidence (DI%)[4] means on eggplant root culture isolates from plants growing in the field soils from southern Alberta, before /after green manures incorporation and potato crops. Data followed by the same letter in each row were not statistically different ($p \geq .05$).

Field	No Green Manure	Wheat	CFPM 101	Sorghum Sudangrass	Vetch/ OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Nightshade	Negative Control
Green manure crop year												
A[5]	0.0 a	0.0 a	0.0 a	0.0 a	20.8 a	12.5 a	0.0 a					0.0 a
B[6]	60.1 a	82.8 a	78.0 a	77.6 a	76.3 a				78.0 a	74.2 a		0.0 b
After green manure incorporation												
A[6]	0.0 a	0.0 a	0.0 a	0.0 a	8.7 a	8.7 a	0.0 a					0.0 a
B[8]	83.3	54.2	62.5	95.8	77.8				100.0	75.0		0.0
C[5]	58.3 a	0.0 b	0.0 b	0.0 b	0.0 a	0.0 b						0.0 b
D[9]	0.0	0.0	0.0	0.0	0.0	0.0	0.0					0.0
E[2,3]	---	---	---	---	---	---		---				
F[2,3]	---	---	---	---	---	---		---				
G[3]	---	---	---	---	---	---					---	
After potato crop year												
A[9]		0.0	0.0	0.0	0.0				0.0	0.0		0.0
B[5]		37.5 a	54.17 a	50.0 a	66.7 a				37.5 a	25.0 a		0.0 a
C[3]	---	---	---	---	---	---						
D[1]	---	---	---	---	---	---	---					---
E[2,5]	95.8 a	100.0 a	50.0 a	70.8 a	75.0 a	75.0 a		50.0 a				79.2 a
F[2,5]	70.8 a	25.0 a	25.0 a	20.8 a	25.0 a	75.0 a		75.0 a				79.2 a
G[5]	----[10]	25.0 a	25.0 a	25.0 a	25.0 a	0.0 a					75.0 a	95.8

[1]Field not planted to potatoes following green manure crop year.
[2]Hail storms in summer (2008 and 2009) affected green manure crops and potato crop in the following year.
[3]No samples were collected prior to incorporation of green manure in 2007 and 2008 or insufficient soil quantities for eggplant bioassays were collected in 2008.
[4] Plant disease incidence means (DI) are based on the percentage of roots evaluated per crop growing on PDA-A and CZA-S agar plates with *Fusarium spp*. on them.
[5]Raw data were used for analysis.
[6]Square root-transformed data were used for analysis with detransformed means presented.
[7]Arcsine-transformed data were used for analysis with detransformed means presented.
[8]Raw data were used for analysis but letters are not in this row because this analysis failed the Bartlett's test for homogeneity (was significant).
[9]No statistical analysis was performed for this row because either because these results were all 0s or only one plant was infected.
[10]There were no potato crops that were planted outside this experimental plot to obtain soil from.

Table 20: *Fusarium spp.* disease incidence (DI%)[4] means on eggplant stem culture isolates from plants growing in the field soils from southern Alberta, before /after green manures incorporation and potato crops. Data followed by the same letter in each row were not statistically different ($p \geq .05$).

Field	No Green Manure	Wheat	CFPM 101	Sorghum Sudangrass	Vetch/ OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Nightshade	Negative Control
Green manure crop year												
A[6]	0.0 a	0.0 a	0.0 a	0.0 a	8.7 a	6.2 a	0.0 a					0.0 a
B[6]	77.6 a	88.7 a	82.9 a	91.5 a	88.2 a				82.9 a	95.7 a		0.0 b
After green manure incorporation												
A[6]	0.0 a	0.0 a	0.0 a	0.0 a	6.2 a	8.7 a	0.0 a					0.0 a
B[7]	93.4 ab	60.4 b	93.4 ab	100.0 a	100.0 a				100.0 a	98.9 a		0.0 c
C[5]	70.8 a	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b						0.0 b
D[8]	0.0	4.2	0.0	0.0	0.0	0.0	0.0					0.0
E[2,3]	---	---	---	---	---	---		---				
F[2,3]	---	---	---	---	---	---		---				
G[3]	---	---	---	---	---	---					---	
After potato crop year												
A[8]		0.0	0.0	0.0	0.0				0.0	0.0		0.0
B[5]		37.5 a	58.3 a	50.0 a	88.9 a				37.5 a	37.5 a		0.0 a
C[3]	---	---	---	---	---	---						
D[1]	---	---	---	---	---	---	---					---
E[2,5]	100.0	100.0 a	41.7 a	75.0 a	75.0 a	75.0 a		50.0 a				29.2 a
F[2,5]	75.0 a	25.0 a	25.0 a	25.0 a	25.0 a	75.0 a		75.0 a				29.2 a
G[5]	----[9]	25.0 bc	25.0 bc	25.0 bc	16.7 bc	0.0 c					70.8 ab	95.8 a

[1]Field not planted to potatoes following green manure crop year.
[2]Hail storms in summer (2008 and 2009) affected green manure crops and potato crop in the following year.
[3]No samples were collected prior to incorporation of green manure in 2007 and 2008 or insufficient soil quantities for eggplant bioassays were collected in 2008.
[4] Plant disease incidence means (DI) are based on the percentage of stems evaluated per crop growing on PDA-A and CZA-S agar plates with *Fusarium spp*. on them.
[5]Raw data were used for analysis.
[6]Square root-transformed data were used for analysis with detransformed means presented.
[7]Arcsine-transformed data were used for analysis with detransformed means presented.
[8]No statistical analysis was performed for this row because either because these results were all 0s or only one plant was infected.
[9]There were no potato crops that were planted outside this experimental plot to obtain soil from.

Table 21: *V. dahliae* disease incidence (DI%)[4] means on eggplant root culture isolates from plants growing in the field soils from southern Alberta, before /after green manures incorporation and potato crops. Data followed by the same letter in each row were not statistically different ($p \geq .05$).

Field	No Green Manure	Wheat	CFPM 101	Sorghum Sudangrass	Vetch/ OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Nightshade	Negative Control
Green manure crop year												
A[9]	0.0	0.0	0.0	0.0	0.0	0.0	0.0					0.0
B[6]	1.95 a	0.0 a	0.0 a	0.0 a	0.0 a				0.0 a	0.0 a		0.0 a
After green manure incorporation												
A[9]	0.0	0.0	0.0	0.0	0.0				0.0	0.0		0.0
B[6]	0.0 a	2.0 a	2.0 a	0.0 a	0.0 a				0.0 a	0.0 a		0.0 a
C[9]	0.0	0.0	0.0	0.0	0.0	0.0						0.0
D[9]	0.0	0.0	0.0	0.0	0.0	0.0	0.0					0.0
E[2,3]	---	---	---	---	---	---		---				
F[2,3]	---	---	---	---	---	---		---				
G[3]	---	---	---	---	---	---					---	
After potato crop year												
A[9]		0.0	0.0	0.0	0.0				0.0	0.0		0.0
B[5]		0.0 a	0.0 a	4.17 a	0.0 a				0.0 a	0.0 a		0.0 a
C[3]	---	---	---	---	---	---						
D[1]	---	---	---	---	---	---	---					---
E[2,5]	0.0 b	20.8 b	0.0 b	0.0 b	0.0 b	0.0 b		0.0 b				79.2 a
F[2,5]	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b		0.0 b				79.2 a
G[9]	----[10]	0.0	0.0	0.0	0.0	0.0					0.0	0.0

[1]Field not planted to potatoes following green manure crop year.
[2]Hail storms in summer (2008 and 2009) affected green manure crops and potato crop in the following year.
[3]No samples were collected prior to incorporation of green manure in 2007 and 2008 or insufficient soil quantities for eggplant bioassays were collected in 2008.
[4] Plant disease incidence means (DI) are based on the percentage of roots evaluated per crop growing on PDA-A and CZA-S agar plates with *V.dahliae on* them.
[5]Raw data were used for analysis.
[6]Square root-transformed data were used for analysis with detransformed means presented.
[7]Arcsine-transformed data were used for analysis with detransformed means presented.
[8]Raw data were used for analysis but letters are not in this row because this analysis failed the Bartlett's test for homogeneity (was significant).
[9]No statistical analysis was performed for this row because either because these results were all 0s or only one plant was infected.
[10]There were no potato crops that were planted outside this experimental plot to obtain soil from.

Table 22: *V. dahliae* disease incidence (DI%)[4] means on eggplant stem culture isolates from plants growing in the field soils from southern Alberta, before /after green manures incorporation and potato crops. Data followed by the same letter in each row were not statistically different ($p \geq .05$).

Field	No Green Manure	Wheat	CFPM 101	Sorghum Sudangrass	Vetch/ OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Nightshade	Negative Control
Green manure crop year												
A[8]	0.0	0.0	0.0	0.0	0.0	0.0	0.0					0.0
B[6]	9.5 a	8.5 a	20.1 a	23.4 a	8.5 a				14.4 a	2.0 a		0.00 a
After green manure incorporation												
A[8]	0.0	0.0	0.0	0.0	0.0				0.0	0.0		0.0
B[5]	50.0 ab	16.7 cd	33.3 bc	37.5 bc	77.8 a				54.2 ab	33.3 bc		0.0 d
C[5]	25.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a						0.0 a
D[8]	0.0	12.5	0.0	0.0	0.0	0.0	0.0					0.0
E[2,3]	---	---	---	---	---	---		---				
F[2,3]	---	---	---	---	---	---		---				
G[3]	---	---	---	---	---	---					---	
After potato crop year												
A[8]		0.0	0.0	0.0	0.0				0.0	0.0		0.0
B[5]		29.2 a	25.0 a	12.5 a	50.0 a				12.5 a	25.0 a		0.0 a
C[3]	---	---	---	---	---	---						
D[1]	---	---	---	---	---	---	---					---
E[2,6]	0.0 c	21.0 ab	2.0 bc	0.0 c	0.0 c	3.4 abc		0.0 c				23.1 a
F[2,5]	4.2 b	0.0 b	4.2 b	0.0 b	0.0 b	4.2 b		0.0 b				29.2 a
G[5]	----[9]	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b					0.0 b	8.3 a

[1]Field not planted to potatoes following green manure crop year.
[2]Hail storms in summer (2008 and 2009) affected green manure crops and potato crop in the following year.
[3]No samples were collected prior to incorporation of green manure in 2007 and 2008 or insufficient soil quantities for eggplant bioassays were collected in 2008.
[4] Plant disease incidence means (DI) are based on the percentage stems of evaluated per crop growing on PDA-A and CZA-S agar plates with *V.dahliae* on them.
[5]Raw data were used for analysis.
[6]Square root-transformed data were used for analysis with detransformed means presented.
[7]Arcsine-transformed data were used for analysis with detransformed means presented.
[8]No statistical analysis was performed for this row because either because these results were all 0s or only one plant was infected.
[9]There were no potato crops that were planted outside this experimental plot to obtain soil from.

Table 23: Rhizoctonia disease incidence (DI%)[4] means on eggplant root culture isolates from plants growing in the field soils from southern Alberta, before /after green manures incorporation and potato crops. Data followed by the same letter in each row were not statistically different ($p \geq .05$).

Field	No Green Manure	Wheat	CFPM 101	Sorghum Sudangrass	Vetch/ OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Nightshade	Negative Control
Green manure crop year												
A[9]	0.0	0.0	0.0	0.0	0.0	0.0	0.0					0.0
B[9]	0.0	0.0	0.0	0.0	0.0				0.0	0.0		0.0
After green manure incorporation												
A[9]	0.0	0.0	0.0	0.0	0.0				0.0	0.0		0.0
B[6]	0.0 a	0.0 a	8.4 a	2.0 a	0.0 a				0.0 a	2.0 a		0.0 a
C[5]	4.17 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a						0.0 a
D[8]	0.0	0.0	0.0	0.0	0.0	0.0	0.0					0.0
E[2,3]	---	---	---	---	---	---		---				
F[2,3]	---	---	---	---	---	---		---				
G[3]	---	---	---	---	---	---					---	
After potato crop year												
A[9]		0.0	0.0	0.0	0.0				0.0	0.0		0.0
B[5]		0.0 a	4.2 a	0.0 a	0.0 a				0.0 a	0.0 a		0.0 a
C[3]	---	---	---	---	---	---						
D[1]	---	---	---	---	---	---	---					---
E[2,5]	8.3 b	0.0 b	0.0 b	0.0 b	4.2 b	4.2 b		4.2 b				79.2 a
F[2,5]	0.0 b	8.3 b	0.0 b	0.0 b	0.0 b	4.2 b		16.7 b				79.2 a
G[,9]	----[10]	0.0	0.0	0.0	0.0	0.0					0.0	0.0

[1]Field not planted to potatoes following green manure crop year.
[2]Hail storms in summer (2008 and 2009) affected green manure crops and potato crop in the following year.
[3]No samples were collected prior to incorporation of green manure in 2007 and 2008 or insufficient soil quantities for eggplant bioassays were collected in 2008.
[4] Plant disease incidence means (DI) are based on the percentage of roots evaluated per crop growing on PDA-A and CZA-S agar plates with rhizoctonia on them.
[5]Raw data were used for analysis.
[6]Square root-transformed data were used for analysis with detransformed means presented.
[7]Arcsine-transformed data were used for analysis with detransformed means presented.
[8]Raw data were used for analysis but letters are not in this row because this analysis failed the Bartlett's test for homogeneity (was significant).
[9]No statistical analysis was performed for this row because either because these results were all 0s or only one plant was infected.
[10]There were no potato crops that were planted outside this experimental plot to obtain soil from.

Table 24: Rhizoctonia disease incidence (DI%)[4] means on eggplant stem culture isolates from plants growing in the field soils from southern Alberta, before /after green manures incorporation and potato crops. Data followed by the same letter in each row were not statistically different ($p \geq .05$).

Field	No Green Manure	Wheat	CFPM 101	Sorghum Sudangrass	Vetch/ OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Nightshade	Negative Control
Green manure crop year												
A[8]	0.0	0.0	0.0	0.0	0.0	0.0	0.0					0.0
B[5]	0.0 a	0.0 a	0.0 a	4.2 a	0.0 a				0.0 a	0.0 a		0.0 a
After green manure incorporation												
A[8]	0.0	0.0	0.0	0.0	0.0				0.0	0.0		0.0
B[6]	0.0 a	0.0 a	0.0 a	0.0 a	2.9 a				2.0 a	0.0 a		0.0 a
C[8]	0.0	0.0	0.0	0.0	0.0				0.0	0.0		0.0
D[8]	0.0	0.0	0.0	0.0	0.0	0.0	0.0					0.0
E[2,3]	---	---	---	---	---	---		---				
F[2,3]	---	---	---	---	---	---		---				
G[3]	---	---	---	---	---	---					---	
After potato crop year												
A[8]		0.0	0.0	0.0	0.0				0.0	0.0		0.0
B[5]		0.0 a	0.0 a	0.0 a	5.6 a				4.2 a	0.0 a		0.0 a
C[3]	---	---	---	---	---	---						
D[1]	---	---	---	---	---	---	---					---
E[2,5]	8.3 b	0.0 b	0.0 b	8.3 b	0.0 b	8.3 b		0.0 b				29.2 a
F[2,6]	16.5 ab	0.0 c	0.0 c	0.0 c	0.0 c	2.0 bc		14.8 ab				23.1 a
G[8]	----[9]	0.0	0.0	0.0	0.0	0.0					0.0	0.0

[1]Field not planted to potatoes following green manure crop year.
[2]Hail storms in summer (2008 and 2009) affected green manure crops and potato crop in the following year.
[3]No samples were collected prior to incorporation of green manure in 2007 and 2008 <u>or</u> insufficient soil quantities for eggplant bioassays were collected in 2008.
[4] Plant disease incidence means (DI) are based on the percentage of stems evaluated per crop growing on PDA-A and CZA-S agar plates with rhizoctonia on them.
[5]Raw data were used for analysis.
[6]Square root-transformed data were used for analysis with detransformed means presented.
[7]Arcsine-transformed data were used for analysis with detransformed means presented.
[8]No statistical analysis was performed for this row because either because these results were all 0s or only one plant was infected.
[9]There were no potato crops that were planted outside this experimental plot to obtain soil from.

Molecular Determination of Verticillium Inoculum - Alberta

Growth of fungal and plant materials for positive controls

Stock cultures of *Verticillium albo-atrum* and *V. dahliae* isolated from potato were axenically stored as a conidial suspension in aqueous glycerol at - 70 ° C. To revive stock cultures, single droplets of thawed suspension were pipetted directly onto potato dextrose agar (PDA). To obtain conidia, the resulting mycelial colonies were maintained at 22 °C in the dark and spores were harvested 4 weeks later in sterile distilled water or colonies were grown in Czapek's broth at 22 °C with shaking. Potato plants of the cultivar Shepody were grown in a growth chamber with a 12 h photoperiod. Day and night temperatures were 22 °C and 18 °C, respectively. Young rooted cuttings from cloned susceptible plants were root-dip inoculated into suspensions of 1.5 x 10^7 conidia of *V. albo-atrum* or *V. dahliae* per ml of sterile distilled water.

Potato leaves, stems and roots were collected for DNA extraction when the cuttings inoculated with *V. albo-atrum* began to develop wilt symptoms. A sample of the leaflets, as well as stem and root segments from the same plant were retained for attempted isolation of the fungi. These plant tissues were surface sterilized by dipping for 30 s in 70% ethanol, followed by 1 min in 0.5% sodium hypochlorite and 3 min of agitation in sterile distilled water. The surface sterilized segments were plated on PDA medium and the plates checked periodically for the growth of *Verticillium*.

Polymerase chain reaction

Genomic DNA from *Verticillium* and potato was extracted by the hexadecyltrimethylammonium bromide (CTAB) method. Filtered *Verticillium* hyphae from mycelial cultures or finely-cut plant pieces were ground to a coarse powder in the presence of liquid nitrogen; 1-3 g of ground tissue was suspended in extraction buffer (1.4M NaCl, 20 mm EDTA, 0.1M Tris-HCI, pH 8.0 containing 1% PVP-40 and 2 % CTAB). The suspension was extracted twice with chloroform/isoamyl alcohol and precipitated with 2 volumes of ethanol containing 2% potassium acetate.

PCR amplification was conducted in 50 µl of PCR buffer containing 0.2 mm bovine serum albumin (BSA), 0.2 mm of each deoxyribonucleotide triphosphate, 12.5 pmol of each oligonucleotide primer, 0.01-0.05 µg of DNA extract and 2g of Taq DNA polymerase (Promega, Madison, Wisconsin). The amplification was performed in a programmable heating block (Ericomp Co., San Diego, California) using 30 reaction cycles consisting of a 1m denaturation step at 94 °C, a 1minute annealing step at 60 °C and a 2 minute elongation step at 72 °C. The DNA products were precipitated with ethanol, resuspended in 25 % formamide dye and analysed on by polyacrylamide gel electrophoresis at 1000 V for 3-4 h. The gels were stained with ethidium bromide prior to photography at 300 nm on a transilluminator.

Verticillium extraction and quantitation from soil

A 10 ml beaker was used to take samples of soil from the each of the replicate samples in a particular crop field. These sub-samples were combined and mixed in a 50 ml conical tube. Approximately 3 gm of each combined sample was weighed out and DNA was extracted. Soil DNA was extracted using the Power Soil DNA Kit (MoBio Laboratories Inc., Carlsbad, CA). To enhance DNA recovery, an alternate lysis method involving incubation at high temp (65°C for 10 min) before beadbeating was performed. Isolated DNA was amplified by PCR using primers specific to the rRNA ITS region of *Verticillium* species (ITS-1F 5'tcaaacttggtcatttagaggaagtaaaagtcg3' and ST-Ve1 5'ccgttgttaaaagttttaatggttcgctaaga3'). PCR was performed for 30 cycles of 1 minute at 94°C, 1 minute at 60°C, and 1 minute at 72°C. Reaction products were run on a 1% agarose gel for 1 hour at 60V and visualized through Ethidium Bromide staining of the gel and transillumination at 300 nM. Verticillium semi-quantitation in soil samples was based on an arbitrary scale of 0-3, zero being equivalent to the negative PCR control and 3 being equivalent to the highly infected positive PCR control.

<u>Cloning and sequencing rDNA</u>

Genomic DNA was prepared from fungal and plant tissues by an adaptation of standardized procedures previously developed for *Verticillium* spp. To extract the nucleic acid, the sample was ground with liquid nitrogen before adding 2 mL of 0.15 M NaCl, 0.1M EDTA, pH 8.0. Proteinase K (Sigma Chemical Co., St. Louis, Missouri) was added (75 g/ml) and the tissues were lysed with sodium dodecylsulphate (1% final concentration) and gentle mixing at 37 °C for 3 h. The suspension was further heated at 60 °C for 30 min and extracted at room temperature with an equal volume of chloroform/isoamyl alcohol (24 :1). The nucleic acid was precipitated from the aqueous phase with ethanol, resuspended in 0.15 M NaCl, 0.1 M EDTA, pH 8.0, treated with RNAase A ribonuclease and re-extracted with chloroform/isoamyl alcohol. To construct genomic libraries, purified *Verticillium* DNA was digested with *Eco*RI endonuclease and cloned into the BlueScript II vector (Stratagene, California). The inserts from such recombinants were sequenced by the dideoxy method using T3 and T7 primers.

<u>Results and Discussion</u>

Analysis of the soil and stem samples with PCR specific for Verticillium spp. rDNA ITS have shown various levels of verticillium. *Verticillium dahliae* was the predominant species in the samples although other *Verticillium* species were detected including *Verticillium albo-atrum* and *Verticillium nigrescens.* Primers were designed for conserved sequences in the ribosomal DNA sequences to obtain the hypervariable sequences of the *Verticillium* species. Soil DNA extractions were successful using the MoBio Ultra Clean Soil DNA Isolation Kit (#12800-50). Maximum yield was obtained from 0.25 gm of soil. Soil samples spiked with verticillium species were positive with the polymerase chain reaction amplifications and greenhouse soil negative. The assay was semi-quantitative in determining the pathogen titres in soil and tissue samples (Figure 1 and Table 1).

We have also examined samples from another AAFC rotation with the developed amplification procedures and found that the levels of the verticillium wilt disease have dropped substantially in a 5-year sustainable rotation even though the pathogen levels remain virtually unchanged (Larney et al 2009). This indicates that other parameters influenced the incidence and severity of the verticillium wilt. Perhaps most interesting and surprising has been the increase in the diversity of the associated microbial populations in soil samples as detected by generic rDNA ITS amplification and sequencing. For example, the presence of a nematophagous fungus and potential biocontrol agent *Plectosphaerella cucumerina* was detected in the 5-year rotation. This likely reduces the nematode populations and severity of the early dying as the nematodes are known to play an important role in early dying disease. In addition, the increased presence of other microbes such as species of *Fusarium* in specific rotations with reduced early dying, indicates that the other microbial organisms may be out-competing the *Verticillium s*pecies and represent an important component in reducing disease levels. Diagnostics developed to assess the microbial population of soils prior to planting should assist in predicting disease levels. Furthermore, beneficial crop rotations and the promotion of beneficial soil microbes should reduce disease levels for verticillium wilt and other plant diseases.

Table 25: Molecular detection and quantification of Verticillium species in soil samples from fields in southern Alberta before and after incorporation of green manures. Data were generated from composite samples for each crop. Verticillium semi-quantitation in soil samples was based on an arbitrary scale of 0 to +++, with zero being equivalent to the negative PCR control and +++ being equivalent to the highly infected positive PCR control.

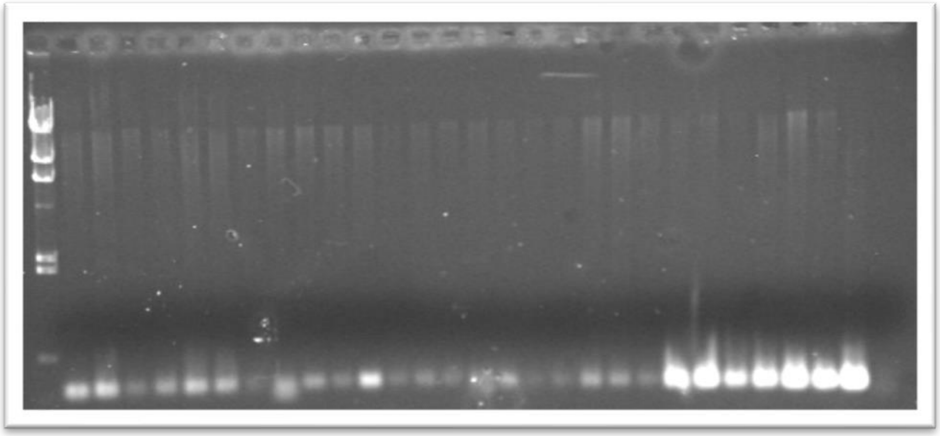
Field	No Green Manure	Wheat	CFPM 101	Sorghum Sudangrass	Vetch/ OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Nightshade
After green manure incorporation											
A											
B											
C	+	++	-	-	-	+	n/a				
D	+	-	-	-	-	-	-				
E[2]	+	++	+	+	+	+		+			
F[2]	+	++	+	+	++	++		++			
G	+	++	++	+	++	+					++
After potato crop year											
A	n/a	+	+	+++	+	+	+				
B	n/a	+++	+	+	-				++	-	
C	+++	+++	+++	+++	+++	+++	n/a				
D[1]	n/a	n/a	n/a	n/a	n/a	n/a	n/a				
E[2]	+	+	+	+	-	-		-			
F[2]	+++	+++	+++	++	+++	+++		+++			
G	n/a	+	+	+	+	+					++

[1]Field not planted to potatoes following green manure crop year.
[2]Hail storms in summer (2008 and 2009) affected green manure crops and potato crop in the following year.

Table 26: Molecular detection and quantification of Verticillium species in potato samples collected from fields in southern Alberta the year following incorporation of green manures. Data were generated from composite samples for each crop. Verticillium semi-quantitation in stem samples was based on an arbitrary scale of - to +++, with zero being equivalent to the negative PCR control and +++ being equivalent to the highly infected positive PCR control.

Field	No Green Manure	Wheat	CFPM 101	Sorghum Sudangrass	Vetch/ OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Nightshade
After potato crop year											
A	n/a	-	-	-	++	-	-				
B	n/a	-	+	-	-				-	-	
C	n/a	+++	+++	+++	+++	+++	n/a				
D[1]	n/a	n/a	n/a	n/a	n/a	n/a	n/a				
E[2]	-	-	-	+	+	-		-			
F[2]	+++	++	+++	+++	+++	++		+++			
G	n/a	n/a	n/a	n/a	n/a	n/a					n/a

[1]Field not planted to potatoes following green manure crop year.
[2]Hail storms in summer (2008 and 2009) affected green manure crops and potato crop in the following year.



Lane 1: Lamda HindIII DNA ladder.
Lanes 2-8: Field G – OPV, CFPM, Sorghum Sudangrass, ryegrass, wheat, nightshade, no green manure.
Lanes 9-12: Field F – OPV, CFPM, Sorghum Sudangrass, ryegrass.
Lanes 13-19: Field E – OPV, CFPM, Sorghum Sudangrass, ryegrass, wheat, teff, non green manure.
Lanes 20-22: Field F – wheat, teff, no green manure
Lanes 23-28: Field C – OPV, CFPM, Sorghum Sudangrass, ryegrass, wheat, no green manure.
Lane 29: Positive control.
Lane 30: Negative control.

Figure 1. Agarose gel showing the amplified ribosomal DNA from soil and plant tissue samples.

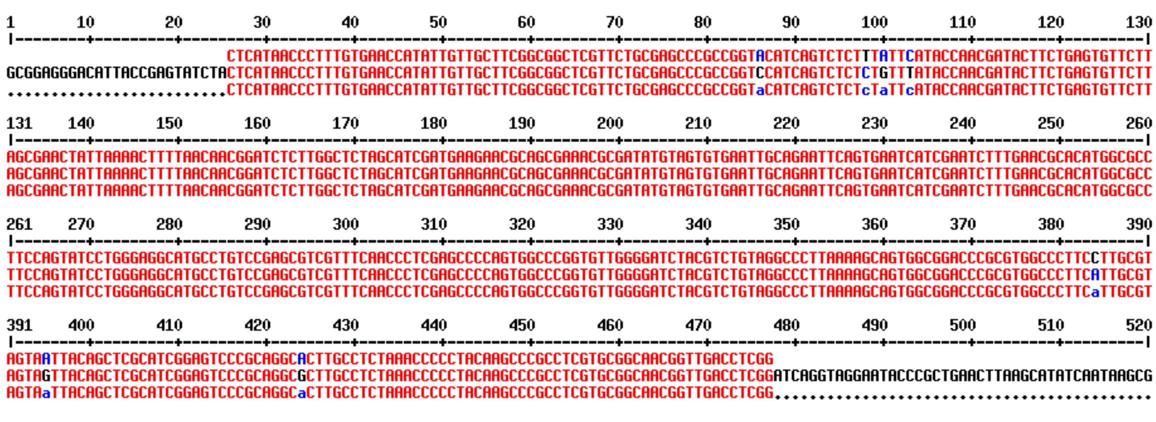


Figure 2. Sequences of the internal transcribed hypervariable sequence from the ribosomal RNA gene of *Verticillium albo-atrum* (top) and *Verticillium dahliae* (middle), and consensus (bottom).

Potato Crop - Alberta

Both fields where green manure plots were grown in 2006 were planted to processing potatoes in 2007 by commercial co-operators: Field A was planted to Shepody (early) potatoes; and Field B was planted to Russet Burbank (late) potatoes. Only one of the fields where green manure plots were grown in 2007 was planted to processing potatoes in 2008: Field C was planted to Russet Burbank potatoes; and Field D was planted to a different crop and no subsequent data was collected from the plots. All three fields where green manure plots were grown in 2008 were planted to potatoes in 2009: Fields E, F and G were planted to Russet Burbank potatoes. Potatoes grown in commercial fields were planted and managed throughout the growing season following each grower's usual practices. Plots were relocated within the potato crop with the help of metal detectors to find corner pins, GPS equipment and measurements from landmarks where possible. Samples were taken from the central area of each plot to ensure data was collected from the treatments area and not at the border of the treatment area.

Potato stem samples were collected from each site prior to harvest, brought back to CDCS, and evaluated for symptoms of vascular discoloration, which are typical of infection by vascular pathogens such as *Verticillium*. Tissue pieces from symptomatic stems were plated onto agar media to confirm the presence of fungal pathogens. Stem samples were also forwarded to AAFC, Lethbridge for molecular diagnosis of *Verticillium* infection.

In 2007, tubers from 6 m strips from the centre of each green-manured area were harvested August 9 from Field A and September 6 from Field B with a one-row Checci digger. Field weights were recorded, and tubers were graded into size categories.

In 2008, tubers from 6 m strips from the centre of each green-manured area were harvested September 8 from Field C with a one-row Checci digger. Field weights were recorded, and tubers were graded into size categories. No potatoes were planted in Field D, so no harvest data was available from this location in 2008.

In 2009, tubers from 6 m strips from the centre of each green-manured area were harvested September 9 from Field E and Field F with a one-row Checci digger. Field weights were recorded, and tubers were graded into size categories. Reglone (1.4 L/ac) was applied September 1 to Field G to facilitate mechanical harvest. Tubers from 6 m strips from the centre of each green-manured area were harvested September 15 from Field G with a one-row Checci digger. Tubers were evaluated at CDCS for yield, grade, specific gravity and internal defects.

Tubers were stored at 8˚C until graded. Tubers were graded into size categories (less than 4 oz., 4 – 6 oz., 6 – 10 oz., and over 10 oz. and deformed). A sample of twenty-five tubers (over 4 oz.) from each replicate was used to determine specific gravity using the weight in air over weight in water method. These tubers were cut longitudinally to assess internal defects.

The data presented here have been statistically analyzed using ANOVA and Tukey's Multiple Comparison Test; (SPSS; $p \leq 0.05$). Statistical summaries are available upon request.

Results

Yield of potatoes was not significantly affected by the presence of a green manure crop in the year prior to potatoes in most site years of the study (Table 27). In Field C, the area of the field without a green manure crop resulted in greater marketable yield compared to areas of the field where green manure crops had been grown. This was likely a result of differences in fertility levels between these areas of the field. Green manure crops may have tied up N while the crops were mineralized, while N would have been more available early in the season for area of the field left fallow. Hail storms in August of 2009 affected the total and marketable yield in Fields E and F compared to yields from other site years. In Field F, total yield of potatoes following CPFM 101 was significantly greater than the total yield following Sorghum Sudan grass, however, no significant differences were observed in the marketable yield between plots.

Few significant differences were observed in specific gravity measurements from potatoes grown in green manured areas of the fields (Table 27). Where specific gravity figures were measured from potatoes grown in areas not planted to green manure, the specific gravities tended to be higher than those measured from potatoes grown in previously green-manured plots. In Field B, the Brassica green manure crops (Oriental mustard and oilseed radish) tended to reduce specific gravity relative to other green manure crops, however these crops were dropped from the study after the 2006 crop year in response to growers' concerns.

Table 27: Yield (ton/ac) and specific gravity of potatoes grown the year after green manure crops were incorporated into soil. Data followed by the same letter in each row were not statistically different ($p \geq 0.05$).

Field	No Green Manure	Wheat	CFPM 101	Sorghum Sudangrass	Vetch/ OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Nightshade
Total Yield											
A	n/a	17.43 a	17.40 a	16.91 a	16.72 a	17.35 a	18.36 a				
B	n/a	18.98 a	18.39 a	16.97 a	17.96 a				17.26 a	15.54 a	
C	19.11 a	15.76 a	17.14 a	16.46 a	15.82 a	17.03 a					
D[1]											
E[2]	9.34 a	8.32 a	8.32 a	8.95 b	8.04 a	7.93 a		8.33 a			
F[2]	7.05 ab	4.64 ab	9.56 a	3.43 b	5.63 ab	6.22 ab		4.81 ab			
G	n/a	33.87 a	35.14 a	35.74 a	35.30 a	36.77 a					34.19 a
Marketable Yield (> 4 oz)											
A	n/a	13.29 a	13.49 a	12.81 a	12.40 a	13.36 a	14.37 a				
B	n/a	11.80 a	11.55 a	10.86 a	11.12 a				10.79 a	9.08 a	
C	15.13 a	11.37 b	12.21 b	11.73 b	11.85 b	12.11 b					
D[1]											
E[2]	5.61 a	3.69 a	6.38 a	1.95 a	3.70 a	3.96 a		3.27 a			
F[2]	2.68 a	2.15 a	2.18 a	2.38 a	2.19 a	1.80 a		2.00 a			
G	n/a	26.91 a	27.28 a	27.26 a	28.58 a	28.47 a					26.89 a
Specific Gravity											
A	n/a	1.085 a	1.082 a	1.089 a	1.087 a	1.087 a	1.091 a				
B	n/a	1.091 a	1.091 a	1.089 ab	1.088 ab				1.086 a	1.082 a	
C	1.095 a	1.089 b	1.091 ab	1.087 b	1.092 ab	1.090 b					
D[1]											
E[2]	1.069 a	1.069 a	1.068 a	1.070 a	1.064 a	1.067 a		1.067 a			
F[2]	1.072 a	1.070 a	1.059 a	1.076 a	1.059 a	1.071 a		1.068 a			
G	n/a	1.085 a	1.085 a	1.086 a	1.084 a	1.085 a		1.085 a			

[1]Field not planted to potatoes following green manure crop year.
[2]Hail storm in August affected yield and grade of potatoes.

Potato Disease Evaluation

Materials and Methods

<u>Fields A and B – Potato crop year</u>

In 2007 staff pulled five stem/roots from the outer areas on each side of a subplot for a total of 10 stems, except for Field B with only 6 stems gathered.. The tops were trimmed off with pruning shears so that each stem length was ca. 30 cm long (12"). These were then placed into labelled 15# poly bags, tied shut and placed into coolers with ice packs, prior to transporting them to CDCS. Upon arrival, they were then placed into a 5°C cooler, until disease ratings were performed. Then the stems for each subplot were washed under running tap water to remove the soil and debris, just prior to disease incidence ratings (DI %) for three pathogens. Disease severities were not rated. Each stem was scanned for rhizoctonia canker and Sclerotinia *spp*.; the presence of each disease was recorded. To perform vascular discoloration DI%, the stem top was cut to locate the vascular bundles and discoloration was noted as positive. For verification, each bundle was scraped longitudinally along the stem in three areas and any discoloration was also recorded as positive. DI% was calculated and the stems were rebagged. All stems from subplots were rated by this protocol and then stored in the 5°C cooler again, until 10 cm stem portions were prepared to send to Dr. Kawchuk, with moist chambers set up at CDCS later.

Moist chamber were prepared by using two 8# labeled plastic bags /subplot with moistened paper towels inside. Stems were trimmed to ca. 15 cm. (6") long and 5 were placed into each bag, which was then inflated, sealed and stored at RT for evaluations ca. 1 week later. Then the stems were examined under a dissecting microscope, for *Verticillium /Fusarium spp.* presence and finally, DI% was calculated for each.

<u>Field C – Potato crop year</u>

In 2008, approximately 2-weeks prior to the potato harvest date, the subplots were rated for early dying disease incidence in plants (DI %), early dying disease severity percentage (DS%) and finally, canopy disease severity (DS). The first rating was performed by rating 25 random potato plants /subplot as positive or negative for early dying/wilt symptoms and then calculating the DI%. Then concurrently on these same 25 plants, the number of stems on each was tabulated, with the diseased stem quantity also recorded. The percentage of infected stems was calculated and this was recorded as DS%. For the canopy DS ratings, each subplot was rated on a scale of 0-5 points where 0 = no early dying or wilt, 1 = <1% early dying or wilt, 2 = 1-10% early dying or wilt, 3 = 11-25% early dying or wilt, 4 = 26-50% early dying or wilt and finally,
5 = >50% early dying or wilt.

As before, 10 stems /subplot were then pulled, trimmed, bagged and transported to a CDCS cooler. Stem evaluations were performed the same as before, except instead of rating for sclerotinia, black dot (*Colletotrichum coccoides*) was rated instead, due to its prevalence on the stems. Also, following these ratings, 10 discs were cut /stem and placed into a labeled Petri dish moist chamber (empty dish lined with moistened filtered paper). These were sealed with Parafilm and left at RT under natural light, until rating them ca. 2-weeks later under a dissecting microscope, for *V. dahlia* presence, with DI% tabulated.

<u>Fields E, F and G – Potato crop year</u>

For 2009, there were no in-field examinations performed in Field F, due to extensive hail damage but Field E had all disease rating done, as per 2007, even though there was moderate hail damage there also. Field G had just canopy ratings performed (DS), as this field had been prematurely top-killed. As in 2008, 10 stems /subplot were gathered, trimmed and bagged, along with the same number from four replications in the farmer's potato field bordering Fields E and F only. Only the green manure plot stems were bagged in Field G though, because potatoes weren't planted in the surrounding field. The laboratory tasks for disease ratings were identical to 2008, with moist chambers for the stem discs set up too.

Results and Discussion

Data for all ratings were summarized and analyzed using the ARM 7 statistical software program (Gylling Data Management, Brookings, SD), with the DI and DS experimental means results presented as either raw or de-transformed data. Duncan's Multiple Range Test ($P \leq 0.05$) was used.

Table 28: Potato canopy and stem evaluations for Fields A-C and E-G
Table 29: Stem moist chamber evaluations for Fields A-C and E-G

With <u>Fields A and B</u>, during the potato crop year (2007), in-field ratings weren't being performed yet, so there isn't any data in the first three columns on Table 1. However, stems were still gathered and rated for both fields, with all data statistically insignificant. <u>Field A</u> was healthier overall than Field B, so stem vascular discoloration DI ratings were from 35% (millet) to 60% (vetch), with both *R. solani* and sclerotia having low ratings: *R. solani* ranged from 0% (millet) to 15% (wheat) and sclerotinia DI was from 7.5% (millet) to 17.5% (wheat, Sudangrass and ryegrass). However, the moist chamber readings (Table 27) showed that all of the crops had severe verticillium DI levels (≥ 88%), with moderate fusarium on the stem discs (65.6% on wheat - 85% on ryegrass). No single crop seemed to show less disease than the others.

<u>Field B</u> DI ratings demonstrated vascular discoloration DI levels from 79.2% (wheat) to 95.8% (radish).
R. solani DI was from 33.3% (millet) to 58.4% (Sudangrass), with sclerotinia from 25% (Sudangrass) to 41.7% (millet, vetch and mustard). The moist chambers showed wheat and Sudangrass with very high verticillium DI ratings (83.3% and 87.5%) but all stem discs from the other four crops grew this pathogen (100% DI). Fusarium developed on the discs at moderately low levels only, ranging from 4.2% (radish) up to 45.8% (Sudangrass).

All <u>Field C</u> ratings were statistically insignificant in 2008, which was the first year that in-field ratings were done. When it received canopy disease severity (DS) evaluations (0-5 point scale), results showed moderate disease /crop, that were from 1.60 (Sudangrass) up to 3.31 (millet). Low to moderate plant early dying /wilt DI ratings ranged from 10.6% (wheat) to 53.5% (millet) and similar levels were found on the individual plant stems during the field ratings (DS% levels were from 15.7%: wheat to 42.0%: millet). Interestingly, millet had the highest rating for both parameters, coinciding with the canopy DS evaluations. When 10 stems /subplot were evaluated in the laboratory, all were diseased with rhizoctonia and black dot, but vascular discoloration showed up in them as 71.4% (Sudangrass) up to 86.4% (wheat). The moist chambers only had verticillium DI ratings performed on them, ranging from ca. 30 – 40.8% for all of the crops. Again, as all Field C data were insignificant, no crop stood out as more beneficial than the others.

By 2009, black dots (*C. coccoides*) was very apparent as a potential cause of early dying and wilt damage in potato crops, so this was included in all stem ratings for <u>Fields E-G</u>. With <u>Field E</u>, all field data were statistically insignificant and were moderately high, likely due to the hailstorm, with the canopy DS ratings from 2.25 (millet) to 3.75 (Sudangrass and teff), plant early dying DI levels from 63% (oat-pear-vetch) to 80% (ryegrass) and the stem early dying DS (%) ranged from 40.4% (oat-pea-vetch) up to 59.4% (ryegrass). When stems lab evaluations were performed, all of the vascular discoloration DI ratings were ≥ 94.9%. Stems actually had significantly less rhizoctonia ($P \leq 0.05$) in the "no green manure" area (DI of 0%) in comparison to the other treatments, ranging from 21.4% (Sudangrass) up to 55.9% (millet). However, black dot was present at insignificant, very low levels on the stems (≤10.2 DI). Moist chamber readings were also not significant, showing stem verticillium DI from 3.6 % (wheat) to 20.8% (ryegrass) with moderate black dot, from 11% (no green manure) up to 38% (ryegrass). No trends were observed.

Field F had extensive hail storm damage, so field readings weren't performed and when the stems were evaluated in the laboratory, most ratings were very high but all were statistically insignificant. For example, the stem vascular discoloration DI values were ≥ 82.5% and black dot levels were ≥ 92.5%. However, rhizoctonia was present on the stems, ranging from 57.5% up to 92.5% DI, so wasn't as prevalent. When the moist chambers were read though, low to moderate verticillium counts were present, ranging from ca. 15% (no green manure and Sudangrass) up to 48.8% (teff), but actually, the black dot levels on the stem discs were slightly less (ca. 65% to 78% DI) than on the outside of the stems, as noted on the previous evaluation.

With Field G, all data were insignificant and only the field canopy ratings were taken, with millet at only 0.5 DS up to 1.5 DS for wheat and these values were the lowest for all of the potato fields in this experiment. This field also had nightshade rather than teff, as one of the green manure crops and also, there was not a "no green manure" potato field surrounding it as a comparison. After the stems were collected though, moderate to high DI levels were observed for vascular discoloration (52.5% to 75%), ratings for rhizoctonia were mid-range (35% to 57.5%) and finally, there were overall high levels of black dot on them (≥77.5%). For the moist chamber examinations, verticillium DI levels were extremely low, with only ryegrass and nightshade growing this fungi (0.9% /0.2%), but again, black dot was severe, with DI% levels of ≥ 88.5%.

In conclusion, although all field data were mostly insignificant, black dot was the most prevalent pathogen in Fields C, E, F and G during the potato crop year (2008 and 2009). However in 2007 for Fields A and B, verticillium was very extensive on the stems but then again, black dot wasn't specifically noted or rated that year. During the last two years for the other fields, verticillium levels were only low to moderate. *R. solani* (rhizoctonia disease) likely was a part of this disease complex, with a wide range of DI results for all fields, and at times, it was nearly as severe a pathogen as black dot. No single green manure crop stood out as potentially beneficial for reducing potato early dying complex.

Table 28: Potato canopy and stem evaluations for Fields A-C and E-G during the Potato Crop Year

Field	Crop	Field ratings[1]			Stem ratings performed in the laboratory[1]			
		Canopy DS (0-5)[3]	Plant early dying DI (%)[4]	Stem early dying DS (%)[5]	Vascular discoloration DI (%)[6]	*R. solani* DI (%)[6]	Sclerotinia DI (%)[6]	Black dot DI (%)[6]
A	**Wheat**	---	---	---	47.5 a[7]	15.0 a[7]	17.5 a[7]	---
	Sudan Grass	---	---	---	47.5 a	7.5 a	17.5 a	---
	Millet	---	---	---	35.0 a	0.0 a	7.5 a	---
	Vetch	---	---	---	60.0 a	7.5 a	10.0 a	---
	Phacelia	---	---	---	40.0 a	2.5 a	12.5 a	---
	Ryegrass	---	---	---	45.0 a	2.5 a	17.5 a	---
B	**Wheat**	---	---	---	79.2 a[7]	37.5 a[7]	33.4 a[7]	---
	Sudan Grass	---	---	---	83.3 a	58.4 a	20.8 a	---
	Millet	---	---	---	87.5 a	33.3 a	41.7 a	---
	Vetch	---	---	---	87.5 a	41.7 a	41.7 a	---
	Mustard	---	---	---	91.7 a	37.5 a	41.7 a	---
	Radish	---	---	---	95.8 a	58.3 a	25.0 a	---
C	**Wheat**	2.31 a[8]	10.6 a[8]	15.7 a[8]	86.4 a[8]	100.0	---	100
	Sudan Grass	1.60 a	17.8 a	23.3 a	71.4 a	100.0	---	100
	Millet	3.31 a	53.5 a	42.0 a	76.4 a	100.0	---	100
	Oat-Pea-Vetch	2.60 a	30.5 a	34.3 a	75.8 a	100.0	---	100
	Ryegrass	2.64 a	29.7 a	30.2 a	81.2 a	100.0	---	100
E[2]	**Wheat**	3.50 a[7]	65.0 a[7]	44.1 a[7]	97.2 a[8]	26.3 a[8]	---	10.0 a[8]
	Sudan Grass	3.75 a	75.0 a	57.0 a	100.0 a	21.4 a	---	6.0 a
	Millet	2.25 a	77.0 a	51.2 a	100.0 a	55.9 a	---	1.3 a
	Oat-Pea-Vetch	2.50 a	63.0 a	40.4 a	97.5 a	35.6 a	---	0.0 a
	Ryegrass	3.50 a	80.0 a	59.4 a	94.9 a	32.1 a	---	0.0 a
	Teff	3.75 a	79.0 a	58.0 a	100.0 a	24.5 a	---	6.3 a
	No green manure	3.00 a	69.0 a	48.2 a	100.0 a	0.0 b	---	10.2 a
F[2]	**Wheat**	---	---	---	95.0 a[7]	67.5 a[7]	---	92.5 a[8]
	Sudan Grass	---	---	---	97.5 a	57.5 a	---	99.4 a
	Millet	---	---	---	82.5 a	70.0 a	---	99.4 a
	Oat-Pea-Vetch	---	---	---	85.0 a	82.5 a	---	99.4 a
	Ryegrass	---	---	---	87.5 a	85.0 a	---	99.4 a
	Teff	---	---	---	92.5 a	70.0 a	---	92.5 a
	No green manure	---	---	---	97.5 a	92.5 a		100.0 a
G[9]	**Wheat**	1.50 a[7]	---	---	55.0 a[7]	37.5 a[7]	---	85.0 a

	Sudan Grass	1.00 a	---	---	70.0 a	35.0 a	---	77.5 a
	Millet	0.50 a	---	---	75.0 a	42.5 a	---	85.0 a
	Oat-Pea-Vetch	0.75 a	---	---	75.0 a	37.5 a	---	95.0 a
	Ryegrass	0.75 a	---	---	57.5 a	40.0 a	---	87.5 a
	Nightshade	0.75 a	---	---	52.5 a	57.5 a	---	85.0 a

[1]Results are the means of four replications. Data followed by the same letter in each row were not statistically different ($p \geq 0.05$).

2Summer hail storms (2008 & 2009) affected green manure crops/potato crop the next year; field rating may not have been done.

[3]Plant canopy early dying disease severity (DS) means are on a 0-5 point scale, where 0 = no early dying or wilt, 1 = <1% early dying or wilt, 2 = 1-10% early dying or wilt, 3 = 10-25% early dying or wilt, 4 = 10-25% early dying or wilt, 5 = >50 % early dying or wilt.

[4]Disease incidence means (DI) are the percentage of plants with early dying and/or wilt symptoms.

[5]Stem disease severity means (DS) are the percentage of stems with early dying and/or wilt symptoms

[6]Disease incidence means (DI) are the percentage of stems with vascular discoloration, *R solani*, sclerotinia or black dot.

[7]Raw data were used for analysis.

[8]Either square root or arcsine-transformed data were used for analysis with detransformed means presented.

[9]Field plant and stems ratings were not performed, as this field was top-killed, prior to this task's scheduled date.

Table 29. Potato stem disc moist chamber evaluations for Fields A-C and E-G during the Potato Crop Year[1].

Field	Crop	Stem ratings performed in the laboratory		
		Verticillium DI (%)[3]	Fusarium DI (%)[3]	Black dot DI (%)[3]
A	**Wheat**	94.4 a[5]	65.6 a[4]	---
	Sudan Grass	93.3 a	80.0 a	---
	Millet	97.3 a	67.5 a	---
	Vetch	88.1 a	77.5 a	---
	Phacelia	99.2 a	72.5 a	---
	Ryegrass	92.6 a	85.0 a	---
B	**Wheat**	83.3 a[4]	20.8 a[4]	---
	Sudan Grass	87.5 a	45.8 a	---
	Millet	100.0 a	29.2 a	---
	Vetch	100.0 a	12.5 a	---
	Mustard	100.0 a	20.8 a	---
	Radish	100.0 a	4.2 a	---
C	**Wheat**	30.0 a[5]	---	---
	Sudan Grass	40.8 a	---	---
	Millet	35.2 a	---	---
	Oat-Pea-Vetch	39.3 a	---	---
	Ryegrass	29.3 a	---	---
E[2]	**Wheat**	3.6 a[4]	---	25.1 a[4]
	Sudan Grass	16.0 a	---	33.3 a
	Millet	18.3 a	---	28.5 a
	Oat-Pea-Vetch	10.5 a	---	19.3 a
	Ryegrass	20.8 a	---	38.0 a
	Teff	6.5 a	---	15.5 a
	No green manure	6.3 a	---	11.0 a
F[2]	**Wheat**	30.5 a[4]	---	71.6 a[4]
	Sudan Grass	15.5 a	---	70.0 a
	Millet	40.5 a	---	65.8 a
	Oat-Pea-Vetch	33.3 a	---	74.5 a
	Ryegrass	42.3 a	---	77.0 a
	Teff	48.8 a	---	73.5 a
	No green manure	15.3 a	---	77.5 a
G	**Wheat**	0.0 a[5]	---	95.8 a[4]

	Sudan Grass	0.0 a	---	96.0 a
	Millet	0.0 a	---	88.5 a
	Oat-Pea-Vetch	0.0 a	---	92.0 a
	Ryegrass	0.9 a	---	96.0 a
	Nightshade	0.2 a	---	93.3 a

[1]Results are the means of four replications. Data followed by the same letter in each row were not statistically different ($p \geq 0.05$).
[2]Hail storms in summer (2008 and 2009) affected green manure crops and potato crop in the next year.
[3]Disease incidence means (DI) are the percentage of stem discs placed into moist chambers with verticillium, fusarium or black dot growing on them. For the various fields, all of these pathogens might not necessarily have been rated, depending upon the year.
[4]Raw data were used for analysis.
[5]Either square root or arcsine-transformed data were used for analysis with detransformed means presented.
[6]Field plant and stems ratings were not performed, as this field was top-killed, prior to this task's scheduled date.

Nematode Analyses – 2007

Dr. Guy Belair analyzed soil samples collected before green manure crops were planted and after incorporation of the green manure crops in 2007. Root lesion nematode (*Pratylenchus neglectus*) populations decreased in three of the crops incorporated at one site, but no significant differences were observed at the second location. *Pratylenchus neglectus* were populations ranged from 27 to over 6500 nematodes per kg of soil. Soil samples from potatoes planted on the 2006 green manured areas were also assessed. Only samples from the oilseed radish plots showed significantly fewer nematodes than areas following other green manure crops (Table 30). To date, no funding has been secured for nematode analyses.

Table 30: Population of root lesion nematodes (*Pratylenchus neglectus*) per kg soil in 2007, 2008 and 2009. Data followed by the same letter in each row were not statistically different ($p \geq 0.05$).

Field	No Green Manure	Wheat	CFPM 101	Sorghum Sudangrass	Vetch/ OPV	Ryegrass	Phacelia	Teff	Oriental Mustard	Oilseed Radish	Nightshade
Green manure crop year											
A											
B											
C	3503 a	3851 a	5466 a	2115 a	6249 a	3267 a					
D	6538 a	760 b	5394 ab	400 b	27 c	1155 ab	1524 ab				
E[2,3]		2000 b	2752 b	2864 b	3472 b	5760 a		1408 b			
F[2,3]		944 a	1680 a	1104 a	2448 a	1568 a		480 a			
G[3]		0 a	32 a	0 a	16 a	32 a					48 a
Potato crop year											
A	n/a	4540 a	2378 a	1846 a	1613 a	3674 a	3248 a				
B	n/a	1679 ab	485 ab	2465 a	3029 a				1639 ab	177 b	
C[3]		2064 a	1825 a	2272 a	736 a	2144 a					
D[1]											
E[2,3]		320 a	160 a	448 a	336 a	416 a		432 a			
F[2,3]		256 b	160 b	960 a	96 b	208 b		176 b			
G[3]		240 a	16 a	288 a	32 a	64 a					64 a

[1]Field not planted to potatoes following green manure crop year.
[2]Hail storms in summer (2008 and 2009) affected green manure crops and potato crop in the following year.
[3]In 2008 and 2009, data was converted from number of nematodes per 50 cm^3 sample to number per kg using a bulk density of 1.25 Mg/m^3.

Nematode Analyses – 2008 - 2009

Materials and Methods:

Soil samples collected before green manure crops were planted and after incorporation of the green manure crops were evaluated for root lesion nematode populations in 2008 and 2009. Soil samples were passed through a 5 mm sieve and then 50 cm^3 subsamples were placed on Baermann pans (16 cm diameter) and incubated for 7 days for nematode extraction as described by Forge and Kimpinski (2007). After extraction, the samples were poured into gridded counting dishes, observed with an inverted microscope, and all plant-parasitic nematodes in each sample were counted. Nematodes in the genus *Pratylenchus* (root-lesion nematodes) were the only potentially damaging nematodes detected, so analyses focused on the abundance of *Pratylenchus* sp. nematodes.

Nematode count data were subjected to blocked one-way analysis of variance, with separate analyses for each site in each year. When the effect of cover crop was significant in the overall ANOVA, Fisher's protected least significant difference (LSD) was used to separate means.

Results and Discussion:

Pratylenchus population densities in samples from Fields E and F were overall considerably greater in 2008 than in 2009 (Table 30). In 2008, populations were significantly greater in the Italian ryegrass plots than under other cover crops in Field E. At both sites, the lowest population densities were under Teff, but the differences between Teff and other cover crops were not statistically significant. The observation from 2008, that teff does not support population build-up of *P. neglectus*, is promising and warrants additional research.

In 2009, population densities tended to be greatest in sudangrass plots in Fields E and F. The overall ANOVA was significant ($P = 0.02$) for Field E, and the sudangrass plots had significantly greater population densities than all other cover crops. Nematode population densities in Field G were very low in both years, and did not respond to cover crop treatments.

Based on the absence of males in the populations observed, it appears likely that the *Pratylenchus* populations at these sites were *P. neglectus*, however, a thorough species identification of the populations has not yet been conducted. *P. neglectus* can reduce growth of potato in a density dependent manner (Olthoff 1990; Umesh and Ferris 1994; Hafez et al. 1999), but there are reports of differences in pathogenicity among geographically distinct populations of *P. neglectus*. (Hafez et al. 1999).

Previous research has demonstrated that forage pearl millet can suppress population buildup of *Pratylenchus penetrans* (Belair et al. 2005). Most prior research on the relationships of root-lesion nematodes with *Verticillium* and potato early dying has been conducted with *P. penetrans* (Rowe and Powelson 2002, Rotenberg et al. 2004). *Pratylenchus neglectus* interaction with *V. dahliae* has not been studied extensively (Rowe and Powelson 2002). Research on the pathogenicity of Alberta *P. neglectus* to potato, with and without *Verticillium*, would improve understanding of the role that these nematodes may be having in Alberta potato fields. Similarly, prior research demonstrating the nematode-suppressive effects of Canadian Forage Pearl Millet (e.g. Belair et al. 2005) was conducted on *P. penetrans*. We did not observe any effects of forage pearl millet on *P. neglectus* in this study. The response of *P. neglectus* to forage pearl millet needs to be studied under more controlled conditions. *P. neglectus* has a wide host range including many small-grain crops, particularly wheat, which limits the range of crops that can be incorporated into rotations. Wheat is known to be an excellent host for *P. neglectus*, fostering buildup of *P. neglectus* population densities, and the development of high *P. neglectus* population densities under wheat preceding potato has been observed in a rotation study at Vauxhall (Forge et al. 2009). It is therefore surprising that there were no apparent effects of wheat on *P. neglectus* population densities in this study. One possible reason for the discrepancy could be the length of time between the cover crops and when soil samples were taken for analyses. Additional research, confirming the host status or suppressive effects of teff, could lead to a useful new tool for managing *P. neglectus* populations.

Saskatchewan Trial

Green Manure Crop Establishment

Materials and Methods (2006):
The trial site featured a Sutherland Series sandy loam soil (pH 8.1, E.C. <1.0 ds, with 3.8% O.M.) and has been in a three-year potato rotation for over 30 years. Animal manure is usually applied prior to growing cereals in the rotation, and potatoes were planted in the trial area in 2005. A cereal crop was initially planted in this area in 2006, but was ploughed up at the seedling stage, a month prior to planting the green manure. A randomized block design was used with four replicates of the six treatments that were planted in 8 x 8 m^2 plots, with 2 m pathways between each plot. Prior to seeding, soil was sampled from the trial area to a depth of 12 inches. Four samples were taken at random from each of the four quadrants of the plot (total of 16 samples) and the four quadrant samples were combined to give four composite sample. These samples were stored in a cold room and will be evaluated when funds are available.

The summer seeding date was used to determine if growers could grow a green manure crop following harvest of a winter cereal crop. This field used for this project (scab field) has been used for studying potato production for over 30 years. High levels of disease inoculum for a range of potato diseases are present in the soil. Black scurf (*Rhizoctonia solani*), common scab (*Streptomyces scabies*) and powdery scab (*Spongospora subterranea*) infections are common on tubers grown in this field.

Six green manure crops (Table 31) were seeded on July 11th 2006, in one of the potato field plots managed by the University of Saskatchewan. Heavy seeding rates were used to ensure a dense stand. Granular Furadan was applied to the radish and mustard plots immediately after seeding, to control flea beetles. A light rain (0.3”) occurred prior to planting and the soil was moist but workable. The plots were harrowed once each way immediately after seeding to lightly bury the seed of the green manure crops. The seed bed was moist, and irrigation was applied as necessary to maintain conditions conducive to plant establishment.

Table 31: Green manure crops planted in Saskatchewan plots in 2006.

Crop	***Species & Cultivar***	***Rate***
Oriental Mustard	Cutlass	20 lbs/ac
Oilseed Radish		20 lbs/ac
Sorghum Sudan Grass	Grazex	40 lbs/ac
Woolly Pod Vetch		20 lbs/ac
Canadian Forage Pearl Millet	CFPM Hybrid 101	20 lbs/ac
Hard Red Spring Wheat		240 lbs/ac

2007
Another set of green manure plots was planted July 11th, 2007 in the previously described Scab field. As the woolly vetch treatment had failed to establish in the 2006/2007 trial it was replaced with a green pea/oat/woolly vetch mixture. The 2007 green manure trial area was again heavily infested with pigweed, which had emerged prior to planting the green manure plots on July 11th. In an attempt to control this pigweed population the plot area was cultivated prior to seeding and harrowed again after the seed was broadcast. Despite this effort at weed control an extremely heavy pigweed infestation was present by the end of July. Buctril M (bromoxynil) was applied to the non-brassica plots in an

effort to control this red root pigweed but the level of weed control achieved was not sufficient to allow for adequate establishment of the green manure crops. Consequently, all the green manure plots were ploughed down. Better weed control strategies need to be in place before planting green manure crops in weedy locations.

2008

Green manure plots were established successfully in 2008 at two locations on the University of Saskatchewan field sites to compensate for the lost crops in 2007. The green manure plots were established at two sites on the University of Saskatchewan farm, one in the Main potato field and the other in the previously described Scab field. The Main field and the Scab fields are generally similar in soil type and chemistry. However the Main field has only a limited history of potato production. Levels of soil-borne disease such as *Rhizoctonia* and scab are consequently much lower at this site than in the Scab field.

In 2008, all six green manure treatments were seeded in the Main field, in 6 m^2 plots, using a five row drill seeder at 8 inch row spacing on July 17th. This promoted more uniform crop establishment and allowed for hoeing of weeds if necessary. The 5 treatments plots were seeded to pearl millet, sorghum sudan grass, or a mixture of oat/pea/vetch; allowed to develop a natural population of pigweed; or treated with a mustard meal application, watered and covered for 24 hours. The plots were 18 x 18 ft. Emergence was noted on 23rd July. Weeds developed quickly and on August 5th Buctril M (0.4L/acre) was applied to the millet, sorghum Sudan grass, and bare plot to be used for mustard meal application. The mixture seeded plot was hoed to remove weeds. A randomised block design replicated 4 times was used.

Mustard meal was applied to the bare plots on 15th August at 2lbs meal/100 sq. ft, with a hand-held fertilizer applicator. The plots were watered with ¼' overhead irrigation before covering with clear plastic for 48hr.
The fresh weight of material and the amount of dry matter was estimated for the plots in mid September, and the plots were then flail mowed and immediately cultivated, to incorporate the green material.

The second site used was the Main potato field where there is natural inoculum of Rhizoctonia but low levels of scab inoculum. The same protocols were followed for the trial at the Main site, but the treatments were seeded as follows: 1) spring wheat/pearl millet mixture; 2) sorghum sudan grass; 3) a mixture of oat/pea/vetch; 4) oilseed radish; 5) oriental mustard; or 6) allowed to develop a natural population of pigweed. No mustard meal was applied. Buctril M and hoeing were used for weed control in plots other than treatment 6.

The green manure crops used in 2008 were oat/pea/vetch mixture, oriental mustard (cultivar Cutlass), pearl millet/ spring wheat mixture, sorghum Sudan grass, oilseed radish and an unseeded check plot. In the Scab field, the three green manure treatments tested were millet, sorghum and pea/oat/vetch mixture. No oilseed crops were sown at this site because the weed population in this field was very high, and using only monocot crops allowed the plots to be sprayed with the herbicide Buctril M. A fourth treatment used in the Scab field was an unseeded plot which was not sprayed with herbicide, and which was allowed to develop into a pigweed plot. A fifth treatment in the Scab field involved applying mustard meal to the surface of the plot. Mustard meal is being promoted as a potential herbicide but the thiocyanates present in the meal may also control certain soil-borne diseases. The mustard meal was obtained from Peacock Industries (1 Rheinland Road, Hague, Sk. S0K 1X0).

The green manure plots were seeded in mid-July 2008. Over 0.5 inch of rain fell several days after seeding – this assisted in the establishment of the plots. Seedling emergence was noted five days after seeding.

By the end of July all the green manure crops had emerged. Weed development, mainly red root pigweed, was observed in the unseeded plots, particularly in the Scab field. In early August, the millet and sudan grass plots in both fields and the unseeded plots in the Main field were sprayed with Buctril M applied at 0.4L/acre. No herbicide was applied to the unseeded plot in the Scab field, and it consequently developed a significant population of red root pigweed. Herbicide was applied to the mustard meal plot, eight days prior to the meal application. On August 15th mustard meal was applied to the surface of the unseeded plot in the Scab field at the supplier recommended rate of 2 lb/100 ft^2 (Peacock Industries, 2008 pers. comm.). The plots treated with the mustard meal were overhead irrigated with approximately ¼ inch of water and then covered with sheets of clear plastic for 48 hours. Watering "activates" the mustard meal causing it to release volatile organic compounds such as isothiocyanates which have disease control properties. Covering the treated area with a clear tarp seals in these volatiles, thereby increasing their efficacy. This treatment significantly reduced weed growth in these plots – whether this reflected toxic effects of the mustard meal or overheating due to the presence of the clear plastic cover could not be determined.

The green manure crops were incorporated into the soil in the third week in September, before any of the crops had developed mature seed, and the maximum amount of green material had been produced. Recommended timing for incorporation of Brassica green manure crops is at bolting, when flowers are beginning to form, but no seed has been produced yet. The plant material was shredded with a flail mower and then a rotovator was used to incorporate the green manure into the soil to a depth of 15 cm. In the Main field the wheat plants had begun to fill heads and the vetch was still flowering at the time of plough down. The weedy plots had well developed pigweed plants present at plough down and weeds were also present in the brassica plots. Millet and sudan grass plants had developed leaf spot disease, but this did not appreciably reduce green matter production. In the Scab field the pigweed populations were high in the non-planted "weed" plots, while in the plots treated with mustard meal only a very few, small weed plants were present. The plant material present in two 0.5 m^2 areas per plot, chosen at random, was removed and weighed in both fields, to estimate the amount of green material produced and incorporated.

Green Manure Biomass

2006

By July 20th 2006, the two *Brassica* species had emerged, the wheat seedlings were well established, the millet and sorghum were just emerging, and there was no sign of the vetch. Red root pigweed (*Amaranthus retroflexus*) became a serious weed competitor in all plots by August 4th . A tank mix of the herbicides dicamba (Banvel; 0.125 lb ai/acre) + 2,4-D amine (0.36lb ai/acre) was applied to the cereal plots on August 10th in an effort to control the red root pigweed problem. The herbicide treated pigweed started to wilt by August 15th. At the time of the herbicide treatment there was very little woolly vetch established - these plots were not treated with the herbicide, and were subsequently used as a "pigweed" check. No herbicides were available for pigweed control in the *Brassica* species, and consequently some pigweed was present in these plots. The oriental mustard was flowering by August 8th. The *Brassica* species flowered and formed pods before incorporation of the plots on September 6th. On September 6th a rototiller was used to incorporate the green manure material into the soil to a depth of 15 cm.

Scab Field 2008

The oat/pea/vetch mixture produced the most green matter at plough down in the Scab field, while the mustard meal treatments produced the least organic matter (Table 32). The presence of significant weed populations in the non-manured control treatment did result in the addition of substantial organic matter in those plots.

Table 32. Weight of plant material present in 1m^2 of green manure plots prior to incorporation in the Scab field in 2008.

Green Manure treatment in 2008	Wt. of green plant material /m^2 (kg)
Oat/pea/vetch mixture	4.0 a*
Sorghum Sudan Grass	3.1 b
CFPM 101	2.8 b
No green manure, weedy control	1.6 c
Mustard meal incorporation	0.7 d

*Means followed by the same letter are not significantly different at *p*<.1, using the Student's t-test.

The soil organic matter content of the scab field in mid-June of 2009 ranged from 3.4% in the no green manure weedy control plots to a high of 4.7% in the plots that had been green manured to pea/oat/vetch in the previous season. The relative amounts of organic matter detected in the plots in 2009 roughly corresponds to the relative amount of biomass produced by the green manure crops during the previous year (no manure lowest, oat/pea/vetch mixture highest).

Main Field 2008

The oriental mustard and pea/oat vetch green manures produced the most green manure biomass at plough down in 2008, while the oilseed radish produced the least biomass (Table 33). As a result of weed growth the control plots received a substantial amount of green biomass.

Table 33. Weight of plant material present in 1m^2 of green manure plots prior to incorporation in the Main field in 2008.

Green Manure treatment in 2008	Wt. of green plant material /m^2 (kg)
Oriental mustard	3.9 a*
Pea/oat/vetch mixture	3.7 ab
No manure, weedy control	2.8 bc
Millet	2.3 c
Sorghum	2.1 c
Oilseed radish	1.2 d

*Means followed by the same letter are not significantly different at p<.1, using the Student's t test.

The soil organic matter content of the Main field in mid-June of 2009 ranged from a low of 1.9% in the no manure weed control plots to a high of 2.2% in the plots previously green manured with oilseed radish and millet plots. There was no apparent correlation between the amount of biomass produced by the green manure crop and the soil organic matter content recorded in the Main field plots during the subsequent cropping season.

Potato Crop - Saskatchewan
In spring of 2007, potatoes were seeded into the green manure plots in the third week of May. Prior to seeding, additional nitrogen was applied to the trial site at 25 lbs/acre Two 6m rows of Russet Burbank (E2) and Alpha (E4) potatoes were seeded at 24 pieces per row, with a guard row between the two cultivars and on either side of each plot. The short rows (6m) helped ensure that the potatoes were only planted in a green manure area (the original green manure plots being 8m x 8m). These two cultivars were chosen because Alpha is a white-skinned potato which makes it easy to detect surface diseases such as black scurf and scab and Russet Burbank is a netted potato commonly grown by the industry.
The potato plots were managed using standard procedures for irrigation and hilling. The plots were top-killed at the beginning of September and harvested three weeks later. The harvested tubers were graded into size categories (**small** < 44 mm, **medium** 44-88 mm, **large** > 88 mm) and weights were determined in each size category. The weight of medium sized tubers was recorded as the marketable yield. The tubers were stored at 6°C until disease assessments were made on a sample of 30 washed tubers. The incidence (percentage) of tubers infected with black scurf, common and powdery scab was recorded for both cultivars. The severity of the infections (% of tubers with more than 5% of surface area infected) was also assessed. This severity threshold was based on CFIA tuber quality standards that allow 10% of tubers with light scab or *Rhizoctonia* infection to be present in a graded sample, or 5% of tubers with moderate infection.

In 2009, the green manure plots from 2008 were seeded with two potato cultivars, Alpha (E3) and Norland (E2). Norland is a popular red, table potato that is susceptible to both common and powdery scab as well black scurf. A sample of the seed potatoes planted in 2008 was evaluated for disease prior to planting. The Alpha seed had very low levels of *Rhizoctonia* (13% incidence), low levels of black dot (*Colletotrichum coccodes*) and a high incidence (69%), with low severity (2.1% of tuber surface covered with disease) of silver scurf (*Helminthosporium solani*). The Norland seed had higher levels of *Rhizoctonia* (100% incidence, 4.4% average surface area infected), slight dry rot, and very low and no levels of black dot and silver scurf respectively. The two cultivars were seeded into the green manure plots, with three 6m rows of Alpha and three rows of Norland in each plot. This resulted in 6m by 6m potato plots within the original green manure plots. Approximately 1m wide pathways separated each plot. The potatoes were planted on 12th May and were hilled approximately one month later. The 2009 potato plots were managed using standard potato growing practices for weed control and irrigation.

The soil organic matter content of the main and scab fields was tested in early June of 2009 to determine the impact of the green manure treatments on soil organic matter content during the subsequent cropping season.

Results:
The 2007 potato crop appeared healthy with no obvious problems with disease or insects. There were no visually apparent effects of the green manure treatments on the potato crop.

The green manure treatments had no significant impact on any aspect of the yields of either cultivar in 2007 (data not shown). Yields in this trial were comparable to other trials conducted by the U of S in adjacent plot areas in 2007.

In 2009, the total and marketable yield of Alpha tubers in the Scab field was highest in the plots that did not receive manure (weedy control), and was lowest in the plots where the mustard meal was applied the previous season. As root damage ratings due to *Rhizoctonia* were highest in the mustard meal plots, a depression in yield in this treatment would be expected – as damaged root systems are less capable of absorbing nutrients and water. However the Sorghum green manure plots also showed a low yield, despite having low *Rhizoctonia* root damage ratings. The Sorghum plots had the second highest levels of plant material incorporation in the Scab field so it is possible that Sorghum as a green manure had a depressant effect on potato yield in this field.

Table 34. Effect of green manure treatments on yield of Alpha tubers in the Scab field in 2009.

Green Manure Treatment	Yield (kg/6m row)	
	Total yield	Marketable yield
No manure, weedy control	22.8 a *	17.2 a
Millet	21.2 ab	15.9 ab
Pea/oat/vetch mixture	20.9 abc	14.8 abc
Sorghum	17.6 bc	12.5 bc
Mustard meal incorporation	17.4 c	11.6 c

*Means followed by the same letter are not significantly different at $p<.1$, using the Student's t test.

The green manure treatments had no significant effects on yields of either Alpha or Norland in the Main field in 2009 (data not shown).

Rhizoctonia and Scab

There were significantly lower levels of *Rhizoctonia* on the Russet Burbank tubers grown in plots following the mustard, pigweed and wheat green manure treatments in comparison with the sorghum green manure (Table 35). The overall levels of Rhizoctonia on the Russet Burbank crop were high, with all plots having at least 50% of the tubers with some black scurf infection. The levels of common scab in the Russet Burbank plots were much lower; very little scab developed in the pigweed plots, and the highest scab levels were seen in the plots previously sown to millet.

Table 35. Incidence of black scurf and common scab on Russet Burbank tubers harvested from plots previously sown to different green manure crops.

Green Manure Treatment	Incidence of Rhizoctonia (% tubers infected)	Incidence of common scab (% tubers infected)
Oriental mustard	55.0 b*	5.8 ab
Oilseed radish	66.0 ab	5.0 ab
Sorghum	88.5 a	9.0 ab
Redroot pigweed	55.0 b	0.8 b
Millet	66.5 ab	12.5 a
Wheat	60.0 b	3.3 ab
Coefficient of Variance	*22.3*	*106.2*

*values followed by the same letter are not significantly different at $P = 0.1$ using the Duncan's Multiple Range test.

The average disease levels on the Alpha tubers were lower for both black scurf and common scab than on the Russet Burbank tubers. The levels of common scab on the Alpha crop were too low for analysis of significance. This is surprising as usually disease is more obvious on the smooth-skinned white Alpha tubers than on russet type cultivars. The incidence of Rhizoctonia on the Alpha tubers was again lowest in the pigweed plots (Table 36) and highest in the millet and wheat plots. There was no significant difference in the severity of diseases on the tubers.

Table 36. Incidence black scurf on Alpha tubers harvested from plots previously sown to different green manure crops.

Green Manure Treatment	Incidence of Rhizoctonia (% tubers infected)
Oriental mustard	34.8 ab*
Oilseed radish	31.5 ab
Sorghum	43.3 ab
Redroot pigweed	12.5 b
Millet	51.5 a
Wheat	49.0 a
Coefficient of Variance	*52.6*

*values followed by the same letter are not significantly different at $P = 0.1$ using the Duncan's Multiple Range test.

At the end of August 2009, underground stems were sampled from three potato plants per row. The level of *Rhizoctonia* damage to these stems was assessed in both the Scab and Main field, using the rating scheme shown in Appendix 1. In the Scab field, powdery scab gall production on the root samples taken in late August was also assessed (rating scheme in Appendix 1). Late blight was observed in the Scab field at this time and the fields were top killed using a flail mower to prevent spread of this disease. The plots were harvested in mid-September and the total and marketable weights of tubers harvested from each 6m row were recorded as previously described. Disease ratings were made on 30 tuber samples taken at random from each bag of harvested tubers, using the previously described disease rating systems.

Statistical Analyses - All data were tested for fit to a normal distribution and if necessary data were transformed before analysis. The analysis of incidence of *Rhizoctonia* infection of Alpha tubers in the Scab field was conducted after log transformation to normalize the data.

In 2009, the incidence of powdery scab on the Alpha tubers and black scurf on the Norland tubers in the Scab field was significantly higher in the mustard meal treated plots than in the plots with no green manure treatment (Table 37). When the mustard meal was applied in 2008 there was a significant reduction in the weed density in these plots. It is possible that the increase in disease seen the next season was due to a lack of organic matter incorporation in the mustard meal treated plots, rather than any effect of the meal on the disease organisms present in the soil. There was no significant reduction in either powdery scab or black scurf when comparing incorporation of specific green manures against the disease levels seen in the non-manured plots. Although the non-manured plots had significantly less green material incorporated than the green manured plots (Table 4), there was more plant material incorporated in these "control" plots than in the mustard meal treated plots , as the mustard meal had suppressed weed growth.

Table 37 . Effect of green manure treatments on disease incidence on Alpha and Norland tubers in the Scab field in 2009.

Green Manure treatment	Alpha		Norland
	Incidence* of Powdery scab	Incidence of Black scurf	Incidence of Black scurf
Mustard meal	38.0a **	65.6 a	64.0 a
Pea/oat/vetch mixture	29.0 ab	22.7 b	39.8 ab
Millet	24.3 b	28.2 b	50.8 ab
No manure, weedy control	19.0 b	38.8 ab	36.8 b
Sorghum	16.0 b	33.0 b	57.5 ab

*incidence = % of 30 tubers infected with disease.
**Means followed by the same letter are not significantly different at *P*<.1, using the Student's t test.

There appeared to be a relationship between the incidence of black scurf on Alpha tubers in the Scab field and the amount of green plant material incorporated in the previous year (Table 38). As the amount of plant material incorporated during the previous cropping season increased the incidence of black scurf infection in the subsequent Alpha crop decreased.

Table 38. Relationship between incidence of Black scurf on Alpha tubers and weight of green material incorporated the previous year, in the Scab field.

Statistic	**Measure**
Linear fit	% Rhizoc = (GM wt (kg/m2) - 3.5)*-33.3
RSquare	0.22
Prob>F	0.037

The ranking of the level of *Rhizoctonia* on the stolon samples taken from the Alpha plants in the Scab field (Table 39) was roughly similar to the incidence of disease subsequently found on the tubers.

The green manure treatments had no significant effect of on powdery scab gall production on the roots sampled in August.

Table 39. Average rating of Rhizoctonia infection of roots of Alpha plants in the Scab field in 2009.

Green Manure Treatment	Average root rating
Mustard meal incorporation	1.6 a*
Pea/oat/vetch mixture	1.55 ab
No manure, weedy control	1.48 ab
Millet	1.3 bc
Sorghum	1.03 c

*Means followed by the same letter are not significantly different at p<.1, using the Student's t test.

In the Main field Alpha crop the lowest levels of black scurf were found in the non-manured plots, while significantly higher levels of black scurf were observed in the Sorghum, millet and rapeseed green manure plots. There was no correlation between the weights of green manure added in the previous season versus disease levels observed in the Alpha crop grown in the Main field (Table 40).

Table 40. Effect of green manure treatments on disease incidence on Alpha tubers in the Main field in 2009.

Green Manure Treatment	Incidence* of Black scurf
Sorghum	81.8 a**
Millet	77.6 ab
Rapeseed	75.0 ab
Pea/oat/vetch mixture	70.8 abc
Oilseed radish	66.0 bc
No manure crop	55.8 c

*incidence = % of 30 tubers infected with disease.
**Means followed by the same letter are not significantly different at $p<.1$, using the Student's t test.

The green manure treatments had little impact on disease levels in the Norland plots grown in either the Main or Scab fields. The only significant effect was on the incidence of *Rhizoctonia* infection in the Scab field, where the highest levels of infection were found in the mustard meal treated plots and the lowest in the weedy, non-manured control plots (Table 37). Both these plots had relatively little organic matter incorporated in 2008 (Table 32), so the high levels of disease in the mustard meal treated plots cannot be explained wholly by the lack of incorporation of green material in this instance. There was no effect of green manure on the levels of common or powdery scab on the Norland potatoes but there was a significant relationship between the weight of green material incorporated and the incidence of common scab (Table 41).

Table 41. Relationship between incidence of common scab on Norland tubers and weight of green material incorporated the previous year, in the Scab field.

Statistic	**Measure**
Linear fit	% Common scab = (Gm wt (kg/m) - 0.96)*25.0
RSquare	0.32
Prob>F	0.0099

As the amount of plant material incorporated as green manure increased so did the incidence of common scab in the next cropping season.

Discussion:

Contrary to expectations, none of the green manure treatments provided any consistent advantage to the subsequent potato crop – either in the form of yield enhancement or by providing a consistent degree of protection against soil-borne diseases such as scab or *Rhizoctonia*. In a study by Ochiai et al (2007), no green manure treatment resulted in statistically significant improvement of potato yield compared with the non-amended control. Where significant yield or disease effects of green manure treatments were observed, most commonly the non-manured weedy control treatments produced the "best" results (higher yields and/or less disease).

There are several possible explanations for these unexpected results.

The organic matter being added to the plots as a function of the green manure treatments may have exacerbated problems with soil-borne diseases such as scab and *Rhizoctonia*. The relationship between soil organic matter content and potato disease is complex (Conn and Lazarovits 1998). Soil organic matter derived from residues of previous crops or applied as animal manure may serve as an alternate food source for potentially pathogenic species like *Streptomyces* – but it may also enhance populations of microbes antagonistic to potential pathogens (Conn and Lazarovits, 1998). Soils rich in organic matter tend to better retain soil moisture – and diseases such as *Rhizoctonia* and powdery scab thrive under cool moist conditions. However, if enhanced soil organic matter increases the supply of available water to the plant this would be expected to enhance both yields and crop health. It is noteworthy that the amount of organic matter added to the soils by even the most productive green manure treatments used in this study (ca. 4 kg fresh weight/m = ca 4t/ha of dry organic matter) would have added relatively little to the total organic matter content already present in a typical sandy loam soil (ca. 2% O.M. = 45 t/ha). The soil at the Scab fields used in these trials had a long history of animal manure applications prior to its use in this potato research – and consequently this site has an unusually high level of residual soil organic matter content for a sandy loam soil type (ca 3-4%). This would have further diluted any contribution to the total soil organic matter pool made by the short-term green manure treatments used in this study.

The fact that the mustard meal treatments which supplied the least organic matter also tended to have the highest incidence of disease would suggest a potential association between disease and soil organic matter content. However this theory is contradicted by the fact that the weedy control treatment that had most frequently limited disease in the subsequent potato crop did not add as much organic matter to the soil as several of the green manure treatments – yet those green manure treatments had provided little benefit to the health of subsequent crops. This would suggest that positive effects of the weedy control treatments are working independently of any biomass contribution.

Another possibility is that the weeds present in the non-manured “control” treatments were actually serving as bio-control agents for disease. Red root pigweed (*Amaranthus retroflexus*) was the dominant weed species in the non-manured control plots in all tests. We could find no indications in the research literature of red root pigweed having any demonstrated biocontrol potential or any use as a green manure crop. Instead red root pigweed is widely considered to be a host for a number of insects, nematodes and viral diseases which may cause problems in potato crops.

While red root pigweed is widely distributed and common in cultivated fields across North America its prevalence in this trial is largely due to the manner in which the study was performed. Red root pigweed thrives in moist, nutrient rich soils and is therefore common at sites with a history of potato production. Red root pigweed requires recently disturbed soil and warm conditions to germinate. Preparing the green manure plots for seeding by rotovating in mid-summer and then irrigating after seeding would have provided the required conditions.

Management of the green manure plots in a manner that insured that only the selected green manure crop got established was problematic in this study. Red root pigweed represented a significant portion of the total biomass produced in all the green manure treatments tested – including the no green manure “control” treatment. This tended to confound and/or obscure any green manuring effects. Selection of faster growing, aggressive, green manure crops, coupled with more appropriate crop management practices, including use of selective herbicides, are required in order to achieve a vigorous and pure stand of the green manure crop. A “clean” control treatment where all weeds are controlled throughout the green manure crop period should also be included in any future studies.

A number of studies have found significant variation in the efficacy of green manures for disease control, and possible reasons for this variation have been proposed. Charron and Sams (1999) suggested that the stage of maturation of plant material of *Brassica* species and climatic factors may have an effect on the toxicity, and hence the fungicidal properties, of incorporated green manure material. Kirkegaard and Sarwar (1998) also concluded that the timing of incorporation of the green manure was important, depending on the susceptible stage of the pathogen and the need for suppression to last long enough to provide protection for the following crop. Zasada et al. (2003) concluded that consistent and reliable soilborne pest management with Brassicaceous amendments will not be achieved without a better understanding of the biological and chemical components involved. Ultimately designing pest management systems for specific target organisms will be based on integration of much information (Zasada et al. 2003).

Economics

Green manure crops were planted at rates recommended in the literature or by the supplier. At the recommended rates, the costs per acre to plant green manure crops ranged from $12 to $96. No additional fertilizer was supplied to the green manure crops. Supplemental irrigation may be required and some pest control may be necessary on all but the cleanest fields to ensure a healthy stand of biomass. Incorporation of the green manure crop was necessary. Production of the green manure crops in this study represented a significant cost – in terms of inputs (seed, fuel and labor), but most importantly it tied up high value land in a largely non-productive function for at least a portion of a cropping season. At the outset of the trial, commodity prices were low and growers were willing to entertain the idea of sacrificing a cereal crop to grow a green manure crop if the yield of the subsequent potato crop could be increased by even 1 to 2 ton per acre. As the trial progressed, commodity prices improved and the economic realities are now not conducive to adopting a green manure approach unless a consistent and significant improvement in potato yield was assured. In areas, such as the Pacific Northwest U.S.A., where soil fumigants are employed to control soil-borne potato diseases, the costs of a green manure crop are less than the costs of fumigation. In western Canada, longer crop rotations, and the inclusion of organic matter via compost or cereal crops, have proven to be more realistic approaches to reducing potato early dying (Larney et al. 2009). Davis et al. (2010) suggest several scenarios for including green manure crops in potato rotations that offer an income stream for the green manure year. These strategies include growing a forage crop and plowing in re-growth, double cropping short season crops, under-seeding an income crop to a green manure among others. Our intention was to first determine whether any of the green manure crops proposed for our area would effectively reduce potato early dying, then to develop an agronomic plan to work these crops into a potato production system. As none of the green manure crops evaluated significantly improved potato yield or reduced potato early dying, work on the logistics of the rotation are not relevant at this time. As the green manure treatments provided little obvious benefit in terms of yields or crop quality for the subsequent potato crop it is very doubtful whether this practice makes economic sense under the conditions encountered in this study. As noted by Cherr et al. (2006), green-manure based systems may provide alternatives to current approaches to crop production; however, the use of green manures may not be economically justified without the provision of multiple services such as nutrient supply, pest and weed control, and improvement of soil characteristics for crop production.

Discussion

Although an informal survey of potato fields affected by potato early dying indicated the involvement of *Verticillium* spp., the data from this report suggests that *Verticillium* is not the most significant pathogen causing premature death in Alberta potato fields. In fact, other pathogens, such as *Colletotrichum coccoides,* may play a larger role than was previously acknowledged. Davis et al. (2001) found that apical stem populations of *C. coccoides* correlated significantly with wilt severity in southeastern Idaho in one year of their study. The soil dilution assays employed during this study were specific to *V. dahliae* and did not provide information regarding other *Verticillium* spp. Several species of *Verticillum* were detected in molecular analyses and the role of these other species is not as well documented in the literature. While PED is caused primarily by *V. dahliae* in most areas of the United States, *V. albo-atrum* may be the dominant pathogen in cooler production areas of the most northern U.S. states and southern Canada, where average soil temperatures during the growing season rarely exceed 25˚C (Rowe and Powelson 2002).

Another thing that became apparent as the trial progressed, was that commercial fields with a history of potato production may not always present a risk of potato early dying as the length of crop rotations and other crops included in the rotations greatly affect both soil inoculum and infection rates in subsequent potato crops. Generally, rotation crops can reduce soil-borne pathogens by any (or all) of three different mechanisms: (i) by serving to interrupt or break the host-pathogen cycle; (ii) by altering soil physical , chemical or biological characteristics; and (iii) by indirect inhibition of pathogens (Larkin and Honeycutt 2006). In contrast, benefits of green manure include: improved soil condition, increased organic matter, improved water penetration, reduction of some diseases, reduced nematode population, and increased availability of nutrients (Davis et al. 2010). These benefits may not become apparent after a single green manure event, however, recent work reported by Davis et al (2010) reports that once a suppressive effect has been established in a soil, a single green manure treatment is sufficient to re-establish the effect.

The ultimate goal of using green manures to manage soil-borne diseases is to reduce yield losses caused by pathogens (Ochiai et al. 2007). Benefits of green manure crops often cannot always be correlated with reduced populations of *V. dahliae*, but can often be explained by reduced infections by *V. dahliae* (Davis et al. 2010). In fact, Davis et al. (1999) reported that *V dahliae* soil inoculum density was not a reliable predictor of Verticillium wilt or potato yield potential, but root colonization was highly correlated with both. In our study, we did not evaluate root colonization of potatoes by *Verticillium* spp., but further work in this area may be informative. Disease reduction has been highly correlated with changes in microbial activities as evidenced by increases in non-pathogenic *Fusarium* spp (Davis et al 1994, 1996). In Idaho, *Fusarium equiseti* (a type culture of *F. avenaceum*) populations have consistently been shown to correlate inversely with Verticillium wilt (Davis et al 2004) and may be useful as an indicator of biologically suppressive soils. In this study, *Fusarium* species were recovered from soil samples from cooperator fields, but it is not clear if these organisms contributed to the risk of early dying or if they represent suppressive soils as documented in other published studies. Wiggins and Kinkel (2005) studied soil microbial communities in green manure amended fields and indicated that green manure treatments may contribute to active management of the pathogen inhibitory activity of the Streptomycete community to achieve plant disease control for Vertilcillium wilt and potato scab. Assays for Stretomycete communities were out of scope for this study.

Although funding was not secured for root lesion nematode analyses, the efforts of two collaborating AAFC scientists contributed to our knowledge in these areas. In other studies, specific green manures, such as sorghum Sudan grass, have been shown to suppress populations of two species of root knot nematode, *Melodogyne chitwoodi* (Mojtachedi et al. 1993) and *M. hapla* (Viaene and Abawi 1998). Much of what is understood about interactions between root lesion nematodes and Verticillum wilt is based on data from regions where *P. penetrans* is the dominant and aggressive species. Root lesion nematodes in Alberta potato fields appear to be *Pratylenchus neglectus* rather than *P. penetrans*. Davis et al. reported in 2001 that studies in Idaho have not shown a relationship between *P. neglectus* and either wilt or potato yield. Amankwa et al (2006) indicated that CPFM is a poor host for *P. penetrans* compared to winter rye, but there was no mention of *P. neglectus*. In this study, no suppressive effect of sorghum Sudan grass or CFPM was observed with root lesion nematodes, however, the green manure crop, teff, did not appear to support a root lesion nematode population. Additional work with teff as a green manure or in rotation may prove beneficial.

Collaborating with researchers in Saskatchewan enabled us to determine whether the use of green manures may be beneficial in reducing the incidence or severity of *Rhizoctonia*, or common or powdery scab. While biomass of incorporated green manure has been associated with suppressive effects on some pathogens, the volume of biomass may also contribute to greater issues with common scab and black scurf. Crop rotation, rather than green manure plow down may provide better control. Larkin and Honeycutt (2006) reported that a rotation study in Maine demonstrated that incidence and severity of stem and stolon canker and black scurf of potato were reduced for most rotations, especially when potato followed canola, barley or sweet corn. No positive impact on potato yield or quality was observed as a result of green manure incorporation in the Saskatchewan fields.

Conclusions

As a result of this preliminary study using green manure crops in potato production systems in western Canada, we learned that potato early dying is likely a result of the interaction between several potato pathogens, but is not always caused by *Verticillium dahliae*. These pathogens may include other *Verticillium* spp., *Colletotrichum coccodes, Alternaria alternata*, and *Fusarium* spp. Several of the green manure crops studied can be effectively grown and incorporated in western Canada within a short growing season. These crops could be utilized as an under-seeded crop or planted following an early harvest of silage or field peas. Management of the green manure crops will influence the effectiveness of these crops to reduce disease, or increase yield. Agronomic work and pest control work may be required to establish green manure crops with sufficient biomass. We know that root lesions nematodes are present in potato production systems in western Canada, but little is known about the prevalent species, *Pratylenchus neglectus*, or its effect on potato yield. If these root lesion nematodes play a role in potato early dying in western Canada, more work is required to determine which potato pathogens are synergistically affected by the root lesion nematode populations. One green manure crop, *Erogrostis tef*, did not appear to support *P. neglectus* populations in potato fields. No consistent reduction in disease was noted as a result of incorporating green manure crops in Saskatchewan fields infested with scab and black scurf. Green-manure based systems may provide alternatives to current approaches to potato production; however, the use of green manures do not seem economically justified at this time. An integrated management approach that includes longer crop rotations will likely results in more consistent control of soil-borne pathogens at this time.

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Presentations

A field day was organized to show growers, agronomists and other potato industry personnel how the green manure plots looked August 9, 2006. Several growers indicated that they had planted green manure fields. There was some interesting discussion on the best timing and method of incorporation. Also, many individuals were speculating on how each crop might be worked in to a rotation strategy for potato production.

Posters were presented at the Annual General Meeting of the Potato Growers of Alberta in 2008, 2009 and 2010.

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