CHARACTERIZATION AND DETECTION OF BACTERIAL PATHOGENS OF COMMON BEAN

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Katie Lea Nelson

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Pathology

August 2022

Fargo, North Dakota

North Dakota State University
Graduate School

Title

CHARACTERIZATION AND DETECTION OF BACTERIAL PATHOGENS OF COMMON BEAN

By

Katie Lea Nelson

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

SUPERVISORY COMMITTEE:

Julie Pasche
Chair

Zhaohui Liu

Guiping Yan

Juan Osorno

Approved:

September 26, 2022
Date

Jack Rasmussen
Department Chair

ABSTRACT

Common bacterial blight (CBB) (*Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* pv. *fuscans* (*Xff*), halo blight (*Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*)), and bacterial brown spot (*P. syringae* pv. *syringae* (*Pss*)), are yield-limiting diseases of common bean in North Dakota and Minnesota. The objectives of this research were to optimize a multiplex quantitative PCR (qPCR) assay for rapid detection and quantification of four bacterial pathovars in common bean seed, leaf, and pod tissue; determine the aggressiveness of *Xap* and *Xff* isolates; and determine the race types of *Psp* isolates in North Dakota and Minnesota. A fourplex qPCR assay was optimized, and novel primers and probe were designed for pathovar-specific detection of *Pss.* Significant differences were observed in *Xap/Xff* aggressiveness across isolates evaluated and in susceptibility of the differential lines (P <.0001). The 60 *Psp* isolates were identified as Race 6, the predominant race in the region.

ACKNOWLEDGEMENTS

First, I would like to thank my advisor, Dr. Julie Pasche, for her endless guidance, advice, patience, and encouragement during my master's research. Your continuous support and expertise were the pillars to my work, and I appreciate your patience in allowing me to learn and grow. I would also like to express gratitude to Dr. Zhaohui Liu, Dr. Guiping Yan, and Dr. Juan Osorno for your participation on my graduate committee. Your cooperation and insight into my master's degree has been beneficial, and I thank you for your contribution to my professional career.

A special thank you to the members, faculty, and staff of the Plant Pathology department for their help with my research. Thank you to Dr. Kristin Simons, Dr. Amanda Beck-Okins, Robin Lamppa, Dr. Kimberly Zitnick-Anderson, Claire Poore-Fonseka, and Dimitri Fonseka for the assistance in my work and countless lessons of plant pathology. I learned so much within the pulse lab, and it is thanks to you all. Many thanks to Roshan Sharma-Poudel, Ipsita Mallik, and Kishore Chittem for their input and guidance in my research.

Last and most importantly, I want to thank all of my family and friends who have encouraged me throughout all aspects of life and while pursuing my degree. To my parents, Lyndon and Becky, thank you for the unconditional love and support at all times. Thank you to my sisters, Lyndsie and Halie, for always being my role models. You four have been my biggest fans throughout my personal and professional development, and I'll never be able to express how much it means to me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

LIST OF APPENDIX FIGURES

Figure Page

LITERATURE REVIEW

Common Bean

Common bean (*Phaseolus vulgaris* L.) is one of the most important legume crops grown worldwide (Singh et al., 1991; Kelly, 2010). It is a great source of nutrition containing high amounts of protein, fiber, vitamins and minerals. Beans originated over 7,000 years ago in south-central Mexico/Guatemala and later spread to North and South America where it had two centers of domestication: the Middle American and Andean regions, resulting in two associated gene pools (Bitocchi et al., 2013; Bitocchi et al., 2014; Gepts et al., 1986; Mamidi et al., 2011; Schmutz et al., 2014; Zizumbo-Villarreal and Colunga-GarcíaMarín, 2010). Beans from these centers of domestication can be further divided into seven races including the Durango/Jalisco complex, Mesoamerica, and Guatemala (Middle America) and Chile, Nueva Granada, and Peru (Andean) (Singh et al., 1991; Wallace et al., 2018), varying in leaf morphology, seed size, seed shape, pod morphology, stem thickness, internode length, plant habit, allozyme type, and phaseolin type. Local landraces exemplify regional preferences in seed type. For example, in Venezuela and Guatemala, black beans are favored; in Colombia and Honduras, red; in Peru and Mexico, cream, tan, or black; and in Brazil, black or tan striped are favored by local consumers (Schwartz et al., 2005).

Common Bean Production

Worldwide, the common bean (*Phaseolus vulgaris* L.) is grown in a number of countries, and its production is led by Brazil, United States, Tanzania, Mexico, and Uganda (FAOSTAT, 2020; "*Phaseolus* Beans – Crop Vulnerability Statement (September 2020)", 2020). North Dakota has been the leading US producer since 1991. In 2020, North Dakota growers planted approximately 270,000 hectares of common bean. Minnesota ranked second in production

planting 111,000 hectares, followed by Michigan (85,000 ha) and Nebraska (50,000 ha) (USDA-NASS, 2022). The main market classes grown in the United States are pinto, navy, black, dark and light red kidney and great northern. North Dakota is the leading US producer of pinto (68% total acres planted) and black (37%) beans, and Minnesota is the top producer of kidney beans (67%) (USDA-NASS, 2018).

Bacterial Blight Disease Complex

Common bean may be affected negatively by bacterial pathogens occurring either individually or in a complex. Common bacterial blight (CBB), bacterial brown spot (BS), and halo blight (HB) are the three most common bacterial diseases observed on common bean. Bacterial blight has been observed in nearly 100% of fields surveyed for disease in North Dakota and Minnesota for over a decade. Additionally, a grower survey conducted in North Dakota and Minnesota has ranked CBB as one of the three worst diseases for bean growers over the past six years (Knodel et al., 2016-2021). When susceptible cultivars are planted, yield losses from these pathogens can exceed 40% (Opio et al., 1996).

Environmental conditions, amount of inoculum, and cultivar susceptibility are all factors that affect bacterial disease severity (Schuster et al., 1983). When infected seed is planted, bacteria are transferred from seed to seedling through the vascular system (Schwartz et al., 2005). Contaminated seed is an important source of inoculum for local and global dissemination of the pathogen (Mutlu et al., 2008). In addition, infected plant residue that is not incorporated can be a source of inoculum. Other means of infection are natural openings or wounds created by heavy wind, rain, hail, equipment, irrigation, animals, insects and other diseased plants. Heavy infection leads to pathogen colonization of pods and seeds. Incidence of seed infection by the CBB pathogen, *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), has been correlated with CBB

incidence in a seed production field (Valarini et al., 1992). A positive correlation between seed symptoms and the population of *Xap* per seed also has been reported (Opio et al., 1993).

In addition to yield reductions, a reduction in seed quality is also common under high disease pressure. Seed may be rotted, discolored, or shriveled resulting in an unmarketable product. Germination and vigor may be greatly reduced in infected seed (Harveson and Schwartz, 2007). Bacteria can survive longer than the seed is viable (Dreo et al., 2003), and *Xap* was recovered from 15-year-old bean seeds (Schuster and Sayre, 1967).

Symptoms are similar among these bacterial diseases at early stages of infection and can be observed on leaves, pods and seed within four to 10 days after infection (Schwartz et al., 2005). Early symptoms include water-soaked spots on the underside of the leaf. Common bacterial blight lesions enlarge to appear necrotic with a raised edge and a chlorotic border. They are typically found on interveinal tissue or along leaf margins. Bacterial brown spot lesions are much smaller and more circular than CBB. These necrotic spots coalesce and may fall out, similarly to CBB. Halo blight lesions are comparable in size to brown spot, but they have a large yellow-green border, or halo. Pod symptoms are difficult to distinguish at all stages of development and include water-soaking that further develops into circular, red-brown, sunken lesions. Bacterial oozing may be present under humid conditions.

Common Bacterial Blight

Common bacterial blight (CBB) is caused by the gram-negative, rod-shaped bacterium *Xanthomonas axonopodis* pv. *phaseoli (Xap)* Smith 1987 (Vauterin et al., 1995) and *Xanthomonas fuscans* subsp. *fuscans (Xff)* Burkholder (Starr & Burkholder, 1942). *Xap* and *Xff* grow as yellow, convex colonies on media. *Xff* produces a diffusible brown pigment due to secretion and oxidation of homogentisic acid, distinguishing it from *Xap* when cultured on

nutrient media (Goodwin and Sopher, 1994). *Xap/Xff* are oxidase negative, catalase positive, produce acid from several sugars, hydrolyze starch, and proteolyze milk (Schwartz et al., 2005). Aside from common bean, the primary host, *Xap* and *Xff* may naturally infect other legume species including lima bean (*Phaseolus lunatus* L.), tepary bean (*P. acutifolius* A. Gray), runner bean (*P. coccineus* L.), moth bean (*Vigna aconitifolia* Jacq.), mung bean (*Phaseolus aureus* L.), velvet bean (*Mucuna pruriens* L.), and hyacinth bean (*Lablab purpureus* L.). (EPPO, 2019). *Xap* and *Xff* are distributed worldwide and registered as quarantine pests in Africa, Asia, and Europe. Damage caused by these bacterial pathogens is more severe in warm, humid or wet conditions at temperatures around 26 to 32˚C.

Genetic and pathogenic variation has been reported among isolates of *Xap* and *Xff* on several common bean lines (Duncan et al., 2011; Ekpo and Saettler, 1976; Lopez et al., 2006; Mahuku et al., 2006; Mkandawire et al., 2004; Mutlu et al., 2008; Opio et al., 1996; Zapata, 1997). Ekpo and Saettler identified variation among eight *Xap* and seven *Xff* isolates based upon disease reactions in six common bean cultivars (Ekpo and Saettler, 1976). Similarly, Zapata et al. used a differential set of common and tepary bean lines to describe distinct races of *Xap* among bacterial isolates from Costa Rica, Cuba, Dominican Republic, and Puerto Rico (Zapata et al., 1985). Mkandawire et al. grouped *Xap* into three distinct genotypes, two found in East Africa, and the third group containing isolates from East Africa and the rest of the world, based upon RFLP analyses and rep-PCR (Mkandawire et al., 2004).

Genetic analysis and pathogenicity tests indicated that *Xff* isolates were more closely related than *Xap* isolates, and *Xff* was genetically different from *Xap* (Lopez et al., 2006). Cultivars representing the two common bean gene pools were tested for common bacterial blight reactions with 26 *Xap* and 11 *Xff* isolates (Lopez et al., 2006). Spanish *Xap* isolates were placed

into a large cluster with New World *Xap* isolates, and two clusters of *Xff* were identified comprising of one with African isolate and the other with New World (North America, Mexico, Central America, South America, and the Caribbean) isolates (Lopez et al., 2006). Rep-PCR and RAPD results indicated that *Xff* isolates were genetically different from *Xap* (Mutlu et al., 2008). Out of 69 *Xap* and 15 *Xff* isolates, the Caribbean and South American isolates showed the largest variation in pathogenicity across 12 inoculated bean lines. The *Xff* isolates were more aggressive than *Xap*, and African *Xff* isolates were most aggressive. Alavi et al. grouped *Xap* and *Xff* isolates into four genetically distinct lineages by fluorescent amplified fragment length polymorphism: six *Xap* into cluster 1, seven *Xff* into cluster 2, two *Xap* into cluster 3, and six *Xap* into cluster 4 (Alavi et al., 2008). More recently, isolates of *Xap* and *Xff* were divided into three pathovars: one for *Xap*, one for *Xff* and one for isolates collected from hyacinth beans (*Lablab purpureus* L.) based on multilocus sequence analysis (MLSA) (Tugume et al., 2018). In contrast, Jara et al. did not identify a diverse pathogen population among 12 *Xap* and eight *Xff* isolates (Jara et al., 1999).

Bacterial Brown Spot

Bacterial brown spot is caused by the fluorescent bacterium *Pseudomonas syringae* pv. *syringae* van Hall (*Pss*). The pathogen is gram-negative, rod-shaped, and motile with polar flagella. When cultured on artificial growth media, colonies are cream-colored, white, or translucent and grow optimally at 28 to 30˚C. *Pss* produces a bacteriocin known as syringacin W-1 in the host plant (Schwartz et al., 2005). It is unique when compared to most *P. syringae* pathovars in its ability to cause disease in over 180 species of plants in several unrelated genera including stone fruits, pome fruits, other woody hosts such as willow, crop plants such as bean, and grasses such as wheat (Bradbury 1986). Some hosts include common bean, faba bean (*Vicia*

faba L.), lima bean (*Phaseolus lunatus* L.), pea (*Pisum sativum* L.), soybean (*Glycine max* L.), and cowpea (*Vigna unguiculata* L.) (Schwartz et al., 2005). Brown spot is favored by temperatures of around 26 to 29˚C.

Halo Blight

Halo blight is caused by *Pseudomonas savastanoi* pv. *phaseolicola* (Burkholder) Young et al. (*Psp*). It has a similar host range to *Pss, Xap,* and *Xff. Psp* is closely related to *Pss*, but it produces phaseolotoxin, causing a yellow to lime-green halo around the lesion (Schwartz et al., 2005). Infection and toxin production of *Psp* are favored by high humidity and temperatures ranging from 18 to 23˚C.

Nine races of *Psp* have been recognized on the basis of the differential reactions to eight common bean lines. Races 1 and 2 were first identified and are now distributed in Europe (Taylor, 1970; Wharton, 1967), New Zealand (Hale and Taylor, 1973), North America (Guthrie and Fenwick, 1967; Patel and Walker, 1965), Latin America (Buruchara and Pastor-Corrales, 1981) and Africa (Kinyua et al., 1981; Msuku, 1986). A third race was described from Africa after it caused a characteristic hypersensitive reaction on two bean cultivars which had been previously susceptible to races 1 and 2 (Mabagala and Saettler, 1992; Taylor and Teverson, 1985). Tests later evaluating 175 *Psp* isolates representative of various geographical regions worldwide identified a more complex race structure comprised of nine subdivisions of races 1, 2, and 3. Subdivisions based upon reactions on eight differential lines include races 1, 2, 5, 6 and 7 distributed worldwide, races 3 and 4 found in East/Central Africa, and races 8 and 9 in Southern Africa (Taylor et al., 1996). Among *Psp* isolates evaluated, 32% were race 6, 19% race 4, and 12% race 1. Race 6 was widespread throughout Africa, North America, Latin America, and Europe (Taylor et al., 1996; Lamppa et al., 2002; Rico et al., 2003; Félix-Gastélum et al., 2016).

Race 4 was found in seven countries in Africa, and race 1 is distributed worldwide including Africa, Latin America, North America, Europe, and New Zealand. Race 2 and race 7 are widespread throughout 13 and 11 countries worldwide, respectively. Races 5 and 8 included isolates from Africa, and races 3 and 9 from Africa and Colombia. Studies conducted in North Dakota evaluated race-types among a total of 161 *Psp* isolates including: 148 collected in 2000, six in 1995, three in 1997 and ten in 1999. Among 161 isolates, 148 were identified *Psp* race 6, concluding race 6 as the most prevalent in North Dakota (Lamppa et al., 2002).

Detection

Bacterial blight is an economically important and widespread disease in common bean production, especially certified seed. Seed certification provides a practical and reliable method to verify and maintain pathogen-free seed. Reliable detection of bacterial blight pathogens is critical as seeds are an important source of inoculum for outbreaks, and bacteria can be transmitted from seeds to seedlings at a high frequency (Valarini et al., 1992). Several approaches have been used for detecting and identifying these seedborne pathogens including the dome test (Venette et al. 1987), indirect immunofluorescence microscopy and ELISA (Wong, 1991), PCR (Audy et al., 1994; Bultreys and Gheysen, 1999; Sato et al., 1997; Toth et al., 1998), q-PCR (Cho et al., 2010; He, 2010; Xu and Tambong, 2011) and culturing seed soak fluid (Grimault et al., 2018). The seed soak method and the dome test are the only two of the published methods to have been accepted as routine diagnostic tools for seed health testing (Grimault et al., 2018; Venette et al., 1987).

The dome test (NDSSD WI-DL208), used by the North Dakota State Seed Department and other seed certification programs including the Minnesota Crop Improvement Association and Manitoba Agriculture, is based upon visualization of water-soaked spots on 13-day old bean

leaves grown in cake containers (Venette et al., 1987). The average lesion number on a plant corresponds to a diagram value in which the seed sample passes or fails. It is impossible to distinguish the four bacterial pathogens on the basis of early symptoms using the dome test, thus making it a non-specific test. Furthermore, this test is outdated as it has been used for over three decades. Another method of diagnosing bacterial blight of common bean is via pathogen isolation from seed soaking and plating the suspension onto semi-selective Milk Tween (MT) and *Xanthomonas campestris* pv. *phaseoli* XCP1 (adapted from McGuire et al., 1986) media (Grimault et al., 2018). Bacterial concentration (CFU/mL) is calculated from a countable plate containing target bacteria. Plating seed soak delivers a more specific result than the dome test, however the presence of non-target organisms on semi-selective media is common.

PCR and qPCR assays have been developed for detection and quantification of *Xap* and *Xff* in common bean seed (Audy et al., 1994; He, 2010). Conventional PCR has been used to detect pure isolates of *Pss* (Bultreys and Gheysen, 1999), and primer sets were designed for conventional PCR amplification of *Psp* in soaked bean seed (Schaad et al., 1995; Sato et al., 1997). The first qPCR assays for *Pss* and *Psp* (Xu and Tambong, 2011) and *Psp* (Cho et al., 2010) have been published for detection of pure bacterial isolates. Both assays offer higher sensitivity compared to conventional PCR. Simultaneous quantification of *Xap*, *Xff*, *Pss*, and *Psp* with conventional or qPCR has not yet been carried out in pure culture or plant tissue such as common bean seed, pod, and leaf material. A specific assay with multiplex detection of the four bacterial pathogens is useful because they are not easily distinguished by early visual examination.

Management

Management of the bacterial blight complex in common beans is most effective using an integrated pest management (IPM) program including planting disease-free seed, crop rotation with non-host crops for at least two years, soil incorporation of crop debris, host resistance, seed treatments and foliar bactericides (Schwartz et al., 2005). Reducing the amount of available inoculum is significant in decreasing blight disease pressure and is commonly done with tilling the previous year's infested residue and implementing a two-year crop rotation with non-host crops. Planting healthy seed may prevent or delay early infection. Limiting the use of irrigation is effective by preventing water splash that can lead to bacteria entering the plant. Copper and peroxide-based foliar bactericides and a registered streptomycin seed treatment can be used, but they are not commonly applied by growers in the region given variable efficacy and costs (Knodel et al., 2016-2021). Bactericides should be applied before symptoms appear for best efficacy (Schwartz et al., 2005).

Genetic resistance is the most effective and efficient tool to provide durable management of bacterial blights (Zapata et al., 1985; Duncan et al., 2011; Schwartz et al., 2005; Singh and Miklas, 2015), however it is often challenging to incorporate into common bean lines (Jung et al., 2003; Singh and Munoz, 1999; Webster et al., 1980). The quantitative resistance to bacterial blights is highly variable depending on environmental conditions, disease pressure, genetics, and plant maturity (Kelly et al., 2003; Miklas et al., 2006c; Durham et al., 2013). Additionally, a line may be resistant to one bacterial isolate but susceptible to others (Aggour et al., 1989; Duncan et al., 2011; Mkandawire et al., 2004; Mutlu et al., 2008; Zapata, 1997; Zapata and Beaver, 2005).

Summary

Common bean production in North Dakota and Minnesota is an extremely important industry. Common bacterial blight, halo blight, and brown spot have been reported throughout North Dakota and Minnesota bean growing regions and are known to cause substantial yield loss. Pathogenic variation within and among common blight pathogens *Xap* and *Xff* has been reported, thus it would be prudent to test the response of common bean lines to a pathogen population in North Dakota and Minnesota. Additionally, nine races occur within *Psp*, with race 6 as most prevalent in this area; therefore, testing responses of beans to local *Psp* isolates is important before using them in breeding programs. Current seed testing methods for the diagnosis of bacterial blight in the region are outdated and non-specific. Additionally, these traditional methods are time consuming and labor intensive. It is imperative to continue making advancements in pathogen detection especially in seed certification programs. Molecular detection holds the ability to specifically quantify pathogens in plant tissue, and simultaneous quantification of *Xap, Xff, Pss,* and *Psp* is unavailable. Pathogen detection is critical in disease management, particularly with seed-borne pathogens. Therefore, a qPCR assay has the potential to provide certified seed growers with reliable, sensitive results. Furthermore, assessing the pathogenic variation among the bacteria in this disease complex can aid in disease management.

Literature Cited

Adams, M. W., Kelly, J. D., and Saettler, A. W. 1988. A gene for resistance to common blight (*Xanthomonas campestris* pv. *phaseoli*). Annu. Rep. Bean Improv. Coop. 31:73–74.

Aggour, A. R., and Coyne, D. P. 1989. Heritability, phenotypic correlations, and associations of the common blight disease reactions in beans. J. Am. Soc. Hortic. Sci. 114:828–833.

Alavi, S. M., Sanjari, S., Durand, F., Brin, C., Manceau, C., and Poussier, S. 2008. Assessment of the genetic diversity of *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas fuscans* subsp. *fuscans* as a basis to identify putative pathogenicity genes and a type III secretion system of the SPI-1 family by multiple suppression subtractive hybridizations. Appl. Environ. Microbiol. 74:3295-3301.

Arnold, D. L., Lovell, H. C., Jackson, R. W., and Mansfield, J. W. 2011. *Pseudomonas syringae* pv. *phaseolicola.* From 'has bean' to supermodel. Mol. Plant Pathol. 12:617-627.

Audy, P., Laroche, A., Saindon, G., Huang, H. C., and Gilbertson, R. L. 1994. Detection of the bean common blight bacteria, *Xanthomonas-campestris* pv *phaseoli* and *x-c phaseoli* var *fuscans*, using the polymerase chain-reaction. Phytopathology. 84:1185-1192.

Bitocchi, E., Bellucci, E., Giardini, A., Rau, D., Rodriguez, M., Biagetti, E., Santilocchi, R., Spagnoletti Zeuli, P., Gioia, T., Logozzo, G., Attene, G., Nanni, L., and Papa, R. 2013. Molecular analysis of the parallel domestication of the common bean (*Phaseolus vulgaris*) in Mesoamerica and the Andes. New Phytol. 197:300–313.

Bitocchi, E., Nanni, L., Bellucci, E., and Papa, R. 2012. Mesoamerican origin of the common bean (*Phaseolus vulgaris* L.) is revealed by sequence data. Proc. Natl. Acad. Sci. USA 109:E788–E796.

Borkar, S. G., and Yumlembam, R. A. 2017. Bacterial diseases of crop plants. 1st edition. Taylor & Francis Group, Boca Raton, FL. p. 1-594.

Bradbury, J. F 1986. Guide to Plant Pathogenic Bacteria. Wallingford, UK: CAB International.

Buruchara, R. A., and Pastor-Corrales, M. A. 1981. Variation, virulence of *Pseudomonas syringae* pv. *phaseolicola* on beans in Colombia. In: Proceedings of the Fifth International Conference on Plant Pathogenic Bacteria, Cali, Columbia: Centro International de Agricultura Tropical. 341–51.

Bultreys, A., and Gheysen, I. 1999. Biological and molecular detection of toxic lipodepsinonapeptide-producing *Pseudomonas syringae* isolates and PCR identification in plants. Appl Environ Microbiol. 65:1904-1909.

CABI. 2007. Distribution maps of plant diseases. Wallingford, UK: CAB International. April. Map 401 (Edition 4).

Casper-Lindley, C., Dahlbeck, D., Clark, E. T., and Staskawicz, B. J. 2002. Direct biochemical evidence for type III secretion-dependent translocation of the AvrBs2 effector protein into plant cells. Proc. Natl. Acad. Sci. USA. 99:8336–8341.

Chataika, B. Y. E., Bokosi, J. M., Chirwa, R. M., and Kwapata, M. B. 2011. Inheritance of halo blight resistance in common bean. African Crop Sci. J. 9:325-333.

Cho, M. S., Jeon, Y. H., Kang, M. J., Ahn, H., Baek, H., Na, Y. W., Choi, Y. M., Kim, T. S., and Park, D. S. 2010. Sensitive and specific detection of phaseolotoxigenic and nontoxigenic isolates of *Pseudomonas syringae.* pv. *phaseolicola* by TaqMan real-time PCR using site-specific recombinase gene sequences. Microbiol. Res. 165:565-572.

Claflin, L. E., Vidaver, A. K., and Sasser, M. 1987. MXP, a semi-selective medium for *Xanthomonas campestris* pv. *phaseoli*. Phytopathology. 77:730-734.

Coyne, D. P., and Schuster, M. L. 1983. Genetics of and breeding for resistance to bacterial pathogens in vegetable crops. HortScience. 18:30–36.

Curland, R. D., Gao, L., Bull, C. T., Vinatzer, B. A., Dill-Macky, R., Van Eck, L., and Ishimaru, C. A. 2018. Genetic diversity and virulence of wheat and barley isolates of *Xanthomonas translucens* from the upper midwestern United States. Phytopathology. 108:443-453.

Dreo, T., Demsar, T., and Ravnikar, M. 2003. Quarantine of *Xanthomonas campestris* pv. *phaseoli* in beans. Zbornik predavanj in referatov 6. Slovenskega Posvetovanje o Varstvu Rastlin, Zrece, Slovenije. 4-6:123-127.

Duncan, R. W., Singh, S. P., and Gilbertson, R. L. 2008. Identification and inheritance of a new source of halo blight resistance in common bean. Annu. Rep. Bean. Improv. Coop. 51:48-49.

Duncan, R. W., Singh, S. P., and Gilbertson, R. L. 2011. Interaction of common bacterial blight bacteria with disease resistance quantitative trait loci in common bean. Phytopathology. 101:425–435.

Duncan, R. W., Lema, M., Gilbertson, R. L., and Singh, S. P. 2014. Registration of common bean pinto US14HBR6 resistant to race 6 of the halo blight pathogen, *Pseudomonas syringae* pv. *phaseolicola*. J. Plant Reg. 8:53-56.

Duncan, R.W., Lema, M., Gilbertson, R. L., and Singh, S. P. 2014. Registration of common bean pinto US14HBR6 resistant to race 6 of the halo blight pathogen, *Pseudomonas syringae* pv. *phaseolicola*. J. Plant Reg. 8:53-56.

EPPO. 2019. EPPO Global Database (available online). https://gd.eppo.int.

Félix-Gastélum, R., Maldonado-Mendoza, I. E., Navarrete-Maya, R., Olivas-Peraza, N. G., Brito-Vega, H., and Acosta-Gallegos, J. A. 2016. Identification of *Pseudomonas syringae* pv. *phaseolicola* as the causal agent of halo blight in yellow beans in northern Sinaloa, Mexico. Phytoparasitica. 44:369–378.

Food and Agriculture Organization of the United Nations. 2020. FAOSTAT statistical database. [Rome]:FAO.

Fourie, D. 2002. Bacterial diseases of Dry beans in South Africa with special reference to common Bacterial blight and its control. P.h.D thesis submitted to the Faculty of Natural and Agricultural Sciences, Department of Microbiology and plant pathology, University of Pretoria.

Gepts, P., Osborn, T., Rashka, K. and Bliss, F. 1986. Phaseolin-protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris*): evidence for multiple centers of domestication. Econ. Bot. 40:451–468.

Gepts, P., and Debouck, D. G. 1991. Origin, domestication, and evolution of the common bean (*Phaseolus vulgaris* L.). In: van Schoonhoven, A., Voysest, O. (eds.). Common beans: research for crop improvement. Commonwealth Agricultural Bureaux International, Wallingford, United Kingdom. p. 7-53.

Goodwin, P. H., and Sopher, C. R. 1994. Brown pigmentation of *Xanthomonas campestris* pv. *phaseoli* associated with homogentisic acid. Canadian Journal of Microbiology. 40:28-34.

Grimault, V., Olivier, V., Rolland, M., Darrasse, A., and Jacques, M. A. 2018. Detection of *Xanthomonas* axonopodis pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* in *Phaseolus vulgaris* (bean) seed. International Rules for Seed Testing. 7-021.

Gurtler, V., and Barrie, H. D. 1995. Typing of *Staphylococcus-aureus* isolates by PCR-amplification of variable-length 16s-23s rDNA spacer regions - characterization of spacer sequences. Microbiology-Uk. 141:1255-1265.

Guthrie, J. W., and Fenwick, H. S. 1967. Pathogenicity of Idaho isolates of *Pseudomonas phaseolicola*. Plant Dis. Rep. 51:591–3.

Hale, C. N., and Taylor, J. D. 1973. Races of *Pseudomonas phaseolicola* causing halo-blight of beans in New Zealand. New Zealand J. Agric. Res. 16:147–9.

Harveson, R. M., and Schwartz H. F. 2007. Bacterial diseases of dry edible beans in the central high plains. Plant Health Prog. PHP-2007-0125-01-DG.

He, Y. 2010. Improved seed health tests for *Xanthomonas axonopodis* pv. *phaseoli* in common bean. Graduate Theses and Dissertations. Ames, IA. 11565.

He, Y., and Munkvold, G. P. 2012. Comparison of extraction procedures for detection of *Xanthomonas axonopodis* pv. *phaseoli* in common bean seed. Plant Pathol. 61:837-843.

He, R., Liu, P., Jia, B., Xue, S., Want, X., Hu, J., Shoffe, Y. A., Gallipoli, L., Mazzaglia, A., Balestra, G. M., and Liwu, Z. 2019. Genetic diversity of *Pseudomonas syringae* pv. *actinidiae* isolates from different geographic regions in China. Phytopathology. 109:347-357.

Jara, C., Mahuku, G., Teran, H., and Singh, S. P. 1999. Reaction of common bean lines VAX 4, VAX 5, and VAX 6, derived from interspecific hybridization and gene pyramiding, to 20 *Xanthomonas campestris* pv. *phaseoli* isolates of different geographical origins. Annu. Rep. Bean Improv. Coop. 42:1-2.

Jones, J. D. G., and Dangl, J. L. 2006. The plant immune system. Nature. 444:323–329.

Jung, G., Ariyarathne, H. M., Coyne, D. P., and Nienhuis, J. 2003. Mapping QTL for bacterial brown spot resistance under natural infection in field and seedling stem inoculations in growth chamber in common bean. Crop Sci. 43:350-357.

Karavina, C., Mandumbu, R., Parwada, C., and Tibugari, H. 2011. A review of the occurrence, biology and management of common bacterial blight. J. Agric. Sci. Technol. 7:1459-1474.

Kelly, J. D., Gepts, P., Miklas, P. N., and Coyne, D. P. 2003. Tagging and mapping of genes and QTL and molecular marker-assisted selection for traits of economic importance in bean and cowpea. Field Crops Res. 82 135–154.

Kelly, J. D. 2010. The story of bean breeding. Michigan: Michigan State University.

Kinyua, G. K., Mukunya, D. M., and Van Breukelen, E. M. 1981. Studies on genetic resistance of beans to *Pseudomonas phaseolicola* in Kenya. In: Proceedings of the Fifth International Conference on Plant Pathogenic Bacteria, Cali, Columbia: Centro International de Agricultura Tropical. 352–7.

Knodel, J. J., Beauzay, P. B., Endres, G. J., Franzen, D. W., Kandel, H. J., Markell, S. G., Osorno, J. M., Pasche, J. S., and Zollinger, R. K. 2016. 2015 Dry bean grower survey of production, pest problems and pesticide use. North Dakota State University Extension Service. E1802.

Knodel, J. J., Beauzay, P. B., Endres, G. J., Franzen, D. W., Kandel, H. J., Markell, S. G., Osorno, J. M., Pasche, J. S., and Zollinger, R. K. 2017. 2016 Dry bean grower survey of production, pest problems and pesticide use. North Dakota State University Extension Service. E1841.

Knodel, J. J., Beauzay, P. B., Endres, G. J., Franzen, D. W., Kandel, H. J., Markell, S. G., Osorno, J. M., Pasche, J. S., and Zollinger, R. K. 2018. 2017 Dry bean grower survey of production, pest problems and pesticide use. North Dakota State University Extension Service. E1884.

Knodel, J. J., Beauzay, P. B., Endres, G. J., Franzen, D. W., Ikley, J. T., Kandel, H. J., Markell, S. G., Osorno, J. M., and Pasche, J. S. 2019. 2018 Dry bean grower survey of production, pest problems and pesticide use. North Dakota State University Extension Service. E1902.

Knodel, J. J., Beauzay, P. B., Endres, G. J., Franzen, D. W., Ikley, J. T., Kandel, H. J., Markell, S. G., Osorno, J. M., and Pasche, J. S. 2020. 2019 Dry bean grower survey of production, pest problems and pesticide use. North Dakota State University Extension Service. E1952.

Knodel, J. J., Beauzay, P. B., Endres, G. J., Franzen, D. W., Ikley, J. T., Kandel, H. J., Markell, S. G., Osorno, J. M., and Pasche, J. S. 2021. 2020 Dry bean grower survey of production, pest problems and pesticide use. North Dakota State University Extension Service. E2014.

Lahman, L. K., and Schaad, N. W. 1985. Evaluation of the “dome test” as a reliable assay for seedborne bacterial blight pathogens of beans. Plant Dis. 69:680-683.

Lamppa, R. S. ,Gross, P. L., and del Rio, L. E. 2002. Races of *Pseudomonas syringae* pv. *phaseolicola* in North Dakota. Ann Bean Improv Coop. 45:104-106.

Lindgren, P. B., Peet, R. C., and Panopoulos, N. J. 1986. Gene cluster of *Pseudomonas syringae* pv. *phaseolicola* controls pathogenicity of bean plants and hypersensitivity of non-host plants. J. Bacteriol. 168:512–522.

López, R., Asensio, C., and Gilbertson, R. L. 2006. Phenotypic and genetic diversity in isolates of common blight bacteria (*Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) in a secondary center of diversity of the common bean host suggests multiple introduction events. Phytopathology. 96:1204–1213.

Mabagala, R. B, and Saettler, A. W. 1992. The role of weeds in survival of *Pseudomonas syringae* pv. *phaseolicola* in Northern Tanzania. Plant Dis. 76:683–6.

Mahuku, G. S., Jara, C., Henríquez, M. A., Castellanos, G., and Cuasquer, J. 2006. Genotypic characterization of the common bean bacterial blight pathogens, *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* by rep-PCR and PCR-RFLP of the ribosomal genes. J. Phytopathol. 154:35–44.

Mamidi, S., Rossi, M., Annam, D., Moghaddam, S., Lee, R., Papa, R., and McClean, P. 2011. Investigation of the domestication of common bean (*Phaseolus vulgaris*) using multilocus sequence data. Funct. Plant Biol. 38:953–967.

Maringoni, A. C., Kimati, H., and Kurozawa, C. 1998. Comparison between two extraction methods of *Xanthomonas campestris* pv. *phaseoli* from bean seeds. Pesquisa Agropecuaria Brasileira. 33:659-664.

Miklas, P. N., Fourie, D., Trapp, J., Davis, J., and Myers, J. R. 2014. New loci including conferring resistance to halo bacterial blight on chromosome pv04 in common bean. Crop Sci. 54:2099-2108.

Miklas, P. N., Fourie, D., Wagner, J., Larsen, R. C., and Mienie, C. M. S. 2009. Tagging and mapping *Pse-1* gene for resistance to halo blight in common bean differential cultivar UI-3. Crop Sci. 49:41-48.

Miklas, P. N., Singh, S. P., Terán, H., Kelly, J. D., and Smith, J. R. 2011. Registration of common bacterial blight resistant cranberry dry bean germplasm line USCR-CBB-20. J. Plant Reg. 5:98–102.

Miklas, P. N., Smith, J. R., and Singh, S. P. 2006a. Registration of common bacterial blight resistant dark red kidney bean germplasm line USDK-CBB-15. Crop Sci. 46:1005–1006.

Miklas, P. N., Smith, J. R., and Singh, S. P. 2006b. Registration of common bacterial blight resistant white kidney bean germplasm line USWK-CBB-17. Crop Sci. 46:2338–2339.

Miklas, P. N., Kelly, J. D., Beebe, S. E., and Blair, M. W. 2006c. Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. Euphytica. 147:105–131.

Mkandawire, A. B. C., Mabagala, R. B., Guzman, P., Gepts, P., and Gilbertson, R. L. 2004. Genetic diversity and pathogenic variation of common blight bacteria (*Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) suggests coevolution with the common bean. Phytopathology. 94:593–603.

Mohr, T. J., Liu, H., Yan, S., Morris, C. E., Castillo, J. A., Jelenska, J., and Vinatzer, B. A. 2008. Naturally occurring nonpathogenic isolates of the plant pathogen *Pseudomonas syringae* lack a type III secretion system and effector gene orthologues. Journal of Bacteriology. 190:2858-2870.

Moser, P. E., Cown, L., Massoth, H., and Murray, G. 1994. A semi-selective medium planting assay for detection and differentiation of bacterial blight pathogens of bean and pea. Annu Rep Bean Improv Coop. Fort Collins, CO. 37:23-24.

Msuku, W. A. B. 1986. Pathogenic variation and virulence of isolates of *Pseudomonas syringae* pv. *phaseolicola* in Malawi. Annual Report of the Bean Improvement Cooperative. 28:125–6.

Mutlu, N., Vidaver, A. K., Coyne, D. P. Steadman, J. R. Lambrecht, P.A. and Reiser, J. 2008. Differential pathogenicity of *Xanthomonas campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans*) stains on bean genotypes with common blight resistance. Plant Dis. 92:546–554.

Opio, A. F., Teri, J. M., and Allen, D. J. 1993. Studies on seed transmission of *Xanthomonas campestris* pv. *phaseoli* in common beans in Uganda. Afr. Crop Sci. J. 1:59-67.

Opio, A. F., Allen, D. J., and Teri, J. M. 1996. Pathogenic variation in *Xanthomonas campestris* pv. *phaseoli*, the causal agent of common bacterial blight in *Phaseolus* beans. Plant Pathol. 45:1126-1133.

Patel, P. N., and Walker, J. C. 1965. Resistance in *Phaseolus* to halo-blight. Phytopathology. 55:889–94.

Phaseolus Beans – Crop Vulnerability Statement (September 2020). 2020. Retrieved from https://www.ars-grin.gov/documents/cgc/cvs/2020-Phaseolus%20Crop%20Vulnerability%20Statement.pdf.

Porch, T. G., Cichy, K., Pastor-Corrales, M. A., Grusak, M. A., Beaver, J. S., Hart, J., Fourie, D., Estevez de Jensen, C., Nchimbi, S., and Miklas, P. N. 2016. Characterization and application of the Andean diversity panel for the improvement of common bean productivity in Sub-Saharan Africa. Poster session presented at the meeting of the Pan-African Grain Legume & World Cowpea Conference, 2016 February 28 – March 4, Livingstone, Zambia.

Prosen, D., Hatziloukas, E., Schaad, N. W., and Panopoulos, N. J. 1993. Specific detection of *Pseudomonas syringae* pv. *phaseolicola* DNA in bean seed by polymerase chain reaction-based amplification of a phaseolotoxin gene region. Phytopathology. 83:965–970.

Rico, A., López, R., Asensio, C., Aizpún, M. T., Asensio-S-Manzanera, M. C., and Murillo, J. 2003. Nontoxigenic strains of *Pseudomonas syringae* pv. *phaseolicola* are a main cause of halo blight of beans in Spain and escape current detection methods. Phytopathology. 93:1553–1559.

Rudolph, K. 1990. Toxins as taxonomic features. Methods in Phytobacteriology. Ed. Z. Klement, K. Rudolph and D. C. Sands. 251-267.

Saettler, A. W. 1989. Common bacterial blight. Bean Production Problems in the Tropics, 2nd ed. H. F. Schwartz and M. A. Pastor-Corrales, eds. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. p. 261-283.

Sato, M., Watanabe, K., Yazawa, M., Takikawa, Y., and Nishiyama, K. 1997. Detection of new ethylene-producing bacteria, *Pseudomonas syringae* pvs. *cannabina* and *sesami*, by PCR amplification of genes for the ethylene-forming enzyme. Phytopathology. 87:1192-1196.

Schaad, N. W., Cheong, S. S., Tamaki, S., Hatziloukas, E., Panopoulos, N. J. 1995. A combined biological and enzymatic amplification (BIO-PCR) technique to detect *Pseudomonas syringae* pv. *phaseolicola* in bean seed extracts. Phytopathology. 85:243–8.

Schmutz, J., McClean, P. E., Mamidi, S., Wu, G. A., Cannon, S. B., Grimwood, J., ... & Jackson, S. A. 2014. A reference genome for common bean and genome-wide analysis of dual domestications. Nat. Genet. 46:707-713.

Schuster, M. L. and Sayre, R. M. 1967. A coryneform bacterium induces purple-colored seed and leaf hypertrophy of *Phaseolus vulgaris* and other leguminosae. Phytopathology. 57:1064-&.

Schuster, M. L., and Coyne, D. P. 1981. Biology, epidemiology, genetics and breeding for bacterial pathogens of *Phaseolus vulgaris* L. Hortic. Rev. (Am. Soc. Hortic. Sci.) 3:28–58.

Schuster, M. L., Coyne, D. P., Behre, T., and Leyna, H. 1983. Sources of *Phaseolus* species resistance and leaf and pod differential reactions to common blight. HortScience. 18:901–903.

Schwartz, H. F., Steadman, J. R., Hall, R., and Forester, R. I. 2005. Compendium of bean diseases. 2nd edition. American Phytopathological Society, St. Paul, MN. p. 46-50.

Seal, S. E., Jackson, L. A., Younge, J. P. W., and Daniels, M. J. 1993. Differentiation of *Pseudomonas-solanacearum*, *Pseudomonas-syzygii*, *Pseudomonas-pickettii* and the blood-disease bacterium by partial 16s-ribosomal-RNA sequencing - construction of oligonucleotide primers for sensitive detection by polymerase chain-reaction. J. Gen. Microbiol. 139:1587-1594.

Singh, S. P., and Muñoz, C. G. 1999. Resistance to common bacterial blight among *Phaseolus* species and common bean improvement. Crop Sci. 39:80–89.

Singh, S. P., Gepts, P., and Debouck, D. 1991. Races of common bean (*Phaseolus vulgaris*, Fabaceae). Econ. Bot. 45:379-396.

Singh, S. P., and Miklas, P. N. 2015. Breeding common bean for resistance to common blight: a review. Crop Sci. 55:971–984.

Starr, M. P., and Burkholder, W. H. 1942. Lipolytic activity of phytopathogenic bacteria determined by means of spirit blue agar, and its taxonomic significance. Phytopathology. 32:598-604.

Sutton, M. D., and Wallen, V. R. 1970. Epidemiological and ecological relationship of *Xanthomonas phaseoli* and *X. c*. var *fuscans* on bean in Southern Ontario, 1961–1968. Can. J. Bot. 48:1329–1334.

Taylor J. D. 1970. Bacteriophage and serological methods for the identification of *Pseudomonas phaseolicola* (Burkh.) Dowson. Ann. Appl. Biol. 66:387– 95.

Taylor, J. D., Innes, N. L., Dudley, C. L., and Griffiths, W. A. 1978. Sources and inheritance of resistance to halo-blight of *Phaselous* beans. Ann. Appl. Biol. 90:101-110.

Taylor, J. D., and Teverson, D. M. 1985. Halo-blight of *Phaseolus* bean. Report of the National Vegetable Research Station for 1984. 87.

Taylor, J. D., Teverson, D. M., Allen, D. J., and Pastor-Corrales, M. A. 1996. Identification and origin of races of *Pseudomonas syringae* pv. *phaseolicola* from Africa and other bean growing areas. Plant Pathol. 45:469-478.

Teràn, H., Lema, M., Webster, D., and Singh, S. P. 2009. 75 years of breeding pinto bean for resistance to diseases in the United States. Euphytica. 167:341-351.

Teverson, D. M. 1991. Genetics of pathogenicity and resistance in the halo-blight disease of beans in Africa. Ph.D. diss., Univ. of Birmingham, Birmingham, UK. p. 205.

Toth, I. K., Hyman, L. J., Taylor, R., and Birch, P. R. J. 1998. PCR-based detection of *Xanthomonas campestris* pv. *phaseoli* var. *fuscans* in plant material and its differentiation from *X. c.* pv. *phaseoli*. J. App. Microbiol. 85:327-336.

Tugume, J. K., Tusiime, G., Sekamate, A. M., Buruchara, R., and Mukankust, C. M. 2018. Diversity and interaction of common bacterial blight disease-causing bacteria *Xanthomonas* spp.) with *Phaseolus vulgaris* L. Crop J. 7(2019):1-7.

USDA-NASS Quick Stats. 2019. United States Department of Agriculture National Agricultural Statistic Service. https://www.nass.usda.gov/Quick_Stats/

Valarini, P. J., and Menten, J. O. M. 1992. Methodology for detection of *Xanthomonas campestris* pv. *phaseoli* in bean seeds. Fitopatol. Bras. 17:373-383.

Valarini, P. J., Menten, J. O. M., and Lollato, M. A. 1992. Incidence of the common bacterial blight in the field and transport of *Xanthomonas campestris* pv. *phaseoli* through bean seeds, obtained by different methods. Summa Phytopathologica. 18:160-166.

Vauterin, L., Hoste, B., Kersters, K., and Swings, J. 1995. Reclassification of *Xanthomonas*. Int. J. Syst. Bacteriol. 45(3):472-489.

Venette, J. R., Lamppa, R. S., Albaugh, D. A., and Nayes, J. B. 1987. Presumptive procedure (dome test) for detection of seed-borne bacterial pathogens in dry beans. Plant Dis. 71:984-990.

Vidaver, A. K. 1993. *Xanthomonas campestris* pv. *phaseoli*: cause of common bacterial blight of bean. In J. G. Swings and E. L. Civerolo (ed.), *Xanthomonas.* Chapman and Hall, London, United Kingdom. 40-44.

Viteri, D. M., Teran, H., Asensio-S-Manzanera, M. C., Asensio, C., Porch, T. G., and Miklas, P. N. et al. 2014. Progress in breeding andean common bean for resistance to common bacterial blight. Crop Sci. 54 2084–2092.

Walcott, R. R., and Gitaitis, R. D. 2000. Detection of *Acidovorax avenae* subsp *citrulli* in watermelon seed using immunomagnetic separation and the polymerase chain reaction. Plant Dis. 84:470-474.

Wallace, L., Arkwazee, H., Vining, K., Myers, J. R. 2018. Genetic diversity within snap beans and their relation to dry beans. Genes (Basel). 9:587.

Webster, D. M., Temple, S. R., and Schwartz, H. F. 1980. Selection for resistance to *Xanthamonas phaseoli* in dry beans. Crop Sci. 20:519-522.

Wharton, A. L. 1967. Detection of infection by *Pseudomonas phaseolicola* (Burkh.) Dowson in white-seeded dwarf bean seed stocks. Ann. Appl. Biol. 60:305–12.

Wong, W. C. 1991. Methods for recovery and immunodetection of *Xanthomonas campestris* pv *phaseoli* in navy bean seed. J. Appl. Bacteriol. 71:124-129.

Zapata, M. 1997. Identification of *Xanthomonas campestris* pv. *phaseoli* races in *Phaseolus vulgaris* leaves. Agron. Mesoam. 8:44–52.

Zapata, M., Freytag, G. F., and Wilkinson, R. 1985. Evaluation for bacterial blight resistance in beans. Phytopathology. 75:1032-1039.

Zapata, M., and Beaver, J. S. 2005. Differential leaf reaction of common bean lines to pathogenic races of *Xanthomonas axonopodis* pv. *phaseoli* from Costa Rica, Nicaragua, and Puerto Rico. Annu. Rep. Bean Improv. Coop. 48:102–103.

Zizumbo-Villarreal, D. and Colunga-GarcíaMarín, P. 2010. Origin of agriculture and plant domestication in West Mesoamerica. Genet. Resour. Crop Evol. 57:813–825.

CHAPTER 1: AN OPTIMIZED MULTIPLEX QPCR ASSAY TO DETECT BACTERIAL BLIGHT PATHOGENS IN COMMON BEAN

Abstract

Common bacterial blight, caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* pv. *fuscans* (*Xff*), halo blight, caused by *P. savastanoi* pv. *phaseolicola* (*Psp*) and bacterial brown spot, caused by *Pseudomonas syringae* pv. *syringae* (*Pss*), are known to cause losses to common bean production. These four bacterial pathogens infect seed, leaf and pod tissue of susceptible hosts when conditions are favorable, causing reductions in seed yield and quality. One management tool for assessing risk of seed-borne bacterial infection in beans is routine seed health testing. This study established and optimized a sensitive and robust multiplex qPCR assay using specific primers and probes for detecting and quantifying *Xap, Xff, Psp* and *Pss* in a seed-soak suspension, leaf tissue and pod tissue. Primers and probes to detect *Xap, Xff* and *Psp* were obtained from previous literature, and to our knowledge, this is the first pathovar-specific primer pair and probe to quantify *Pss*. Validation was successful using inoculated greenhouse and field-grown plant samples. Results indicate a significant positive correlation (r = .85 (P = 0.0007) (Fargo 2017), r = .56 (P = 0.0033) (Oakes 2016)) between International Seed Testing Association (ISTA) protocol and qPCR detection of *Xap* from seed in field trials. This research indicates the multiplex qPCR assay is a useful tool for quantifying *Xap* in common bean seed, leaf, and pod tissue. Further validation is needed for detection of *Xff*, *Psp*, and *Pss* to utilize this test for certified seed testing. Comparisons of the qPCR assay to traditional plating as well as dome test scores are needed for validation.

Introduction

The common bean (*Phaseolus vulgaris* L.) is the most cultivated legume worldwide. It holds a high nutritional value from protein, fiber, folate, iron, potassium and magnesium. Currently, North Dakota leads the United States in the production of multiple market classes of common bean, accounting for 35% of total production (USDA-NASS 2019). Major bacterial diseases affecting common bean include common bacterial blight (CBB), brown spot and halo blight, typically seen as a complex in North Dakota and Minnesota. CBB is caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and its variant *Xanthomonas fuscans* subsp. *fuscans* (*Xff*), brown spot is caused by *Pseudomonas syringae* pv. *syrinage* (*Pss*), and halo blight is caused by *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*). Yield losses from these pathogens can exceed 40% (Opio et al. 1996).

Management of the bacterial blight complex includes an effective integrated pest management (IPM) approach. Incorporating infested residue into the soil and utilizing a minimum two-year crop rotation reduces the amount of bacterial inoculum in the field (Schwartz et al. 2005). Planting certified, disease-free seed can help prevent or delay early infection of seedlings (Bailey et al. 2003). Foliar bactericides and streptomycin seed treatments are available, although not commonly applied. An effective method in reducing yield loss is the use of partial host resistance (Vandemark et al., 2008; Singh and Schwartz, 2010; Viteri et al., 2014a; Viteri et al., 2014b; Viteri and Singh, 2014; Singh and Miklas, 2015).

All bean seed eligible for certification in the large common bean producing region of North Dakota are required to be tested for the presence of bacterial pathogens. Bacterial blights present a major challenge in the production of certified common bean seed. In 2019, 10 fields were marked as failures due to bacterial blight which accounted for 43% of fields inspected.

Between 2016 and 2020, 21 fields were marked as failures due to bacterial blight. The current routine evaluation of *Xap, Xff, Psp,* and *Pss* in North Dakota bean seed samples for certification depends on visual examination of water-soaked lesions on infected leaves via the dome test (NDSSD WI-DL208) (Venette et al., 1987). While this test has served the bean industry for many years, the implementation of newer technology has the potential to improve common bean seed certification.

The dome test is calculated by taking the average lesion number percent on 30 (13-day-old) plants and converting the percentage to a value on a scale of 0 to 7. Samples receiving a score of 5 or higher fail the test. The dome test requires extensive time and labor to complete, identification of the bacterial species present is not readily completed and contamination can be a confounding issue. At times, the dome containers used in the test produce optimal conditions for fungal growth, obfuscating bacterial water soaking. Hence, a more rapid, specific, and sensitive method is needed to identify and quantify the pathogens causing bacterial blight diseases on common bean. Growers have relied on the dome test for seed certification since 1985, but call to question its reliability based on observations made over many growing seasons. Newer technology is needed for accurate, sensitive and rapid results (Lahman and Schaad 1985).

Bacterial blight has also been diagnosed from bean seed via pathogen isolation by the International Seed Testing Association (ISTA) (Grimault, 2018). This test is carried out by plating seed extract from an overnight sterile-saline soak onto semi-selective media for *Xanthomonas* and *Pseudomonas*. Quantifying pathogens via plating is resource-intensive and time consuming, and saprophytic microorganisms may interfere with target bacterial growth. This leads to a misrepresentation of pathogen quantification. To reduce time and labor, molecular PCR-based diagnostic methods have been used; however, validation with seed soaking

is needed for standardized use of these tests. Simplex qPCR assays have been designed for *Xap*, *Xff* (He 2010) and *Psp* (Cho et al., 2010). A qPCR assay to detect *Pss* also has been developed; however, it amplifies DNA from a number of *Pseudomonas syringae* pathovars including *Psp* (Xu and Tambong 2011). This method is more rapid, sensitive, and specific than traditional plating and the dome test, but it is more cost-intensive. Multiplex qPCR is used with the benefit of rapidly detecting two or more pathogens but can come at a higher cost than simplex assays. Another factor to consider in using multiplex is the presence of inhibitors and lower efficiency values. Assays of this method have been established in other plant pathogens including bacterial species in tomato (Strayer et al., 2016), seedborne fungal pathogens in soybeans (Ciampi-Guillardi et al., 2020; Hosseini et al., 2021), fungal pathogens of wheat (Abdullah et al., 2018), fungal berry pathogens (Malarczyk et al., 2020), and others. A multiplex qPCR assay has not been established for *Xap, Xff, Psp,* and *Pss.* The first objective in this research is to develop a multiplex qPCR assay to simultaneously detect and quantify all four bacterial pathogens in bean seed, leaf and pod material.

Materials and Methods

Bacterial isolate preparation

Pure cultures of *Xap, Xff, Psp,* and *Pss* from the American Type Culture Collection (ATCC®, Manassas, VA, U.S.A.) were used for optimization of the multiplex qPCR assay (Table 1.1). Non-target bacterial organisms were used for specificity among the four primer and probe sets. The isolates were cultured on nutrient media (8.0g/L BD Difco™ nutrient broth, 14.0g/L agar), grown for 48 hours at room temperature, and identified with standard biochemical tests (Schaad, 1988). *Xff* was identified by observing brown pigmentation of the nutrient media, and *Pss* and *Psp* were confirmed with a negative reaction to the oxidase test.

Table 1.1. Bacterial isolates used for specificity and detection of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), *X. fuscans* pv. *fuscans* (*Xff*), *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*) and *P. syringae* pv. *syringae* (*Pss*) by quantitative polymerase chain reactions

Bacterial species/isolates	Source	Year of collection
Xanthomonas axonopodis pv. *phaseoli* 30	North Dakota	1994
Xap 68	Minnesota	1991
Xap 79	Minnesota	1991
Xap 1	University of Nebraska	-
Xap 20	ATCC[a]	-
Xap 27 (*X. fuscans* pv. *fuscans*)	ATCC	-
Xap 28	ATCC	-
Bacillus subtillis	Sigma	-
Erwinia herbicola	North Dakota	1992
Pseudomonas fluorescens	-	-
P. putida	-	-
P. viridaflava	North Dakota	-
Xanthomonas campestris pv. *campestris*	Idaho	-
X. campestris pv. *translucens*	Idaho	-
X. campestris pv. *diffenbachiae*	Hawaii	-
X. campestris pv. *sojense*	North Dakota	1989
Erwinia amylovora	-	-
Escherichia coli	Sigma	-
Agrobacterium tumefaciens	University of Florida	-
Curtobacterium flaccumfaciens pv. *flaccumfaciens*	North Dakota	1995
Pseudomonas syringae pv. *syringae*	ATCC	-
P. savastanoi pv. *phaseolicola*	ATCC	-
P. syringae pv. *tomato*	University of Florida	-
P. syringae pv. *lachrymans*	University of Florida	-
P. syringae 83 H_2O	-	-
P. syringae pv. *glycinea* 66 m2	North Dakota	1989

[a]ATCC = American Type Culture Collection
-, unknown

Optimization of simplex and multiplex qPCR assays

Standard curves were established in duplicate with reference cultures *Xap* (10199), *Xff* (11766), *Psp* (BAA-978D), and *Pss* (19310) obtained from the ATCC. Previously published primers and probes were obtained for the target organisms *Xap, Xff* (He 2010), *Pss* (Xu and Tambong 2011), and *Psp* (Cho et al., 2010) (Table 1.2). Evaluations uncovered low specificity of

Pss primers detecting a number of *P. syringae* pathovars including target *Pss* and non-target *Psp*; therefore, a novel *Pss* primer set was designed from a custom bash script using local alignment tools and Linux syntax (Table 1.2). The selected primer pair was developed based on a unique bifunctional protein *glmU* gene (Psyr_5119). The *glmU* gene functions as a substrate for two important biosynthetic pathways: peptidoglycan and lipid A synthesis; and therefore, serves as an effective target (Mengin-Lecreulx and van Heijenoort, 1993; Slawomir et al., 2006). Specificity of each primer set in a simplex assay was confirmed by melt curve analysis against isolates of 27 related and non-related bacterial species (Table 1.1).

Table 1.2. Sequences of qPCR primers and probes for detection and quantification of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), *X. fuscans* pv. *fuscans* (*Xff*), *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*), and *P. syringae* pv. *syringae* (*Pss*)

Target	Primer/probe	Sequence (5'-3')	Tm[a] (˚C)	Amplicon (bp)	Reference
Xap	Xap F	CGCAGATCACCATCAACGAA	55.4	~200	He (2010)
Xap	Xap R	CAACCCCGCGCTGTTC	57.5		
Xap	Xap Probe	CAAGCAACGCGCTCA	53.7		
Xff	Xapf F	CCTTGTGGAACACGCTAGCA	57.7	~200	He (2010)
Xff	Xapf R	GCGAAGCATCAGCTTGATTG	54.8		
Xff	Xapf Probe	GCCTGCCTCAACAGAAAATGTGCA	62.3		
Psp	SSRP_F	GACGTCCCGCGAATAGCAATAATC	57.9	183	Cho et al., (2010)
Psp	SSRP_R	CAACGCCGGCGCAATGTCG	63.0		
Psp	SSRP_P Probe	TGACGTGACACTCGCCGAGCTGCA	66.5		
Pss	Pss F	TCGATATCAATGTGATTCTTGAGG	52.6	235	This study
Pss	Pss R	TTCTTGAGCTCGACAAAGTTACC	55.0		
Pss	Pss Probe	TGTCCTTGATGACGCAGTTCGGAC	61.1		

[a] Melting temperature

Genomic DNA was extracted with Qiagen QIAprep® Spin Miniprep Kit (Qiagen, Hilden, Germany) from bacteria grown on a shaker for 48 h in nutrient broth (8.0g/L BD Difco™ nutrient broth). Limit of detection of simplex qPCR was determined with extracted DNA on Invitrogen Qubit™ 4 fluorometer (Thermo Fisher, Waltham, Massachusetts) and performed using six, ten-fold serial dilutions of each target organism. Detection sensitivity of the simplex and multiplex assays of bacterial cells were performed using six, ten-fold serial dilutions from pure culture of each target organism. Bacterial concentration (CFU/mL) was determined via plating the dilution series on nutrient media (8.0g/L BD Difco™ nutrient broth, 14.0g/L agar). Bacterial cells from pure culture were lysed prior to PCR by placing them on ice for 5 min, boiling for 5 min, and placing on ice again for 5 min. Standard curves were generated by plotting log bacterial CFU/mL against quantification cycle (Ct). A Ct value >35 was considered negative based upon primer sensitivity (limit of detection), a non-treated control (NTC) of nuclease-free water, and healthy plant tissue. Standard curves for the multiplex assay were generated by combining the dilution series of each target organism thoroughly mixed with a vortex prior to combining to obtain a homogenous solution.

All qPCR reactions were performed using the Bio-Rad CFX96 Touch Real-time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A.). Simplex assays were carried out using the Bio-Rad SsoAdvanced Universal SYBR Green Supermix with 2.0 µL template and 250 nM of each forward and reverse primer. Multiplex assays were carried out using the Bio-Rad iQ™ Multiplex Powermix with 2.0 µL template and 250 nM of each primer and 125 nM of each probe. A no-DNA template (PCR grade water) was used as a negative control (NTC) and cells from pure *Xap, Xff, Pss,* and *Psp* suspensions were used as positive controls. Fluorophores on each probe were assigned as: HEX on *Xap*, FAM on *Xff*, Texas Red on

Psp, and Cy5 on *Pss*. The cycling conditions for simplex and multiplex assays were as follows: incubation at 95˚C for 3 min; 39 cycles of 95˚C for 10 s and 60˚C for 20 s; and 72˚C for 30 s followed by melting curve analysis using the default settings to evaluate the amplification specificity. The Ct was determined with Bio-Rad CFX Manager Software V3.1 default settings. Amplification efficiencies (E) were calculated from the slope (m) of a plot of Ct (y-axis) and log of DNA (x-axis) ($E = 10^{(1/-m)}–1$). Target amplification was confirmed with melt curve analysis and gel electrophoresis. Efficiencies of lysed cells and extracted DNA were compared with simplex and multiplex qPCR.

To begin validation of the qPCR assays, bacteria was quantified in seed samples amended with known quantities of target bacterial cells. Six 300g seed samples were obtained from the North Dakota State Seed Department. Following the ISTA method, seed was surface sterilized with 0.525% $NaClO^-$ (10% household bleach solution) for 30 sec, rinsed with sterile water for 30 sec, placed into a one-gallon plastic bag with 750 mL sterile saline + 0.15 µL Tween20 and soaked overnight at 20 to 22˚C on a shaker at 80 RPM. A 1.0 mL subsample of the liquid suspension was diluted ten-fold six times. Identification of possible *Xap* and *Xff* was done by spread-plating 100 µL of each dilution on semi-selective media Medium for *Xanthomonas phaseoli* (MXP; K_2HPO_4 (0.8 g/L), KH_2PO_4 (0.6 g/L) yeast extract (0.7 g/L), soluble potato starch (8.0 g/L), potassium bromide (10. g/L), glucose (1.0 g/L), and agar (15.0 g/L)) (Claflin et al., 1987). Identification of *Pss* and *Psp* was conducted by spread-plating 100 µL of each dilution on Difco™ Pseudomonas Agar F (VWR® 90003-352) (38.0 g/L Pseudomonas Agar F, 16 mL/L cephalexin (200 mg in 100 mL H2O) and 10 mL/L cycloheximide (200 mg in 100 mL H_2O).Cephalexin monohydrate (4 mL of 200 mg in 100 mL of H_2O), 10 mL cycloheximide (200 mg in 100 mL H_2O), 10 mL kasugamycin hemisulfate (200 mg in 100 mL of H_2O and 10 mL

gentamycin sulfate (20 mg in 100 mL of H_2O) were added to autoclaved and cooled ($40^{O}C$) media to give final concentrations of 15, 20, 20, and 2 µg/mL, respectively.). Plates were incubated at room temperature for 48 h and observed for bacterial growth. After confirming no growth on all plates for all dilutions, negative seed-soak subsamples were amended with each target organism (100 µL each).

Bacterial quantification in leaf and pod tissue

Simplex and multiplex assays were also validated using infected leaf and pod tissue generated under greenhouse conditions. Primary leaves of six plants of susceptible dark red kidney bean cv. Charlevoix were individually inoculated with *Psp* and *Pss*. Primary leaves of six plants of susceptible pinto bean cv. UI-114 were inoculated with *Xap* and *Xff* (Mutlu et al., 2008). A bacterial suspension of 10^8 CFU/mL was forced into a 1 cm diameter area on both sides of the midrib of primary leaves and also sprayed on the adaxial side until runoff using a Paasche airbrush set at 20 psi. Inoculated plants were incubated at 25 to 28˚C at >90% relative humidity (RH) for 24 h and returned to the greenhouse at average temperatures of 25˚C ± 2˚C. Disease reaction of leaves were recorded after 10 days using a 1 to 5 disease severity scale (Innes et al., 1984), where 1, red-brown necrotic spots in the area of maximum inoculation; 2, red-brown necrosis with a trace of water-soaking; 3, some necrosis but more watersoaking that is confined to the area of maximum inoculation; 4, water-soaked lesions <1 mm diameter distributed randomly over abaxial side of leaf; and 5, watersoaked lesions 1-3 mm in diameter distributed at random over the leaf underside. Six pods of susceptible dark red kidney bean cv. Charlevoix were each individually inoculated with *Psp* or *Pss*, and six pods of susceptible pinto bean cv. UI-114 were each individually inoculated with *Xap* or *Xff*. Pods were injured with sandpaper and dipped in 10^8 CFU/mL bacterial suspension of each bacterial pathogen onto six separate pods (6

pods × 4 bacterial isolates = 24 samples). Inoculated beans were incubated at >90% relative humidity for 24 h and returned to the greenhouse at average temperatures of 29˚C. A plastic bag was placed around the pods to maintain humid conditions favorable for bacterial colonization. Disease reaction was recorded after 10 days using a 1 to 9 disease severity scale (Mills and Silbernagel, 1992), where 1 = no water-soaking (WS) at inoculated region, 2 = no WS with trace of necrosis, 3 = slight WS (1-2 mm) turns necrotic, 4 = slight WS (1-2 mm) turns necrotic and transitory systemic chlorosis, 5 = moderate WS (2-4 mm) strong necrosis, 6 = moderate WS (2-4 mm) trace necrosis, 7 = moderate WS (2-4 mm) trace necrosis and leaf systemic chlorosis, 8 = severe WS (> 4 mm) no necrosis, 9 = severe WS (> 4 mm) no necrosis and severe leaf systemic chlorosis).

Leaves and pods were harvested 10 days post-inoculation when water-soaking and symptoms were observed. For each of the four targeted bacteria, six leaf and six pod samples containing green tissue (1 cm^2) and water-soaked lesions near the inoculated region were soaked with 1 mL sterile water for two minutes and macerated with a sterile scalpel. The supernatant was pipetted into a 1 mL tube. Bacterial cells were lysed using the ice:boil:ice method previously described (Optimization of simplex and multiplex qPCR assays). For the multiplex qPCR, 100 µL of each sample containing one target was combined in one tube and vortexed to form a mixture of all four pathogens for a total of six samples and then prepared into six, serial 10-fold dilutions. qPCR was carried out in simplex and multiplex assays using conditions previously described.

Bacterial quantification in field-grown seed

The simplex *Xap* qPCR assay was validated using diseased seed from field trials conducted in Oakes in 2016 and Fargo in 2017. Trials assessing the efficacy of 12 bactericidal

products for treatment of common bacterial blight had varying levels of *Xap* across all treatments based upon common bacterial blight disease severity ratings (Beck, 2018). In all trials, common bean plants were sandblasted at 70 to 90% bloom and inoculated with 1 x 10^8 bacterial cell suspension of *Xap* with a backpack sprayer at 20 gallons per acre. A total of 96 seed samples from field trials were measured, sterilized, soaked and prepared for plating onto semi-selective media for isolation of *Xap* with methods described above (Grimault et al., 2018). Seed samples were also prepared and *Xap* was quantified using the qPCR assay with methods described above.

To compare the results obtained from the qPCR assay with the traditional dome test, a total of 42 common bean seed samples were obtained from the North Dakota State Seed Department (NDSSD). These were the total samples that had been submitted to the NDSSD in 2019 for seed certification. The dome test was carried out by the NDSSD following standard procedures (Venette et al., 1987). Briefly, enough seed from a single sample to fill 100 mL beaker was poured into a 1 L sterile flask. Seeds were sterilized with dish soap and 0.525% $NaClO^-$ (10% bleach solution), rinsed and germinated. Germinated seeds were planted into cake dome containers to create humid conditions. Eleven days after planting, 30 representative plants with primary leaves were evaluated for percentage water-soaking on the underside of the leaves. The average lesion percentage was taken from the 30 plants, and the percentage was transformed to a value of 0-7. If the grade was a four or below, the sample passed. Samples assigned a value of 5 or greater failed. To compare the multiplex qPCR assay to the dome test, samples were weighed, sterilized, soaked, and prepared for qPCR with methods described above.

Statistical analyses

All qPCR reactions performed for validation of simplex and multiplex assays were run in duplicate, and standard curve for the development of novel *Pss* simplex assay was run in

triplicate. The PROC CORR procedure in SAS (version 9.4, Statistical Analysis System; SAS Institute, Cary, NC) was used to compare the bacterial concentration (CFU/mL) of *Xap, Xff, Pss,* and *Psp* estimated by qPCR and the number of *Xap, Xff, Pss,* and *Psp* colonies from traditional plating on selective media for each pathogen from bacterial-amended seed soak samples, inoculated leaf and pod tissue and field seed. PROC CORR also was used to compare bacterial DNA quantified by qPCR and rating results from the dome test.

Results

Optimization of simplex and multiplex qPCR assays

Specificity of all primer pairs assessed on DNA from a collection of related and non-related bacterial species uncovered no amplification with non-target organisms (Appendix A; Table A.1). Amplification of target pathogens was confirmed with single melt-peak (Appendix A; Figure A.1). Target DNA was quantified in a linear range over six orders of magnitude to a limit of detection (LOD) of 220, 400, 650 and 170 fg DNA/µl for *Xap, Xff, Psp,* and *Pss,* respectively. Standard curve simplex efficiencies established from lysed cells of *Xap, Xff, Psp,* and *Pss* were 109.1, 105.4, 100.8 and 95.3%, respectively, and R^2 values were 0.99 for all primers/probes (Figure 1.1). Efficiencies of lysed cells of *Xap, Xff, Psp,* and *Pss* in the standard curve of optimized multiplex assays were 100.9, 103.1, 90.1 and 94.4%, respectively, and R^2 values were ≥ 0.95 for all primers/probes (Figure 1.2).

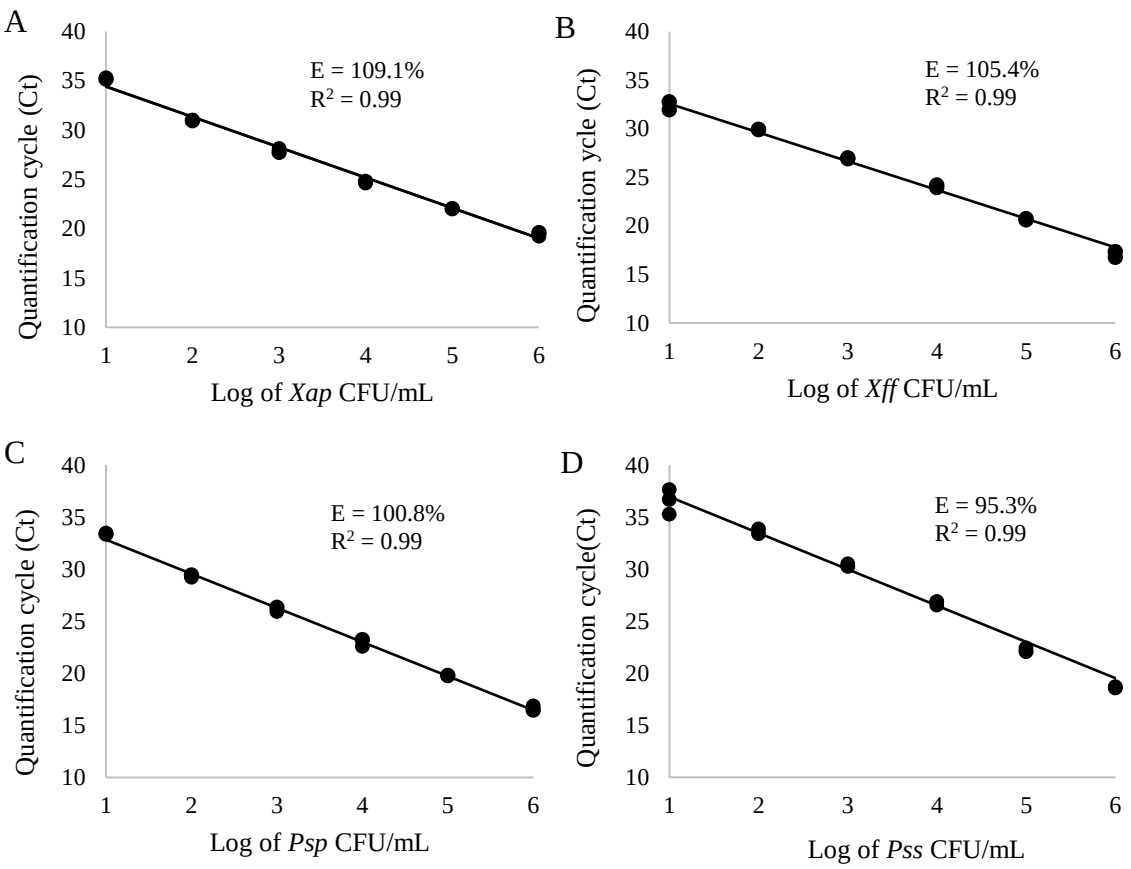

Figure 1.1. Standard curves of $\log_{10}$ cell suspension concentration, colony forming units (CFU)/ml, and cycle threshold (Ct) across six, 10-fold serial dilutions for simplex qPCR. (A) Detection of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), (B) *X. fuscans* pv. *fuscans* (*Xff*), (C) *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*), (D) *P. syringae* pv. *syringae*, (*Pss*). Starting quantities of the dilutions were 2.1×10^8 (*Xap*), 1.4×10^8 (*Xff*), 1.4×10^8 (*Psp*), and 3.8×10^8 (*Pss*) CFU/mL

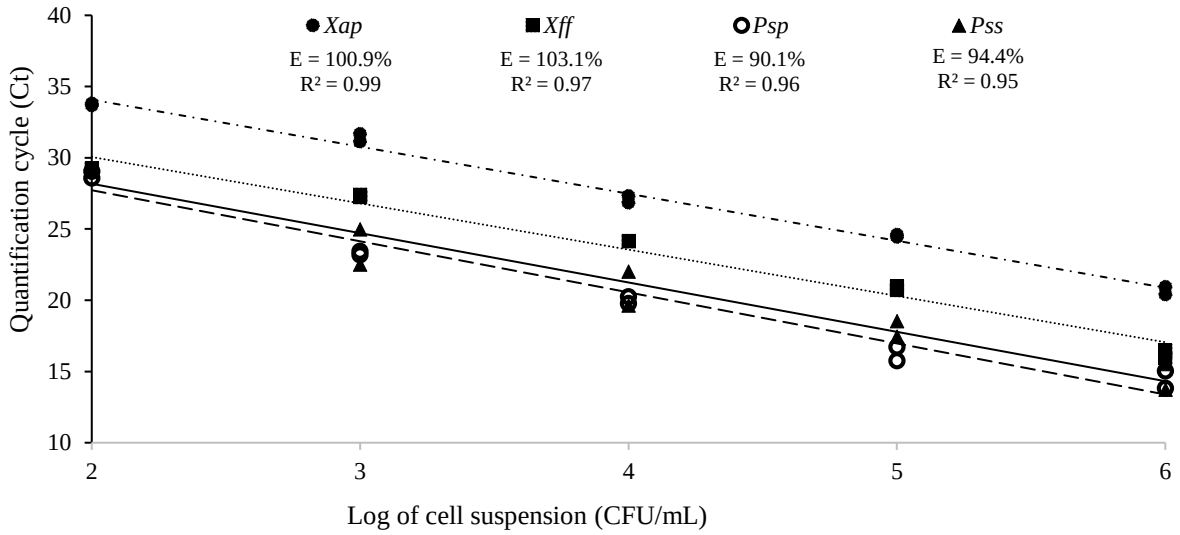

Figure 1.2. Standard curves of $\log_{10}$ cell suspension concentration, colony forming units (CFU)/ml, and cycle threshold (Ct) across five, 10-fold serial DNA dilutions for multiplex qPCR detection of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), *X. fuscans* pv. *fuscans* (*Xff*), *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*), and *P. syringae* pv. *syringae* (*Pss*)

Six seed samples from the North Dakota State Seed Department with no detected bacterial pathogens based on the dome test and qPCR were amended with 100 µl of each of the four target pathogens. Efficiency values of the simplex assay detecting target DNA in six seed samples were 110.2, 106.6, 102.1 and 99.9% for *Xap, Xff, Psp,* and *Pss*, respectively, and R^2 values were ≥ 0.99 for all primers/probes. The multiplex assay had efficiency values of 106.9, 108.9, 91.3 and 86.9% for *Xap, Xff, Psp* and *Pss*, respectively, and R^2 values were ≥ 0.94 for all primers/probes.

Detection frequency of the 1:10 dilutions ranged from 67% to 100% (Figure 1.3). All bacteria were detected at 100% in the 1:100 and 1:1,000 dilutions. At 1:10,000, detection of *Xap* and *Pss* fell to 67% and at 1:100,000 *Pss* was not detected, *Psp* and *Xap* were detected at 67% and *Xff* was still detected in 100% of the samples.

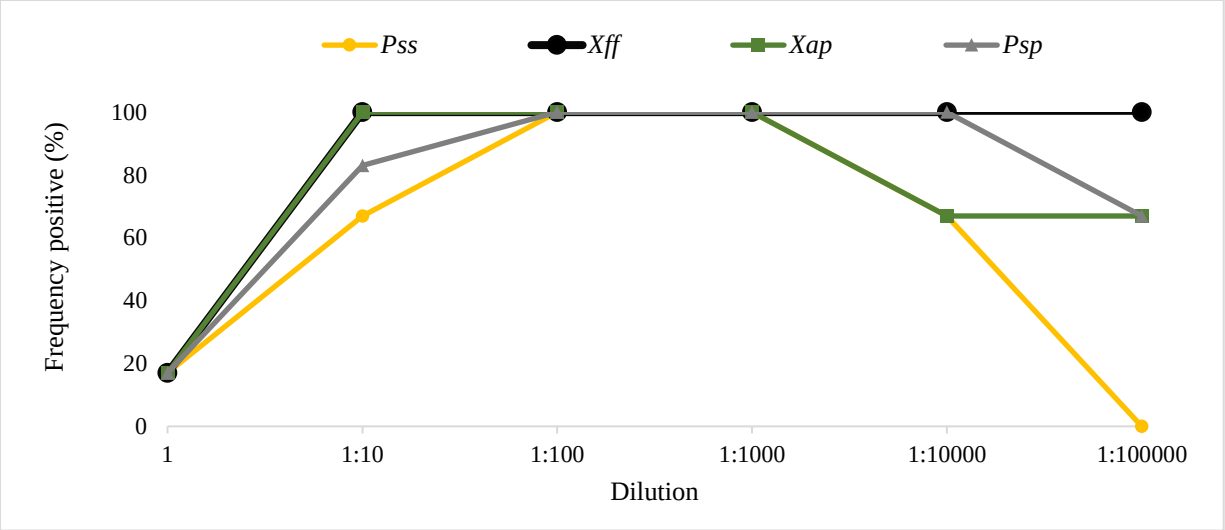

Figure 1.3. Detection frequency (%) of positive *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), *X. fuscans* pv. *fuscans* (*Xff*), *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*), *P. syringae* pv. *syringae* (*Pss*) in multiplex qPCR from six seed soak solution samples amended with bacteria

Bacterial quantification in leaf and pod tissue

Leaves and pods from greenhouse-inoculated plants exhibited variable levels of bacterial blight symptoms (Appendix A; Table A.4, A.5). The simplex and multiplex assays detected and quantified four bacterial pathogens in six leaf and six pod samples inoculated in the greenhouse.

Detection frequency by the multiplex assay was 100% for *Xap*, *Xff*, and *Psp* in the non-diluted, 1:10, and 1:100 dilutions from leaf samples inoculated under greenhouse conditions. Detection of *Xap*, *Xff*, and *Psp* ranged from 67% to 100% in dilutions greater than 1:1,000. *Pss* was detected 17% of the time in the non-diluted, and at 1:1,000. Pss was detected at 17% at 1:10, and Pss was not detected at 1:10,000 and 1:100,000. *Pss* was detected highest at 83% frequency in the 1:10 dilution followed by 67% in 1:100 dilution. No detection of *Pss* was found in the 1:10,000 and 1:100,000 dilutions. The 1:10 dilution provided the best detection frequency for all targets (Figure 1.4).

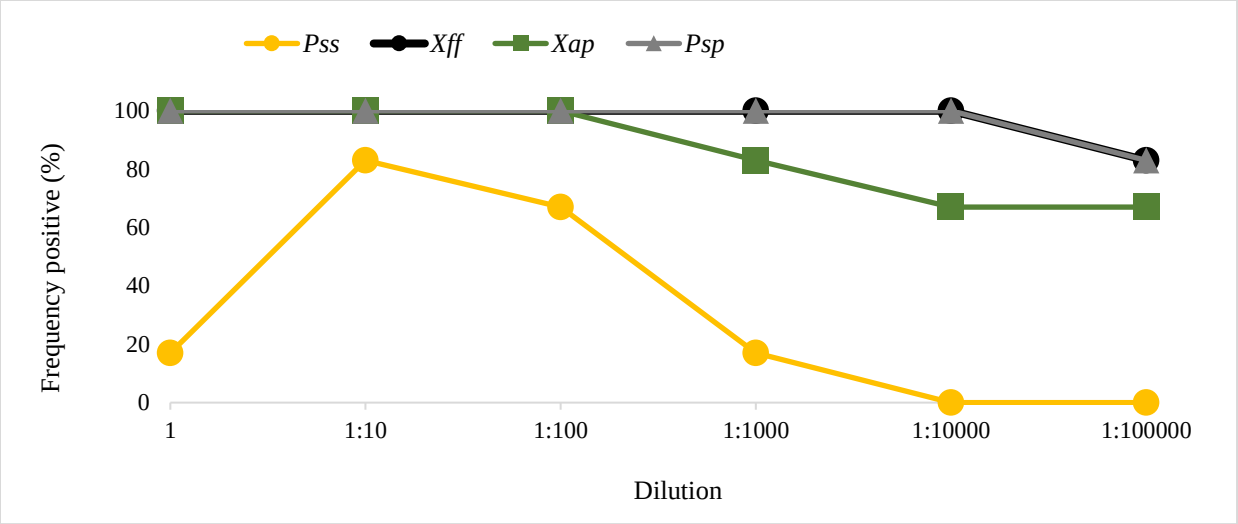

Figure 1.4. Detection frequency (%) of positive *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), *X. fuscans* pv. *fuscans* (*Xff*), *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*), *P. syringae* pv. *syringae* (*Pss*) in multiplex qPCR from six inoculated leaf samples

Detection frequency of *Xap*, *Xff*, and *Psp* was 100% for the non-diluted samples. *Xff* was detected in 100% of pod samples at all dilutions by the multiplex qPCR. Detection of *Xap* in multiplex qPCR of pod was 100% for the non-diluted, 1:10, and 1:100 dilutions. At 1:1,000, detection of *Xap* was 83% and fell to 67% in the 1:10,000 and 1:100,000 dilutions. Detection of *Psp* fell from 100% in the non-diluted to 83% in the 1:10 dilutions and 67% in the 1:100 dilutions. *Psp* was detected at 17% in the 1:1,000 dilutions and was not detected at 1:10,000 and 1:100,000. Detection of *Pss* was 17% for the non-diluted and dilutions 1:10, and 1:100 and was not detected at 1:1,000, 1:10,000, and 1:100,000. The non-diluted sample provided the best detection frequency for all targets (Figure 1.5).

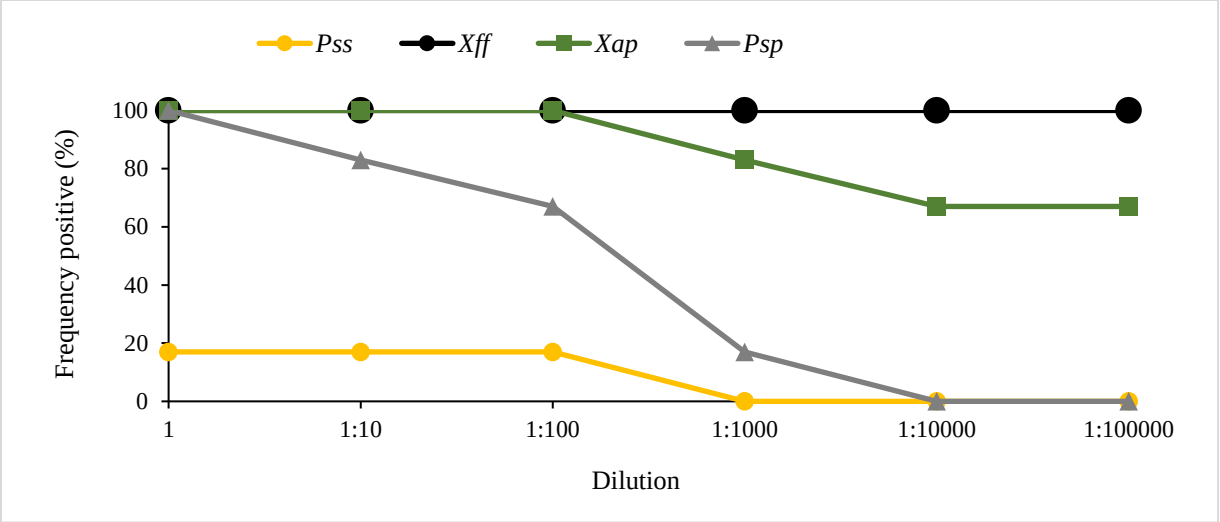

Figure 1.5. Detection frequency (%) of positive *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), *X. fuscans* pv. *fuscans* (*Xff*), *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*), *P. syringae* pv. *syringae* (*Pss*) in qPCR from six inoculated pod samples

Bacterial quantification in field-grown seed

The multiplex assay detecting *Xap* was validated using seed samples obtained from field trials conducted in Oakes 2016 and Fargo 2017. The 96 seed samples were diluted 1:10 and used to compare the qPCR assay to traditional plating methods using selective media. A positive and significant correlation between qPCR quantification and CFU/mL as determined via plate counts was observed for *Xap* in field trial Fargo 2017 ($r = 0.85$, $P = .0007$) and Oakes 2016 ($r = 0.56$, $P = .0033$) (Figure 1.6).

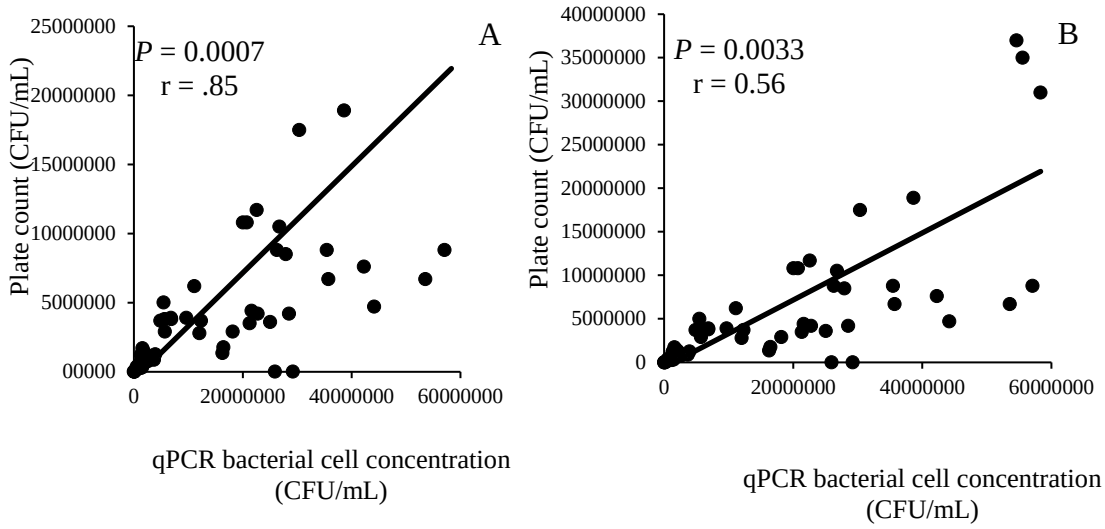

Figure 1.6. Pearson correlation between CFU/mL generated from plating and results from the 1:10 sample dilution qPCR. Bacterial dilutions were generated from common bean seeds grown in field trial conducted in Fargo in 2017 (A) and Oakes in 2016 (B) inoculated with *Xanthomonas axonopodis* pv. *phaseoli* in treatments exhibiting varying levels of common bacterial blight

The multiplex qPCR assay was compared to the dome test for further validation. A Pearson correlation was tested between bacterial concentration quantified by qPCR and dome test results of 42 non-diluted seed samples (r = 0.18, *P* = .2437) (Figure 1.7).

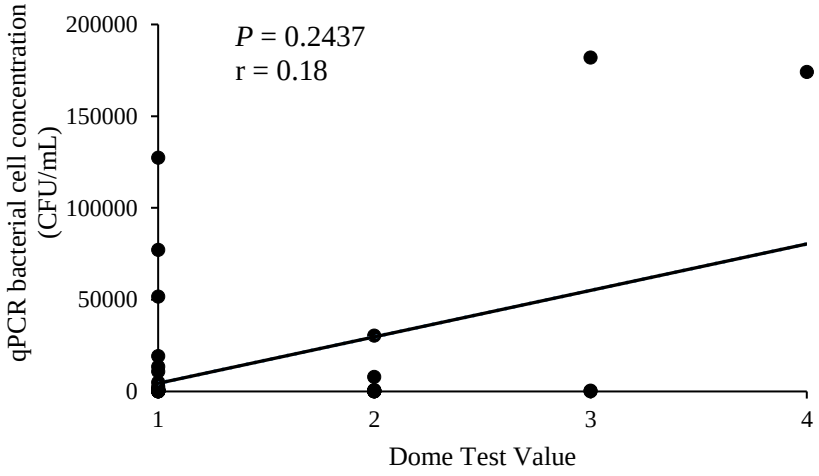

Figure 1.7. Pearson correlation between Dome Test severity ratings and multiplex qPCR for all four bacterial pathogens of common bean

Discussion

As a result of this project, a multiplex quantitative PCR assay was optimized for direct detection of *Xap, Xff, Psp,* and *Pss* in seed, leaf, and pod tissue to overcome limitations of previous studies. This study confirmed pathovar-specificity of qPCR primers and probes to detect *Xap, Xff* and *Psp* developed in previous studies (Cho et al., 2010; He, 2010). Primers to detect *Pss* in had been previously developed for qPCR; however, cross-reaction to other *P. syringae* pathovars was observed in this study and was reported in the original study (Xu and Tambong, 2011). Therefore, a novel primer and probe set were designed to detect *Pss*. It is challenging to distinguish *P. syringae* pathovars based on the sequences of toxin, avirulence, 16S rRNA genes or repetitive elements because *P. syringae* is highly heterogeneous, comprised of 57 pathovars (Gardan et al., 1997; Volksch and Weingart, 1997). The novel *Pss* primers developed here amplify a 235 bp target sequence in *glmU* region. The *glmU* gene functions as a substrate for two important biosynthetic pathways: peptidoglycan and lipid A synthesis; and therefore,

serves as an effective target (Mengin-Lecreulx and van Heijenoort, 1993; Slawomir et al., 2006). The evaluations of 18 non-target bacteria confirm that hypothesis. All primers use the same concentration and cycling parameters, including annealing temperature, making them amenable to a multiplex assay. The multiplex assay simultaneously detected and differentiated *Xap, Xff, Psp,* and *Pss* under the experimental conditions evaluated here. The standard curves developed for simplex and multiplex detection of *Xap, Xff, Psp* and *Pss* from pure culture confirmed the linearity of the quantification process between exponential increases in bacterial concentration and cycle threshold values.

The qPCR assay allows for rapid results and reduced labor compared to the dome test, which requires 14 days to generate results. PCR can result in the overestimation of cell counts due to amplification of DNA from non-viable cells; however, this has not been a limiting factor in the quantification of other pathogens (Reichert-Schwillinsky et al., 2009; Ross and Somssich, 2016; Hilje-Rodriguez et al., 2020; Cullen et al., 2001). For example, qPCR quantification standards were correlated to number of CFU under low-stress conditions when testing *Listeria monocytogenes* (Reichert-Schwillinksy et al., 2009). In addition, detection of *Pseudomonas syringae* in *Arabidopsis thaliana* L. with qPCR was highly comparable to plate counting (Ross and Somssich, 2016). A *Botrytis cinerea* and *Trichoderma atroviride*-specific multiplex qPCR quantified fungi in inoculated blackberries (Hilje-Rodriguez et al., 2020). Another species-specific qPCR assay detected *Helminthosporium solani* in potato tubers and soil (Cullen et al., 2001).

Assay inhibition was observed with detection of all target bacteria from non-diluted seed soak suspensions and with detection of *Pss* from leaf and pod samples. This inhibition could also be due to high bacterial levels, or inhibiting compounds in the plant tissue. Various compounds

in plant tissue are common to inhibit a PCR reaction (Wilson, 1997; Mancini et al., 2016). To overcome this problem, PCR inhibitors may be removed with various methods. Fungal DNA inhibitors have been removed with commercial DNA extraction kits (Ma and Michailides, 2007) allowing the detection of several fungal pathogens from seeds, such as *Ascochyta rabiei* from chickpea seeds (Phan et al., 2002), *L. maculans* from canola seeds (Chen et al., 2010), and *Botrytis* spp. from onion seeds (Chilvers et al., 2007). PCR inhibitors may also be removed by diluting samples (Demeke and Jenkins, 2010). When testing bacteria in plant tissue, diluting samples 1:10, 1:100 or even 1:1000 was effective in reducing inhibitors and increasing assay efficacy in simplex and multiplex qPCR. Disease severity ratings in the greenhouse showed that not all leaves and pods had the same level of infection for each pathogen, which may be explanatory of low detection of *Pss* in leaf and pod tissue. *Pss* infection on pod tissue was limited, with little to no disease symptoms. Extracted DNA did not amplify with better efficiency compared to lysed bacterial DNA, thus it may be beneficial to use lysed DNA to reduce time and financial resources. Our validation steps with pure bacteria, greenhouse inoculated plants and field-derived samples indicate that the assays derived here are generally effective. Our comparisons with the dome test indicate that this method may be fraught with false positive and false negative results. However, further evaluations are needed to validate this multiplex qPCR assay for seed certification purposes.

Identifying the relationship between *Xap* concentration quantified by qPCR and as determined by seed soak-plating aids in the validation of this assay to establish a reliable method for commercial seed testing of common bacterial blight, the most prevalent of the three bacterial blights on common bean. However, further steps are needed to develop a relationship between test results and in-field disease development are needed to validate the multiplex assay for this

purpose. Scores from the dome test and total bacterial DNA quantities determined by qPCR from the same seed samples were correlated with r = 0.18 (P = 0.2437). Based on grower observations, the question remains if this lack of correlation is due to the dome test not being an accurate and reliable method for assessing pathogen levels in seed samples for certification in North Dakota.

The purpose of the dome test is to evaluate the potential risk from planting a seed lot (Venette et al., 1987). The value of this newly developed multiplex qPCR assay is the direct and individual quantification of the four bacterial pathogens that cause common bacterial blight, brown spot, and halo blight. This assay is currently not validated for use in seed certification. Additional research is required to correlate qPCR results with risks of common bacterial blight, brown spot, and halo blight development in the field.

Acknowledgements

Thank you to the North Dakota Department of Agriculture for providing funding, the North Dakota State Seed Department for providing seed, and Dr. Kristin Simons, Dr. Amanda Beck-Okins, Robin Lamppa, Dr. Roshan Sharma Poudel, and Ipsita Mallik for assistance in conducting this research.

Literature Cited

Abdullah, A. S., Turo, C., Moffat, C. S., Lopez-Ruiz, F. J., Gibberd, M. R., Hamblin, J., and Zerihun, A. 2018. Real-time PCR for diagnosing and quantifying co-infection by two globally distributed fungal pathogens of wheat. Front. Plant Sci. 9:1086.

Bailey, K. L., Gossen, B. D., Gugel, R. K., and Morrall, R. A. A. 2003. Diseases of field crops in Canada. 3rd ed. University Extension Press, University of Saskatchewan, Saskatoon, SK. 290 pp.

Beck, A. L. 2018. Characterization of pea seed-borne mosaic virus, efficacy of foliar applications for common bacterial blight management in dry beans and impact of common bacterial blight on prostrate and upright beans. Graduate Theses and Dissertations. North Dakota State University.

Brandfass, C., and Karlovsky, P. 2008. Upscaled CTAB-based DNA extraction and real-time PCR assays for *Fusarium culmorum* and *Fusarium graminearum* DNA in plant material with reduced sampling error. Int. J. Mol. Sci. 9:2306-2321.

Chen, G., Wu, C., Li, B., Su, H., Zhen, S., An, Y. 2010. Detection of *Leptosphaeria maculans* from imported canola seeds. J. Plant Dis. Prot. 117:173–6.

Chilvers, M., Du Toit, L., Akamatsu, H., Peever, T. 2007. A real-time, quantitative PCR seed assay for *Botrytis* spp. that cause neck rot of onion. Plant Dis. 91:599–608.

Cho, M. S., Jeon, Y. H., Kang, M. J., Ahn, H., Baek, H., Na, Y. W., Choi, Y. M., Kim, T. S., and Park, D. S. 2010. Sensitive and specific detection of phaseolotoxigenic and nontoxigenic isolates of *Pseudomonas syringae.* pv. *phaseolicola* by TaqMan real-time PCR using site-specific recombinase gene sequences. Microbiol. Res. 165:565-572.

Ciampi-Guillardi, M., Ramiro, J., Heliosa Duarte de Moraes, M., Coan Goldoni Barbieri, M., and Massola Jr, N. S. 2020. Multiplex qPCR assay for direct detection and quantification of *Colletotrichum truncatum*, *Corynespora cassiicola*, and *Sclerotinia sclerotiorum* in soybean seeds. Plant Dis. 104:3002-3009.

Claflin, L. E., Vidaver, A. K., and Sasser, M. 1987. MXP, a semi-selective medium for *Xanthomonas campestris* pv. *phaseoli*. Phytopathology. 77:730-734.

Cullen, D. W., Lees, A. K., Toth, I. K., and Duncan, J. M. 2001. Conventional PCR and real-time quantitative PCR detection of Helminthosporium Solani in soil and on potato tubers. Eur. J. Plant Pathol. 107:387-398.

Gardan, L., Shafif, H., and Grimont, P. A. D. 1997. DNA relatedness among pathovars of *Pseudomonas syringae* and related bacteria. In: Rudolph, K. Burr, T. J. Mansfield, J. W. Stead, D. Von Kietzell, J. Vivian, A. editors. *Pseudomonas syringae* pathovars and related pathogens. London, United Kingdom: Kluwer Academic Publishers. p. 445–8.

Grimault, V., Olivier, V., Rolland, M., Darrasse, A., and Jacques, M. A. 2018. Detection of *Xanthomonas* axonopodis pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* in *Phaseolus vulgaris* (bean) seed. International Rules for Seed Testing. 7-021.

Ha, Y., Fessehaie, A., Ling, K. S., Wechter, W. P., Keinath, A. P., and Walcott, R. R. 2009. Simultaneous detection of *Acidovorax avenae* subsp. *citrulli* and *Didymella bryoniae* in cucurbit seed lots using magnetic capture hybridization and real-time polymerase chain reaction. Phytopathology. 99:666-678.

He, Yiqing. 2010. Improved seed health tests for *Xanthomonas axonopodis* pv. *phaseoli* in common bean. Graduate Theses and Dissertations. Iowa State University, Ames. 11565.

Hilje-Rodriguez, I., Albertazzi, F. J., Rivera-Coto, G., and Molina-Bravo, R. 2020. A multiplex qPCR TaqMan-assay to detect fungal antagonism between *Tichoderma atroviride* (Hypocreaceae) and *Botrytis cinerea* (Sclerotiniaceae) in blackberry fruits using a *de novo* tef1-α- and an IGS- sequence based probes. Biotechnol Rep. 27:e00447.

Hosseini, B., Voegele, R. T., Link, T. I. 2021. Establishment of a quadruplex real-time PCR assay to distinguish the fungal pathogens *Diaporthe longicolla*, *D. caulivora*, *D. eres*, and *D. novem* on soybean. PLoS ONE 16(9): e0257225.

Innes, N. L., Conway, J., and Taylor J. D. 1984. Resistance to halo-blight in the Cambridge accessions V4604 and V4058 of *Phaseolus* beans. Ann. Appl. Biol. 105:307-314.

Knodel, J. J., Beauzay, P. B., Endres, G. J., Franzen, D. W., Ikley, J. T., Kandel, H. J., Markell, S. G., Osorno, J. M., and Pasche, J. S. 2020. 2019 Dry bean grower survey of production, pest problems and pesticide use. North Dakota State University Extension Service. E1952.

Ma, Z., Michailides, T. J. 2007. Approaches for eliminating PCR inhibitors and designing PCR primers for the detection of phytopathogenic fungi. Crop Prot. 26:145–61.

Malarczyk, D. G., Panek, J., and Frac, M. 2020. Triplex real-time PCR approach for the detection of crucial fungal berry pathogens – *Botrytis* spp., *Colletotrichum* spp. and *Verticillium* spp. Int. J. Mol. Sci. 21:8469.

Mancini, V., Murolo, S., and Romanazzi, G. 2016. Diagnostic methods for detecting fungal pathogens on vegetable seeds. Plant Pathol. 65:691-703.

Mbofung, G. C. Y., and Pryor, B. M. 2010. A PCR-based assay for detection of *Fusarium oxysporum* f. sp. *lactucae* in lettuce seed. Plant Dis. 94:860-866.

Mengin-Lecreulx, D., and van Heijenoort, J. 1993. Identification of the *glmU* gene encoding N-acetylglucosamine-1- phosphate uridyltransferase in *Escherichia coli*. J. Bacteriol. 175:6150-6157.

Mills, L. J., and Silbernagel, M. J. 1992. A rapid screening technique to combine resistance to halo blight and bean common mosaic virus in *Phaseolus vulgaris* L. Euphytica 58:201-208.

Mutlu, N., Vidaver, A. K., Coyne, D. P., Steadman, J. R., Lambrecht, P.A., and Reiser, J. 2008. Differential pathogenicity of *Xanthomonas campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans*) stains on bean genotypes with common blight resistance. Plant Dis. 92:546–554.

Opio, A. F., Allen, D. J., and Teri, J. M. 1996. Pathogenic variation in *Xanthomonas campestris* pv. *phaseoli*, the causal agent of common bacterial blight in *Phaseolus* beans. Plant Pathol. 45:1126-1133.

Phan, H., Ford, R., Bretag, T., Taylor, P. 2002. A rapid and sensitive polymerase chain reaction (PCR) assay for detection of *Ascochyta rabiei*, the cause of ascochyta blight of chickpea. Australas. Plant Pathol. 31:31–9.

Reichert-Schwillinsky, F., Pin, C. Dzieciol, M., Wagner, M., and Hein, I. 2009. Stress- and growth rate-related differences between plate count and real-time PCR data during growth of *Listeria monocytogenes*. Appl. and Environ. Microbiol. 75:2132-2138.

Ross, A., Somssich, I. E. 2016. A DNA-based real-time PCR assay for robust growth quantification of the bacterial pathogen *Pseudomonas syringae* on *Arabidopsis thaliana*. Plant Methods. 12:48.

Schaad, N. W. 1988. Laboratory guide for identification of plant pathogenic bacteria. 2nd ed. American Phytopathological Society, St. Paul. p. 164.

Singh, S. P., and Schwartz, H. F. 2010. Breeding common bean for resistance to diseases: a review. Crop. Sci. 50:2199–2223.

Singh, S. P., and Miklas, P. N. 2015. Breeding common bean for resistance to common blight: a review. Crop Sci. 55:971-984.

Slawomir, M., Gabriel, I., and Olchowy, J. 2006. Enzymes of UDP-GlcNAc biosynthesis in yeast. Yeast 23:1-14.

Strayer, A. L., Jeyaprakash, A., Minsavage, G. V., Timilsinia, S., Vallad, G. E., Jones, J. B., and Paret, M. L. 2016. A multiplex real-time PCR assay differentiates four *Xanthomonas* species associated with bacterial spot of tomato. Plant Dis. 100:1660-1668.

USDA-NASS Quick Stats. 2019. United States Department of Agriculture National Agricultural Statistic Service. https://www.nass.usda.gov/Quick_Stats/

Vandemark, G. J., Fourie, D. & Miklas, P. N. 2008. Genotyping with real-time PCR reveals recessive epistasis between independent QTL conferring resistance to common bacterial blight in dry bean. Theor Appl Genet. 117:513–522.

Venette, J. R., Lamppa, R. S., Albaugh, D. A., and Nayes, J. B. 1987. Presumptive procedure (dome test) for detection of seed-borne bacterial pathogens in dry beans. Plant Dis. 71:984-990.

Viteri, D. M., Cregan, P. B., Trapp, J., Miklas, P. N., and Singh, S. P. 2014a. A new common bacterial blight resistance QTL in VAX 1 common bean and interaction of the new QTL, SAP6, and SU91 with bacterial strains. Crop Sci. 54:1598–1608.

Viteri, D. M., Terán, H., Asensio-S.-Manzanera, M. C., Asensio, C., Porch, T. G., Miklas, P. N. and Singh, S. P. 2014b. Progress in breeding Andean common bean for resistance to common bacterial blight. Crop Sci. 54:2084–2092.

Viteri, D. M., and Singh, S. P. 2014. Response of 21 common beans of diverse origins to two strains of the common bacterial blight pathogen, *Xanthomonas campestris* pv. *phaseoli*. Euphytica. 200:379–388.

Volksch, B., and Weingart, H. Comparison of ethylene-producing *Pseudomonas syringae* isolates isolated from kudzu (*Pueraria lobata*) with *Pseudomonas syringae* pv. *phaseolicola* and *Pseudomonas syringae* pv. *glycinea*. Eur. J. Plant Pathol. 103:795–802.

Wilson, I. G. 1997. Inhibition and facilitation of nucleic acid amplification. Appl. Environ. Microbiol. 63:3741–3751.

Xu, R., and Tambong, J. T. 2011. A TaqMan real-time PCR assay targeting the cytochrome o ubiquinol oxidase subunit II gene for detection of several pathovars of *Pseudomonas syringae*. Canad. J. Plant Pathol. 33(3):318-331.

CHAPTER 2: EVALUATION OF *XANTHOMONAS AXONOPODIS* PV. *PHASEOLI* AND *X. FUSCANS* PV. *FUSCANS* AGGRESSIVENESS AND GENETIC DIVERSITY IN NORTH DAKOTA AND MINNESOTA

Abstract

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* and *X. fuscans* pv. *fuscans*, is the most damaging bacterial disease to common bean (*Phaseolus vulgaris* L., in North Dakota and Minnesota, resulting in yield losses and reductions in seed quality. The pathogenic variation and genetic diversity of *Xap* and *Xff* have been examined, but those studies only included two isolates from North Dakota and none from Minnesota. In 2018 and 2019, CBB symptomatic leaves were sampled from 77 common bean fields in North Dakota and Minnesota. Xanthomonad-like bacteria were isolated from these leaves and characterized based upon colony characteristics, casein hydrolysis, brown pigmentation, qPCR with *Xap* and *Xff* specific primers and pathogenicity on susceptible common bean. Fifty-four isolates of *Xap* and 3 isolates of *Xff* obtained from 38 fields were determined to be pathogens of common bean based on results from greenhouse inoculations. Each isolate was inoculated onto a differential set of eight common bean lines to determine aggressiveness. An interaction was found between isolate and line, suggesting that host resistance to single isolate may not be effective on other isolates. Four housekeeping genes (*dnaK, atpD, gyrB,* and *rpoD*) were sequenced from *Xap* isolates collected from 1990 to 2018. Analysis of these four phylogenetic trees characterized three groups of *Xap* based on genetic similarity. A better understanding of the aggressiveness and genetic makeup of *Xap/Xff* isolates from the region will aid in the development of management strategies, including cultivars with CBB resistance.

Introduction

Approximately 441,000 hectares of common bean were planted in North Dakota and Minnesota in 2020. North Dakota common bean production reached an economic value of $200 million USD in 2019, and production in Minnesota valued $130 million USD (USDA-NASS, 2020). Common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* pv. *fuscans* (*Xff*), remains a consistent disease threat to beans in this leading common bean production region. According to common bean growers in the Northarvest region (Eastern North Dakota and Minnesota), bacterial blights, particularly common bacterial blight (CBB), are among the most damaging diseases (Knodel et al., 2020). When susceptible cultivars are planted, yield losses from these pathogens can exceed 40% (Opio et al., 1996). Yield loss is partially a result of reduced seed size and quality, but a reduction in seed number is a larger factor (Boersma et al., 2015).

Common bacterial blight symptoms can be seen throughout the plant (Schwartz et al., 2005). Leaf lesions appear as water-soaked spots that enlarge to form necrotic lesions surrounded by a chlorotic zone of yellow tissue. Lesions may be found on leaf margins and interveinal. Severely infected plants appear burnt due to coalescing lesions, and plants mature unevenly. Symptoms appear initially on pods as water-soaked spots, and develop into sunken, brown lesions (Schwartz et al., 2005). Seeds may have obvious symptoms of discoloration and reduced size; however, infected seed may be asymptomatic. Infected seed and debris serve as primary inoculum sources for *Xap* and *Xff*. While *Xap* and *Xff* are mainly seed-borne, bacteria also enter the plant naturally through the stomata (Rudolph, 1993). It takes as little as 10 to 14 days from initial infection until secondary spread can occur under favorable conditions (Schwartz et al.,

2005). Secondary infection is aided by bacterial dispersal from wind, hail, rain splash, machinery, excessive irrigation, animals and people.

Cultural methods such as a minimum two-year crop rotation and the incorporation of infected debris are used to manage CBB. Other methods include the use of a streptomycin seed treatment, copper- or hydrogen-based foliar bactericides, and planting partially resistant varieties. Partial resistance to CBB has been incorporated into varieties and some research has uncovered a significant bacterial isolate × resistance QTL interaction (Viteri et al., 2014). Therefore, an understanding of pathogenic variation within the population is important in screening for resistance and it is advised to use bacterial isolates with differing aggressiveness to inoculate primary and trifoliate leaves and pods to ensure the identification of stable sources of genetic resistance (Singh and Miklas, 2015).

The aggressiveness of *Xap* and *Xff* has been previously evaluated for isolates worldwide (Ekpo and Saettler, 1976; Lopez et al., 2006; Mutlu et al., 2008; Singh and Miklas, 2015; Zapata, 1997, 1985; Zapata and Vidaver, 1987). Isolates of *Xap* from South America, Central America, the Caribbean, Africa, North America, the Middle East, Europe and Australia have been identified as genetically distinct and differentially aggressive on lines of common bean (Lopez et al., 2006; Mahuku et al., 2006; Mkandawire et al., 2004). The aggressiveness of three *Xap* isolates from North Dakota were evaluated; however, the isolates date back to 1995 and 1999 (Mutlu et al., 2008). More extensive knowledge of these pathogens in this region is needed, and it will assist breeders in their efforts to screen for CBB resistant material. Therefore, the objective of this study was to evaluate the aggressiveness of *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas fuscans* pv. *fuscans* isolates collected from North Dakota and western Minnesota.

Materials and Methods

Isolation and identification of *Xap* and *Xff* isolates

A total of 44 and 33 commercial and research plot fields were sampled in 2018 and 2019, respectively, in North Dakota and Minnesota. Leaf samples exhibiting bacterial blight symptoms were taken at each point in a W-pattern for a total of 25 leaves per field. A green portion of the leaf (1 cm^2) surrounding the lesion was macerated in sterile distilled water and plated onto a bacterial blight differential medium (BBD; Na-Deoxycholate (250mg), Proteose Peptone No. 3 (2.5g), yeast extract (0.5g), glycerol (2.5 mL) and agar (7.5g) and water (250mL) in one bottle; dry milk (10g) and water (250mL) in another bottle) (Moser et al. 1994). After autoclaving, cooling and mixing the contents of the two bottles, cycloheximide (40 mg), cephalexin (10 mg) and vancomycin (5 mg) were added. Identification of purified, isolated bacteria was confirmed via morphology of yellow colonies and a zone of casein hydrolysis on the BBD media. Distinguishing *Xap* from *Xff* was done by transferring bacterial colony to nutrient agar (BD Difco™ nutrient broth (8g/L and agar (14g/L)). Bacteria that turned media pigment brown was determined to be *Xff.* Additionally, bacterial species was confirmed using species specific qPCR (He 2010). Reactions were performed using the Bio-Rad CFX96 Touch Real-time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A.). Master Mix of the qPCR included Bio-Rad SsoAdvanced Universal SYBR Green Supermix with 2.0 µL bacterial cell suspension and 250 nM of each forward and reverse primer (He, 2010). A no-DNA template (PCR grade water) was used as a negative control and cells from pure *Xap* and *Xff* suspension were used as a positive control. The cycling conditions were as follows: incubation at 95˚C for 3 min; 39 cycles of 95˚C for 10 s and 60˚C for 20 s.

Pathogenic variation

Pathogenicity of *Xap* and *Xff* isolates were confirmed on susceptible pinto bean cultivar UI-114 (Mutlu et al., 2008). Plants were inoculated using a Paasche airbrush at 20 psi when plants were at the primary leaf stage. Inoculum was forced into a small area (1 cm diameter) on both sides of the midrib of primary leaves and sprayed on the adaxial side until runoff (Taylor et al., 1996). Inoculated plants were incubated at >90% relative humidity for 24 h and returned to the greenhouse at average temperatures of 29˚C. Disease reaction was recorded 10 days post-inoculation. Isolates that caused water-soaking and chlorosis on the border of the inoculated regions were considered pathogenic and saved for further aggressiveness evaluations. Aggressiveness-typing of *Xap* and *Xff* was carried out on eight common bean lines: VAX 6, G22247, XAN 159, Wilkinson 2, Pompadour J-19, Tars-VCR 43, UI-114, and GN Nebraska #1 (Mutlu et al., 2008) and evaluated with a disease reaction rating scale (Aggour et al., 1989) (Figure 2.1), where 1 = complete resistance (no necrotic lesion and/or chlorosis); 2-4 = high to low resistance (inoculated leaf area has necrotic lesion and/or chlorosis); 5-6 = low to moderate susceptibility (inoculated leaf area has chlorosis); 7-9 = high to severe susceptibility (inoculated area has severe chlorosis and has spread among leaf). Data were transformed from non-parametric to parametric by calculating the average percentage from both ends of each rating scale value. Rating scale values were transformed to percent disease severity as follows: 1 = 0%, 2 = 6.75%, 3 = 19.25%, 4 = 32.25%, 5 = 45.25%, 6 = 58.25%, 7 = 71.25%, 8 = 84.25%, and 9 = 95.5%. The trial was repeated and conducted in a completely random design with three replicates with a positive control of *Xap* 91-5, an aggressive *Xap* isolate used in previous common bacterial blight field studies in North Dakota, and a negative water control. Analyses of data from the isolate aggressiveness study was conducted using SAS version 9.4 (SAS Institute, Cary, NC). An

analysis of variance (ANOVA) was conducted to compare disease reaction of isolates across lines and an interaction between isolate and line.

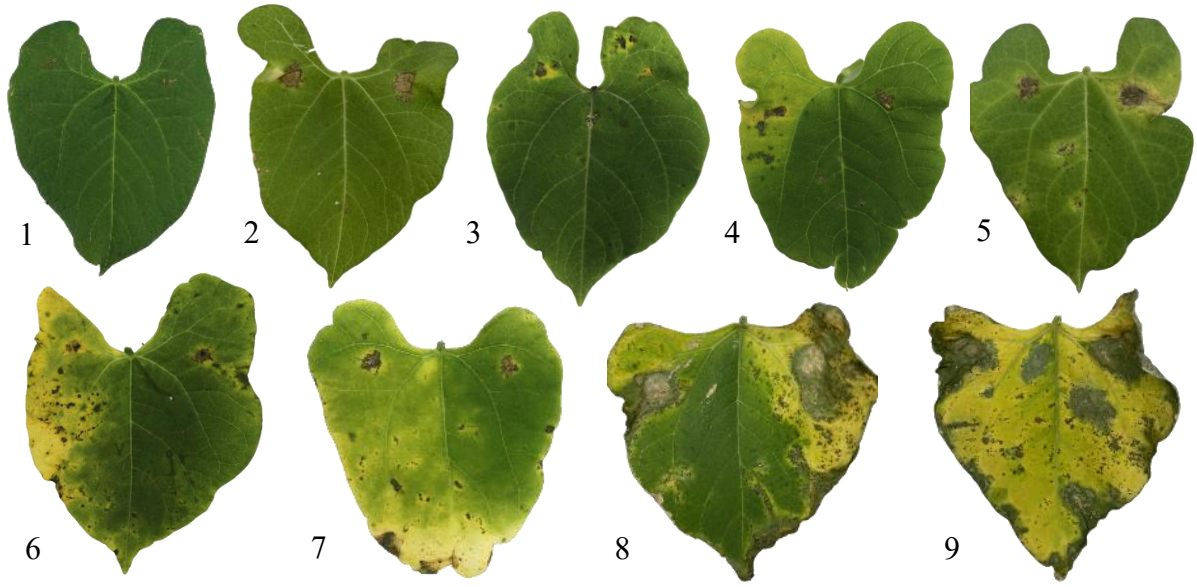

Figure 2.1. Common bacterial blight disease severity scale where 1 = complete resistance; 2-4 = high to low resistance; 5-6 = low to moderate susceptibility; 7-9 = high to severe susceptibility (adapted from Aggour et al., 1989)

Genetic diversity of *Xap/Xff* isolates

Sixty-nine isolates of *Xap* and *Xff* were obtained in 1990-2018 from common bean field leaves in North Dakota and American Type Culture Collection (ATCC) (Table B.4). This study was conducted in 2018, therefore no 2019 isolates were evaluated. Identity of the isolates was confirmed by culturing in nutrient media (8g/L BD Difco™ nutrient broth, 15g/L agar), brown media pigmentation (*Xff*), and qPCR (He, 2010). Housekeeping genes *atpD, dnaK, efp, fyuA, glna, gyrB, and rpoD* of confirmed *Xap* isolates were amplified using PCR (Table 2.1) (Mhedbi-Hajri et al., 2013). Assays for amplification of the housekeeping genes were first optimized on American Type Culture Collection (ATCC) isolates of *Xap* (*Xap1*, *Xap*20, *Xap*27(*Xff*), *Xap*28). The bacterial cells were lysed using the freeze-thaw boiling method. PCR reactions were run on

a Bio-Rad T100™ Thermal Cycler with amended conditions as follows: incubation at 95°C for 3 min; 39 cycles of 95°C for 30 s, and 62°C for 30 s, 72°C for 2 min, and 72°C for 10 min. The reaction for the amplification of each housekeeping gene of each bacterial isolate was amended from the previous study by mixing the following in a 20 µL reaction: 5 µL 5x Promega GoTaq® Green Master Mix, 0.5 µL 10 µM dNTP, 0.4 µL10 µM forward primer, 0.4 µL 10 µM reverse primer, 0.2 µL Go Taq Polymerase, and 2 µL template of suspended bacterial cells. The PCR products were run on 1% agarose gel with GelRed Nucleic Acid Stain (Biotium, Inc. Fremont, CA 10,000× in water) at 80 V for one hour and visualized using the AlphaImager™ program to confirm amplification and amplicon size.

Table 2.1. Primers for housekeeping genes used for genetic diversity of *Xanthomonas axonopodis* pv. *phaseoli/Xanthomonas fuscans* pv. *fuscans* isolates (Mhedbi-Hajri et al., 2013)

Locus	Sequence of forward (F) primer (5'-3')	Sequence of reverse (R) primer (5'-3')	Amplicon (bp)
atpD	GGGCAAGATCGTTCAGAT	GCTCTTGGTCGAGGTGAT	868
dnaK	GGTATTGACCTCGGCACCAC	ACCTTCGGCATACGGGTCT	1034
efp	TCATCACCGAGACCGAATA	TCCTGGTTGACGAACAGC	445
fyuA	ACCATCGACATGGACTGGACC	GTCGCCGAACAGGTTCACC	963
glna	ATCAAGGACAACAAGGTCG	GCGGTGAAGGTCAGGTAG	1094
gyrB	TGCGCGGCAAGATCCTCAAC	GCGTTGTCCTCGATGAAGTC	1051
rpoD	ATGGCCAACGAACGTCCTGC	AACTTGTAACCGCGACGGTATTCG	1313

The amplified housekeeping genes were sequenced by McLab Sequencing, South San Francisco, CA (Molecular Cloning Laboratories). Four phylogenetic trees were constructed based on the sequences of four housekeeping genes: *dnaK*, *atpD*, *gyrB*, and *rpoD*. Primers targeting gene *glna* were omitted because PCR amplification resulted in two amplified sequences rather than one. Sequences of genes *fyuA* and *efp* were omitted because they were not successfully amplified in enough isolates to provide useful comparisons. Construction of phylogenetic trees was done as follows: The evolutionary history was inferred using the

Maximum Likelihood method and Tamura-Nei mode (Tamura and Nei, 1993). The bootstrap consensus tree, inferred from 1000 replicates (Felsenstein, 1985), was taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50 percent bootstrap replicates were collapsed. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. This analysis involved 69 nucleotide sequences. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018). Based upon similar grouping in the four phylogenetic trees, three clusters were named in the analysis to represent all 69 isolates.

Results

Isolation and identification of *Xap* and *Xff* isolates

Thirty-one isolates of *Xap* were collected from 24 fields in 2018, and 23 isolates of *Xap* were collected from 14 fields in 2019 (Appendix B; Table B.1, B.2, B.3). Isolates of *Xap* were recovered from seven North Dakota counties including Cass, Foster, Grand Forks, Steele, Traill, Walsh, and Wells and three counties in Minnesota, Becker, Hubbard and Otter Tail. *Xff* was recovered from Cass and Wells counties in North Dakota. Two isolates of *Xff* were collected from two fields in 2018, and one isolate of *Xff* was collected in 2019. Two of the three *Xff* isolates were isolated from NDSU research fields in Fargo, ND in 2018 and 2019. Bacterial isolations were impeded by non-target bacterial growth on BBD medium, a medium also selective for common bean pathogens *Pseudomonas syringae* pv. *syringae* and *P. savastanoi* pv. *phaseolicola*. Isolates of *Xap/Xff* exhibited convex and smooth, yellow-colored colonies and hydrolyzed casein on BBD medium. Isolates of *Xff* produced a brown pigment on nutrient agar

media. Additionally, all bacterial isolates were successfully amplified with *Xap* and *Xff* specific qPCR (He, 2010).

Pathogenicity and aggressiveness

All *Xap* and *Xff* isolates tested were pathogenic to susceptible common bean 'UI-114'. Ten days after inoculation, bean lines used to evaluate aggressiveness exhibited varying symptoms of water-soaking and/or chlorosis near the inoculated area, similar to those observed under field conditions. Significant differences were observed in aggressiveness across isolates evaluated and in susceptibility of the differential lines (P <.0001) (Table 2.2). Additionally, a significant interaction between isolate and bean line was observed (P <.0001), indicating that the isolates varied in aggressiveness on each line. Inoculation with all *Xap* and *Xff* isolates resulted in greater than 60% severity on UI-114, with a maximum of 95.5% (Table 2.3). Severity on the other bean lines ranged from 1% to nearly 90%. Mean CBB severity for each isolate across all lines ranged from 31% to nearly 70%. The mean percent disease severity caused by *Xap* did not differ significantly from *Xff* across all lines (data not shown). Across all differential lines, the three *Xff* isolates were more aggressive than 45 of 54 *Xap* isolates tested (Table 2.3).

Table 2.2. Summary of analysis of variance of common bacterial blight (CBB) reaction on eight common bean lines with 57 isolates of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* pv. *fuscans* (*Xff*) under greenhouse conditions

Source of variation	Degrees of freedom	Mean square	Probability
Rep	5	769.2773	0.0002
Isolate	57	4734.5403	<.0001
Line	7	102107.8152	<.0001
Isolate × line	399	1776.5758	<.0001
Error	2315	159.0988	

Table 2.3. Severity of common bacterial blight (CBB) in six plants of each common bean differential line to 57 isolates of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* pv. *fuscans* (*Xff*) collected from common bean fields in 2018 and 2019

	Disease severity (%)								
Isolate	UI-114	GNNE #1	VAX 6	Wilk 2	Xan159	Tars VCR 43	Pompadour J 19	G22247	Mean
Control	88	86	82	52	28	39	19	12	51
ND18-2	88	65	26	11	17	30	39	2	35
ND18-3	82	63	78	26	26	12	16	12	39
ND18-5.1	71	83	58	32	17	17	9	9	37
ND18-5.2	69	80	50	24	11	24	34	32	40
ND18-7.1	86	82	69	26	30	37	52	37	52
ND18-7.2	94	79	58	24	19	28	32	26	45
ND18-7.3	92	84	69	52	24	24	47	4	49
ND18-8	94	73	71	45	30	21	58	3	50
ND18-10.1	90	73	24	47	26	69	26	10	46
ND18-10.2	96	80	34	43	73	37	41	8	51
ND18-11	92	82	88	73	37	41	11	13	55
ND18-15	94	77	82	43	43	65	24	1	54
ND18-16	92	79	73	54	19	69	19	28	54
ND18-18	90	84	58	71	10	77	32	17	55
MN18-19	77	17	80	41	54	19	1	35	41
ND18-20	73	50	79	43	45	13	4	36	43
ND18-21.1	96	69	45	17	56	69	65	63	60
ND18-23	60	43	69	28	28	52	58	58	50
ND18-24.1	69	73	43	17	43	43	32	32	44
ND18-24.2	88	65	39	58	22	45	32	39	49
ND18-25	92	50	62	65	56	37	56	52	59
ND18-26	84	45	37	30	50	76	52	56	54
ND18-27	92	77	86	41	41	69	65	63	67
ND18-28.1	84	82	37	50	83	50	78	78	68
ND18-28.2	86	84	58	63	75	43	71	71	69
ND18-28.3	92	69	56	63	63	34	58	69	63
ND18-29	88	78	32	67	56	26	65	43	57
ND18-30	84	43	62	82	69	60	78	73	69
ND18-33	84	37	32	34	34	28	60	60	46
ND18-35	92	37	63	41	34	45	56	43	51
ND18-37	82	52	50	37	54	54	54	39	53
ND19-3	94	50	58	50	19	78	34	10	49
ND19-5	90	39	54	60	28	58	37	17	48
ND19-6	88	45	63	60	22	58	32	9	47

Table 2.3. Severity of common bacterial blight (CBB) in six plants of each common bean differential line to 57 isolates of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* pv. *fuscans* (*Xff*) collected from common bean fields in 2018 and 2019 (continued).

	Disease severity (%)								
Isolate	UI-114	GNNE #1	VAX 6	Wilk 2	Xan159	Tars VCR 43	Pompadour J 19	G22247	Mean
ND19-9	80	34	58	73	19	56	52	13	48
ND19-10.1	86	24	58	75	22	56	37	8	46
ND19-10.2	88	30	45	65	34	69	58	15	51
ND19-10.3	90	26	56	56	24	71	19	6	44
ND19-11.1	86	30	19	45	28	63	28	11	39
ND19-11.2	69	15	22	28	11	71	39	6	33
ND19-12.1	86	19	43	56	26	71	54	6	45
ND19-12.2	90	30	34	67	34	17	58	15	43
ND19-14	82	19	19	60	56	65	24	11	42
ND19-16.1	94	17	22	52	17	52	26	6	36
ND19-16.2	84	45	41	56	43	69	63	32	54
ND19-18.1	94	50	54	56	26	63	43	43	54
ND19-18.2	84	19	32	58	30	24	22	15	36
ND19-19.1	86	30	50	54	32	30	13	11	38
ND19-19.2	76	15	45	26	13	50	22	6	32
ND19-19.3	78	19	45	22	11	52	13	9	31
ND19-20.1	78	22	43	56	52	24	15	9	37
ND19-20.2	76	24	41	58	45	17	11	8	35
ND19-21	78	30	39	41	15	21	30	32	36
ND19-24.1	79	52	45	37	17	32	43	9	39
ND18-21.2*	88	71	69	28	79	63	78	69	68
ND18-44*	92	73	58	50	63	58	67	52	64
ND19-24.2*	88	65	58	56	34	54	41	39	54
CV	11	25	28	24	35	27	32	44	26
Mean	85	52	52	47	36	46	40	27	48
Range	60-96	15-86	19-88	11-82	10-83	12-77	1-78	1-78	31-69

**Xanthomonas fuscans* pv. *fuscans*

Genetic variation of *Xap* and *Xff* isolates

Sequences of four housekeeping genes (*atpD*, *dnaK*, *gyrB* and *rpoD*) were included in the analyses. Genes of all isolates were not amplified; therefore, separate phylogenetic trees were

constructed using concatenated sequences of all the amplified housekeeping genes. All four phylogenetic trees displayed similar grouping of the *Xap* isolates (Figures 2.2, 2.3, 2.4, and 2.5). The phylogenetic tree constructed from amplicons of target gene *atpD* included 53 isolates (Figure 2.2), tree of target gene *dnaK* included 50 isolates (Figure 2.3), gene *gyrB* included 47 isolates (Figure 2.4), and gene *rpoD* included 52 isolates (Figure 2.5). In total, 69 isolates of *Xap* and *Xff* were used to create a consensus of three phylogenic groupings based on the four phylogenetic trees (Table 2.4). Cluster 1 was the largest with 33 isolates and contained most of the older isolates from 1990-1995, along with one isolate from 2016 and four from 2018. Cluster 2 contained 19 isolates including all four *Xff* isolates along with other isolates, six of which were collected in 2018 (4 *Xap*, 2 *Xff*). Cluster 3 was the smallest and contained 17 isolates, all collected in 2018.

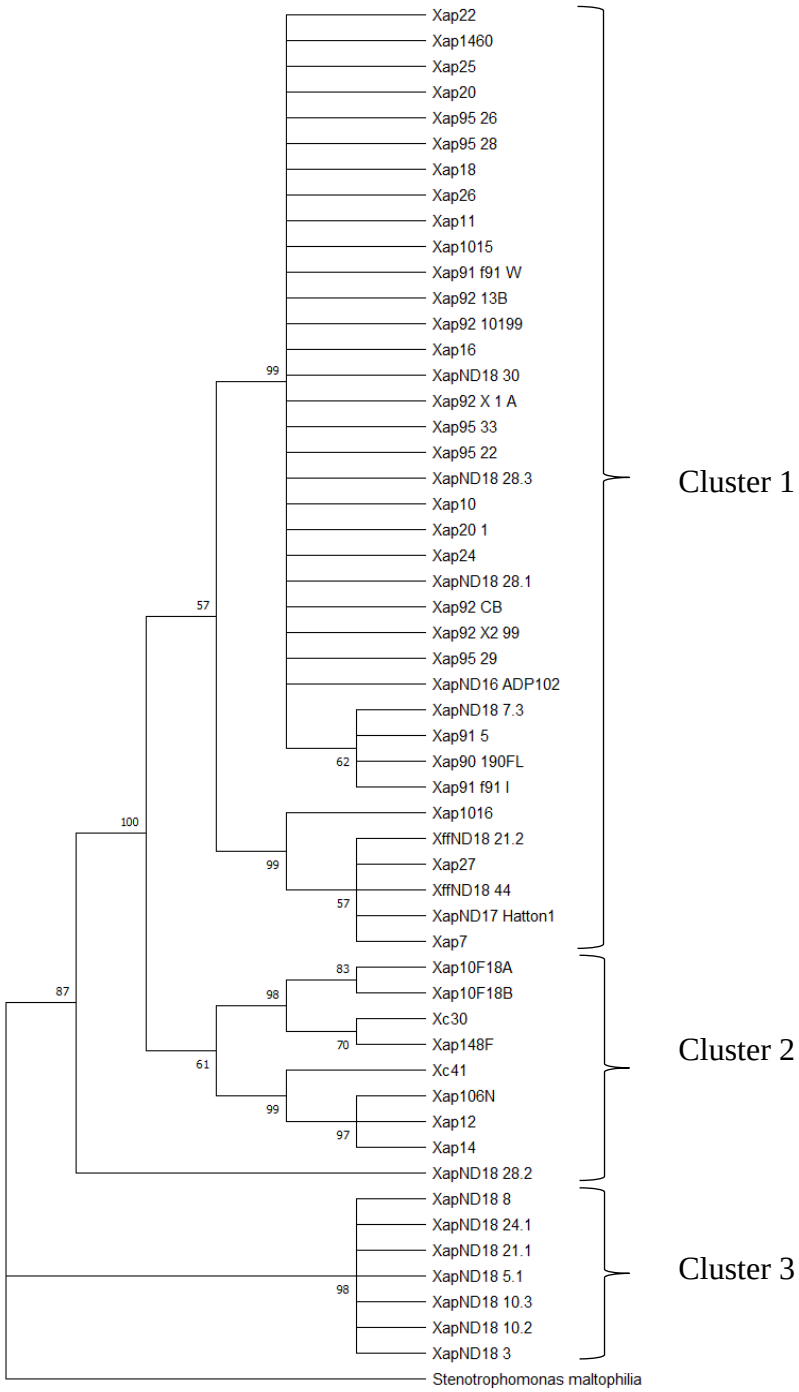

Figure 2.2. Phylogenetic tree of 53 isolates of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* pv. *fuscans* (*Xff*) based on sequencing a portion of the *atpD* gene. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches

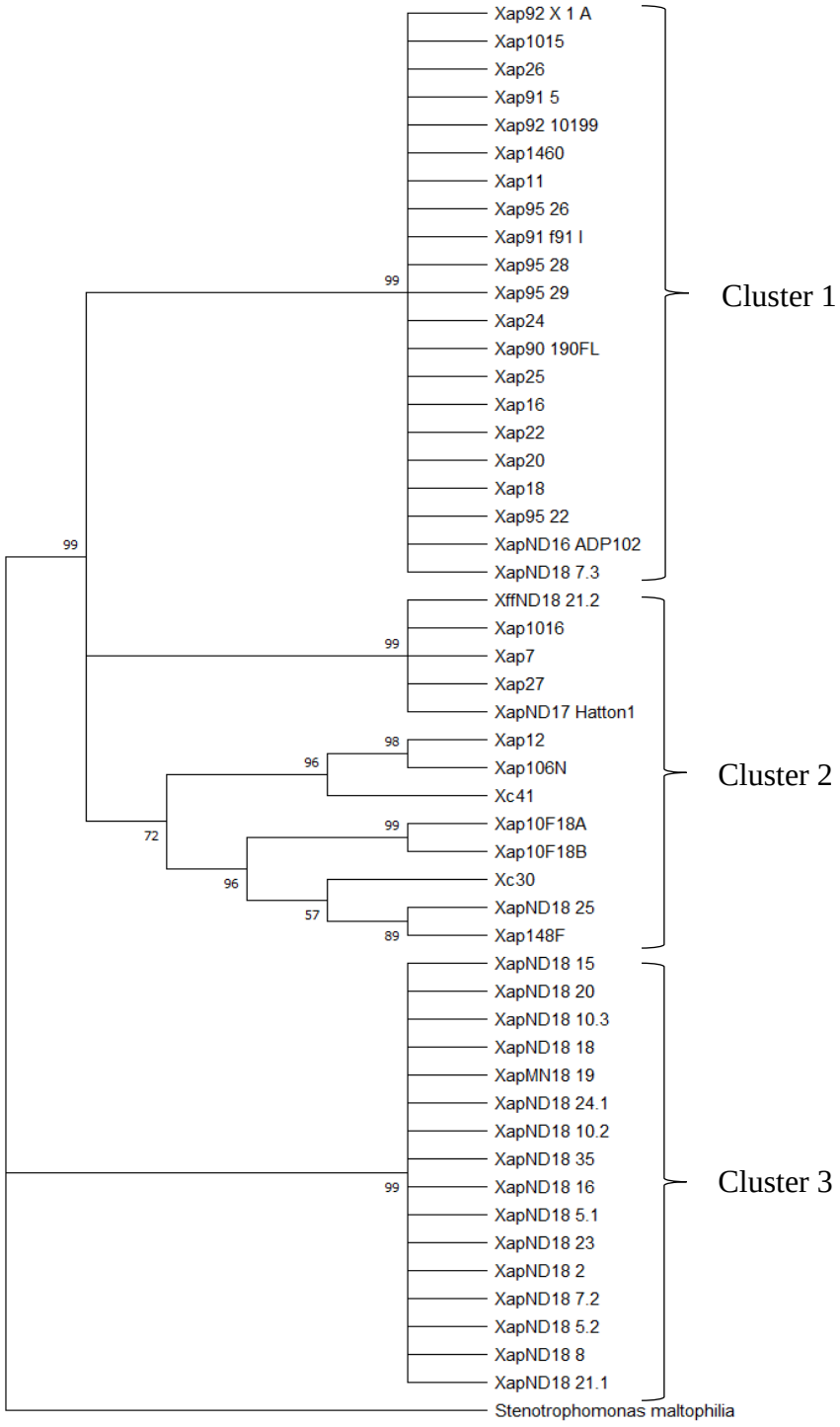

Figure 2.3. Phylogenetic tree of 50 isolates of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* pv. *fuscans* (*Xff*) based on the *dnaK* primer set. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown next to the branches

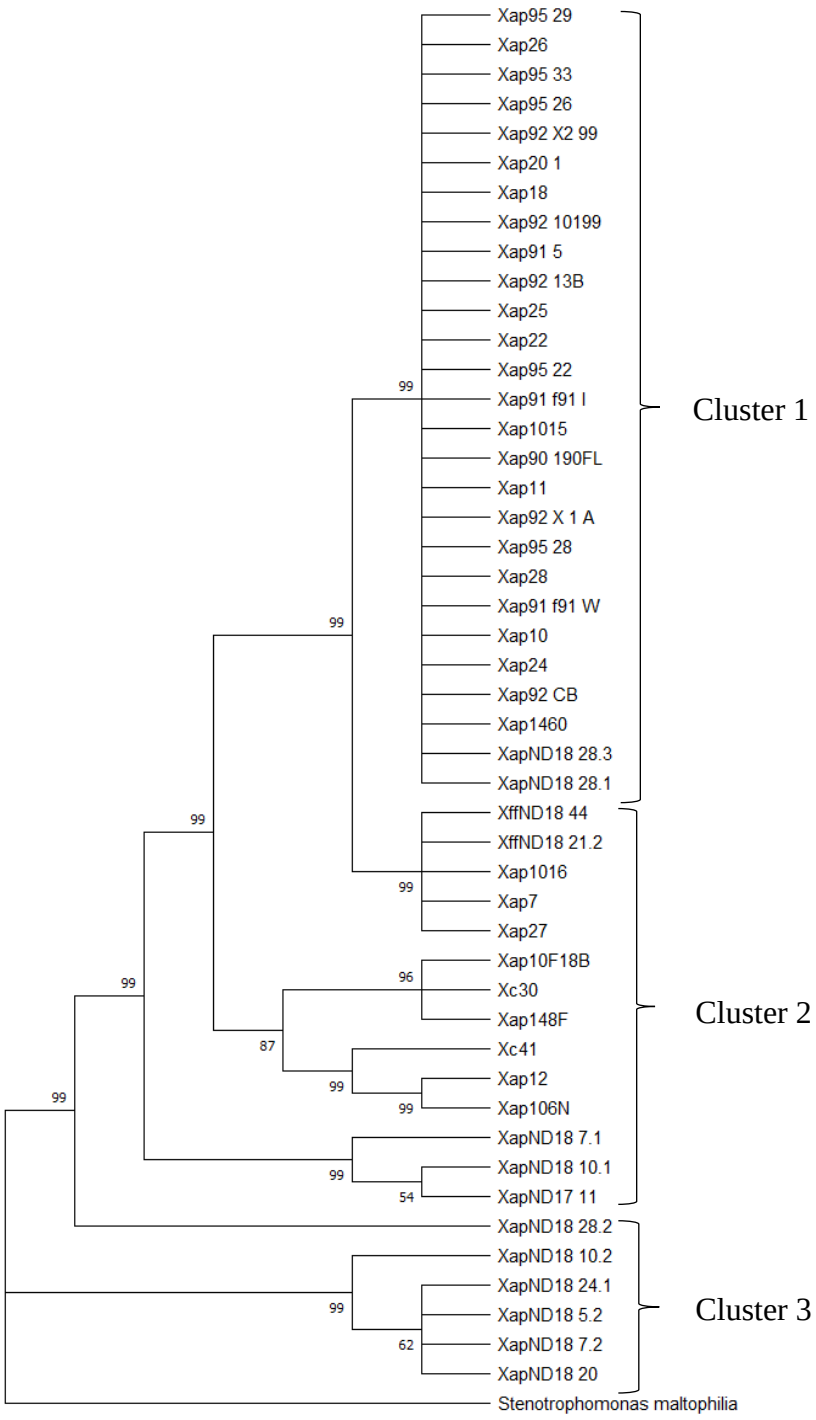

Figure 2.4. Phylogenetic tree of 47 *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* pv. *fuscans* (*Xff*) isolates based on the *gyrB* primer set. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown next to the branches

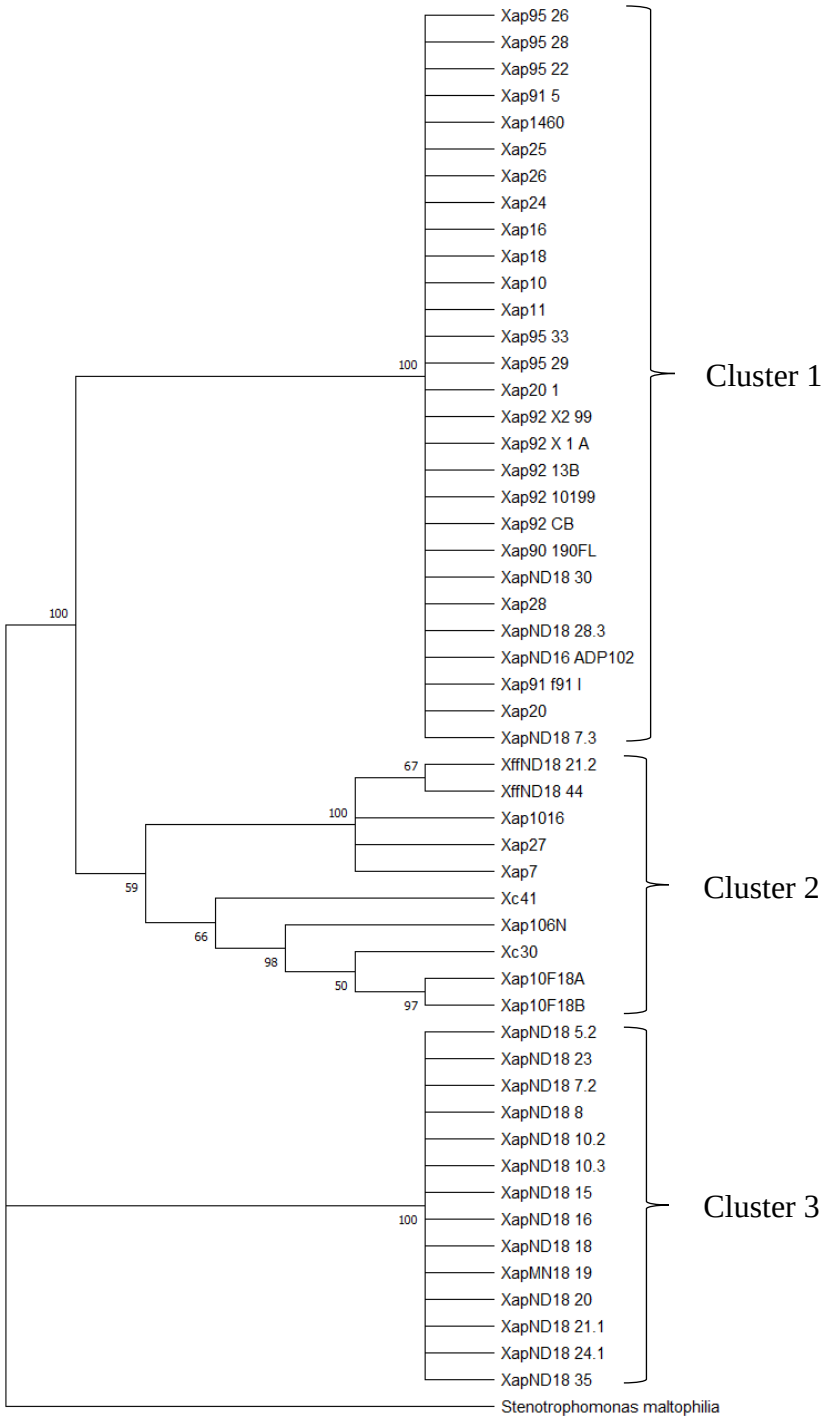

Figure 2.5. Phylogenetic tree of 52 *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* pv. *fuscans* (*Xff*) isolates based on the *rpoD* primer set. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown next to the branches

Table 2.4. Three clusters of 69 isolates of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* pv. *fuscans* (*Xff*) based on a consensus of four phylogenetic trees

Cluster 1	Year	Cluster 2	Year	Cluster 3	Year
Xap10	1992	Xap1016	---	XapND18 10.2	2018
Xap1015	---	Xap106N	---	XapND18 10.3	2018
Xap11	1992	Xap10F18A	---	XapND18 15	2018
Xap1460	---	Xap10F18B	---	XapND18 16	2018
Xap16	1992	Xap12	1992	XapND18 18	2018
Xap18	1992	Xap14	1992	XapMN18 19	2018
Xap20[a]	---	Xap148F	---	XapND18 2	2018
Xap20 1	---	Xap27 (Xff)[a]	---	XapND18 20	2018
Xap22	1992	Xap7	1992	XapND18 21.1	2018
Xap24	1992	XapND17 11	2017	XapND18 23	2018
Xap25	1992	XffND17 Hatton1	2017	XapND18 24.1	2018
Xap26	1992	XapND18 10.1	2018	XapND18 3	2018
Xap28[a]	---	XapND18 25	2018	XapND18 35	2018
Xap90 190FL	1990	XapND18 28.2	2018	XapND18 5.1	2018
Xap91 5	1991	XapND18 7.1	2018	XapND18 5.2	2018
Xap91 f91 I	1991	Xc30	---	XapND18 7.2	2018
Xap91 f91 W	1991	Xc41	---	XapND18 8	2018
Xap92 10199	1992	XffND18 21.2	2018	XapND18 35	2018
Xap92 13B	1992	XffND18 44	2018		
Xap92 CB	1992				
Xap92 X 1 A	1992				
Xap92 X2 99	1992				
Xap95 22	1995				
Xap95 26	1995				
Xap95 28	1995				
Xap95 29	1995				
Xap95 33	1995				
XapND18 28.1	2018				
XapND18 28.3	2018				
XapND18 30	2018				
XapND18 7.3	2018				
ADP102	2016				

[a] ATCC = American Type Culture Collection

---, unknown

Discussion

North Dakota and Minnesota are arguably the most important regions to study the pathogen diversity of CBB bacteria because this is the largest bean production region in the United States and environmental conditions favoring these pathogens are common. The current research confirms that among the two causal bacteria of CBB, *Xap* and *Xff*, *Xap* was by far most prevalent. This was the first documented report of *Xff* in North Dakota and Minnesota. Previously, *Xff* has been isolated in Nebraska, Michigan, and Wisconsin as well as across the world including Canada, South America, Africa, Australia, and Europe (Mkandawire et al., 2004; Mutlu et al., 2008). While colony morphology, biochemical tests, and qPCR were utilized to identify the bacteria, pathogenicity tests confirmed that these isolates were CBB-causing Xanthomonads. Performing pathogenicity tests on susceptible bean cultivars to ensure these bacteria cause CBB is critical prior to continuing with additional experiments as contamination can occur during the isolation process.

The 57 *Xap/Xff* isolates evaluated during this research varied in aggressiveness on eight common bean lines ranging from disease severity of 0 to 95.5% and an interaction was observed between isolate and differential. Some isolates that were highly aggressive on one differential were less aggressive on another, suggesting that pathogen resistance in one bean line is not effective against other isolates. This information is critical in the development of common bean cultivars with resistance to *Xap*/*Xff*. The results generated during this study indicate that the selection of pathogen isolates will influence phenotypic results; therefore, it is highly recommended that isolates be screened across this differential set prior to use in phenotyping, and that a combination of isolates highly aggressive on most or all of the differentials be used.

Xff was confirmed for the first time during this research in the most important common bean growing region in the US. In North Dakota, *Xff* was found in Cass and Wells County. Mutlu et al. 2008 found that all 15 *Xff* isolates originating from Africa, North America, South America, Australia, and Europe were more aggressive than 69 *Xap* isolates from the same regions across all 13 bean lines. The mean disease reaction was 44% and 31% for *Xff* and *Xap*, respectively; however, Duncan et al. 2011 reported differing results while screening five *Xap* and three *Xff* isolates across 28 common bean lines. Isolates originated from Wisconsin, Puerto Rico, and Malawi as well as an additional *Xap* isolate from Tanzania. Mean disease rating was 3.9 and 4.5 (1 to 9 scale) for *Xap* and *Xff*, respectively, across all lines. In the current study, the three *Xff* isolates tested were more aggressive than 80% of *Xap* isolates based on mean disease severity across all lines. More isolates of *Xff* should be obtained and evaluated from the region to generate sufficient conclusions concerning aggressiveness.

Most of the historical isolates of *Xap/Xff* grouped together in Cluster 1 (Mhedbi-Hajri et al., 2013); however additional historical isolates were placed in Cluster 2. Genetic variation is known to be caused by molecular mechanisms including: spontaneous mutations from DNA replication, repair of DNA mismatches, transposition, site-specific recombination, and horizontal DNA acquisition (Scortichini et al., 2001). It is likely that variations due to some of these mechanisms had been occurring in the *Xap/Xff* population in North Dakota and Minnesota. The gene flow within *Xap/Xff* could favor the emergence of new, genetically distinct isolates by horizontal transfer of virulence-associated genes (Mhedbi-Hajri et al., 2013). The few newer isolates placed into Cluster 1 may have failed to diverge due to geographic isolation or lack of selective pressure. Based upon phylogenetic grouping, more genetic diversity was observed in *Xap* than *Xff*, similarly to previous findings (Mahuku et al., 2006; Mkandawire et al., 2004).

However, only three *Xff* isolates were tested in this study. A larger pathogen population of *Xff* should be tested for evaluating diversity within the pathovar.

Fifty-four isolates of *Xap* and three isolates of *Xff* obtained from 38 fields were pathogenic to common bean based on results from greenhouse inoculations and varied in aggressive across eight common bean lines. The interaction observed between isolate and line suggests that host resistance to single isolate may not be effective on other isolates. Four housekeeping genes (*dnaK*, *atpD*, *gyrB*, and *rpoD*) were sequenced from *Xap* isolates collected from 1990 to 2018. Analysis of these four phylogenetic trees characterized three groups of *Xap* based on genetic similarity. Overall, this study provided information regarding the prevalence of *Xap* and *Xff* in common bean growing regions of North Dakota and Minnesota and suggests that the use of this isolate collection from the region can assist in CBB resistance screening for the development of common bean cultivars.

Literature Cited

Aggour A. R., Coyne, D. P., Vidaver, A. K., and Eskiridge, K. M. 1989. Transmission of the common blight pathogen in bean seed. J. Am. Soc. Hortic. Sci. 114:1002-1008.

Boersma, J. G., Hou, A. Gillard, C. L., McRae, K. B., and Conner, R. L. 2015. Impact of common bacterial blight on the yield, seed weight and seed discoloration of different market classes of dry beans (*Phaseolus vulgaris* L.). Can. J. Plant Sci. 95:703-710.

Curland, R. D., Gao, L., Bull, C. T., Vinatzer, B. A., Dill-Macky, R., Van Eck, L., and Ishimaru, C. A. 2018. Genetic diversity and virulence of wheat and barley isolates of *Xanthomonas translucens* from the upper midwestern United States. Phytopathology 108:443-453.

Duncan, R. W., Singh, S.P., and Gilbertson, R. L. 2011. Interaction of common bacterial blight bacteria with disease resistance quantitative trait loci in common bean. Phytopathology 101:425–435.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791.

Gilbertson, R. L., Otoya, M. M., Pastor-Corrales, M. A., and Maxwell, D. P. 1991. Genetic diversity in common blight bacteria is revealed by cloned repetitive DNA sequences. Annu. Rep. Bean Improv. Coop. 34:37-38.

Harveson, R. M. and Schwartz, H. F. 2007. Bacterial Diseases of Dry Edible Beans in the Central High Plains. Plant Management Network.

He, Y. 2010. Improved seed health tests for *Xanthomonas axonopodis* pv. *phaseoli* in common bean. Graduate Theses and Dissertations. Ames, IA. 11565.

He, R., Liu, P., Jia, B., Xue, S., Want, X., Hu, J., Shoffe, Y. A., Gallipoli, L., Mazzaglia, A., Balestra, G. M., and Liwu, Z. 2019. Genetic diversity of *Pseudomonas syringae* pv. *actinidiae* isolates from different geographic regions in China. Phytopathology. 109:347-357.

Hildebrand, D. C., Palleroni, N. J., and Schroth, M. N. 1990. Deoxyribonucleic acid relatedness of 24 xanthomonad isolates representing 23 *Xanthomonas campestris* pathovars and *Xanthomonas fragariae*. J. Appl. Bacteriol. 68:263-269.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35:1547-1549.

López, R., Asensio, C., and Gilbertson, R. L. 2006. Phenotypic and genetic diversity in isolates of common blight bacteria (*Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) in a secondary center of diversity of the common bean host suggests multiple introduction events. Phytopathol. 96:1204–1213.

Mahuku, G. S., Jara, C., Henríquez, M. A., Castellanos, G., and Cuasquer, J. 2006. Genotypic characterization of the common bean bacterial blight pathogens, *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* by rep-PCR and PCR-RFLP of the ribosomal genes. J. Phytopathol. 154:35–44.

Mhedbi-Hajri, N., Hajri, A., Boureau, T., Darrassee, A., Durand, K., Brin, C., Fischer-Le Saux, M., Manceau, C., Poussier, S., Pruvost, O., Lemaire, C., and Jacques, M. 2013. Evolutionary history of the plant pathogenic bacterium *Xanthomonas axonopodis*. PLoS ONE: 8(3):e58474.

Mkandawire, A. B. C., Mabagala, R. B., Guzmán, P., Gepts, P., and Gilbertson, R. L. 2004. Genetic diversity and pathogenic variation of common blight bacteria (*Xanthomonas campestris* pv. *phaseoli* and *Xanthomonas campestris* pv. *phaseoli* var. *fuscans*) suggests pathogen coevolution with the common bean. Phytopathol. 94:593-603.

Moser, P. E., Cown, L., Massoth, H., and Murray, G. 1994. A semi-selective medium planting assay for detection and differentiation of bacterial blight pathogens of bean and pea. Annu Rep Bean Improv Coop. Fort Collins, CO. 37:23-24.

Mutlu, N., Vidaver, A. K., Coyne, D. P., Steadman, J. R., Lambrecht, P.A., and Reiser, J. 2008. Differential pathogenicity of *Xanthomonas campestris* pv. *phaseoli* and *X. fuscans* subsp. *fuscans*) stains on bean genotypes with common blight resistance. Plant Dis. 92:546–554.

Opio, A. F., Allen, D. J., and Teri, J. M. 1996. Pathogenic variation in *Xanthomonas campestris* pv. *phaseoli*, the causal agent of common bacterial blight in *Phaseolus* beans. Plant Pathol. 45:1126-1133.

Pedraza, F., Gallego, G. Beebe, S., and Tohme, J. 1997. Marcadores SCAR y RAPD para la resistencia a la bacteriosis comun (CBB). In: S.P. Singh and O. Voysest, editors, Taller de mejoramiento de frijol para el siglo XXI: Bases para una estrategia para America Latina. CIAT, Cali, Colombia. p. 130–134.

Rudolph, K. 1993. Infection of the plant by *Xanthomonas*. In: Swings JG, Civerolo EL (eds) Xanthomonas. Chapman & Hall, London, p. 193–264

Schwartz, H. F., Steadman, J. R., Hall, R., and Forester, R. I. 2005. Compendium of bean diseases. 2nd edition. American Phytopathological Society, St. Paul, MN. p. 46-50.

Scortichini, M., Marchesi, U., di Prospero P. 2001. Genetic diversity of *Xanthomonas arboricola* pv. *juglandis* (synonyms: *X. campestris* pv. *juglandis*; *X. juglandis* pv. *juglandis*) isolates from different geographical areas shown by repetitive polymerase chain reaction genomic fingerprinting. J. Phytopathol. 149:325–332.

Silva, L. O., Singh, S. P., and Pastor-Corrales, M. A. 1989. Inheritance of resistance to common bacterial blight in common bean. Theor. Appl. Genet. 78:619–624.

Singh, S. P., and Muñoz, C. G. 1999. Resistance to common bacterial blight among *Phaseolus* species and common bean improvement. Crop Sci. 39:80–89.

Tamura, K. and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10:512-526.

Taylor, J. D., Teverson, D. M., Allen, D. J., and Pastor-Corrales, M. A. 1996. Identification and origin of races of *Pseudomonas syringae* pv. *phaseolicola* from Africa and other bean growing areas. Plant Pathol. 45:469-478.

Tugume, J. K., Tusiime, G., Sekamate, A. M., Buruchara, R., and Mukankust, C. M. 2018. Diversity and interaction of common bacterial blight disease-causing bacteria *Xanthomonas* spp.) with *Phaseolus vulgaris* L. Crop J. 7(2019):1-7.

Vauterin, L., Rademarker, J., and Swings, J. 2000. Synopsis on the taxonomy of the genus *Xanthomonas*. Phytopathol. 90:677-682.

Viteri, D. M., Cregan, P. B., Trapp, J., Miklas, P. N., and Singh, S. P. 2014. A new common bacterial blight resistance QTL in VAX 1 common bean and interaction of the new QTL, SAP6, and SU91 with bacterial strains. Crop Sci. 54:1598–1608.

Yu, Z. H., Stall, R. E., and Vallejos, C. E. 1998. Detection of genes for resistance to common bacterial blight of beans. Crop Sci. 38:1290–1296.

CHAPTER 3: IDENTIFICATION OF *PSEUDOMONAS SAVASTANOI* PV. *PHASEOLICOLA* RACE-TYPES IN NORTH DAKOTA AND MINNESOTA

Abstract

Halo blight, caused by *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*), affects common bean in the top-producing region of North Dakota and Minnesota in the United States. Races (1-9) of *Psp* are present worldwide, and race 6 is the most common. North Dakota isolates of *Psp* found in 1995-2000 were identified as mostly race 6, but race 2 was also present as well as an uncharacterized race. In 2018 and 2019, leaves with characteristic halo blight symptoms were sampled from fields in North Dakota and Minnesota. Pseudomonad-like bacteria were isolated from these leaves and characterized based upon colony characteristics, biochemical tests, *Psp* specific qPCR primers and pathogenicity tests on a susceptible bean cultivar. Race-types were evaluated by inoculating 60 *Psp* isolates onto a differential set of eight bean cultivars. All isolates were identified as race 6. As this race is virulent on all differentials in the set, it is important that the set be expanded in order to explore new races of this pathogen as diversity likely is present in the population that is not being identified in North Dakota and Minnesota as well as across all common bean growing regions.

Introduction

Halo blight (*Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*)) is a seed-borne bacterial disease of common bean that has a worldwide distribution. North Dakota and Minnesota common bean growers have reported observations of halo blight in fields over the past six years (Knodel et al., 2016-2021). A total amount of 12,634 acres were reported by growers in 2020 as halo blight as one of the worst three disease problems in the Northarvest region (Knodel et al., 2021). In the United States, halo blight has caused reductions in pod and seed quality and yield

(Saettler, 1991; Schwartz, 1989). Under favorable conditions of halo blight, crop losses can reach up to 45% (Singh and Schwartz, 2010). The bacterium survives on common bean seed and debris, infecting seedlings early in the growing season. Bacteria may also enter through natural openings such as the stomata or hydathodes or through wounds when heavy storms occur. Infection symptoms on leaves begin as water-soaked spots that turn into black to brown lesions surrounded by a yellow to lime-green halo (Schwartz et al., 2005). The halo surrounding the lesion is indicative of the presence of phaseolotoxin, a toxin all pathogenic isolates of *Psp* produce. Diseased pods have red to brown lesions that appear water-soaked, and bacterial ooze may sometimes be observed when relative humidity is high. Symptomatic seed appears shriveled and discolored, but seed infected with *Psp* can also be asymptomatic. Wind, rain splash, hail, excessive irrigation, machinery, animals and people can spread bacteria, causing secondary infections. Halo blight is most damaging under cooler temperatures of 18 to 22˚C, and therefore, is more commonly observed earlier in the growing season.

An integrated pest management (IPM) approach is taken to manage halo blight. Cultural methods include planting disease-free seed, a minimum two-year crop rotation and the incorporation of residue into the soil. Chemical methods include the application of streptomycin seed treatments and foliar bactericides, although these tactics are not often used by growers. The most recommended management tool is planting resistant varieties (Arnold et al., 2011; Rivas et al., 2005; Agarwal and Sinclair, 1997).

The pathogen is variable phenotypically, therefore *Psp* has been characterized into nine races based on reactions on eight differential cultivars (Taylor et al., 1996). Races 1, 2, 5, 6 and 7 are distributed worldwide, with race 6 most common. Races 3 and 4 have been found in East/Central Africa and races 8 and 9 in Southern Africa. Isolates of *Psp* from North Dakota

have been determined to be predominately race 6, however these date back to 1995-2000 (Lamppa et al., 2002). Races of *Psp* present in Minnesota have not been determined. Consequently, the objective of this study was to identify the physiological races of *Psp* isolates collected from North Dakota and Minnesota common bean fields in 2018 and 2019. These results will benefit breeding programs when screening against the most common and virulent races of *Psp*.

Materials and Methods

Isolation and identification of bacterial pathovars

A total of 44 and 33 fields were sampled in 2018 and 2019, respectively, in North Dakota and Minnesota. Leaf samples exhibiting bacterial blight symptoms were taken at each point in a W-pattern for a total of 25 leaves per field. Diseased leaves were macerated in water and plated onto a bacterial blight differential medium (BBD; Na-Deoxycholate (250mg/250mL), Proteose Peptone No. 3 (2.5g/250mL), yeast extract (0.5g/250mL), glycerol (2.5 mL/250mL) and agar (7.5g/250mL) in one bottle; dry milk (10g/250mL) in another bottle) (Moser et al., 1994). After autoclaving, cooling and mixing the two bottles, cycloheximide (40 mg), cephalexin (10 mg) and vancomycin (5 mg) were added. Identification of isolated bacteria was confirmed via morphology, fluorescence of bacteria on King's B medium and the absence of a zone of hydrolysis on BBD media. Additional confirmation was done with a negative oxidase reaction with tetra-methyl-p-phenylenediamine (Schaad, 1988) and qPCR (Cho et al., 2010).

Race determination

Pathogenicity and aggressiveness of *Psp* isolates were confirmed on susceptible dark red kidney bean cv. Charlevoix (Stadt and Saettler, 1981). Plants were inoculated with each *Psp* isolate from 24-48 hour old colonies using a Paasche airbrush set at 20 psi when plants were at

the primary leaf stage. Inoculum of 10^8 CFU/mL adjusted with a spectrophotometer was forced into a small area (1 cm diameter) on both sides of the midrib of the abaxial side of both primary leaves and also sprayed on the adaxial side until runoff. Inoculated plants were incubated at >90% relative humidity for 24 h and returned to the greenhouse at average temperatures of 21˚C with a 16-hour light cycle. Disease reaction was recorded after 10 days using a 1 to 5 halo blight disease severity scale adapted from previous evaluations (Innes et al., 1984), where 1 = red-brown necrotic spots in the area of maximum inoculation); 2 = red-brown necrosis with a trace of water-soaking; 3 = some necrosis but more watersoaking that is confined to the area of maximum inoculation; 4 = water-soaked lesions <1 mm diameter distributed randomly over abaxial side of leaf; and 5 = watersoaked lesions 1-3 mm in diameter distributed at random over the leaf underside (Figure 3.1).

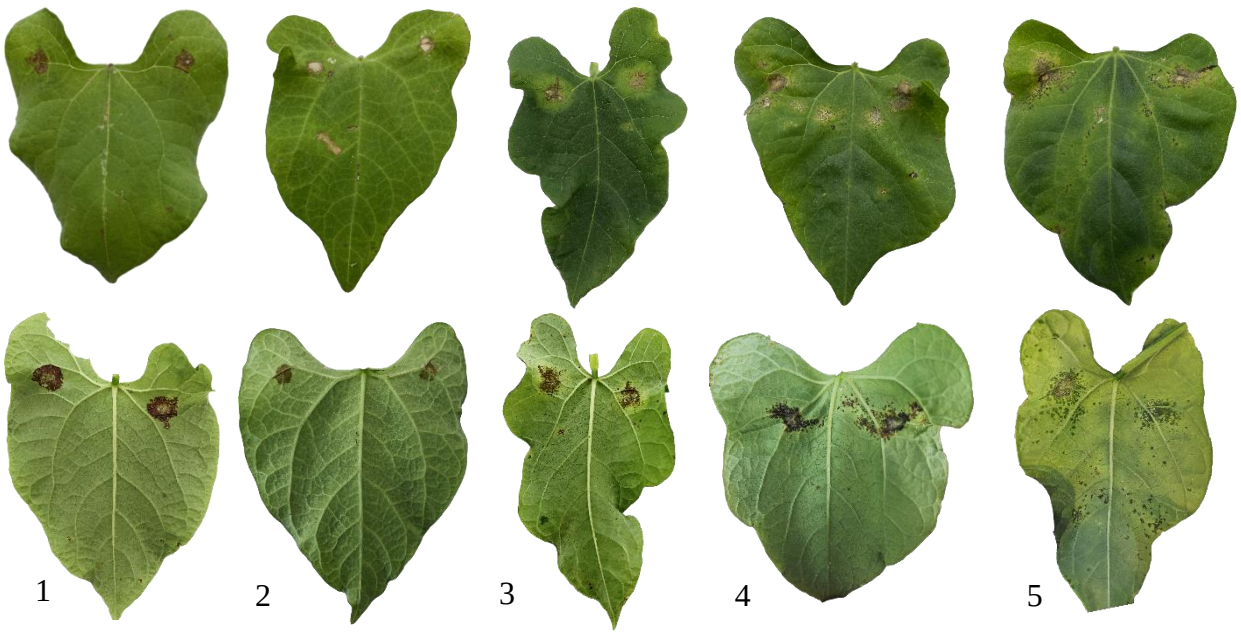

Figure 3.1. Halo blight disease severity scale of upper- and under-side of leaf where 1 = red-brown necrotic spots in the area of maximum inoculation; 2 = necrosis with a trace of water-soaking; 3 = some necrosis but more water-soaking; 4 = water-soaked lesions <1 mm diameter distributed randomly; 5 = water-soaked lesions 1-3 mm in diameter distributed at random covering leaf (adapted from Innes et al., 1984)

Isolates were considered avirulent if a disease rating of 1 to 2 was observed, and isolates that received ratings of 3 to 5 were considered virulent. Greenhouse trials assessing race-types of *Psp* were conducted using a completely randomized design (CRD) and performed twice. The trial contained three replicates with a positive control of *Psp* Race 6 and a negative control with water. Race-type was determined using a differential set of eight hosts: Canadian Wonder, A52, A53, A43, 1072, Tendergreen, Red Mexican UI-3, and 196-B (Taylor et al., 1996) (Table 3.1). Isolates were also inoculated onto cultivars Rojo, Vista, and VAX 3 to test for halo blight tolerance and resistance. Plants were inoculated using the method described above for pathogenicity testing.

Table 3.1. Differential set for race determination of *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*) (Taylor et al., 1996)

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

[a] = + : compatible (virulent), − : incompatible (avirulent)

Results

Race-typing of *Psp*

Thirty isolates of *Psp* were collected from 19 fields in 2018, and 30 isolates were collected from 15 fields in 2019 (Table C.1, Table C.2). Samples in 2018 were collected from five North Dakota counties: Grand Forks, Steele, Traill, Walsh, and Wells and Otter Tail County in Minnesota. Samples in 2019 were collected from five North Dakota counties: Cass, Foster, Grand Forks, Traill and Wells and three Minnesota counties: Becker, Hubbard and Otter Tail. All isolates exhibited convex, smooth, and opaque white/cream colored colonies that did not create a zone of hydrolysis on BBD medium. All isolates produced a fluorescent pigment on King's B medium and were oxidase negative. Previously developed *Psp*-specific qPCR successfully amplified a product from all bacterial isolates (Cho et al., 2010).

All *Psp* isolates tested were pathogenic to common bean 'Charlevoix.' Ten days after inoculation, bean plants displayed watersoaking and lesions surrounded by a yellow-green halo similar to those observed under field conditions. Greenhouse temperatures were an average of 20˚C and an average 46% relative humidity. All 60 *Psp* isolates examined during the study were identified as race 6 (Table 3.2). Bacterial isolates were virulent on all cultivars in the differential set. This is the first report of *Psp* race 6 in Minnesota and North Dakota counties Cass and Wells.

Table 3.2. Race identification and mean disease severity for 60 isolates of *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*) collected from North Dakota and Minnesota common bean fields in 2018 and 2019

	Differential set reactions of *Psp*								
Psp isolate	CW	A52	TG	UI3	1072	A53	A43	196-B	Race
Control	4.5	3.8	3.8	4.0	4.5	4.3	4.3	3.8	6
Psp1-ND18	5.0	4.8	4.7	4.5	5.0	4.8	5.0	5.0	6
Psp11.1-ND18	3.7	3.7	4.2	3.5	4.2	4.5	4.0	3.8	6
Psp11.2-ND18	3.3	3.3	3.0	3.0	3.3	3.0	3.0	3.2	6
Psp14.1-ND18	3.2	3.8	3.8	3.8	4.3	4.3	3.7	4.2	6
Psp14.2-ND18	4.0	4.5	3.3	4.0	4.5	4.3	4.5	4.2	6
Psp16-ND18	3.7	3.7	3.5	3.5	3.7	3.8	4.2	3.7	6
Psp17-ND18	4.7	4.0	3.2	3.8	4.3	4.7	4.3	3.8	6
Psp19.1-ND18	4.8	5.0	4.7	4.3	4.3	5.0	4.5	4.5	6
Psp19.2-ND18	4.5	3.7	3.5	3.8	3.8	3.7	4.0	3.5	6
Psp21.1-ND18	3.2	3.7	3.3	4.0	3.8	3.0	3.0	3.2	6
Psp21.2-ND18	4.5	3.7	3.5	4.7	5.0	4.0	3.5	4.0	6
Psp21.3-ND18	4.2	3.5	3.0	3.3	3.5	3.3	3.0	4.2	6
Psp22-ND18	3.7	3.3	3.2	3.7	3.0	4.0	3.2	3.7	6
Psp24.1-ND18	3.3	3.3	3.5	4.3	3.5	3.0	3.0	3.2	6
Psp24.2-ND18	4.2	4.0	3.5	4.2	4.7	3.2	3.2	4.0	6
Psp25.1-ND18	3.0	3.3	3.2	3.0	3.5	3.2	3.0	3.0	6
Psp25.2-ND18	3.3	3.2	3.0	4.0	4.8	3.7	3.2	4.7	6
Psp25.3-ND18	3.0	3.0	3.0	4.5	4.7	3.0	3.0	4.2	6
Psp26-ND18	3.2	3.2	3.0	3.2	3.8	3.0	3.0	3.3	6
Psp27.1-ND18	3.3	3.0	3.0	3.5	4.2	3.0	3.2	3.3	6
Psp27.2-ND18	3.3	3.2	3.0	3.2	3.8	3.0	3.3	3.5	6
Psp29-ND18	3.0	3.0	3.2	3.5	3.3	3.0	3.0	3.2	6
Psp31.1-ND18	3.0	3.2	3.2	3.3	4.8	3.0	3.2	3.2	6
Psp31.2-ND18	3.5	3.0	3.2	3.2	4.0	3.0	3.2	3.0	6
Psp34-ND18	3.2	3.5	3.0	3.3	3.7	3.2	3.2	3.2	6
Psp35-ND18	3.3	3.3	3.0	3.3	4.2	3.2	3.5	3.7	6
Psp36-ND18	3.5	3.0	3.2	3.2	3.5	3.8	3.2	3.3	6
Psp38-ND18	3.3	3.0	3.0	3.0	3.2	3.0	3.2	3.0	6
Psp39.1-ND18	3.0	3.3	3.0	3.3	3.0	3.3	3.2	3.2	6
Psp39.2-ND18	3.7	3.0	3.0	3.3	4.0	3.2	3.3	3.3	6
Psp1-ND19	4.5	3.7	3.2	4.5	5.0	3.7	3.8	3.3	6
Psp4.1-ND19	4.7	3.3	3.3	4.8	4.7	3.8	3.7	4.0	6
Psp4.2-ND19	5.0	3.8	3.0	4.7	4.8	3.7	4.8	4.3	6
Psp4.3-ND19	3.7	4.3	3.0	4.8	4.3	3.7	4.7	4.3	6
Psp8-ND19	3.2	3.2	3.0	4.7	4.7	3.7	4.0	4.0	6

Table 3.2. Race identification and mean disease severity for 60 isolates of *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*) collected from North Dakota and Minnesota common bean fields in 2018 and 2019 (continued).

	Differential set of reactions of *Psp*								
Psp isolate	CW	A52	TG	UI3	1072	A53	A43	196-B	Race
Psp9.1-ND19	3.7	3.3	3.3	4.8	4.5	4.0	4.7	4.0	6
Psp9.2-ND19	4.0	3.7	3.0	4.7	4.3	3.0	4.7	4.7	6
Psp10.1-ND19	3.2	4.3	3.3	4.7	4.3	3.7	4.7	4.7	6
Psp10.2-ND19	3.3	4.0	3.2	4.7	4.2	3.0	3.7	4.3	6
Psp10.3-ND19	3.2	4.3	3.0	4.7	4.3	3.2	4.3	5.0	6
Psp13.1-ND19	3.3	3.3	3.0	5.0	5.0	3.2	4.3	3.3	6
Psp13.2-ND19	3.0	4.0	4.3	4.5	5.0	3.2	3.7	3.3	6
Psp16.1-ND19	3.7	4.0	3.3	5.0	4.7	3.5	3.7	4.3	6
Psp16.2-ND19	4.2	3.8	3.8	4.2	3.7	3.8	4.3	4.5	6
Psp16.3-ND19	4.3	3.3	3.8	4.0	3.5	4.7	4.5	3.8	6
Psp17-ND19	4.2	3.8	3.8	4.5	3.5	4.5	4.0	4.0	6
Psp22-ND19	3.7	3.3	3.5	4.7	3.7	4.0	3.8	3.5	6
Psp24.1-ND19	3.8	3.8	3.7	4.2	3.8	4.2	3.5	3.8	6
Psp24.2-ND19	3.8	3.0	3.8	4.2	4.0	4.2	4.2	4.0	6
Psp24.3-ND19	4.0	3.2	3.7	3.8	3.3	4.0	4.0	3.3	6
Psp3.1-MN19	4.3	3.8	3.7	4.0	4.0	4.2	4.0	3.8	6
Psp3.2-MN19	3.7	3.7	3.7	4.0	3.8	3.5	4.5	4.0	6
Psp4.1-MN19	3.7	4.2	4.5	4.5	3.8	4.2	3.7	3.8	6
Psp4.2-MN19	4.0	3.7	3.8	4.5	4.0	4.0	4.3	4.5	6
Psp6.1-MN19	4.0	3.3	3.8	4.5	3.8	4.2	4.5	3.7	6
Psp6.2-MN19	3.8	4.0	4.0	4.5	3.8	4.3	4.2	3.8	6
Psp6.3-MN19	4.0	4.0	4.2	4.2	4.0	3.5	4.3	3.7	6
Psp7-MN19	3.7	4.3	4.0	4.3	3.7	4.2	3.7	3.8	6
Psp9.1-MN19	4.0	4.2	4.8	4.0	3.8	3.3	3.5	3.8	6
Psp9.2-MN19	3.7	4.0	4.2	4.2	3.5	3.8	4.3	3.8	6

[a] Control = *Psp*2000-13

CW = Canadian Wonder, A52 = ZAA54, TG = Tender Green, UI3 = Red Mexican, 1072 = 1072, A53 = ZAA55, A43 = ZAA12, 196-B = Guatemala B-196

Rating scale = 1 to 5: infection grades 1,2 = avirulent; 3,4,5 = virulent

Discussion

The Northarvest region of North Dakota and Minnesota is an important region to study the pathogen diversity of the halo blight bacteria because of its ranking as the largest bean production area in the United States. *Psp*-like bacteria was selected based upon colony characteristics. Biochemical tests, qPCR and pathogenicity tests, were used to confirm that these isolates were *Psp*, the causal agent of halo blight. Confirming pathogenicity is the only way to be certain that each isolate indeed is the causal agent of halo blight as morphological, and even biochemical and PCR tests can be misleading.

All isolates of *Psp* collected in 2018 and 2019 were Race 6, coinciding with those previously reported for most isolates previously collected from North Dakota (Lamppa et al., 2002). Race 6, considered the most prevalent race of *Psp* worldwide (Taylor et al., 1996), also has been previously reported in Nebraska (Ariyarathe et al., 2001). Recently, races 2 and 6 were found in Alberta, Saskatchewan and Manitoba, Canada (Chatterton et al., 2016). Race 2, considered the most common race in Latin America (Taylor et al., 1996), was identified for the first time in the US on samples collected in 2000 from North Dakota counties Sargent, Foster and Stutsman (Lamppa et al., 2002). Race 8 was identified from a sample from a North Dakota field of unknown location in that same study, the first report of its presence in North America (Lamppa et al., 2002). Race 8 has been previously identified in Africa (Taylor et al., 1996). Race 2 and 8 were not found during the current study, possibly due to *Psp* isolated in different counties than previously sampled, the environment, differences in pathogen aggressiveness, or resistance to these races in currently grown cultivars.

Race 6 is virulent on all of the lines in the current differential set; therefore, the differential set for determining races of *Psp* needs to be expanded to capture the true pathogen

variability. Validation of the eight differentials over 20 years ago only included 18 isolates of *Psp* from North America (Taylor et al., 1996). The current differential set of bean lines requires the inclusion of novel lines to explore the existence of potential new pathogen races. Cultivars Vista, Rojo and Vax 3 have been reported to have resistance to halo blight, but exhibited susceptible reactions to 2018 and 2019 isolates of Psp evaluated in the current study. Based on this result, other lines of common bean with reported halo blight resistance should be evaluated for inclusion in an expanded differential set, such as race 6 resistant pinto germplasm US14HBR6 (Duncan et al., 2014).

Halo blight is damaging to common bean worldwide and controlling the inoculum of this pathogen is challenging. Disease-free seed and resistant cultivars are recommended for managing halo blight. The selection of resistant varieties is typically the best management strategy, and streptomycin seed treatments or use of foliar bactericides can increase input cost and have variable results (Schwartz et al., 2005). Therefore, the identification of *Psp* races is important in understanding how these pathogens will interact with common bean cultivars grown here, and is particularly important for screening breeding material and the development of new cultivars, especially with resistance to *Psp* race 6. Sources of resistance to race 6 include a single major-effect QTL HB4.2 conferring race-nonspecific resistance and race-specific resistance with QTL HB5.1 (Tock et al., 2017).

Literature Cited

Agarwal, V. K., and Sinclair, J. B. 1997. Principles of seed pathology. Boca Raton: CRC. 538 p.

Ariyarathe, H. M., Coyne, D. P., Vidaver, A. K., and Eskridge, K. M. 2001. Pathogenic variation in *Pseudomonas syringae* pv. *phaseolicola* isolates on common beans. Bean Improv. Coop. 44:129-130.

Arnold, D. L., Lovell, H. C., Jackson, R. W., and Mansfield, J. W. 2011. *Pseudomonas syringae* pv. *phaseolicola*: from 'has bean' to supermodel. Mol. Plant Pathol. 12:617–627.

Chatterton, S., Balasubramanian, P. M., Erickson, R. S., Hou, A., McLaren, D. L., Henriquez, M. A., and Conner, R. L. 2016. Identification of bacterial pathogens and races of *Pseudomonas syringae* pv. *phaseolicola* from dry bean fields in Western Canada. Can. J. Plant Pathol. 38(1):41–54.

Cho, M. S., Jeon, Y. H., Kang, M. J., Ahn, H., Baek, H., Na, Y. W., Choi, Y. M., Kim, T. S., and Park, D. S. 2010. Sensitive and specific detection of phaseolotoxigenic and nontoxigenic isolates of *Pseudomonas syringae.* pv. *phaseolicola* by TaqMan real-time PCR using site-specific recombinase gene sequences. Microbiol. Res. 165:565-572.

Coyne, D. P., Schuster, M. L., and Shaugnessy, L. 1966. Inheritance of resistance to halo blight and common blight bacteria in *Phaseolus vulgaris* variety cross. Plant Dis. 50(1):29-32.

Duncan, R. W., Lema, M., Gilbertson, R. L., and Singh, S. P. 2014. Registration of common bean pinto US14HBR6 resistant to race 6 of the halo blight pathogen, *Pseudomonas syringae* pv. *phaseolicola*. J. Plant Regist. 8:53-56.

Hill, K., Coyne, D. P., and Schuster, M. L. 1972. Leaf, pod and systemic chlorosis reactions in *Phaseolus vulgaris* to halo blight controlled by different genes. J. Amer. Soc. Hort. Sci. 97:494-498.

Innes, N. L., Conway, J., and Taylor J. D. 1984. Resistance to halo-blight in the Cambridge accessions V4604 and V4058 of *Phaseolus* beans. Ann. Appl. Biol. 105:307-314.

Knodel, J. J., Beauzay, P. B., Endres, G. J., Franzen, D. W., Kandel, H. J., Markell, S. G., Osorno, J. M., Pasche, J. S., and Zollinger, R. K. 2016. 2015 Dry bean grower survey of production, pest problems and pesticide use. North Dakota State University Extension Service. E1802.

Knodel, J. J., Beauzay, P. B., Endres, G. J., Franzen, D. W., Kandel, H. J., Markell, S. G., Osorno, J. M., Pasche, J. S., and Zollinger, R. K. 2017. 2016 Dry bean grower survey of production, pest problems and pesticide use. North Dakota State University Extension Service. E1841.

Knodel, J. J., Beauzay, P. B., Endres, G. J., Franzen, D. W., Kandel, H. J., Markell, S. G., Osorno, J. M., Pasche, J. S., and Zollinger, R. K. 2018. 2017 Dry bean grower survey of production, pest problems and pesticide use. North Dakota State University Extension Service. E1884.

Knodel, J. J., Beauzay, P. B., Endres, G. J., Franzen, D. W., Ikley, J. T., Kandel, H. J., Markell, S. G., Osorno, J. M., and Pasche, J. S. 2019. 2018 Dry bean grower survey of production, pest problems and pesticide use. North Dakota State University Extension Service. E1902.

Knodel, J. J., Beauzay, P. B., Endres, G. J., Franzen, D. W., Ikley, J. T., Kandel, H. J., Markell, S. G., Osorno, J. M., and Pasche, J. S. 2020. 2019 Dry bean grower survey of production, pest problems and pesticide use. North Dakota State University Extension Service. E1952.

Knodel, J. J., Beauzay, P. B., Endres, G. J., Franzen, D. W., Ikley, J. T., Kandel, H. J., Markell, S. G., Osorno, J. M., and Pasche, J. S. 2021. 2020 Dry bean grower survey of production, pest problems and pesticide use. North Dakota State University Extension Service. E2014.

Mills, L. J., and Silbernagel, M. J. 1992. A rapid screening technique to combine resistance to halo blight and bean common mosaic virus in *Phaseolus vulgaris* L. Euphytica 58:201-208.

Moser, P. E., Cown, L., Massoth, H., and Murray, G. 1994. A semi-selective medium planting assay for detection and differentiation of bacterial blight pathogens of bean and pea. Annu Rep Bean Improv Coop. Fort Collins, CO. 37:23-24.

Rivas, L. A., Mansfield, J., Tsiamis, G., Jackson, R. W., and Murillo, J. 2005. Changes in race-specific virulence in *Pseudomonas syringae* pv. *phaseolicola* are associated with a chimeric transposable element and rare deletion events in a plasmid-borne pathogenicity island. App. and Environ. Microbiol. 71(7):3778–378.

Schaad, N. W. 1988. Laboratory guide for identification of plant pathogenic bacteria. 2nd ed. American Phytopathological Society, St. Paul. 164 p.

Schwartz, H. F., Steadman, J. R., Hall, R., and Forester, R. I. 2005. Compendium of bean diseases. 2nd edition. American Phytopathological Society, St. Paul, MN. p. 46-50.

Singh, S. P., and Schwartz, H. F. 2010. Breeding common bean for resistance to diseases. A review. Crop Sci. 50:2199–2223.

Singh, S. P., and Miklas, P. N. 2015. Breeding common bean for resistance to common blight: a review. Crop Sci. 55:971-984.

Stadt, S. J., and Saettler, A. W. 1981. Effect of host genotype on multiplication of *Pseudomonas phaseolicola*. Phytopathology. 71:1307-1310.

Taylor, J. D., Teverson, D. M., Allen, D. J., and Pastor-Corrales, M. A. 1996. Identification and origin of races of *Pseudomonas syringae* pv. *phaseolicola* from Africa and other bean growing areas. Plant Pathol. 45:469-478.

Tock, A. J., Fourie, D., Walley, P. G., Holub, E. B., Soler, A., Cichy, K. A., Pastor-Corrales, M. A., Song, Q., Porch, T. G., Hart, J. P., Vasconcellos, R. C. C., Vicente, J. G., Barker, G. C., and Miklas, P. N. 2017. Genome-wide linkage association mapping of halo blight resistance in common bean to race 6 of the globally important bacterial pathogen. Front. Plant Sci. 8:1-17.

APPENDIX A: QPCR ASSAY RESULTS

Table A.1. Amplification of bacterial DNA species showing primer set specificity for *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), *Xanthomonas fuscans* subsp. *fuscans* (*Xff*) (He 2010), *Pseudomonas syringae* pv. *syringae* (*Pss*) (this study) and *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*) (Cho et al., 2010)

	qPCR reaction			
Isolate name / identity	Xap	Xff	Pss	Psp
Xanthomonas axonopodis pv. *phaseoli* 30	-	-	-	-
Xap 68	-	-	-	-
Xap 79	-	-	-	-
Xap 1	+	-	-	-
Xap 20	+	-	-	-
Xap 27 (*Xff*)	-	+	-	-
Xap 28	+	-	-	-
Bacillus subtilis	-	-	-	-
Erwinia herbicola	-	-	-	-
Pseudomonas fluorescens	-	-	-	-
P. putida	-	-	-	-
P. viridaflava	-	-	-	-
Xanthomonas campestris pv. *campestris*	-	-	-	-
X. campestris pv. *translucens*	-	-	-	-
X. campestris pv. *diffenbachiae*	-	-	-	-
X. campestris pv. sojense	-	-	-	-
Erwinia amylovora	-	-	-	-
Escherichia coli	-	-	-	-
Agrobacterium tumefaciens	-	-	-	-
Curtobacter flaccumfaciens pv. *flaccumfaciens*	-	-	-	-
Pseudomonas syringae pv. *syringae* 19	-	-	+	-
Pseudomonas savastanoi pv. *phaseolicola* 17	-	-	-	+
P. syringae pv. *tomato*	-	-	-	-
P. syringae pv. *lachrymans*	-	-	-	-
P. syringae pv. *helianthi*	-	-	-	-
P. syringae 83	-	-	-	-
P. syringae pv. *glycinea* 66 m2	-	-	-	-

(+) = amplification (Ct value < 35), (-) = no amplification

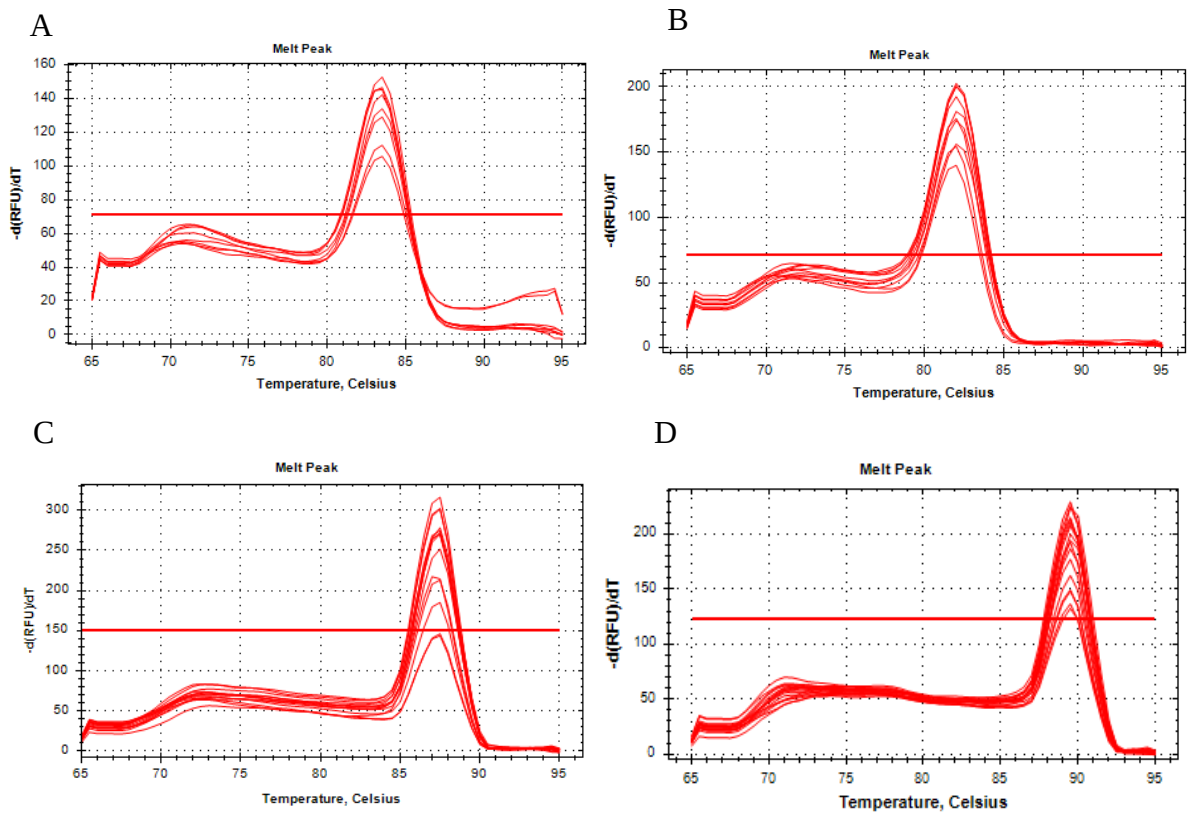

Figure A.1. PCR melt curves for *Xanthomonas axonopodis* pv. *phaseoli* (83.5˚C) (A), *Xanthomonas fuscans* subsp. *fuscans* (82˚C) (B), *Pseudomonas savastanoi* pv. *phaseolicola*, (87.5˚C) (C), *Pseudomonas syringae* pv. *syringae*, (89.5˚C) (D), the causal agents of bacterial blight of common bean. Single peaks indicate specific primer binding for each organism

Table A.2. Summary of analysis of variance of common bacterial blight (CBB) *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) for plating and multiplex qPCR in Fargo 2017 field trial

Source of variation	Degrees of freedom	Mean Squares	F Value	P Value
Model	14	$6.11x10^{14}$	3.15	0.0007
Error	70	$1.94x10^{14}$		
Corrected Total	84			

Table A.3. Summary of analysis of variance of common bacterial blight (CBB) *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) for plating and multiplex qPCR in Oakes 2016 field trial

Source of variation	Degrees of freedom	Mean Squares	F Value	P Value
Model	14	34788735774	2.71	0.0033
Error	66	12830575075		
Corrected Total	80			

Table A.4. Disease severity rating of common bacterial blight (CBB), halo blight, and brown spot inoculated with *Xanthomonas axonopodis* pv. *phaseoli* (CBB), *X. fuscans* pv. *fuscans* (CBB (2), *Pseudomonas savastanoi* pv. *phaseolicola* (halo blight), and *Pseudomonas syringae* pv. *syringae* (brown spot) onto six plants (two primary leaves) in the greenhouse

Sample	CBB on Cv. UI-114	CBB (2) on Cv. UI-114	Halo blight on Cv. Charlevoix	Brown spot on Cv. Charlevoix
1	3	5	3	2
2	4	4	4	3
3	3	4	4	3
4	2	3	3	2
5	4	3	2	3
6	5	3	4	4

1 to 2 = no disease symptoms, 3 to 5 = disease symptoms (1 = red-brown necrotic spots in the area of maximum inoculation; 2 = red-brown necrosis with a trace of water-soaking; 3 = some necrosis but more watersoaking that is confined to the area of maximum inoculation; 4 = water-soaked lesions <1 mm diameter distributed randomly over abaxial side of leaf); and 5 = watersoaked lesions 1-3 mm in diameter distributed at random over the leaf underside (Innes et al., 1984))

Table A.5. Disease severity rating of common bacterial blight (CBB), halo blight, and brown spot inoculated with *Xanthomonas axonopodis* pv. *phaseoli* (CBB), *X. fuscans* pv. *fuscans* (CBB (2)), *Pseudomonas savastanoi* pv. *phaseolicola* (halo blight), and *P. syringae* pv. *syringae* (brown spot) onto six pods in the greenhouse

Sample	CBB on Cv. UI-114	CBB (2) on Cv. UI-114	Halo blight on Cv. Charlevoix	Brown spot on Cv. Charlevoix
1	2	3	2	2
2	2	2	3	2
3	6	2	3	3
4	3	3	2	3
5	5	6	5	2
6	3	4	5	2

1 to 2 = no disease symptoms, 3 to 8 = disease symptoms; where 1 = no water-soaking (WS) at inoculated region, 2 = no WS with trace of necrosis, 3 = slight WS (1-2 mm) turns necrotic, 4 = slight WS (1-2 mm) turns necrotic and systemic chlorosis, 5 = moderate WS (2-4 mm) strong necrosis, 6 = moderate WS (2-4 mm) trace necrosis, 7 = moderate WS (2-4 mm) trace necrosis and systemic chlorosis, 8 = severe WS (> 4 mm) no necrosis, 9 = severe WS (> 4 mm) no necrosis and severe systemic chlorosis (Mills and Silbernagel, 1992)

APPENDIX B: *XAP* AND *XFF* AGGRESSIVENESS RESULTS

Table B.1. Common bean fields in North Dakota and Minnesota sampled for bacterial blight during 2018 and 2019

Field ID	Year	County	Date	Location (GPS)
ND1	2018	Traill	12-Jul	47.4980-97.0434
ND2	2018	Traill	12-Jul	47.5839-97.0412
ND3	2018	Traill	12-Jul	47.5923-97.0392
ND4	2018	Traill	12-Jul	47.5921-96.9628
ND5	2018	Traill	12-Jul	47.6469-97.0341
ND6	2018	Traill	12-Jul	47.6501-96.9621
ND7	2018	Traill	12-Jul	47.7237-97.0205
ND8	2018	Grand Forks	12-Jul	47.7469-97.0114
ND9	2018	Grand Forks	12-Jul	47.7374-96.9813
ND10	2018	Grand Forks	12-Jul	47.7527-97.9978
ND11	2018	Grand Forks	12-Jul	47.7738-96.9954
ND12	2018	Grand Forks	12-Jul	47.7738-97.6254
ND13	2018	Grand Forks	12-Jul	47.7444-97.6881
ND14	2018	Grand Forks	12-Jul	47.7446-97.7817
ND15	2018	Traill	12-Jul	47.6378-97.7245
ND16	2018	Steele	12-Jul	47.5207-97.6953
ND17	2018	Steele	12-Jul	47.5264-97.4321
ND18	2018	Steele	12-Jul	47.5482-97.3823
MN19	2018	Otter Tail	24-Jul	46.5953-95.5730
ND20	2018	Wells	8-Aug	47.4853-99.3948
ND21	2018	Wells	8-Aug	47.5828-99.4152
ND22	2018	Wells	8-Aug	47.6203-99.4160
ND23	2018	Wells	8-Aug	47.6507-99.4156
ND24	2018	Wells	8-Aug	47.6457-99.5541
ND25	2018	Wels	8-Aug	47.7022-99.6220
ND26	2018	Wells	8-Aug	47.7134-99.6187
ND27	2018	Wells	8-Aug	47.7457-99.6169
ND28	2018	Wells	8-Aug	47.7456-99.5878
ND29	2018	Wells	8-Aug	47.7311-99.5108
ND30	2018	Wells	8-Aug	47.6849-99.5121
ND31	2018	Wells	8-Aug	47.6449-99.3943
ND32	2018	Wells	14-Aug	47.5999-97.1223
ND33	2018	Wells	14-Aug	47.6212-97.3922
ND34	2018	Wells	14-Aug	47.6118-973891
ND35	2018	Traill	14-Aug	47.7362-97.3677
ND36	2018	Traill	14-Aug	47.7376-97.3664
ND37	2018	Traill	14-Aug	47.7447-97.5883
ND38	2018	Walsh	14-Aug	48.2378-97.5339
ND39	2018	Walsh	14-Aug	48.2842-97.5573

Table B.1. Common bean fields in North Dakota and Minnesota sampled for bacterial blight during 2018 and 2019 (continued).

Field ID	Year	County	Date	Location (GPS)
ND40	2018	Traill	14-Aug	48.3950-97.5357
ND41	2018	Traill	14-Aug	48.4250-97.5356
ND42	2018	Traill	14-Aug	48.4416-97.5356
ND43	2018	Traill	14-Aug	48.4673-97.4481
ND44	2018	Cass	14-Aug	46.9008-96.8134
ND1	2019	Foster	11-Jul	47.3634-99.2494
ND2	2019	Foster	11-Jul	47.3651-99.2495
ND3	2019	Foster	11-Jul	47.3674-99.2460
ND4	2019	Foster	11-Jul	47.3674-99.2460
ND5	2019	Foster	11-Jul	47.3753-99.2788
ND6	2019	Foster	11-Jul	47.3937-99.3701
ND7	2019	Wells	11-Jul	47.3931-99.3755
ND8	2019	Wells	11-Jul	47.3922-99.3794
ND9	2019	Wells	11-Jul	47.3913-99.3891
ND10	2019	Wells	11-Jul	47.4302-99.3085
ND11	2019	Traill	11-Jul	47.5267-97.1022
ND12	2019	Traill	16-Jul	47.3163-97.0009
ND13	2019	Traill	16-Jul	47.3207-96.5650
ND14	2019	Traill	16-Jul	47.3388-96.5644
ND15	2019	Grand Forks	16-Jul	47.3640-96.5648
ND16	2019	Grand Forks	16-Jul	47.4036-96.5533
ND17	2019	Grand Forks	16-Jul	47.4069-97.0147
ND18	2019	Grand Forks	16-Jul	47.4687-97.0919
ND19	2019	Grand Forks	16-Jul	47.4478-97.2246
ND20	2019	Grand Forks	16-Jul	47.4382-97.2731
ND21	2019	Traill	24-Jul	47.3999-97.2720
ND22	2019	Traill	24-Jul	47.3849-97.2336
ND23	2019	Traill	24-Jul	47.3686-97.1963
ND24	2019	Cass	24-Jul	46.9008-96.8134
MN1	2019	Otter Tail	24-Jul	46.3825-95.4428
MN2	2019	Otter Tail	24-Jul	46.3909-95.4137
MN3	2019	Otter Tail	24-Jul	46.3956-95.3924
MN4	2019	Otter Tail	24-Jul	46.3997-95.3136
MN5	2019	Otter Tail	24-Jul	46.4065-95.3632
MN6	2019	Otter Tail	24-Jul	46.3989-95.3084
MN7	2019	Hubbard	24-Jul	46.5268-95.0389
MN8	2019	Hubbard	24-Jul	46.5533-95.0511
MN9	2019	Becker	24-Jul	46.5531-95.1231
MN10	2019	Becker	24-Jul	46.5526-95.1386

Table B.2. Isolates of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) collected from North Dakota and Minnesota common bean fields in 2018 and 2019

ID	Year	County	Field	Isolate	Date	Location (GPS)
XapND18-2	2018	Traill	2	2	12-Jul	47.5839-97.0412
XapND18-3	2018	Traill	3	3	12-Jul	47.5923-97.0392
XapND18-5.1	2018	Traill	5	5	12-Jul	47.6469-97.0341
XapND18-5.2	2018	Traill	5	5	12-Jul	47.6469-97.0341
XapND18-7.1	2018	Traill	7	7.1	12-Jul	47.7237-97.0205
XapND18-7.2	2018	Traill	7	7.2	12-Jul	47.7237-97.0205
XapND18-7.3	2018	Traill	7	7.3	12-Jul	47.7237-97.0205
XapND18-8	2018	Grand Forks	8	8	12-Jul	47.7469-97.0114
XapND18-10.1	2018	Grand Forks	10	10.1	12-Jul	47.7527-97.9978
XapND18-10.2	2018	Grand Forks	10	10.1	12-Jul	47.7527-97.9978
XapND18-11	2018	Grand Forks	11	11	12-Jul	47.7738-96.9954
XapND18-15	2018	Traill	15	15	12-Jul	47.6378-97.7245
XapND18-16	2018	Steele	16	16	12-Jul	47.5207-97.6953
XapND18-18	2018	Steele	18	18	12-Jul	47.5482-97.3823
XapMN18-19	2018	Otter Tail	19	19	24-Jul	46.5953-95.5730
XapND18-20	2018	Wells	20	20	8-Aug	47.4853-99.3948
XapND18-21.1	2018	Wells	21	21.1	8-Aug	47.5828-99.4152
XapND18-23	2018	Wells	23	23	8-Aug	47.6507-99.4156
XapND18-24.1	2018	Wells	24	24.1	8-Aug	47.6457-99.5541
XapND18-24.2	2018	Wells	24	24.2	8-Aug	47.6457-99.5541
XapND18-25	2018	Wells	25	25	8-Aug	47.7022-99.6220
XapND18-26	2018	Wells	26	26	8-Aug	47.7134-99.6187
XapND18-27	2018	Wells	27	27	8-Aug	47.7457-99.6169
XapND18-28.1	2018	Wells	28	28.1	8-Aug	47.7456-99.5878
XapND18-28.2	2018	Wells	28	28.2	8-Aug	47.7456-99.5878
XapND18-28.3	2018	Wells	28	28.3	8-Aug	47.7456-99.5878
XapND18-29	2018	Wells	29	29	8-Aug	47.7311-99.5108
XapND18-30	2018	Wells	30	30	8-Aug	47.6849-99.5121
XapND18-33	2018	Wells	33	33	14-Aug	47.6212-97.3922
XapND18-35	2018	Traill	35	35	14-Aug	47.7362-97.3677
XapND18-37	2018	Traill	37	37	14-Aug	47.7447-97.5883
XapND19-3	2019	Wells	3	3	11-Jul	47.3674-99.2460
XapND19-5	2019	Wells	5	5	11-Jul	47.3753-99.2788
XapND19-6	2019	Wells	6	6	11-Jul	47.3937-99.3701
XapND19-9	2019	Wells	9	9	11-Jul	47.3913-99.3891
XapND19-10.1	2019	Wells	10	10.1	11-Jul	47.4302-99.3085
XapND19-10.2	2019	Wells	10	10.2	11-Jul	47.4302-99.3085
XapND19-10.3	2019	Wells	10	10.3	11-Jul	47.4302-99.3085
XapND19-11.1	2019	Traill	11	11.1	16-Jul	47.5267-97.1022

Table B.2. Isolates of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) collected from North Dakota and Minnesota common bean fields in 2018 and 2019 (continued).

ID	Year	County	Field	Isolate	Date	Location
XapND19-11.2	2019	Traill	11	11.2	16-Jul	47.5267-97.1022
XapND19-12.1	2019	Traill	12	12.1	16-Jul	47.3163-97.0009
XapND19-12.2	2019	Traill	12	12.2	16-Jul	47.3163-97.0009
XapND19-14	2019	Traill	14	14	16-Jul	47.3388-96.5644
XapND19-16.1	2019	Grand Forks	16	16.1	16-Jul	47.4036-96.5533
XapND19-16.2	2019	Grand Forks	16	16.2	16-Jul	47.4036-96.5533
XapND19-18.1	2019	Grand Forks	18	16.3	16-Jul	47.4687-97.0919
XapND19-18.2	2019	Grand Forks	18	18	16-Jul	47.4687-97.0919
XapND19-19.1	2019	Grand Forks	19	19.1	16-Jul	47.4478-97.2246
XapND19-19.2	2019	Grand Forks	19	19.2	16-Jul	47.4478-97.2246
XapND19-19.3	2019	Grand Forks	19	19.3	16-Jul	47.4478-97.2246
XapND19-20.1	2019	Grand Forks	20	20.1	16-Jul	47.4382-97.2731
XapND19-20.2	2019	Grand Forks	20	20.2	16-Jul	47.4382-97.2731
XapND19-21	2019	Traill	21	21	24-Jul	47.3999-97.2720
XapND19-24.1	2019	Cass	24	24.1	24-Jul	46.9008-96.8134

Table B.3. Isolates of *Xanthomonas fuscans* subsp. *fuscans* (*Xff*) collected from North Dakota and Minnesota common bean fields in 2018 and 2019

ID	Year	County	Field	Isolate	Date	Location
XffND18-21.2	2018	Wells	21	21.2	8-Aug	47.5828-99.4152
XffND18-44	2018	Cass	44	44	14-Aug	46.9008-96.8134
XffND19-24.2	2019	Cass	24	24.2	24-Jul	46.9008-96.8134

Table B.4. Isolates of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* pv. *fuscans* (*Xff*) collected from North Dakota and Minnesota common bean fields

ID	Year	County	Field	Date	Location (GPS)	Source
ADP102	2016	---	---	---	---	ND field leaf
XffND17-Hatton	2017	Traill	---	---	---	ND field leaf
XapND17-11	2017	---	11	---	---	ND field leaf
XapND18-2	2018	Traill	2	12-Jul	47.5291-96.9629	ND field leaf
XapND18-3	2018	Traill	3	12-Jul	47.5612-96.9631	ND field leaf
XapND18-5.1	2018	Traill	5	12-Jul	47.6166-96.9622	ND field leaf
XapND18-5.2	2018	Traill	5	12-Jul	47.6166-96.9622	ND field leaf
XapND18-7.1	2018	Traill	7	12-Jul	47.7011-96.9561	ND field leaf
XapND18-7.2	2018	Traill	7	12-Jul	47.7011-96.9561	ND field leaf
XapND18-7.3	2018	Traill	7	12-Jul	47.7011-96.9561	ND field leaf
XapND18-8	2018	Grand Forks	8	12-Jul	47.7158-96.9702	ND field leaf
XapND18-10.1	2018	Grand Forks	10	12-Jul	47.7483-96.9809	ND field leaf
XapND18-10.2	2018	Grand Forks	10	12-Jul	47.7483-96.9809	ND field leaf
XapND18-10.3	2018	Grand Forks	10	12-Jul	47.7483-96.9809	ND field leaf
XapND18-15	2018	Traill	15	12-Jul	47.7450-97.8659	ND field leaf
XapND18-16	2018	Steele	16	12-Jul	47.5207-97.6953	ND field leaf
XapND18-18	2018	Traill	18	12-Jul	47.4987-97.2194	ND field leaf
XapMN18-19	2018	Otter Tail	19	24-Jul	46.5953-95.5730	MN field leaf
XapND18-20	2018	Wells	20	8-Aug	47.4865-99.4171	ND field leaf
XapND18-21.1	2018	Wells	21	8-Aug	47.5828-99.4152	ND field leaf
XffND18-21.2	2018	Wells	21	8-Aug	47.5828-99.4152	ND field leaf
XapND18-23	2018	Wells	23	8-Aug	47.6507-99.4156	ND field leaf
XapND18-24.1	2018	Wells	24	8-Aug	47.6457-99.5541	ND field leaf
XapND18-28.1	2018	Wells	28	8-Aug	47.7456-99.5878	ND field leaf
XapND18-28.2	2018	Wells	28	8-Aug	47.7456-99.5878	ND field leaf
XapND18-28.3	2018	Wells	28	8-Aug	47.7456-99.5878	ND field leaf
XapND18-30	2018	Wells	30	8-Aug	47.6849-99.5121	ND field leaf
XapND18-35	2018	Traill	35	14-Aug	47.7362-97.3677	ND field leaf
XffND18-44	2018	Cass	44	14-Aug	46.9008-96.8134	ND field leaf
Xap10F18A	---	---	---	---	---	---
Xap10F18B	---	---	---	---	---	---
Xap28	---	---	---	---	---	ATCC 10198
Xap90-190FL	1990	---	---	---	---	ND field leaf
Xap91-f91-W	1991	Cass	---	---	---	ND field leaf
Xap91-f91-I	1991	Cass	---	---	---	ND field leaf
Xap92-10199	1992	---	---	---	---	ND field leaf
Xap92-CB	1992	---	---	---	---	ND field leaf
Xap92-13B	1992	---	---	---	---	ND field leaf
Xap92-X-1-A	1992	---	---	---	---	ND field leaf

Table B.4. Isolates of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* pv. *fuscans* (*Xff*) collected from North Dakota and Minnesota common bean fields (continued).

ID	Year	County	Field	Date	Location (GPS)	Source
Xap92-X2-99	1992	---	---	---	---	ND field leaf
Xap95-29	1995	---	---	---	---	ND field leaf
Xap95-33	1995	---	---	---	---	ND field leaf
Xap20	---	---	---	---	---	ATCC 10199
Xap 20-1	---	---	---	---	---	---
Xap7	1992	---	---	---	---	ND field leaf
Xap10	1992	---	---	---	---	ND field leaf
Xap11	1992	---	---	---	---	ND field leaf
Xap12	1992	Foster	---	---	---	ND field leaf
Xap14	1992	---	---	---	---	ND field leaf
Xap16	1992	---	---	---	---	ND field leaf
Xap18	1992	---	---	---	---	ND field leaf
Xap22	1992	---	---	---	---	ND field leaf
Xap24	1992	---	---	---	---	ND field leaf
Xap25	1992	---	---	---	---	ND field leaf
Xap26	1992	---	---	---	---	ND field leaf
Xap27 (*Xff*)	---	---	---	---	---	ATCC 19315
Xc30	---	---	---	---	---	---
Xc41	---	---	---	---	---	---
Xap-106N	---	---	---	---	---	---
Xap-148F	---	---	---	---	---	---
Xap-1015	---	---	---	---	---	---
Xap-1016	---	---	---	---	---	---
Xap-1460	---	---	---	---	---	---
Xap91-5	1991	---	---	---	---	ND field leaf
Xap95-22	1995	---	---	---	---	ND field leaf
Xap95-26	1995	---	---	---	---	ND field leaf
Xap95-28	1995	---	---	---	---	ND field leaf
Xap95-29	1995	---	---	---	---	ND field leaf
Xap 95-33	1995	---	---	---	---	ND field leaf

---, unknown

Table B.5. Summary of analysis of variance of common bacterial blight (CBB) reaction on eight common bean lines with 57 isolates of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* pv. *fuscans* (*Xff*) under greenhouse conditions

Source of variation	**Degrees of freedom**	**Mean square**	**Probability**
Rep	5	3846.3865	0.0002
Isolate	57	269868.7995	<.0001
Line	7	714754.7066	<.0001
Isolate × line	399	708853.7408	<.0001

APPENDIX C: *PSP* RACE TYPING RESULTS

Table C.1. Common bean fields in North Dakota and Minnesota sampled for bacterial blight during 2018 and 2019

Field ID	Year	County	Date	Location (GPS)
ND1	2018	Traill	12-Jul	47.4980-97.0434
ND2	2018	Traill	12-Jul	47.5839-97.0412
ND3	2018	Traill	12-Jul	47.5923-97.0392
ND4	2018	Traill	12-Jul	47.5921-96.9628
ND5	2018	Traill	12-Jul	47.6469-97.0341
ND6	2018	Traill	12-Jul	47.6501-96.9621
ND7	2018	Traill	12-Jul	47.7237-97.0205
ND8	2018	Grand Forks	12-Jul	47.7469-97.0114
ND9	2018	Grand Forks	12-Jul	47.7374-96.9813
ND10	2018	Grand Forks	12-Jul	47.7527-97.9978
ND11	2018	Grand Forks	12-Jul	47.7738-96.9954
ND12	2018	Grand Forks	12-Jul	47.7738-97.6254
ND13	2018	Grand Forks	12-Jul	47.7444-97.6881
ND14	2018	Grand Forks	12-Jul	47.7446-97.7817
ND15	2018	Traill	12-Jul	47.6378-97.7245
ND16	2018	Steele	12-Jul	47.5207-97.6953
ND17	2018	Steele	12-Jul	47.5264-97.4321
ND18	2018	Steele	12-Jul	47.5482-97.3823
MN19	2018	Otter Tail	24-Jul	46.5953-95.5730
ND20	2018	Wells	8-Aug	47.4853-99.3948
ND21	2018	Wells	8-Aug	47.5828-99.4152
ND22	2018	Wells	8-Aug	47.6203-99.4160
ND23	2018	Wells	8-Aug	47.6507-99.4156
ND24	2018	Wells	8-Aug	47.6457-99.5541
ND25	2018	Wels	8-Aug	47.7022-99.6220
ND26	2018	Wells	8-Aug	47.7134-99.6187
ND27	2018	Wells	8-Aug	47.7457-99.6169
ND28	2018	Wells	8-Aug	47.7456-99.5878
ND29	2018	Wells	8-Aug	47.7311-99.5108
ND30	2018	Wells	8-Aug	47.6849-99.5121
ND31	2018	Wells	8-Aug	47.6449-99.3943
ND32	2018	Wells	14-Aug	47.5999-97.1223
ND33	2018	Wells	14-Aug	47.6212-97.3922
ND34	2018	Wells	14-Aug	47.6118-973891
ND35	2018	Traill	14-Aug	47.7362-97.3677
ND36	2018	Traill	14-Aug	47.7376-97.3664
ND37	2018	Traill	14-Aug	47.7447-97.5883
ND38	2018	Walsh	14-Aug	48.2378-97.5339
ND39	2018	Walsh	14-Aug	48.2842-97.5573
ND40	2018	Traill	14-Aug	48.3950-97.5357

Table C.1. Common bean fields in North Dakota and Minnesota sampled for bacterial blight during 2018 and 2019 (continued).

Field ID	Year	County	Date	Location (GPS)
ND41	2018	Traill	14-Aug	48.4250-97.5356
ND42	2018	Traill	14-Aug	48.4416-97.5356
ND43	2018	Traill	14-Aug	48.4673-97.4481
ND44	2018	Cass	14-Aug	46.9008-96.8134
ND1	2019	Foster	11-Jul	47.3634-99.2494
ND2	2019	Foster	11-Jul	47.3651-99.2495
ND3	2019	Foster	11-Jul	47.3674-99.2460
ND4	2019	Foster	11-Jul	47.3674-99.2460
ND5	2019	Foster	11-Jul	47.3753-99.2788
ND6	2019	Foster	11-Jul	47.3937-99.3701
ND7	2019	Wells	11-Jul	47.3931-99.3755
ND8	2019	Wells	11-Jul	47.3922-99.3794
ND9	2019	Wells	11-Jul	47.3913-99.3891
ND10	2019	Wells	11-Jul	47.4302-99.3085
ND11	2019	Traill	11-Jul	47.5267-97.1022
ND12	2019	Traill	16-Jul	47.3163-97.0009
ND13	2019	Traill	16-Jul	47.3207-96.5650
ND14	2019	Traill	16-Jul	47.3388-96.5644
ND15	2019	Grand Forks	16-Jul	47.3640-96.5648
ND16	2019	Grand Forks	16-Jul	47.4036-96.5533
ND17	2019	Grand Forks	16-Jul	47.4069-97.0147
ND18	2019	Grand Forks	16-Jul	47.4687-97.0919
ND19	2019	Grand Forks	16-Jul	47.4478-97.2246
ND20	2019	Grand Forks	16-Jul	47.4382-97.2731
ND21	2019	Traill	24-Jul	47.3999-97.2720
ND22	2019	Traill	24-Jul	47.3849-97.2336
ND23	2019	Traill	24-Jul	47.3686-97.1963
ND24	2019	Cass	24-Jul	46.9008-96.8134
MN1	2019	Otter Tail	24-Jul	46.3825-95.4428
MN2	2019	Otter Tail	24-Jul	46.3909-95.4137
MN3	2019	Otter Tail	24-Jul	46.3956-95.3924
MN4	2019	Otter Tail	24-Jul	46.3997-95.3136
MN5	2019	Otter Tail	24-Jul	46.4065-95.3632
MN6	2019	Otter Tail	24-Jul	46.3989-95.3084
MN7	2019	Hubbard	24-Jul	46.5268-95.0389
MN8	2019	Hubbard	24-Jul	46.5533-95.0511
MN9	2019	Becker	24-Jul	46.5531-95.1231
MN10	2019	Becker	24-Jul	46.5526-95.1386

Table C.2. Isolates of *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*) collected from North Dakota and Minnesota common bean fields in 2018 and 2019

ID	Year	County	Field	Isolate	Date	Location
PspND18-1.1	2018	Traill	1	1	12-Jul	47.4980-97.0434
PspND18-11.1	2018	Grand Forks	11	11.1	12-Jul	47.7738-96.9954
PspND18-11.2	2018	Grand Forks	11	11.2	12-Jul	47.7738-96.9954
PspND18-14.1	2018	Grand Forks	14	14	12-Jul	47.7447-97.7817
PspND18-16.1	2018	Steele	16	16	12-Jul	47.5207-97.6953
PspND18-17.1	2018	Traill	17	17	12-Jul	47.5264-97.4321
PspND18-19.1	2018	Otter Tail	19	19.1	24-Jul	46.5953-95.5730
PspND18-19.2	2018	Otter Tail	19	19.2	24-Jul	46.5953-95.5730
PspND18-21.1	2018	Wells	21	21.1	8-Aug	47.5828-99.4152
PspND18-21.2	2018	Wells	21	21.2	8-Aug	47.5828-99.4152
PspND18-21.3	2018	Wells	21	21.3	8-Aug	47.5828-99.4152
PspND18-22.1	2018	Wells	22	22	8-Aug	47.6203-99.4160
PspND18-24.1	2018	Wells	24	24.1	8-Aug	47.6457-99.5541
PspND18-24.2	2018	Wells	24	24.2	8-Aug	47.6457-99.5541
PspND18-25.1	2018	Wells	25	25.1	8-Aug	47.7022-99.6220
PspND18-25.2	2018	Wells	25	25.2	8-Aug	47.7022-99.6220
PspND18-25.3	2018	Wells	25	25.3	8-Aug	47.7022-99.6220
PspND18-26.1	2018	Wells	26	26	8-Aug	47.7134-99.6187
PspND18-27.1	2018	Wells	27	27.1	8-Aug	47.7457-99.6169
PspND18-27.2	2018	Wells	27	27.2	8-Aug	47.7457-99.6169
PspND18-29.1	2018	Wells	29	29	8-Aug	47.7311-99.5108
PspND18-31.1	2018	Wells	31	31.1	8-Aug	47.6449-99.3943
PspND18-31.2	2018	Wells	31	31.2	8-Aug	47.6449-99.3943
PspND18-34.1	2018	Wells	34	34	14-Aug	47.6118-97.3891
PspND18-35.1	2018	Grand Forks	35	35	14-Aug	47.7362-97.3677
PspND18-36.1	2018	Grand Forks	36	36	14-Aug	47.7376-97.3664
PspND18-38.1	2018	Walsh	38	38	14-Aug	48.2378-97.5339
PspND18-39.1	2018	Walsh	39	39.1	14-Aug	48.2378-97.5573
PspND18-39.2	2018	Walsh	39	39.2	14-Aug	48.2378-97.5573
PspND19-1	2019	Foster	1	1	11-Jul	47.3634-99.2494
PspND19-4.1	2019	Foster	4	4.1	11-Jul	47.3674-99.2460
PspND19-4.2	2019	Foster	4	4.2	11-Jul	47.3674-99.2460
PspND19-4.3	2019	Foster	4	4.3	11-Jul	47.3674-99.2460
PspND19-8	2019	Wells	8	8	11-Jul	47.3937-99.3701
PspND19-9.1	2019	Wells	9	9.1	11-Jul	47.3913-99.3891
PspND19-9.2	2019	Wells	9	9.2	11-Jul	47.3913-99.3891
PspND19-10.1	2019	Wells	10	10.1	11-Jul	47.4302-99.3085
PspND19-10.2	2019	Wells	10	10.2	11-Jul	47.4302-99.3085
PspND19-10.3	2019	Wells	10	10.3	11-Jul	47.4302-99.3085

Table C.2. Isolates of *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*) collected from North Dakota and Minnesota common bean fields in 2018 and 2019 (continued).

ID	Year	County	Field	Isolate	Date	Location
PspND19-13.1	2019	Traill	13	13.1	16-Jul	47.3207-96.5650
PspND19-13.2	2019	Traill	13	13.2	16-Jul	47.3207-96.5650
PspND19-16.1	2019	Grand Forks	16	16.1	16-Jul	47.4036-96.5533
PspND19-16.2	2019	Grand Forks	16	16.2	16-Jul	47.4036-96.5533
PspND19-16.3	2019	Grand Forks	16	16.3	16-Jul	47.4036-96.5533
PspND19-17	2019	Traill	17	17	16-Jul	47.4069-97.0147
PspND19-22	2019	Traill	22	22	16-Jul	47.3849-97.2336
PspND19-24.1	2019	Cass	24	24.1	24-Jul	46.9018-96.8177
PspND19-24.2	2019	Cass	24	24.2	24-Jul	46.9018-96.8177
PspND19-24.3	2019	Cass	24	24.3	24-Jul	46.9018-96.8177
PspMN19-3.1	2019	Otter Tail	3	3.1	24-Jul	46.3956-95.3924
PspMN19-3.2	2019	Otter Tail	3	3.2	24-Jul	46.3956-95.3924
PspMN19-4.1	2019	Otter Tail	4	4	24-Jul	46.3997-95.3136
PspMN19-4.2	2019	Otter Tail	4	4.2	24-Jul	46.3997-95.3136
PspMN19-6.1	2019	Otter Tail	6	6.1	24-Jul	46.3989-95.3084
PspMN19-6.2	2019	Otter Tail	6	6.2	24-Jul	46.3989-95.3084
PspMN19-6.3	2019	Otter Tail	6	6.3	24-Jul	46.3989-95.3084
PspMN19-7	2019	Hubbard	7	7	24-Jul	46.5268-95.0389
PspMN19-9.1	2019	Becker	9	9.1	24-Jul	46.5531-95.1231
PspMN19-9.2	2019	Becker	9	9.2	24-Jul	46.5531-95.1231