ASSESSMENT OF BACTERIAL BLIGHT PATHOGENS PREVALENT ON DRY BEAN AND IDENTIFICATION OF SOURCES OF RESISTANCE TO RHIZOCTONIA ROOT ROT IN NORTH DAKOTA

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

ABSTRACT

Chang, Yen-Wei, M.S., Department of Plant Pathology, College of Agriculture, Food Systems, and Natural Resources, North Dakota State University, November 2011. Assessment of Bacterial Blight Pathogens Prevalent on Dry Bean and Identification of Sources of Resistance to Rhizoctonia Root Rot in North Dakota. Major Professor: Dr. Rubella S. Goswami.

Bacterial blights and root rots are two major diseases affecting dry edible bean (*Phaseolus vulgaris* L.) production in North Dakota and Minnesota (Venette and Lamey 1998). Bacterial blights of dry bean are commonly caused by three bacterial pathogens, namely *Pseudomonas syringae* pv. *phaseolicola* (*Psp*), *Pseudomonas syringae* pv. *syringae* (*Pss*) and *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), which can appear either together or independently under natural conditions. The bacterial portion of this study involved ascertaining the extent of incidence of bacterial blight in the major dry bean production areas of ND through surveys, determining the prevalence of *Psp* and *Pss* and screening a collection of commercial varieties from different market classes for resistance to these two bacterial pathogens. In this study, more than 50% of the fields surveyed in all the major dry bean producing counties, including Grand Forks, Pembina, Traill and Walsh from year 2008 to 2010, were found to have been affected by bacterial blight. Among the bacterial diseases, bacterial brown spot (caused by *Pss*) appeared to be the most prevalent. Representative isolates for both *Psp* and *Pss* randomly selected from the survey were used for pathogenicity tests and confirmed to be pathogenic. Race typing of the *Psp* isolates confirmed the presence of the races 6 and 8 in the field samples with race 6 being the most common. Susceptibility of the 11 varieties used in this study was also variable. Difference in aggressiveness was observed among the *Psp* isolates.

Root rot of dry bean can be caused by a number of pathogens including *Fusarium*, *Rhizoctonia*, *Macrophomina*, *Sclerotium*, *Thielaviopsis*, and *Pythium* (Singh 1999). Among these, *Rhizoctonia* and *Fusarium* have been reported to be most common in ND and MN. Previous studies have been conducted to identify sources of partial resistance to *F. solani* f.sp. *phaseoli* (the most prevalent *Fusarium* species on dry beans) and *F. graminearum,* within currently available commercial varieties. The goal of this part of the project was to evaluate these varieties for their susceptibility to *Rhizoctonia solani*. Greenhouse trials as well as inoculated and non-inoculated field trials were conducted in 2009 and 2010 using *R. solani* AG 2-2 and AG 4 isolates. These trials demonstrated that the black bean variety Eclipse and the small red bean variety VAX3 were partially resistant to both these AG groups. These varieties have also been reported to have resistance to Fusarium root rot pathogens and therefore would be ideal for inclusion in breeding programs for incorporation of root rot resistance.

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INTRODUCTION

Dry edible bean (*Phaseolus vulgaris* L.) is believed to have in two primary centers of origin, Middle America and the southern Andes, and was introduced around the United States by about 2300 B.P (Before Present) (Brown 2006, CIAT 1991). At that time, bean, corn and squash were commonly cultivated together by the Native Americans. They believed beans could provide sufficient nutrients and would give long-term soil fertility (Eames-Sheavly 1993). Today, dry bean is not only used for its ability to fix atmospheric nitrogen but also for its nutritional value. It has recently been introduced into food pyramid as meat substitute (Lucier 2000). Dry bean is high in protein and contains phosphorus, iron, vitamin B1 and fiber due to which it has been often referred as the "Nearly perfect food" (CIAT 2007).

Furthermore, dry bean has become one of the most economically important legumes in the US. An average of 31 percent of the US dry bean supplies were exported annually from year 2008/09 to 2010/11 (Lucier 2011). In 2010, the U.S. produced about 31.8 million hundredweight (cwt) of dry beans. It was ranked as one of the top five dry bean producing countries in 2009 (FAO-FAOSTAT 2011, Lucier 2011). There are at least 40 states in the US where dry beans are cultivated and in 18 of them dry beans are produced on a commercial-scale (Lucier 2011). North Dakota (ND) is currently the largest dry bean producing state in the US followed by Michigan, Minnesota, Nebraska, and Idaho. In 2010 nearly 0.8 million acres of dry bean were planted in the state with pinto and navy beans being the primary market classes (Lucier 2011).

There are several diseases that generally affect dry bean production and seed yield, these include bacterial blights, bacterial wilt, rust, white mold, alternaria blight, bean

common mosaic virus (BCMV), bean yellow mosaic virus (BYMV), anthracnose and root rot (Venette and Lamey 1998). Of these, bacterial blights and root rot are commonly found in North Dakota and Minnesota. Bacterial blights are one of the most economically important foliar diseases of dry beans in ND. They can destroy a bean field within a few days and can cause more than 75 % yield loss under favorable conditions (Venette and Lamey 1998). On the other hand, root rot pathogens such as *Rhizoctonia* and *Fusarium* are also frequently observed in this region. According to Schwartz (2011), potentially 100 % yield loss can occur due to this disease when the conditions are favorable for root rot pathogens.

The research reported in this thesis involved two different diseases of dry beans, bacterial blights and root rot. First part of the research focused on evaluating the prevalence of two of the most common bacterial pathogens (*Pseudomonas syringae* pv. *phaseolicola*, (*Psp*), causing halo blight and *Pseudomonas syringae* pv. *syringae*, (*Pss*), causing brown spot in ND and finding sources of resistance to halo blight within a collection of common varieties. This was conducted through a multi-year field survey covering the major bean producing counties in ND followed by race typing of the *Psp* isolates and varietal screening using different market classes of dry beans. The second part of the research was aimed at identification of varieties that could serve as sources of resistance for both Rhizoctonia and Fusarium root rot. It involved greenhouse and field based screening under both artificial and natural disease pressure.

LITERATURE REVIEW

The host – dry edible bean

Dry edible bean or common bean (*Phaseolus vulgaris* L.), belonging to the family Fabaceae or Leguminosae, is known to be one of the oldest cultivated plants in America (Gepts and Debouck 1991, Broughton et al. 2003). Dry bean was originally domesticated in Central and South America more than 7000 years ago. It entered Southwestern United States around 2300 B.P. (Brown 2006, CIAT 1991). Dry beans evolved from wild-growing vines into major leguminous crops that are now grown and consumed worldwide (Gepts and Debouck 1991). The genus *Phaseolus* has more than 30 different species but only five of them are cultivated, namely *P. acutifolius* A. Gray (tepary bean), *P. coccineus* L. (scarlet runner bean), *P. lunnatus* L. (Lima bean), *P. polyanthus* Greenman (year-long bean), and *P. vulgaris* L. Among all the *Phaseolus* species, *P. vulgaris* L. or dry edible bean is the most widely grown in the world and occupied over 85% of all production area in the world (Singh 2001).

A dry bean plant is able to grow from 0.2 to 0.6 meters (8 - 24 inches) in height and each of the pods contains four to six seeds. The optimal conditions for dry beans to grow are high areas of the tropics with warm temperature which ranges between 15°C and 24°C (CIAT 1991). However, due to their diversity of growth habits, dry beans can adapt to different climates and conditions. For example, they can survive from warm tropics to cold climate regions, from sea level up to beyond 3,000 m.a.s.l. (meters above sea level), and from flat lands to steep slope areas (CIAT 1991). Basically, four different growth habits can be found in common bean. These include Type I – determinate (bush), Type II –

inderterminate (vining or trailing) with upright stem and branches, Type III – indeterminate with prostrate (little or no climbing ability) stem and branches, and Type IV – indeterminate with strong climbing ability (Venette and Lamey1998). Besides, dry bean seeds are commonly categorized into different market classes based on the physical appearance of the seed. Navy, great northern, pinto, cranberry, dark red kidney, pink, small red, small white and black beans are commonly seen or grown in the United States.

According to Food and Agriculture Organization (FAO), the United States (US) was ranked as one of the top five dry bean producing countries in 2009 (after Brazil, India, Myanmar, and China) when it produced over 1.15 million tonnes of common beans (FAO – FAOSTAT 2009). In 2010, the U.S. had approximately 1.91 million acres planted to dry beans and with North Dakota alone accounting for approximately 0.8 million acres of dry bean (Lucier 2011) making it the largest dry bean producer in the U.S. with pinto and navy beans being the major market classes planted. In fact, there was about 11.5 million cwt. of dry bean seeds produced in the state in 2010. The average value for all dry beans was estimated at $26 per hundredweight (cwt) during the 2010/2011 season. A profit of about 0.3 billion dollars was brought to North Dakota in a single year in 2010 (Lucier 2011).

Bacterial blight diseases

In this region, dry beans are commonly infected by three bacterial pathogens, namely, *Pseudomonas syringae* pv. *phaseolicola* (*Psp*) which causes halo blight, *Pseudomonas syringae* pv. *syringae* (*Pss*) which causes bacterial brown spot, and *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) which causes common bacterial blight.

Halo blight. Halo blight is an important seed-borne disease that is prevalent in cool temperate (16 – 23°C) countries such as North America and Europe, or at high altitudes (1500 – 2000 m) in tropical countries with wet conditions (Guven et al. 2004, Taylor et al. 1996, Fourie 2002). In the U.S., Colorado, Michigan, Minnesota, Nebraska, and North Dakota are the states where halo blight infection is commonly found (Teran et al. 2009). Symptoms of the disease include a bright yellow halo surrounding the site of infection. This symptom is caused by a toxin called phaseolotoxin which is produced by *Psp*. Phaseolotoxin is a reversible inhibitor of ornithine carbamoyl transferase (OCTase) in the urea cycle. The enzyme transforms citrulline from carbamoyl phosphate and ornithine and causes deficiency in intracellular pools which leads to chlorosis on the leaves (Rico et al. 2003). Water-soaked spots are also commonly seen on bean leaves. When the susceptible hosts are infected by *Psp*, water-soaked spots will become severe and cover the entire leaf. Halo blight spreads easily under favorable conditions (wet and cool). Spread of the disease may also be facilitated by human activities, rain, and hail. *Psp* can survive in residue from infected plants and is seed transmitted as well. Under experimental conditions, 43% of dry bean yield loss was observed due to halo blight (Fourie 2002). To date, nine races of *Psp*, which are characterized by reactions on eight differential varieties of dry beans, have been identified (Taylor et al. 1996). Among these races, 1, 2, 6, 7, and 8 races were frequently found in the U.S (Taylor et al. 1996).

Bacterial brown spot. Bacterial brown spot can be found in production areas of dry, snap and lima beans around the world. The brown spot pathogen, *Pss,* prefers to grow at moderate temperatures with daily highs less than 30°C (Saettler 1994). Symptoms of bacterial brown spot look similar to those of halo blight. Lesions are commonly small,

circular, and brown. This disease does show water-soaked lesion that later turns brown and becomes necrotic (Saettler 1994). When the conditions are favorable, the lesion size may grow larger than that of halo blight lesions. *Pss* also produces a phytotoxin, called syringomycin. This toxin induces necrosis in the plant tissues through the amphipathic lipopeptide structure that inserts into the bilayer membranes and forms freely permeable pores that allow the entrance of cations (K^+, H^+, and Ca^{2+}) to complete the infection (Bender et al.1999). *Pss* is also a seed-borne pathogen but chances of an epidemic being caused by seed infection as primary inoculum alone are relatively low (Navarro et al. 2007). Nonetheless, bacterial brown spot did cause serious outbreaks in the mid-1960s in snap beans grown in Wisconsin (Fourie 2002). Disease incidence of 100% and yield loss of 55% were reported in Mpumalanga, South Africa on the dry bean cultivar Bonus in 1992 (Serfontein 1994, Fourie 2002). Wind and overhead irrigation are the most important sources for *Pss* dissemination (Navarro et al. 2007).

Common bacterial blight. Common bacterial blight is one of the most prevalent seed-borne diseases of dry bean. The causal agent *Xap* can survive at warmer temperature (28 – 32°C) zones. The first symptoms of common blight are usually found on the leaves. A bright yellow zone surrounding the brown necrotic lesions can be seen on the infected leaves and enlarges under favorable conditions (Robertson and Frazier 1978). Bacterial ooze on the pod lesions is commonly seen under highly wet conditions. The seeds that are infected by *Xap* frequently exhibit butter yellow or brown spots near hilum. However, symptomless (latently infected) seeds occur as well (Zaumeyer and Thomas 1957). Phytotoxins produced by *Xap* are yet to be discovered. However, exocellular enzymes and polysaccharides may be the pathogenicity factors produced by *Xanthomonas* species and

form yellow zone around the lesion (Li et al. 2007). Most of the bean cultivars in ND are either highly or moderately susceptible to common bacterial blight except for an Adzuki bean (*P. angularis*) variety – Erimo (Kandel 2010). This pathogen can cause up to 40% yield losses depending on the bean cultivar and environmental conditions (Abd-Alla et al. 2010). In Michigan, common bacterial blight damaged at least 75% of navy bean fields and caused 10 to 20% yield reduction in 1967 (Saettler 1989, Allen 1998).Wind-blown rain, overhead irrigation, human activities, and vector are the primary mode of spread of the pathogen (Hagedorn 1994).

Disease control

Management of bacterial diseases in beans is challenging and commonly involves using three to four year rotations with non-host crops, improved cultural practices including removal or plowing of volunteer beans that can serve as reservoirs of inoculum and avoiding cultivation under wet conditions (Schwartz 1999).The use of certified seed to ensure reduction of sources of primary inoculum is considered key in the control of these seed borne pathogens. In 1967, Saettler (1989) reported that more than 75% of the fields in Michigan were found to be infected by common bacterial blight and suggested that could be 50% of the growers used certified seeds and 28% of the growers used their own seeds that could have been contaminated. Therefore, certified seed is recommended for all the bean growers to ensure seed is disease free.

The use of resistant varieties is an important management tool. Though resistance to bacterial disease is not common in pinto and light red kidney beans, some varieties with resistance have been identified in other market classes including navy and small white

beans (Schwartz 1999). Zaumeyer and Meiners (1975) conducted several studies aimed at identification of sources of resistance to halo blight in the past and reported that majority of the cultivars were resistant to races 1 and 2 of *Psp*. Recently, Sehirali-90 and Göynük-98 were reported to be either resistant or moderately resistant to five *Psp* races; while, Karacaşehir-90 was able to resist six *Psp* races (Bozkurt and Soylu 2011). Five varieties belonging to different market classes were also identified by Schwartz et al. (2004) as being resistant to halo blight. These are Buckskin (pinto), Burke (pinto), Chase (pinto), Poncho (pinto), Ivory (great northern), Marquis (great northern), and Foxfire (kidney). However, no commercial bean cultivars with complete resistance to all the races of *Psp* have been identified to date.

P. coccineus or runner bean was reported to be a good source of resistance to brown spot (Singh and Schwartz 2010). Other studies have reported that Hystyle (a green bean cultivar), Great Northern 1140 and Tempo possess resistance to bacterial brown spot (Coyne 1969, Harris 2011). Earliwax, Michelite, Processor, Puregold, Sanilac, Saginaw, Tempo, Truegreen and 10 PI lines were tested and reported as tolerant cultivars (Hagedorn et al. 1972). Schwartz et al. (2004) reported some bacterial brown spot resistant cultivars, including Buckskin (pinto), Chase (pinto), and Poncho (pinto).

Most of the commercial varieties of dry beans are susceptible to CBB infection. Only tepary bean (*P. acutifolius*), has been shown to have a high level of resistance to CBB and showed no infection when inoculated in the field (Zaumeyer and Meiners 1975, Rodino et al. 2009). Verano, a white bean, derived from *P. coccineus* and *P. acutifolius* has been reported to resist common bacterial blight and bean golden yellow mosaic virus (Beaver et al. 2008, Zapata et al. 2011). Crossing between common and runner beans

showed low or intermediate resistance (Zaumeyer and Meiners 1975). Another registered great northern common bean cultivar, Coyne, developed by the dry bean breeding program at the University of Nebraska Agriculture Research Division was improved to possess resistance to common bacterial blight and bean common rust (Urrea et al. 2009). Some other varieties believed to possess resistance to CBB include Beryl (great northern), Marquis (great northern), Weihing (great northern), and Foxfire (kidney) (Schwartz et al. 2004). VAX lines were also reported to be highly resistant to CBB (Duncan 2011).

Rhizoctonia root rot

Root rots of dry bean are economically important diseases all around the world. It is often caused by a combination of root pathogens and can become severe under conditions of stress such as excess water, insect injury and drought (Abawi and Pastor Corrales 1990). In this region, root rot is commonly infected by *R. solani* and *Fusarium solani* f.sp. *phaseoli* (Gambhir 2008).

The pathogen. *Rhizoctonia solani* Kühn, teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk, is a basidiomycete fungus which survives primarily as vegetative mycelium or sclerotia. When the mycelium is young, it is colorless, and turns light brown to dark brown as it gets older (Hagedorn 1994). The hyphae usually branch at right angles (90°) and contain a cross-wall (dolipore septum) near the branch point. Hyphae of pathogenic *R. solani* are commonly multinucleate (containing four to eight nuclei per cell) the number of nuclei was often used to determine whether isolates are likely to be pathogenic with the binucleate condition generally associated with non-pathogenic isolates (Janice 2005). Sexual stage or perfect stage of this fungus is less common and rarely found. If a sexual

stage occurs, basidia have a blackish color with barrel-shaped spores. Typically, there are four sterigmata, each of which carries an oval basidiospore (Hagedorn 1994). *R. solani* does not produce conidia (asexual spores). Therefore, classification of *R. solani* often becomes difficult and complicated.

In 1937, Schultz introduced the anastomosis group (AG) concept to plant pathogens. This scheme was to observe the morphology of the hyphal fusion occurring between the isolates. However, this tactic was not widely used until Parmeter et al. reintroduced the concept of 'hyphal anastomosis' to differentiate and identify *Rhizoctonia* in the United States in 1969. Nutrient medium and pathogenicity test on various plant species were the common methods that were used to distinguish *Rhizoctonia* in the early 1960's. There are two hyphal fusion phenomena that commonly occurred between isolates, known as acceptance (self-pairing) or rejection (somatic incompatibility).If hyphal fusion occurred between the isolates, they were believed to belong to the same anastomosis (AG) group. While, if hyphal fusion did not occur, the isolates were thought to belong to different AG groups. Within the AG, there are four hyphal interaction phenotypes scored from C0 to C3. C0 refers to no interaction between the hypha (no contact) and the isolates are characterized into different AG groups. C1 and C2 are vegetative incompatibility reactions. C2 is commonly referred to killing reaction which shows somatic incompatibility occurred. C3 is vegetative compatibility where there is a perfect fusion (Ceresini 1999).

Until recently, 13 AGs that have been identified (AG-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, -12, and -13). Within these 13 AGs, AG-1, -2, -3, -4, -6, -8, and -9 have different subsets (Carling et al. 2002). Among them, AG-1, AG-2 and AG-4 are the most common

root rot pathogens that cause rotting on seeds, roots and hypocotyls on common beans (Muyolo et al. 1993).

Symptoms

R. solani is known to be a soil-borne pathogen that mainly infects seeds, hypocotyls and roots. In the seedling stage, *R. solani* often causes pre-emergence damping-off and in older plants root rot is caused (Reddy et al. 1994). The lesions caused by *R. solani* on the hypocotyls and epicotyls of the plants are usually small, elongated, sunken, water-soaked, reddish-brown to brown in color (Hagedorn 1994, Schwartz 2011). Cankers appear on the hypocotyls and tap roots when the sunken areas age. This results in stunted and premature plant death when it becomes severe. Brownish-black sclerotia may form on the surface of older cankers and serve as overwintering structures. This pathogen generally prefers cool temperatures (15 – 21°C) and wet conditions. Lesions can be reduced significantly when the temperature drops below 9°C on rises above 21°C (Hagedorn 1994).

Although *R. solani* is a soil-borne pathogen, it is also able to invade the aerial portion of the bean plants. 'Web blight' is the term used to refer to the foliar infection caused by the basidiospores of *Thanatephorus cucumeris* (Godoy-Lutz 2010, Schwartz 1994). Web blight is mainly initiated with sclerotia or mycelia that overwinter from the previous season (Ceresini 1999). Symptoms are easily observed on the lower and middle parts of the plant including petioles, stems and young pods. Initial symptoms appear on primary leaves as small brown necrotic spots and cause the leaves to wilt and stick together under favorable conditions (Gálvez et al. 1989). As the infection progresses, dead leaves fall off and the plants appear tattered. The quality of the bean seeds is frequently affected.

A 90% yield reduction due to web blight was reported in Costa Rica in1980 (Galindo et al. 1983). Six of the *R. solani* subgroups are known to cause web blight of dry beans; these include Ag-1-1A, AG-1-IB, AG-1-IE, AG-1-IF, AG 2-2IV, and AG-2-2WB (Godoy-Lutz 2010).

Disease management

There are several ways to manage Rhizoctonia root rot of dry bean. As in the case of bacterial blight, resistant cultivars are sought. Though several breeding lines with some resistance to Rhizoctonia root rot have been reported but they are not widely available for commercial use (Hagedorn 1994) such PI lines include PI 165426, 165435, 109859, and Venezuela 54 (Zaumeyer and Meiners 1975). The use of fungicidal seed treatments is commonly recommended for the management of Rhizoctonia root rot of dry beans and have been widely used in ND since 1996 (Zollinger et al. 1998) . In ND, dry bean seeds are usually treated with Carboxin, Chloroneb, Fludioxonial, or PCNB to prevent and reduce damage due to Rhizoctonia root rot (McMullen and Lamey 2000). According to the 1996 pesticide use survey, approximately 84% of seed was treated with either captan, carboxin, metalaxyl, mefenoxam or combinations of these fungicides (Glogoza et al. 2000).

Rotation with non-host crops is another management option and it is suggested that dry beans should not follow sugar beet, corn or potato. However, the broad host range of *R. solani* makes it difficult to select crops to be included in such rotations. Biocontrol agents have also been demonstrated to be effective in the control of damage due to *R. solani*. *Pseudomonas fluorescens* and *Trichoderma harzianum* are two of the biocontrol agents which have been shown to be capable of suppressing damping-off caused by *R. solani*

(Samavat 2011, Paula Junior et al. 2008). Additionally, cultural practices such as stubble management and disinfection of tools and agricultural equipment are encouraged to reduce inoculum accumulation and carry over. Tillage practices have shown significant effect on reduction of *Rhizoctonia* infection on dry bean (Paula Junior et al. 2008). According to Paula Junior et al. (2008), tillage to a depth of more than 25 cm is recommended to reduce the activity of *R. solani* on dry beans. Finally, use of organic soil amendments like canola meal and waste lime have also been associated with a reduction in the growth of soil-borne pathogens such as *Rhizoctonia, Fusarium* and *Sclerotinia* (Larkin and Griffin 2007).

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CHAPTER 1: PREVALENCE OF *PSEUDOMONAS* SPECIES ASSOCIATED WITH BACTERIAL BLIGHT OF DRY BEANS AND IDENTIFICATION OF SOURCES OF RESISTANCE

Introduction

Dry edible beans are economically important for North Dakota. They are primarily grown in rotation with cereals and valued for their ability to fix atmospheric nitrogen and their nutrition value. In fact, dry beans have been recently introduced into the USDA food pyramid as a meat substitute. Among the total of 1.91 million acres of dry beans planted in the United States, approximately 0.8 million acres had been grown in N.D. in 2010, which earned about 0.3 billion dollars for the state (Lucier 2011).

However, dry bean production in ND is often limited due to diseases caused by bacteria and fungi resulting in lower yield and seed quality. Bacterial blights are one of the most destructive types of foliar diseases in this region, especially in Minnesota and North Dakota. There are primarily three common bacterial blight pathogens, namely, *Pseudomonas syringae* pv. *phaseolicola* (*Psp*) causing halo blight, *Pseudomonas syringae* pv. *syringae* (*Pss*) causing bacterial brown spot, and *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) causing common bacterial blight (CBB) (Harveson and Schwartz 2007, Hagedorn 1994). These pathogens can infect dry bean plants either independently or in combination depending on the field conditions and the prevalence of inoculum of the

pathogens. More than 75% yield loss due to bacterial blight has been reported previously (Venette and Lamey 1998).

Cultural practices such as use clean seeds, removal of volunteer beans and avoiding cultivation under wet conditions are essential steps in controlling bacterial blight diseases (Schwartz 1999). Copper-based bactericides can be used on foliage but they are not recommended to use after bacterial infection is evident (Dillard and Legard 1991). Although there are no dry bean cultivars that can fully resist all the bacterial pathogens, several resistant cultivars are available for controlling the individual bacterial pathogens. The main objectives of this study were determine the prevalence of halo blight and brown spot in ND; evaluating the races of *Psp* prevalent in ND and assessing the ability of different races of *Psp* to infect commercial varieties of different market classes.

Materials and methods

Survey. Disease surveys were conducted in 2008, 2009, and 2010 during the first week of August when the plants were at flowering or early pod filling stage. Over 30 fields were sampled each year in four major bean growing counties of North Dakota, namely Grand Forks, Pembina, Traill and Walsh,. Disease incidence was visually determined on 20 plants at five locations in each field along a W-transect. Representative samples were brought back to the laboratory for pathogen isolation.

Weather data. Weather data from Cavalier, St. Thomas, Grafton, Forest River, Grand Forks, Mayville, and Hillsboro, seven stations located within the four counties, were collected from North Dakota Agricultural Weather Network (NDAWN). Precipitation,

rainfall, and temperature were included in the evaluation of factors affecting for disease development.

Bacterial isolation. Samples were excised from leaves collected from the infected plants. Tissue from the margin of lesions was excised and surface-sterilized by soaking in 10% bleach for 30 seconds, 95% ethanol for 30 seconds, followed by washing in sterile distilled water three times. The tissue sections were macerated and plated on King's B (KB), Bacterial Blight Differential (BBD) and yeast extract-dextrose-calcium carbonate (YDC) media (Schaad et al. 2001). Pure cultures were established and the bacteria were identified through biochemical tests and PCR confirmation.

Bacterial test. Gram reaction: Bacteria were spread and fixed on a clean slide with light heat. Three steps included the use of staining solutions and a decolorizing solvent (e.g., ethyl alcohol). The smear was flooded with crystal violet solution for 60 seconds and then washed with tap water for 3 seconds. Excess water was drained off and the smear was flooded with iodine solution for 60 seconds. After 60 seconds, iodine solution was washed with tap water and excess water was removed. The slide was decolorized with a solvent, ethyl alcohol, for 30 seconds and washed with tap water for 3 seconds. A safranin solution was used for a counterstain for 30 seconds and the slide washed again with tap water for 3 seconds. The gram stain was examined under the microscope. All three bacteria being studied were gram-negative, including *Pseudomonas* and *Xanthomonas* species.

Biochemical test: *Pseudomonas* species are fluorescent under UV-light on KB medium. Therefore, using this medium we were able to differentiate the *Pseudomonas* species from other pathogens. *Pseudomonas* species were tested for oxidase (-), levan (+), and arginine dihydrolase (-). Growth characteristics on BBD medium and pectinase activities were used

to distinguish *Pss* and *Psp* isolates. Culture characteristics on YDC medium, oxidative fermentative reaction (- fermentative), milk proteolyses (+), and pectinase activities (-) were tested for *Xap* isolates (Schaad et al. 2001). A summary of these reactions is included in Appendix I.

PCR protocol. PCR was used for further confirmation of bacterial species. Previously published primer sets were used with slight modifications of PCR conditions. The primers used were X4c and X4e for *Xap* (Audy et al. 1996); B1 and B2 for *Pss* (Sorensen et al. 1998); and the multiplex primers P5.1, P3.1, P3004L, and P3004R for *Psp* (Rico et al. 2006). Either overnight bacterial cultures of isolates from infected leaves were used directly for PCR or the lesion tissues from inoculated leaves were macerated in a drop of sterile distilled water and 1 µl of the extract was used as template for PCR.

Pathogenicity test. The dry bean cultivar Lariat (pinto bean) was used for assessing the pathogenicity of the *Psp* and *Pss* isolates. Five isolates from each year and each species were grown on KB medium (24 - 48 hours, room temperature) and suspended in sterile distilled water. A concentration of $10^7 - 10^8$ cells/ml was used. This was determined by using a spectrophotometer. The bacterial suspensions were sprayed onto the undersurface of primary leaves with a painter's airbrush (20 p.s.i = 138 kPa). Inoculated plants were kept in a humidity chamber (19°C±1°C, RH=100%) for 24 hours before transfer to the greenhouse (23 – 25°C). The plants were rated at 10 days after inoculation (DAI). A modified 0 to 5 rating scale was used to rate *Psp* infection; where 0= no lesion, 1 = red brown necrotic reaction in area of maximum inoculation (highly resistant), 2 = red brown necrotic reaction with trace of water soaking (resistant), 3 = some necrosis but more extensive water-soaking confined to the area of maximum inoculation (slightly

susceptible), 4 = small water-soaked lesion (<1 mm diameter) distributed at random over the leaf undersurface (susceptible) and 5 = larger-water-soaked lesions (1-3 mm diameter) distributed at random over the leaf undersurface (fully susceptible) (Innes et al. 1984, Taylor et al. 1996). The pathogenicity for *Pss* was evaluated only as a positive (lesion) or negative (no lesion) reaction. Some of the infected leaves were saved for PCR confirmation.

Race ID method. Eight differential cultivars were used for *Psp* race typing. Race was determined by the reaction on the cultivars, according to table 1.1 (Taylor et al. 1996).

Table 1.1. Reactions of varieties to the pathogen in the differential set for *Psp* developed for race typing.

Differential	R-genes	Races[a]								
		1	2	3	4	5	6	7	8	9
Canadian Wonder	-	+	+	+	+	+	+	+	+	+
A52 (ZAA54)	4	+	+	+	+	-	+	+	+	+
Tendergreen	3	+	+	-	-	+	+	+	+	+
Red Mexican UI 3	1,4	-	+	+	+	-	+	-	+	-
1072	2	+	-	+	-	-	+	-	+	+
A53 (ZAA 55)	3,4	+	+	-	-	-	+	+	+	+
A43 (ZAA 12)	2,3,4,5	+	-	-	-	-	+	-	-	-
Guatemala 196-B	3,4	-	+	-	-	-	+	-	+	-

[a] = + : compatible (susceptible), − : incompatible (resistant)

Varietal screening. Disease severity due to infection by *Psp* isolates on 10 dry bean varieties belonging to different market classes (Table 1.2) was evaluated under greenhouse conditions following the protocol mentioned above for the pathogenicity tests. The

varieties were inoculated with one negative control and six isolates, four of which belonged to race 6 (08F63, 08F73, 09F3, and 09F65) and two belonged to race 8 (08F69 and 09F61).

Table 1.2. Varieties used in the study and their corresponding market classes.

Genotype	Market Class	Year released and/reference
Avalanche	Navy	Osorno et al. 2008a
Eclipse	Black	Osorno et al. 2009
Lariat	Pinto	Osorno et al. 2010
Matterhorn	Great northern	Kelly et al. 1999b
Maverick	Pinto	Grafton et al. 1997
Navigator	Navy	1995
Red Hawk	Dark red kidney	Kelly et al. 1998
Sedona	Pink	Kelly et al. 2006
Stampede	Pinto	Osorno et al. 2010
T-39	Black	1974
Vista	Navy	1989

Statistical analysis. Data from the *Psp* pathogenicity test and varietal screening were analyzed using SAS (SAS Institute Inc., Cary, NC). Since the rating scale for *Psp* was in ordinal form, the data was analyzed using the nonparametric methodology as described by Shah and Madden 2004. PROC RANK was used to calculate mid-ranks and PROC MIXED was applied to obtain significance levels and test statistics. Macro of LD_CI was used to determine the confidence intervals at 95%.

Results

Foliar disease surveys were conducted in four major dry bean producing counties (Traill, Grand forks, Pembina and Walsh) in ND from year 2008 to 2010. Disease severity varied between fields in each county with some of the surveyed fields being severely infected by the bacterial pathogens. Figure 1.1 shows the average incidence of bacterial blight in the four major bean producing counties in years 2008, 2009 and 2010 with disease

incidence refereeing to the number of fields with disease present in each county. Disease incidence in Traill was the highest in 2008 with a total of 71% of the fields being affected while Walsh county had highest disease incidence in both years 2009 and 2010, with 55% and 89% of the fields surveyed being affected by bacterial blight in each year respectively.

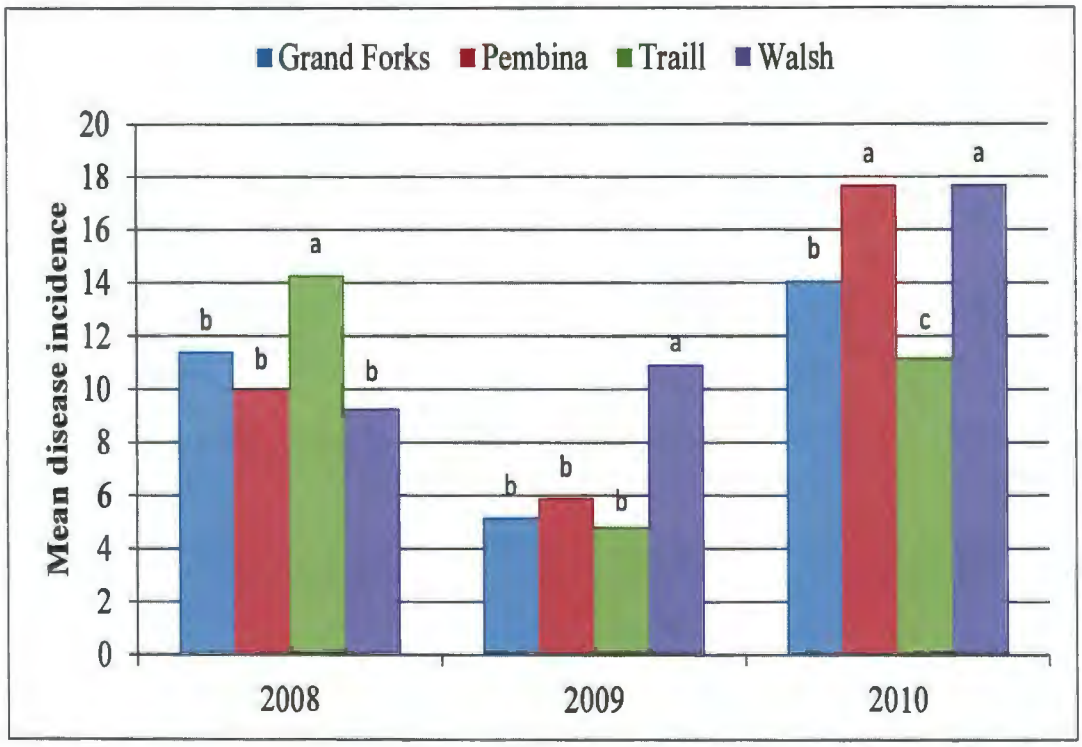

Figure 1.1. Disease incidence in four major dry bean counties between 2008 and 2010. Means followed by the same letters are not significantly different at P=0.05.

Bacterial blight diseases appeared to be prevalent in all fields surveyed with the percentage of infected plants ranging between 16 % and 100 %. Average precipitation and air temperature in the counties was slightly higher in 2010 as compared to 2008 and 2009. In year 2010, the rainfall and precipitation was higher in Pembina and Walsh counties (Table 1.3).

Table 1.3. Weather data from 2008 to 2010.

Year	County	Station	Average			Month of July		
			Precip[a]. May – Aug (mm)	Rainfall (mm)	Temp[b] (°C)	Precip[a]. (mm)	Rainfall (mm)	Temp[b] (°C)
2008	Pembina	Cavalier	203.2	256.8	18	63.5	65.4	19
		St. Thomas	228.6	290.7	18	63.5	64.8	19
	Walsh	Grafton	228.6	263.9	18	63.5	72.5	19
		Forest River	228.6	223.3	19	63.5	70.4	20
	Grand Forks	Grand Forks	228.6	256.3	19	88.9	91.2	20
	Traill	Mayville	279.4	301.2	19	114.3	119.9	20
		Hillsboro	228.6	295.7	19	88.9	118.4	20
2009	Pembina	Cavalier	223.5	177.4	17	25.4	36.2	18
		St. Thomas	223.5	169.6	18	25.4	33.1	18
	Walsh	Grafton	241.3	192.1	17	25.4	32.2	18
		Forest River	241.3	231.1	18	0	40.9	18
	Grand Forks	Grand Forks	152.4	210.3	18	25.4	44.5	19
	Traill	Mayville	165.1	138.4	18	0	34.8	18
		Hillsboro	165.1	204.2	18	25.4	42.9	19
2010	Pembina	Cavalier	266.7	235.0	19	76.2	79.7	21
		St. Thomas	330.2	231.3	20	50.8	90.0	21
	Walsh	Grafton	355.6	285.1	20	76.2	79.9	21
		Forest River	304.8	248.3	20	76.2	85.9	21
	Grand Forks	Grand Forks	266.7	200.2	20	50.8	53.1	21
	Traill	Mayville	292.1	191.8	20	50.8	66.3	21
		Hillsboro	266.7	175.3	20	50.8	66.3	21

[a] = Precipitation, [b] = Temperature

Pathogen isolation and identification. Representative leaf samples were collected from the fields and plated on KB, BBD and YDC medium. Although over 30 fields were sampled, the bacterial pathogens could not be isolated successfully from all the leaf samples plated (Table 1.4). In 2008, 37 fields were surveyed but bacterial pathogens were

isolated only from 34 fields. Among the isolates obtained from these fields, biochemical tests showed that 18 were *Psp* and 19 were *Pss*. The remaining three fields from TraillI had a mix of *Psp* and *Pss*. 22 isolates could be obtained from 2009 samples and 26 isolates from 2010 samples cultured. Among the 2009 isolates, seven were *Psp* and 15 were *Pss*. In 2010, seven isolates from the field samples were *Psp* and 12 were *Pss*. None of the fields had a mixture of two bacterial pathogens in 2009 and only one field in 2010 had both *Pss* and *Psp*. In this study, all of the isolates showed positive amplification using species specific primer sets mentioned in materials and methods. For example, when *Pss* isolate was used as PCR template a single band was obtained on a 1% agarose gel by using B1 and B2 primer sets (representative isolates on PCR confirmation were shown in Appendix II).

Table 1.4. Bacterial isolates obtained from the field samples.

Year	County	Traill	Grand Forks	Pembina	Walsh	Total
2008	Number of field sampled	8	6	12	11	37
	Number of isolates obtained	8	6	9	11	34
	Psp	1	1	6	7	15
	Pss	4	5	3	4	16
	Psp and Pss	3	0	0	0	3
2009	Number of field sampled	12	10	7	5	34
	Number of isolates obtained	11	1	6	4	22
	Psp	2	0	3	2	7
	Pss	9	1	3	2	15
	Psp and Pss	0	0	0	0	0
2010	Number of field sampled	15	5	7	8	35
	Number of isolates obtained	8	3	3	6	20
	Psp	0	1	1	5	7
	Pss	7	2	2	1	12
	Psp and Pss	1	0	0	0	1

Pathogen test. Five isolates of *Pss* and *Psp* from the three years were used for the pathogenicity tests and all of them were able to cause infection on the pinto bean cultivar, Lariat. Disease severity for *Pss* was not measured on a scale but all the isolates were capable of causing disease (positive reaction). The *Psp* isolates evaluated in the study were found to be pathogenic as well (Appendix III). Based on the median rate for disease severity, the five of isolates from 2008 to 2010 evaluated for pathogenicity varied significantly in their ability to cause disease on the dry bean cultivar Lariat (Table 1.5). The isolates F29 (2008), F62 (2009) and F36 (2010) were found to be more aggressive than the other *Psp* isolates with mean rank from 104.50 to 124.38.

Table 1.5. Ability of different *Psp* isolates to cause infection on the susceptible cultivar, Lariat.

Year	Median disease severity	Isolate	Mean rank (LS Mean estimate)	Estimated Relative	95% confidence Interval	
					Lower	Upper
2008	5.0	F29	124.38	.860	.814	.886
	3.0	F53	56.67	.390	.338	.446
	4.0	F69	81.67	.564	.506	.619
	4.0	F101	81.96	.566	.485	.642
	4.0	F121	77.83	.537	.479	.594
	0.0	Negative Control	12.50	.083	-	-
2009	4.5	F39	90.96	.628	.544	.702
	3.0	F42	50.02	.344	.302	.389
	5.0	F62	118.04	.816	.771	.849
	4.0	F68	82.00	.566	.501	.628
	4.0	F69	81.48	.562	.502	.620
	0.0	Negative Control	12.50	.083	-	-
2010	4.0	F15	66.50	.458	.388	.531
	5.0	F16	88.50	.611	.541	.675
	5.0	F18	84.50	.583	.503	.658
	5.0	F36	104.50	.722	.674	.763
	5.0	PH	78.50	.542	.459	.651
	0.0	Negative Control	12.50	.083	-	-

Race typing. In 2008, 11 isolates of *Psp* were tested for determination of race type using the standard differential set suggested by Taylor et al. (1996). In 2009 and 2010, seven and eight isolates were tested for each year, respectively. From the table 1.6, race 6 was considered the most prevalent among all the bacterial isolates. Race 8 was reported only in 2008 and 2009. Overall, among the 17 isolates for which races could be determined, majority were found to belong to race 6. Some isolates which showed intermediate reaction on all the race typing cultivars were grouped as unknowns.

Table 1.6. Race typing for randomly picked isolates from the survey.

Races	2008	2009	2010	Total
6	5	4	4	13
8	3	1	0	4
unknown	3	2	4	9

Varietal screening. The varietal screening (Table 1.7, Appendix IV) results suggest that only Red Hawk was able to resist infection upon inoculation with the some of the isolates, including 08F63, 09F65, and 09F61 with the lowest mean rank being 148.8. The variety Sedona (pink bean) was found to be susceptible to all race 6 *Psp* isolates with a median disease rating and mean ranks that ranged from 396.6 to 538.4. However, there were no statistically significant differences among Lariat, Matterhorn, and Maverick on race 6 isolates. When inoculated with race 8 isolates, however, Sedona was not significantly from Eclipse, Matterhorn, T-39 and Vista genotype.

Table 1.7. Median disease severity on ten varieties as measured on a 1-5 scale when inoculated with a negative control and six isolates of *Psp* belonging to race 6 and 8.

Isolate					
			08F63 (race 6)		
Genotype	Median[a]	Mean rank	Estimated Relative	95% Confidence Interval	
				Lower	Upper
Avalanche	3.0(SS)	388.4	.630	.474	.762
Eclipse	3.0(SS)	411.9	.668	.511	.794
Lariat	4.0(S)	476.1	.772	.618	.875
Matterhorn	3.0(SS)	315.5	.511	.436	.586
Maverick	4.0(S)	476.1	.772	.618	.875
Navigator	3.0(SS)	339.0	.550	.451	.644
Red Hawk	2.0(R)	148.8	.241	.157	.351
Sedona	4.0(S)	443.6	.719	.510	.862
Stampede	3.0(SS)	268.1	.435	.361	.511
T-39	3.0(SS)	324.1	.525	.424	.625
Vista	4.0(S)	420.5	.682	.514	.812
			08F73 (race 6)		
Avalanche	3.0(SS)	224.5	.364	.268	.472
Eclipse	3.0(SS)	268.1	.435	.361	.511
Lariat	4.0(S)	420.5	.682	.514	.812
Matterhorn	4.0(S)	435.9	.707	.511	.847
Maverick	3.0(SS)	356.3	.578	.442	.702
Navigator	3.0(SS)	357.9	.580	.408	.735
Red Hawk	3.0(SS)	270.2	.438	.370	.508
Sedona	4.0(S)	435.9	.707	.511	.847
Stampede	3.0(SS)	388.4	.630	.474	.762
T-39	3.0(SS)	292.0	.473	.457	.490
Vista	4.0(S)	414.1	.671	.442	.839
			09F3 (race 6)		
Avalanche	3.0(SS)	292.0	.473	.457	.490
Eclipse	3.0(SS)	356.3	.578	.442	.702
Lariat	4.0(S)	484.8	.786	.624	.889
Matterhorn	4.0(S)	396.6	.643	.436	.807
Maverick	3.0(SS)	347.6	.564	.445	.675
Navigator	3.0(SS)	324.1	.525	.424	.625
Red Hawk	3.0(SS)	324.1	.525	.424	.625
Sedona	4.0(S)	459.4	.745	.555	.871
Stampede	3.0(SS)	356.3	.578	.442	.702
T-39	3.0(SS)	379.8	.616	.473	.741
Vista	3.5(S)	411.9	.668	.511	.794

Table 1.7. (continued)

Genotype	Median[a]	Mean rank	Estimated Relative	95% Confidence Interval	
				Lower	Upper
		09F65 (race 6)			
Avalanche	3.0(SS)	315.5	.511	.436	.586
Eclipse	3.0(SS)	292.0	.473	.457	490
Lariat	3.0(SS)	451.7	.733	.557	.855
Matterhorn	3.0(SS)	324.1	525	.424	.625
Maverick	4.0(S)	491.0	.796	.680	.877
Navigator	3.0(SS)	292.0	.473	.457	.490
Red Hawk	2.0(R)	148.8	.240	.157	.351
Sedona	4.0(S)	538.4	.873	.819	.913
Stampede	3.0(SS)	362.5	.588	.476	.691
T-39	3.0(SS)	315.5	.511	.436	.586
Vista	3.0(SS)	386.4	.627	.469	.760
		08F69 (race 8)			
Avalanche	3.0(SS)	292.0	.473	.457	.490
Eclipse	3.0(SS)	324.1	.525	.424	.625
Lariat	3.0(SS)	270.2	.438	.370	.508
Matterhorn	3.5(S)	426.8	.692	.551	.804
Maverick	3.0(SS)	394.6	.640	.508	.753
Navigator	3.0(SS)	356.3	.578	.442	.702
Red Hawk	3.0(SS)	356.3	.578	.442	.702
Sedona	3.5(S)	427.2	.693	.513	.828
Stampede	3.0(SS)	244.3	.396	.303	.497
T-39	3.5(S)	444.0	.720	.559	.838
Vista	3.0(SS)	362.5	.588	.476	.691
		09F61 (race 8)			
Avalanche	3.0(SS)	270.2	.438	.370	.508
Eclipse	3.0(SS)	292.0	.473	.457	.490
Lariat	3.0(SS)	356.3	.578	.442	.702
Matterhorn	3.0(SS)	347.6	.564	.445	.675
Maverick	3.0(SS)	356.3	.578	.442	.702
Navigator	3.0(SS)	330.8	.536	.414	.654
Red Hawk	2.0(R)	148.8	.241	.157	.351
Sedona	3.5(SS)	396.6	.643	.436	.807
Stampede	3.0(SS)	292.0	.473	.457	.490
T-39	3.0(SS)	315.5	.511	.436	.586
Vista	3.0(SS)	334.4	.542	.382	.694

Table 1.7. (continued)

Genotype	Median[a]	Mean rank	Estimated Relative	95% Confidence Interval	
				Lower	Upper
			Negative Control		
Avalanche	0.0	44.5	.071	-	-
Eclipse	0.0	44.5	.071	-	-
Lariat	0.0	44.5	.071	-	-
Matterhorn	0.0	44.5	.071	-	-
Maverick	0.0	44.5	.071	-	-
Navigator	0.0	44.5	.071	-	-
Red Hawk	0.0	44.5	.071	-	-
Sedona	0.0	44.5	.071	-	-
Stampede	0.0	44.5	.071	-	-
T-39	0.0	44.5	.071	-	-
Vista	0.0	44.5	.071	-	-

[a] :0 = no lesion; 1 = highly resistant (HR); 2 = resistant(R); 3 = slightly susceptible (SS); 4 = susceptible(S); 5 =fully susceptible (FS)

Discussion

Bacterial blight was found to be present in more than 50% of the fields in the four major dry bean producing counties in North Dakota in 2008, 2009 and 2010. Bacterial brown spot appeared to be the most prevalent of the bacterial diseases particularly in the years 2009 and 2010. The high prevalence of *Pss* and *Psp* in the region could be somewhat influenced by the cool moderate weather and high moisture during these years (Schwartz 1999). This study did not focus on common bacterial blight; however, *Xap* was not isolated as frequently as *Pss* and *Psp*. On comparing the survey and weather data, it appears that counties with higher precipitation such as Walsh and Pembina had higher bacterial blight incidence. Based on bacterial isolations, brown spot appeared to be the most prevalent disease in 2009 and 2010 with more than half the samples being infected by *Pss* and all the *Pss* isolates being found to be pathogenic.

Halo blight was also found in all the counties in ND. Nine, seven and eight isolates of *Psp* were tested for race typing from the 2008, 2009 and 2010 samples respectively. Based on the race typing data, the races 6 and 8 were the most prevalent races in North Dakota. Race 6 has been reported all around the world, except in Asia. Race 8 was thought to be found only in Africa (Taylor et al. 1996) until Lamppa et al. (2002) detected this race in ND and reported race 8 for the first time in North America. To date, there are no commercial common bean varieties known to possess resistance to all the races of *Psp*. Although some pinto and great northern varieties have been previously reported to have resistance to *Psp* (Buckskin, Burke, Chase, Poncho, Ivory, and Marquis), the local commercial pinto varieties evaluated in this study did not demonstrate any resistant type reactions to the *Psp* isolates used. The kidney bean variety Red Hawk was the only one that showed some level of resistance to the different isolates.

Certain isolates could not be classified into races based on the currently available set of differentials. They showed reactions on the differential set which did not match with the races mentioned by Taylor et al. (1996). These isolates which have been classified as belonging to unknown races in this study could potentially represent one or more new races (Table 1.6), however their aspect requires further evaluation. Based on the statistical analysis of the results from the varietal screening, there were no significant differences in the severity of disease caused by isolates from race 6 and race 8 of *Psp*. Certain isolates appeared to be relatively more aggressive than others. This finding suggests that the use of a collection of field isolates from the region for which a variety is being developed may provide more robust results for resistance screening as compared to the use of a single isolate or isolates from a single race. An observation was made that under greenhouse

conditions, some of the *Psp* isolates caused darker lesions on infection on black bean leaves which could possibly be attributed to the reaction of the genotype to the phytotoxin that was produced by different *Psp* isolates.

Overall, this study led to establishing the importance of bacterial diseases, particularly halo blight and brown spot in the dry bean production regions of North Dakota and emphasizing the need for the development and implementation of efficient disease management practices to reduce losses due to these pathogens. It also provided information regarding the prevalent races of the halo blight pathogen, *Psp*, and brought to light the possibility of uncharacterized races being present or developing in the region. The varietal screening study did not lead to the identification of one or more varieties with significant resistance to *Psp* but stressed on the fact that there is variation among isolates and their ability to cause disease on different varieties.

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CHAPTER 2: SCREENING FOR RESISTANCE SOURCES TO RHIZOCTONIA ROOT ROT

Introduction

Roots of dry edible beans (*Phaseolus vulgaris*) have been found to be infected by several pathogenic fungal species including *Fusarium*, *Rhizoctonia*, *Macrophomina*, *Sclerotium*, *Thielaviopsis*, and *Pythium* that can cause root rots (Singh 1999). Among these, *Rhizoctonia solani* is one of the most problematic root rot pathogens that can infect a broad host range, including cotton, dry bean, maize, potato, soybean, sugar beet, and wheat (Carling et al. 2002, Ithurrart et al. 2004, Larkin and Griffin 2007, Lucas et al. 1993, Muyolo et al. 1993). In North Dakota and Minnesota, dry bean root rot is commonly caused by *Rhizoctonia* and *Fusarium* species (Gambhir et al. 2008, NBG 2009). More than 5 - 10% yield losses in the United States and more than 60% yield reduction in Brazil is reported to have been caused by Rhizoctonia root rot in combination with Fusarium root rot (Hagedorn 1994). Among the 13 *Rhizoctonia* anastomosis groups (AGs), AG 1, 2-2, 3, 4, and 5 are known to be commonly found in this region and *R. solani* Ag 2-2 and Ag 4 are the most aggressive on dry bean as well as sugar-beet (Engelkes and Windels 1996, Brantner and Windels 2005).

F. solani f.sp. *phaseoli* is typically considered to be the most common *Fusarium* species associated with root rot of dry beans (Vanette and Lamey 1998). However, recently *F. graminearum* and a few other species have also been found to be associated with the disease (Gambhir et al. 2008, Bilgi et al. 2007). The broad host range of *R. solani* makes it

difficult to control through crop rotation and development of resistant varieties is considered to be a highly desirable trait in varieties grown in root rot prone areas. Previous studies conducted using a set of 11 commercial varieties belonging to different market classes led to the identification of varieties that could serve as potential source of resistance to Fusarium root rot of dry beans caused by *F. solani* f.sp. *phaseoli* and *F. graminearum* (Bilgi et al. 2008, Bilgi et al. 2011). Since, *R. solani* is often found in association with the *Fusarium* species, the goal of this study was to look for resistance to *R. solani* within the same set of varieties in search of a common source of resistance to the two most important root rotting fungi.

Materials and methods

Dry bean genotype. 11 different dry bean varieties belonging to different market classes were included in this study (Table 2.1).

Table 2.1. Varieties of dry edible beans used for assessing root rot resistance and their market classes.

Genotype	Market class	Year released and/or reference
Eclipse	Black	Osorno et al. 2009
Matterhorn	Great northern	Kelly et al. 1999b
Maverick	Pinto bean	Grafton et al. 1997
Montcalm	Dark red kidney	1961
Norstar	Navy	Grafton et al. 1993
Othello	Pinto	Kraft et al. 1995
Red Hawk	Dark red kidney	Kelly et al. 1998
Rojo Chiquito	Small red	Hang et al. 2002
T-39	Black	1974
VAX3	Small red	Singh et al. 2001
Vista	Navy	1989

Disease evaluation. A root rot rating scale from 1 to 9 was used to evaluate the severity of root rot on roots infected with *R. solani* based on tissue discoloration and lesion size. 1= no visible symptom; 3 = light discoloration and no necrotic lesion to maximum of 10% lesions on hypocotyl and root tissues; 5 = Approximately 25% lesions on hypocotyls and root tissue, tissue remains firm and little decay or damage to the root system; 7 = Approximately 50% lesions on hypocotyl and root tissues, root system suffers decay and reduction, and fungal structures are visible; 9 = Approximately 75% or more lesions on hypocotyl and root tissues, root system suffers advanced decay and reduction, and extensive fungal growth (Abawi and Pastor Corrales 1990).

A different root rot rating scale from 1 to 7 was used to evaluate root rot severity due to *Fusarium* species (Schneider & Kelly 2000). 1 = healthy roots with no discoloration of root or hypocotyl and no reduction in root mass; 2 = 0.1- to 0.2-cm small reddish brown lesions at the base of the hypocotyl, with normal root mass and size; 3 = increase in intensity and size and coalescing of localized root/hypocotyl lesions approximately 180° around the stem, with lesions from 0.5 to 1 cm and 10 to 20% root discoloration but no reduction in root mass; 4 = increase in intensity of discoloration and size of hypocotyl lesions, with lesions extending and completely encircling the stem, 5 to 10% root mass reduction, and 95% of the root discoloration; 5 = increasingly discolored and extended hypocotyl lesions, with 100% of the roots intensely reddish-brown and 20 to 50% root reduction; 6 = hypocotyl lesions encircling the stem extending up to 2 cm, intense root mass discoloration, and 50 to 80% root mass reduction; 7 = pithy or hollow hypocotyl with very extended lesions, 80 to 100% root mass reduction, and the root is functionally dead (Schneider & Kelly 2000).

Field inoculum preparation method. Wheat seeds were soaked in sterile distilled water in stainless steel steam table pans for 24 hours, subsequently excess liquid in the pans was drained, and trhe seeds were autoclaved at 121°C and 15 psi. The seeds were autoclaved twice with an hour of cooling in between. Wheat seeds were brought to room temperature prior to inoculation with *R. solani* Ag 2-2 and Ag 4 isolates. The inoculum was stirred with sterile utensils in a laminar flow hood every other day to allow the fungus to grow throughout the contents at room temperature. This wheat inoculum was air dried in the greenhouse before inoculating the fields.

Greenhouse inoculum preparation method. A modified version of wheat inoculum layer method from Bilgi et al. 2008 was used in greenhouse experiments to perform pathogenicity evaluation for *R. solani* Ag 2-2 and Ag 4 on all the bean varieties listed above. Wheat seeds were sterilized as described previously in 2L flasks. After sterilization and cooling to room temperature, Ag 2-2 and Ag 4 isolates were added to the sterile wheat seeds. Flasks were shaken by hand every other day to allow the fungi to grow uniformly throughout the contents. 15g of sterile premium grade coarse dry vermiculite was put into 266 ml plastic drinking cups with water drainage holes at the bottom, followed by 15g of inoculum, then covered with eight grams of vermiculite. Two seeds of the same variety were placed on top of vermiculite layer and covered by another eight grams of vermiculite. Five replicate cups containing seeds of each variety were kept on a tray and watered daily.

Inoculated field trial. Inoculated root rot trials were conducted in research plots at Fargo and Prosper, North Dakota in 2010. The experiment was laid out in a randomized complete block design (RCBD) and each unit was inoculated separately with *F. solani* f.sp. *phaseoli*, *F. graminearum*, *R. solani* AG 2-2 and AG 4 and mixed (all the above) inoculum.

Only four of the bean varieties belonging to different market classes (Eclipse, Norstar, Red Hawk and VAX3) were used for pathogenicity evaluation in this trial. The wheat seed inoculum was applied before the dry beans were planted at the rate of 1.5g per foot per row. The plants were rated at two stages, the seedling stage, 28 days after planting (DAP) and at flowering stage, 56 DAP. Stand counts were taken at 14 DAP and 28 DAP.

Root rot trial under natural disease pressure. Two dry bean root rot trials (natural disease) were conducted at two different locations in Minnesota (Perham and Park Rapids) in 2010. All the dry bean varieties listed above except Othello were planted at both locations. The experiment was also laid out in an RCBD. When the plants were at the seedling stage 4-6 weeks after planting and at flowering or seedpod stage (R1 – R6), 10 roots were randomly sampled from the two outside rows of each experimental unit. Roots were brought to the laboratory for root rot assessment and further investigation.

Pathogen isolation. Infected roots collected from the field were plated on half-strength potato dextrose agar (1/2 PDA) and incubated 4 - 7 days. Sections of root tissues from the margin of lesions on two separate roots were directly plated on PDA and another set of samples from two roots were surface-sterilized by soaking in 10% bleach for 30 seconds, 95% ethanol for 30 seconds followed by three separate washes in sterile distilled water. The surface-sterilized samples were air-dried in a laminar flow hood and then plated on the ½ PDA media. Culture plates (with sterilized and non-sterilized root tissue samples) were kept at room temperature. To obtain a pure culture, single-spore isolation was conducted after two rounds of sub-cultures and *R. solani* colonies were finally grown on a full strength PDA for another 4 – 7 days. Each pure culture was identified based on

morphological characteristics and molecular confirmation was conducted by comparing sequences of the ITS regions with those available in the public databases.

Molecular identification. Fungal DNA was extracted from mycelium of 7 day old pure cultures grown in potato dextrose broth using the Fastprep® instrument (Qbiogene, Irvine, CA) for tissue disruption and the DNeasy™ Plant Tissue mini kit (Qiagen, Valencia, CA) for DNA extraction as per manufacturers' instructions. Polymerase Chain Reaction (PCR) was used to amplify the ITS (Internal transcribed spacer) region of the fungal DNA using the primers set ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') according to White et al. (1990). The amplicons were sent to McLab, CA for sequencing using the same set primers. DNA sequences were edited using the BioEdit software and then analyzed with Blast® software provided by National Center for Biotechnology Information (NCBI).The best matches with more than 97% (Query coverage) and E-value of e-10 or less were considered the most accurate identification for the samples.

Statistical analysis. All the data were analyzed using SAS software Version 9.2 (SAS Institute Inc., Cary, NC). The ordinal root rot severity data were analyzed using the nonparametric methodology as mentioned by Shah and Madden (2004). PROC RANK was used to obtain mid-ranks followed by PROC MIXED to calculate test statistics and significance levels. Confidence intervals 95% were measured using the LD_CI macro described by Shah and Madden (2004). Stand count and yield data were analyzed by analysis of variances (ANOVA) followed with Fisher protected Least Significant Different (LSD) to separate the means.

Results

Based on the weather database, Perham had the highest precipitation and lowest temperature compared to the other locations included in the study in both years 2009 and 2010. The total precipitation was approximately 304 and 374 mm in 2009 and 2010, respectively (Table 2.2). In July 2009, Perham had 63% more precipitation compared to Fargo and Prosper. The average temperature in all the fields was slightly different in both years and ranged between 15 and 21°C. In month of July, when the roots were sampled the temperature ranged between 16 and 22°C. The rainfall data in Perham and Park Rapids was not available in the weather database and were not evaluated.

Table 2.2. Data collected from NDAWN, NOSS and Weather Underground®.

Year	Field	May – Aug			July		
		Precipitation (mm)	Rainfall (mm)	Average Temp (^{0}C)	Precipitation (mm)	Rainfall (mm)	Temp (^{0}C)
2009	Fargo	228.6	142.6	19	38.1	15.9	19
	Prosper	228.6	145.3	18	38.1	24.6	18
	Perham	304.8	-	16	101.6	-	16
	Park Rapids	196.9	-	15	63.5	-	17
2010	Fargo	368.3	255.0	21	114.3	105.1	22
	Prosper	368.3	243.1	20	114.3	103.4	21
	Perham	374.7	-	18	127	-	19
	Park Rapids	323.9	-	19	127	-	20

Green-house based screen. Under greenhouse conditions (Table 2.3), Eclipse, Othello, Rojo Chiquito, and VAX3 appeared to the most resistant varieties among the set of 11 varieties evaluated in this study. In inoculations with *R. solani* Ag 2-2, VAX3 had the lowest disease severity with a mean rank of 39.33 and median disease severity of 3 (Fig. 2.1 and Table 2.3). Matterhorn, Maverick, Montcalm and Norstar were the most

susceptible cultivars with a median disease severity ranging between 7.0 and 9.0. When inoculated with *R. solani* Ag 4, Eclipse, Norstar, Othello, Rojo Chiquito, T-39, and VAX3 appeared to be more resistant than the other varieties with median disease severity ranging from 3.0 to 5.0, however, the differences were not statistically significant. According to results of this screen, Vista was most susceptible among the varieties tested with a median disease severity rated at 8.0 in inoculations with *R. solani* Ag 4.

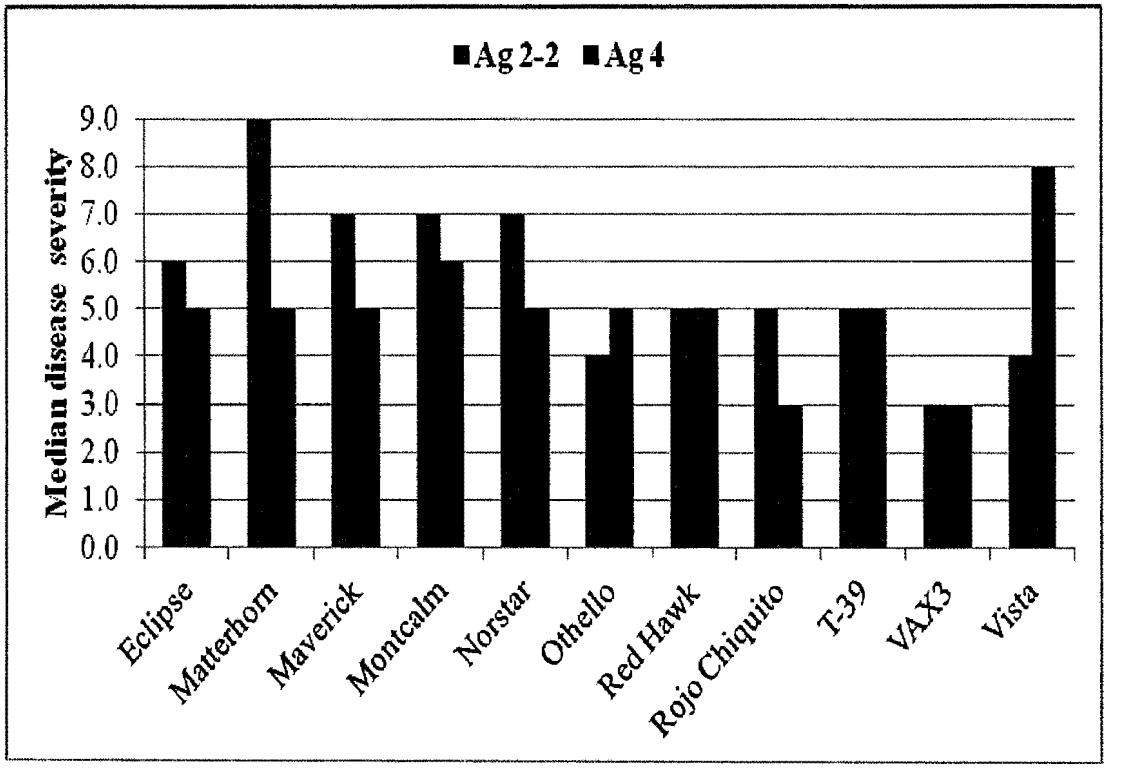


Figure 2.1. Median disease severity for *R. solani* Ag 2-2 and Ag 4 on 11 varieties tested under greenhouse conditions.

Table 2.3. Results from nonparametric analysis of disease severity rating for 11 varieties inoculated with *R. solani* Ag 2-2 and Ag 4 under greenhouse conditions.

Cultivars	Ag 2-2				Ag 4			
	Mean Rank	Estimated Relative	95% Confidence Intervals		Mean Rank	Estimated Relative	95% Confidence Intervals	
			Lower	Upper			Lower	Upper
Eclipse	74.50	.553	.413	.684	45.42	.359	.271	.460
Matterhorn	114.17	.750	.605	.849	83.08	.600	.491	.700
Maverick	103.00	.661	.481	.800	78.33	.561	.444	.670
Montcalm	97.92	.669	.529	.782	83.25	.616	.475	.737
Norstar	101.67	.683	.535	.798	71.00	.516	.353	.676
Othello	47.75	.373	.256	.510	58.17	.440	.319	.569
Red Hawk	62.00	.486	.386	.588	88.17	.614	.485	.726
Rojo Chiquito	59.00	.463	.374	.556	35.58	.304	.219	.408
T-39	74.50	.531	.354	.700	75.83	.546	.400	.684
VAX3	39.33	.320	.215	.451	30.50	.278	.171	.428
Vista	54.00	.429	.289	.582	124.00	.885	.834	.918

Inoculated trial. The inoculated trials were conducted at two locations, Fargo and Prosper in 2010 and involved four varieties and four pathogens, *R. solani* AG 2-2, *R. solani* AG 4, *F. solani* f.sp. *phaseoli* and *F. graminearum*. As mentioned in Table 2.4 and 2.5, Eclipse had the highest stand count at both locations for treatments with all the pathogens except AG 2-2 which did not show any significant stand count differences between the varieties. Norstar and Red Hawk had the lowest stand counts with no significant difference among all the treatments, except on treatments *F. solani* f.sp. *phaseoli* and *F. graminearum* (Table 2.5).

Table 2.4. Stand counts for non-inoculated, *R. solani* Ag 2-2 and *R. solani* Ag 4 treatments in the inoculated trials in Fargo and Prosper conducted in 2010.

Stand Counts*						
Fargo						
	NI		Ag 2-2		Ag 4	
	SC1	SC2	SC1	SC2	SC1	SC2
Eclipse	120.8a	135.5a	20.0a	7.8a	111.5a	126.3a
Norstar	92.5b	97.3b	6.5a	4.3a	68.0b	78.3c
Red Hawk	85.3b	91.0b	8.8a	7.5a	64.8b	81.8c
VAX3	85.8b	97.8b	4.75a	6.5a	74.5b	102.0b
CV	2.3	2.3	2.3	2.3	2.3	2.3
LSD	9.2	7.5	22.7	3.9	16.2	15.5
Prosper						
Eclipse	164.8a	176.0a	1.5a	2.3a	162.5a	161.5a
Norstar	114.5b	125.3b	1.0a	1.5a	110.8c	124.3c
Red Hawk	139.5ab	141.3b	2.0a	1.8a	139.3b	134.5bc
VAX3	130.0b	134.8b	2.3a	1.5a	138.0b	138.8b
CV	2.3	2.3	2.3	2.3	2.3	2.3
LSD	25.4	22.6	1.6	3.4	18.7	11.5

Table 2.5. Stand counts for *F. graminearum*, *F. solani* f.sp. *phaseoli*, and a mixture of these two pathogens with *R. solani* AG 2-2 and AG 4 (Mixed) conducted in Fargo and Prosper in 2010.

Stand Counts*						
Fargo						
	F. gram		*Fsp*		Mixed	
	SC1	SC2	SC1	SC2	SC1	SC2
Eclipse	126.3a	127.8a	118.3a	143.3a	45.8a	49.5a
Norstar	91.3b	101.8b	90.5ab	104.8b	40.8a	39.0a
Red Hawk	79.0c	97.8b	74.8b	92.5b	30.5a	33.3a
VAX3	76.5c	97.5b	64.5b	96.8b	34.3a	63.0a
CV	2.3	2.3	2.3	2.3	2.3	2.3
LSD	10.9	15.8	33.5	14.6	34.6	35.5
Prosper						
Eclipse	173.0a	174.5a	174.8a	182.0a	44.3ab	42.3a
Norstar	115.3c	117.8b	124.5d	130.0b	43.3ab	38.3ab
Red Hawk	143.8b	137.3b	142.5c	145.0b	48.5a	39.8ab
VAX3	142.0b	128.8b	157.5b	166.0a	39.0b	35.0b
CV	2.3	2.3	2.3	2.3	2.3	2.3
LSD	20.9	21.9	13.9	16.6	8.4	6.4

NI = Non-inoculated
SC1 = 14 DAP
SC2 = 28 DAP
* = means with the same letter are not significantly different

In both the Fargo and Prosper trials (Table 2.6 and 2.7), Red Hawk appeared to have the highest disease severity when infected with majority of the pathogens (Fig 2.2 and Fig 2.3). Disease severity on susceptible varieties such as Red Hawk appeared to be higher at the flowering stage compared to the seedling stage. On the other hand, the severity for Eclipse, Norstar, and VAX3 was relatively lower at flowering stage as compared to seedling stage, except on treatment *R. solani* Ag 2-2. In terms of yield (Table 2.8), Eclipse and VAX3 appeared to be the best.

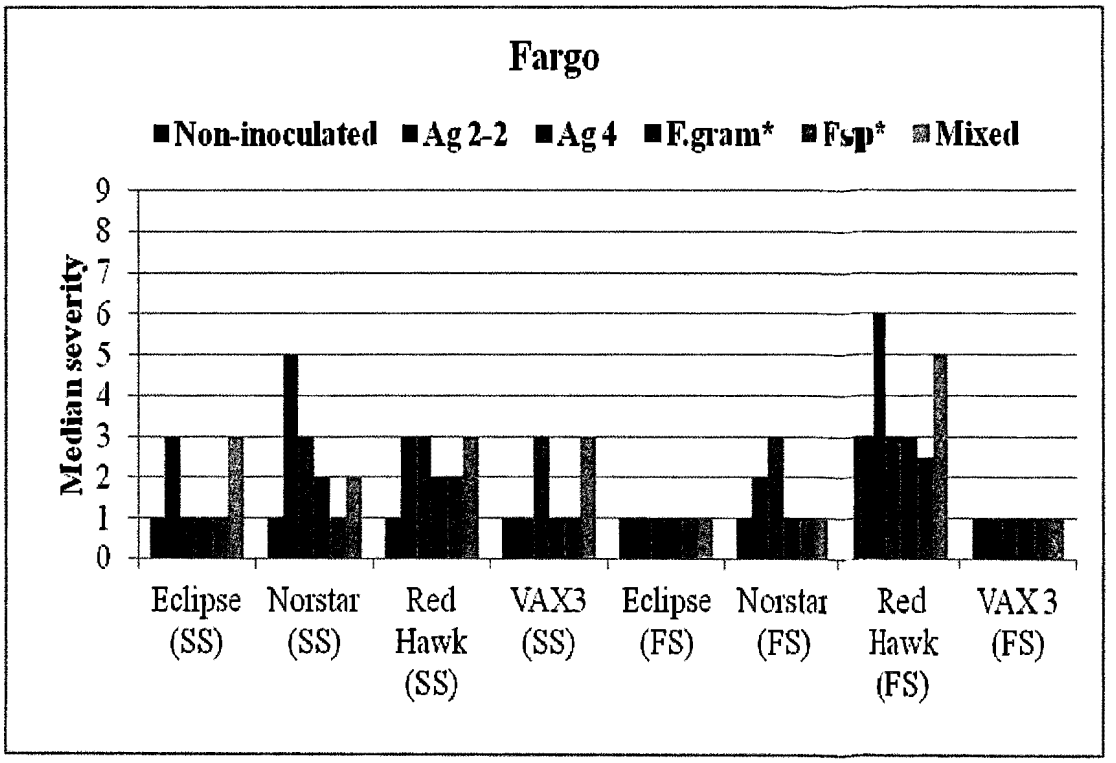
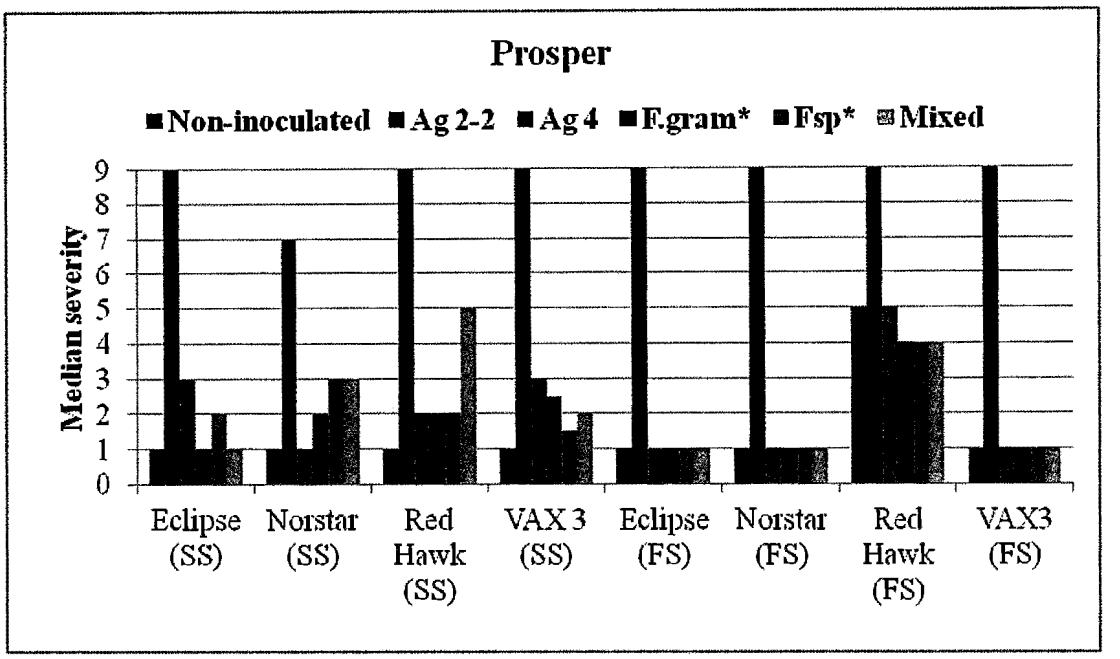


Figure 2.2. Median disease severity on the four varieties used in the inoculated trials at the seedling (SS) and flowering stage (FS) in Fargo.



*= Root rot rating was based on the scale 1 to 7

Figure 2.3. Median disease severity on the four varieties used in the inoculated trials at the seedling (SS) and flowering stage (FS) in Prosper.

Table 2.6. Results from nonparametric analysis of disease severity ratings for four varieties using *R. solani* Ag 2-2 and Ag 4, *F. solani* f.sp. *phaseoli* and *F. graminearum* in inoculated trials at Fargo conducted in 2010.

	Fargo							
	Non-inoculate							
	SS				FS			
Cultivar	Mean rank	Estimated Relative	95% Confidence Intervals		Mean rank	Estimated Relative	95% Confidence Intervals	
			lower	upper			lower	upper
Eclipse	31.00	.477	.420	.534	24.50	.375	.346	.405
Norstar	29.00	.445	.408	.484	24.50	.375	.346	.405
Red Hawk	33.00	.508	.438	.577	52.75	.816	.709	.857
VAX3	37.00	.570	.482	.651	28.25	.434	.374	.497
	R. solani Ag 2-2							
Eclipse	34.09	.525	.415	.631	21.78	.333	.257	.430
Norstar	39.06	.603	.476	.708	34.97	.539	.406	.662
Red Hawk	32.72	.503	.385	.621	47.31	.732	.649	.789
VAX3	24.13	.369	.274	.488	25.94	.398	.317	.490

Table 2.6. (continued)

Cultivar	*R. solani* Ag 4							
	SS				FS			
	Mean rank	Estimated Relative	95% Confidence Intervals		Mean rank	Estimated Relative	95% Confidence Intervals	
			lower	upper			lower	upper
Eclipse	23.19	.355	.265	.470	27.53	.422	.332	.524
Norstar	33.97	.523	.408	.634	35.34	.544	.441	.641
Red Hawk	39.38	607	.502	.697	49.63	.768	.701	.812
VAX3	33.47	515	.391	.636	17.50	.266	.228	.314
	F. graminearum							
Eclipse	24.63	.377	.298	.470	25.16	.385	.348	.425
Norstar	38.78	.598	.491	.691	23.50	.359	.338	.382
Red Hawk	42.06	.649	.536	.737	56.19	.870	.852	.874
VAX3	24.53	.375	.284	.487	25.16	.385	.348	.425
	F. solani f.sp. *phaseoli*							
Eclipse	26.59	.408	.315	.515	22.50	.344	.316	.374
Norstar	32.38	.498	.392	.605	28.16	.432	.364	.505
Red Hawk	40.41	.624	.508	.718	55.13	.854	.811	.868
VAX3	30.63	.471	.367	.579	24.22	.371	.328	.418
	Mixed							
Eclipse	35.22	.543	.414	.662	21.81	.333	.275	.403
Norstar	26.41	.405	.309	.516	26.78	.411	.338	.492
Red Hawk	36.88	.568	.449	.675	53.94	.835	.787	.858
VAX3	31.50	.484	.372	.599	27.47	.421	.342	.509

Trial under natural disease pressure. Trials were conducted under natural disease pressure at two locations in Perham and Park Rapids in 2010. Samples were collected at two stages, the seedling stage and at flowering. The roots were rated for disease severity on a 1-9 scale. Fig 2.4 and Fig 2.5 represent the median disease severity across replications at the Perham and Park Rapid locations in 2010 and the Table 2.9 and Table 2.10 lists the mean ranks of the varieties whereas Table 2.11 puts together the stand counts and yields of the different varieties included in the two trials.

Table 2.7. Results from nonparametric analysis of disease severity ratings for four varieties using *R. solani* Ag 2-2 and Ag 4, *F. solani* f.sp. *phaseoli* and *F. graminearum* in inoculated trials at Prosper conducted in 2010.

Prosper								
Non-inoculated								
	SS				FS			
Cultivar	Mean rank	Estimated Relative	95% Confidence Intervals		Mean rank	Estimated Relative	95% Confidence Intervals	
			lower	upper			lower	upper
Eclipse	32.22	.496	.393	.599	24.22	.371	.328	.417
Norstar	33.34	.513	.412	.613	27.66	.424	.365	.488
Red Hawk	32.88	.506	.398	.613	55.63	.861	.835	.871
VAX3	31.56	.485	.388	.585	22.50	.344	.316	.374
R. solani Ag 2-2								
Eclipse	37.81	.427	.321	.546	33.66	.518	.465	.570
Norstar	27.88	.428	.330	.537	33.56	.517	.462	.570
Red Hawk	43.50	.612	.613	.722	35.50	.547	.512	.581
VAX3	30.81	.484	.377	.574	27.28	.419	.338	.508
R. solani Ag 4								
Eclipse	40.38	.623	.515	.713	23.59	.361	.307	.423
Norstar	26.44	.405	.303	.526	25.69	.394	.326	.469
Red Hawk	28.44	.437	.327	.558	54.13	.838	.483	.861
VAX3	34.75	.535	.420	.644	26.59	.408	.344	.478
F. graminearum								
Eclipse	24.44	.374	.281	.488	26.03	.399	.350	.452
Norstar	33.50	.516	.409	.620	25.50	.391	.354	.430
Red Hawk	34.91	.538	.407	.659	54.47	.843	.737	.869
VAX3	37.16	.573	.454	.678	24.00	.367	.342	.394
F. solani f.sp. *phaseoli*								
Eclipse	32.38	.498	.387	.610	23.00	.352	.328	.377
Norstar	36.03	.555	.440	.661	23.00	.352	.328	.377
Red Hawk	30.81	.474	.355	.598	56.50	.875	-	-
VAX3	30.78	.473	.350	.603	27.50	.422	.374	.472
Mixed								
Eclipse	26.72	.410	.304	.533	27.44	.421	.358	.489
Norstar	31.09	.478	.375	.585	23.50	.359	.330	.391
Red Hawk	42.44	.655	.534	.747	53.31	.825	.732	.859
VAX3	29.75	.457	.351	.571	25.75	.395	.339	.456

Table 2.8. Yields obtained from the four varieties used in the inoculated trials at Fargo and Prosper conducted in 2010.

	Yield (lb/ac)*					
Isolates	Non-inoculated	Ag 2-2	Ag 4	*F. gram*	*Fsp*	Mixed
			Fargo			
Eclipse	1326.3a	674.3a	1157.4a	1254.9a	1284.5ab	917.3a
Norstar	948.4b	378.0ab	1232.0a	1178.3a	1226.0ab	993.2a
Red Hawk	1143.3ab	228.1b	1013.6a	648.5b	1073.6b	794.0a
VAX3	1123.8ab	543.5ab	1053.5a	1176.0a	1425.4a	1129.1a
CV	2.31	2.37	2.26	2.26	2.26	2.31
LSD	237.2	394.3	329.6	336.8	234.9	448.4
			Prosper			
Eclipse	1390.4a	426.8a	1632.1ab	1833.0ab	1662.8ab	1360.9a
Norstar	1330.2a	210.0a	1346.1b	1634.4ab	1604.9ab	1221.3a
Red Hawk	1620.8a	204.3a	1460.8ab	1272.4b	1463.0b	837.6a
VAX3	2251.9a	208.8a	1932.9a	1922.7a	2251.9a	1357.5a
CV	2.26	2.31	2.26	2.26	2.26	2.26
LSD	992.8	387.0	555.2	564.3	651.5	575.0

* = means with the same letter are not significantly different

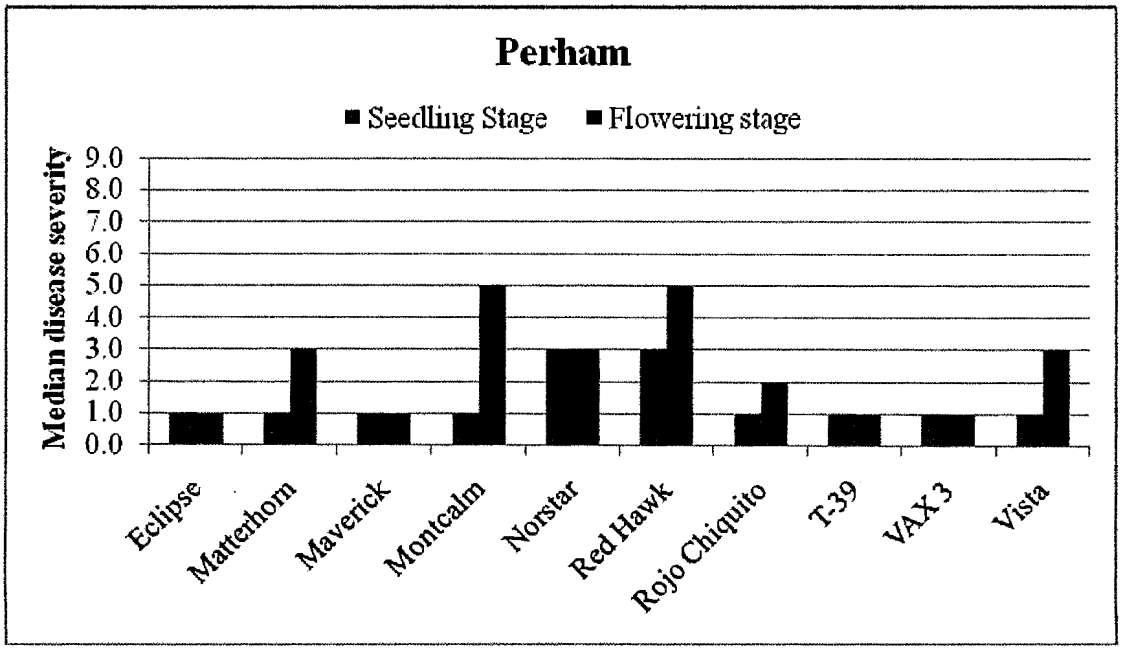


Figure 2.4. Median disease severity on the ten varieties used in the trials under natural disease pressure at Perham.

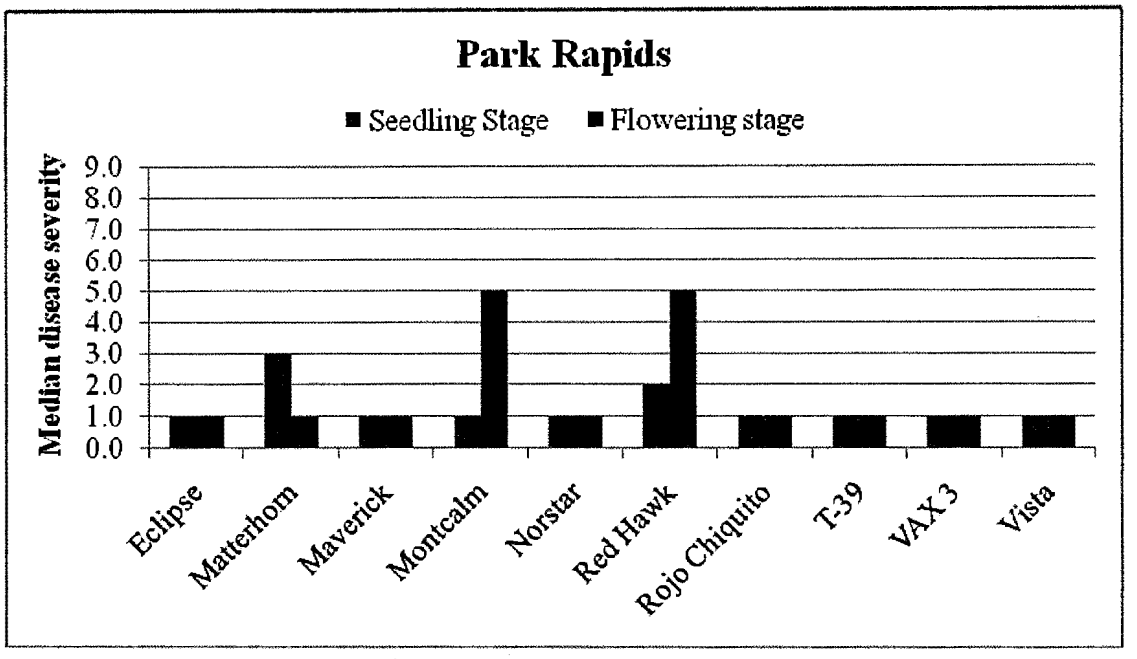


Figure 2.5. Median disease severity on the ten varieties used in the trials under natural disease pressure at Park Rapids.

Table 2.9. Results from nonparametric analysis of disease severity ratings for ten varieties planted under natural disease pressure in Perham.

	Perham							
	Seedling severity				Flowering severity			
Cultivars	Mean Rank	Estimated Relative	95% Confidence Intervals		Mean Rank	Estimated Relative	95% Confidence Intervals	
			Lower	Upper			Lower	Upper
Eclipse	76.88	.477	.372	.585	50.81	.315	.224	.427
Matterhorn	80.97	.503	.382	.624	77.28	.480	.374	.588
Maverick	77.59	.482	.373	.593	72.34	.449	.335	.571
Montcalm	84.28	.524	.403	.641	126.22	.786	.688	.853
Norstar	97.38	.606	.485	.712	81.06	.504	.400	.607
Red Hawk	105.56	.657	.513	.771	141.72	.883	.856	.902
Rojo Chiquito	81.69	.507	.383	.631	68.25	.423	.337	.516
T-39	70.00	.434	.331	.545	54.59	.338	.243	.453
VAX3	66.63	.413	.326	.508	45.56	.282	.224	.351
Vista	64.03	.397	.308	.495	87.16	.542	.464	.617

Table 2.10. Results from nonparametric analysis of disease severity rating for ten varieties planted under natural disease pressure in Park Rapids.

	Park Rapids							
	Seedling severity				Flowering severity			
Cultivars	Mean Rank	Estimated Relative	95% Confidence Intervals		Mean Rank	Estimated Relative	95% Confidence Intervals	
			Lower	Upper			Lower	Upper
Eclipse	60.66	.376	.317	.440	63.00	.391	.346	.438
Matterhorn	102.56	.638	.522	.737	75.00	.466	.389	.544
Maverick	83.94	.522	.414	.626	59.00	.366	.347	.385
Montcalm	77.69	.482	.372	.595	144.00	.897	.882	.908
Norstar	85.78	.533	.418	.644	75.00	.466	.389	.544
Red Hawk	96.31	.599	.483	.710	145.00	.903	.888	.915
Rojo Chiquito	60.66	.376	.317	.440	63.00	.391	.346	.438
T-39	72.72	.451	.350	.558	63.00	.391	.346	.438
VAX3	88.38	.549	.424	.668	59.00	.366	.347	.385
Vista	76.31	.484	.370	.580	59.00	.366	.347	.385

Under the natural disease pressure, the kidney beans Red Hawk and Montcalm appeared to be most affected by root rots. The mean rank of Red Hawk, in both locations was the most susceptible according to these trials, ranged between 96.31 and 145.00 (Table 2.9 and 2.10). The stand counts for Red Hawk were considered low but they were not significantly different from the stand counts for Maverick and T-39 at both locations. The yield of Red Hawk was also the lowest among all the varieties, with 465.3 lb/ac (Table 2.11). In contrast, Eclipse, Maverick, Rojo Chiquito, T-39, and VAX3 were found to be the most resistant varieties with no statistically significant differences among them. Although Vista had the highest stand counts, still VAX3 had the highest yield among the varieties.

Table 2.11. Stand count and yield data for trials under natural disease pressure conducted at Perham and Park Rapids.

Cultivars	Perham		Park Rapids	
	Stand count*	Yield (lb/ac)	Stand count*	Yield (lb/ac)
Eclipse	92.25bc	1028.3	110.25abc	2343.8
Matterhorn	98.75ab	1335.9	106.50bc	1822.8
Maverick	90.00bc	1098.7	102.75bc	1732.0
Montcalm	97.00ab	804.7	107.00bc	1883.0
Norstar	89.75bc	786.6	112.75ab	1579.9
Red Hawk	89.00bc	465.3	95.25c	985.2
Rojo Chiquito	101.50ab	1130.5	105.00bc	2399.4
T-39	78.25c	883.0	99.50bc	2149.7
VAX3	98.50ab	1794.4	103.50bc	2608.2
Vista	113.75a	1226.9	124.25a	1954.5
CV	2.04	25.80	2.04	12.10
LSD	17.10	394.50	15.75	342.10

* = means with the same letter are not significantly different

Discussion

The influence of weather on root rot severity could not be ascertained from this study. However, potential sources of resistance that are effective under both green-house and field conditions were identified. The findings from the green house studies appeared to match field results with varieties like Red Hawk, one of the most susceptible varieties in the green house, being most severely affected by all the pathogens under both conditions. Screening under inoculated conditions suggests that *R. solani* AG 2-2 has major effect on plant stand which is supported by the low stand counts at both the Fargo and Prosper locations. The roots on the plants that survived were also damaged due to infection. However, *R. solani* AG 4 did not affect plant stand to a similar extent though it was capable of causing significant root rot. This finding highlights the differences among the AG groups of *R. solani* not only in their host preferences but also in the way they infect the

same parts of a common host, thereby emphasizing the need for a resistance screening strategy that involves more than one AG group.

One aspect that became apparent in this study was that varieties with higher levels of resistance appeared to have the ability to overcome the effects of root rot to a certain extent as the plants matured. This is based on the observation that in varieties such as VAX3 and Eclipse the disease severity at seedling stage was almost as high as the more susceptible varieties; however, at the flowering stage the disease severity as measured by the extent of root discoloration and root mass loss appeared to reduce. The effect of root rot on yield in these varieties was also lower. Another observation, not apparent in the disease ratings was that there appeared to be a significant amount of secondary root growth in most infections involving *Fusarium* species. This finding is being investigated further.

On comparing our findings with those reported by Bilgi et al., 2008 and 2011 on resistance to Fusarium root rot in the varieties included in our study, we found that the most resistant cultivars in our field trials, Eclipse and VAX3 had also been considered to be resistant to *F. solani* f.sp. *phaseoli* and *F. graminearum*. Therefore, this study has enabled us to identify varieties that could serve as potential sources of resistance to the major root rot causing pathogens in the largest bean growing region in the US.

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SUMMARY

Bacterial blight diseases and root rots of dry beans have been found to affect the cultivation of this crop in North Dakota and Minnesota. The findings reported in this dissertation focus on these two diseases. The study on bacterial diseases was conducted to assess the prevalence of Halo blight (caused by *Pseudomonas syringae* pv *phaseolicola* - *Psp)* and Brown spot (*Pseudomonas syringae* pv *syringae Pss)*, and identify races of the Halo blight pathogen present in ND. A set of commonly available varieties of dry beans were also evaluated for potential resistance to Halo blight. Based on our findings from a three year long survey conducted from 2008-2010, the bacterial diseases appeared to be widespread in the major dry bean growing counties along the Red River Valley that were included in a survey and the disease incidence and pathogen prevalence appeared to be affected by weather conditions. Bacterial brown spot was found to be more prevalent in 2009 and 2010 which could be potentially have been due to weather conditions in these years that were more suitable for the pathogen associated with the disease. All the representative *Pss* and *Psp* isolates obtained from the survey were found to be pathogenic on pinto bean, Lariat. However, the *Psp* isolates varied in their aggressiveness. Race 6 of *Psp* was found to be the most common in this region followed with race 8. Several isolates of *Psp* included in the study exhibited intermediate disease reactions on the current differential set and therefore, could not be assigned to the known races. It is believed that these isolates may represent new races; however, further evaluation has to be conducted. The local pinto varieties studied did not demonstrate any resistance to our *Psp* isolates. Only, the kidney bean variety Red Hawk appeared to have partial resistance to some *Psp* isolates. The findings from this research not only give us information about the prevalence

of the two major bacterial pathogens of dry beans in the region and variability within them, but also suggest that it may be more appropriate to use a collection of *Psp* isolates, preferably from the field in the region for which the varieties are being bred, for use disease resistance screening during the breeding process.

Our studies on root rot of dry beans focused primarily on identifying sources of resistance to Rhizoctonia root rot through green-house and field evaluations of a set of 11 varieties which had previously been assessed for resistance to *Fusarium* species associated with the disease. According to our findings, the kidney bean varieties such as Red Hawk were found to be susceptible to both pathogens under greenhouse and field conditions. *R. solani* AG 2-2 was found significantly affect stand count in the inoculated trials conducted at two locations. *R. solani* Ag 4 however, did not affect stand count as much as *R. solani* Ag 2-2;but it was capable of causing root rot on dry beans. In the study, high level resistance was observed in varieties such as VAX3 and Eclipse. These varieties appeared to have the ability to overcome damage due to root rot as the plants matured, in both inoculated and non-inoculated trials, with disease severity rating at flowering being lower than those at the seedling stage. Overall, the trials suggest that these two varieties, particularly Vax3, could serve as a good source of resistance to both Rhizoctonia and Fusarium root rot and could be incorporated in breeding programs.

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APPENDIX I: BIOCHEMICAL TEST FOR *PSP*, *PSS*, AND *XAP*

Biochemical test for bacteria differential (Shaad et al. 2001).

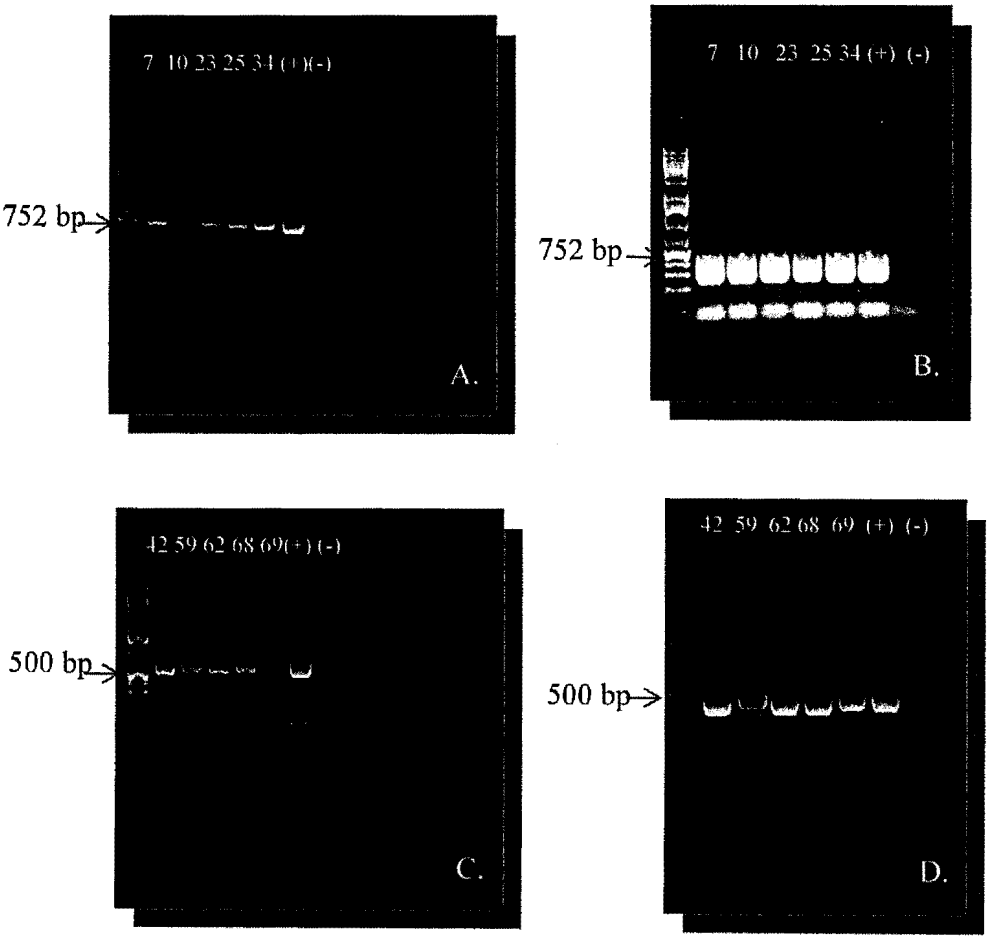
Pathogen	Gram stain	KB[a]	BBD[b]	Levan	Oxidase	Arginine dihydrolase	Pectatelyase	Pactinase activities	
								pH4	pH5
Psp	-	+	-	+	-	-	-	+	-
Pss	-	+	+	+	-	-	-	-	-

[a]= +: fluorescence, -: non-fluorescence
[b]= +: hydrolyze, -: non-hydrolyze

Pathogen	Gram stain	YDC[c]	Oxidative fermentative	Milk proteolyses	Pectinase activities	
					pH4	pH5
Xap	-	yellow	-	+	-	-

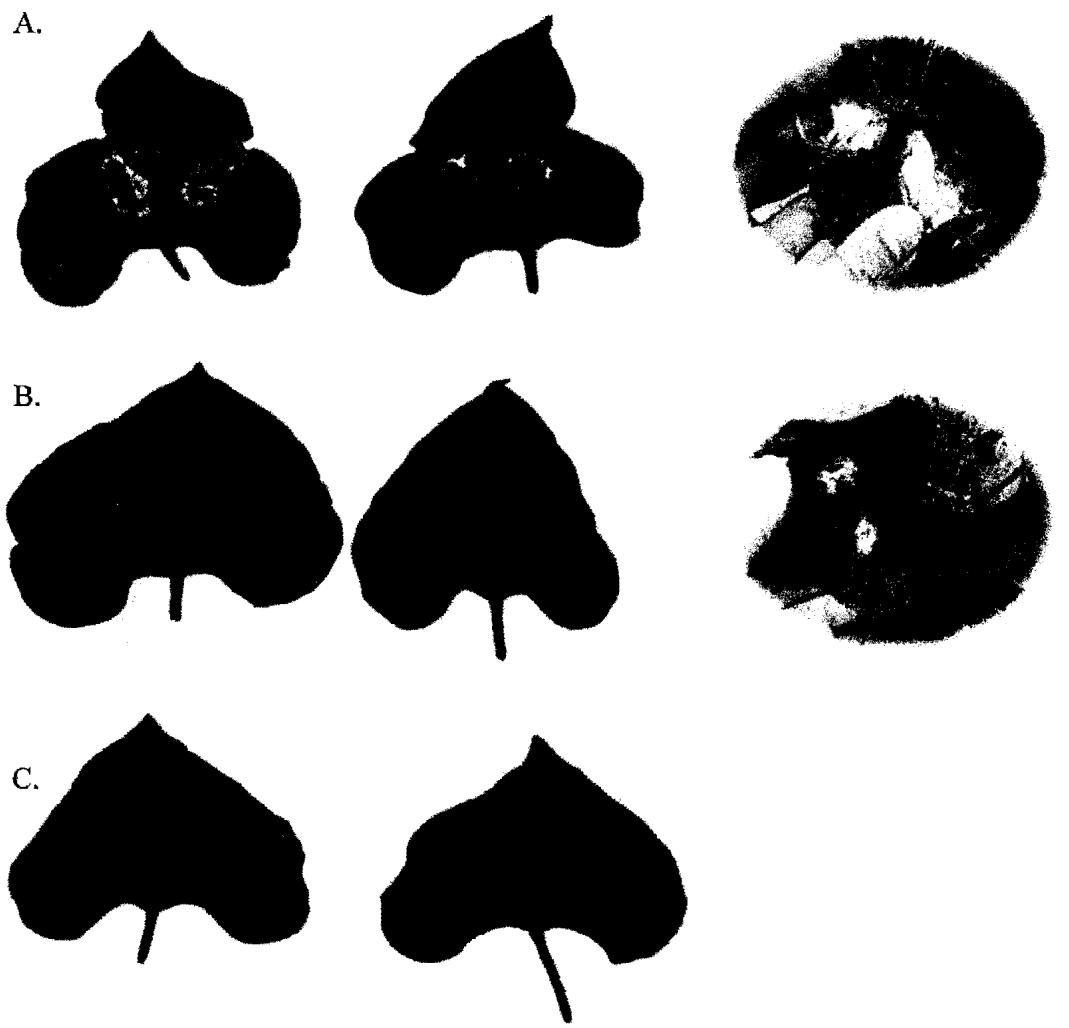
[c] = yellow pigment

APPENDIX II: PCR CONFIRMATION USING SPECIES SPECIFIC PRIMERS FOR *PSP* AND *PSS* USING INFECTED DRY BEAN LEAVES AND BACTERIAL CULTURES AS TEMPLATES



A. PCR using leaf samples infected with *Pss*. B. PCR using *Pss* cultures in nutrient broth (NB). C. PCR using leaf samples infected with *Psp*. D. PCR using *Psp* cultures in nutrient broth (NB).

APPENDIX III: LESION ON THE VARIETY LARIAT CAUSED BY *PSEUDOMONAS* SPECIES AT 10 DAI UNDER GREENHOUSE CONDITIONS



A. Infected by *Pss* isolate B. Infected by *Psp* isolate C. Negative control.

APPENDIX IV: SCREENING FOR RESISTANCE TO *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOLA* ISOLATED FROM DRY BEAN IN LOCAL COMMERCIAL VARIETIES

Type 3 tests of fixed effects.

Effect	Num DF	Den DF	Chi-Square	F value	Pr>ChiSq	Pr> F
trt	6	539	11942.5	1990.4	<.0001	<.0001

Differences of least squares means.

Effect	Trt	trt	Estimate	Standard Error	DF	t value	Pr> |t|
trt	09F65	08F69	1.80	3.74	539	.48	.630
trt	09F65	09F3	-19.53	3.74	539	-5.22	<.001
trt	09F65	09F61	43.41	3.74	539	11.60	<.001
trt	09F65	08F63	-8.56	3.74	539	-2.29	.023
trt	09F65	08F73	4.93	3.74	539	1.32	.188
trt	09F65	Control	311.68	3.74	539	83.30	<.001
trt	08F69	09F3	-21.33	3.74	539	-5.70	<.001
trt	08F69	09F61	41.61	3.74	539	11.12	<.001
trt	08F69	08F63	-10.36	3.74	539	-2.77	.006
trt	08F69	08F73	3.13	3.74	539	0.84	.403
trt	08F69	Control	309.88	3.74	539	82.82	<.001
trt	09F3	09F61	62.94	3.74	539	16.82	<.001
trt	09F3	08F63	10.97	3.74	539	2.93	.004
trt	09F3	08F73	24.46	3.74	539	6.54	<.001
trt	09F3	Control	331.20	3.74	539	88.52	<.001
trt	09F61	08F63	-51.96	3.74	539	-13.89	<.001
trt	09F61	08F73	-38.48	3.74	539	-10.28	<.001
trt	09F61	control	268.27	3.74	539	71.70	<.001
trt	08F63	08F73	13.49	3.74	539	3.60	.0003
trt	08F63	control	320.23	3.74	539	85.59	<.001
trt	08F73	control	306.74	3.74	539	81.98	<.001

Race 6 : 08F73, 09F3, 09F63, 09F65
Race 8 : 08F61, 08F69