

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

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ABSTRACT

PSbMV in field pea has resulted in substantial yield and seed quality losses world-wide and has recently been reported in North Dakota. Traditional management of this virus includes preventative measures such as removal of alternate hosts, planting virus free seed and the use of cultivar resistance. The objectives of this research were to screen field pea cultivars commonly grown in North Dakota for a response to North Dakota PSbMV isolate ND14-1 and ascertain the effect on plant symptoms, seed size and weight, the number of pods and seeds and seed transmission. Two cultivars were identified as highly resistant and one as partially resistant. The results from this study were combined into a risk assessment. Cultivars were categorized based on inherent risk of PSbMV infection, transmission and reduction in total seed weight. Common bacterial blight (CBB) in dry bean is capable of causing substantial yield losses and has been reported in up to 75% of fields in the Northharvest region in the last five years. Current management practices include the use of planting clean seed, crop rotation, partial host resistance and the application of cupric bactericides, although inconsistent for the management of CBB. Growers in this Northharvest region have recently shifted to growing upright (Type II) dry beans rather than prostrate (Type III) dry beans for ease of harvest. The objectives of this research were to evaluate copper products, surface sanitizers and growth promoters for the management of CBB and to discern if Type II dry beans experienced greater yield losses under CBB disease pressure than Type III dry beans. Numerous products were identified that significantly reduced CBB disease severity and spread; however, no significant yield benefit was observed. Across a wide range of disease severity (0-46%), no significant yield losses were observed between high and low disease severity any of the cultivars screened.

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TABLE OF CONTENTS

ABSTRACT.....	iii
ACKNOWLEDGEMENTS.....	iv
LIST OF TABLES.....	x
LIST OF FIGURES.....	xii
LIST OF APPENDIX TABLES.....	xiii
PSBMV LITERATURE REVIEW.....	1
Dry Field Peas.....	1
<i>Pea Seed-borne Mosaic Virus (PSbMV)</i>	2
PSbMV Symptomology.....	3
Economic Impact of PSbMV.....	4
PSbMV Transmission.....	5
PSbMV Pathotyping.....	7
PSbMV Detection.....	7
PSbMV Management.....	8
Summary.....	9
Literature Cited.....	10
CBB LITERATURE REVIEW.....	17
Dry Bean Production.....	17
Origin.....	17
Growth Habits.....	18
Cultivars.....	19
Bacterial Blight of Dry Beans.....	19
Bacterial Blight Complex.....	21
Halo Blight.....	21

Bacterial Brown Spot	22
Common Bacterial Blight.....	22
Management	23
Copper Products	24
Surface Sanitizers	25
Growth Promoters	26
Crop Growth Assessment.....	26
Leaf Area Index	26
Fractional Green Canopy Cover	28
Summary	29
Literature Cited	29
CHAPTER 1: PEA SEED-BORNE MOSAIC VIRUS (PSbMV) RISK ANALYSIS OF FIELD PEA BASED ON SUSCEPTIBILITY AND SEED TRANSMISSION	37
Abstract	37
Introduction	37
Materials and Methods	41
Virus Purification	41
Cultivar Susceptibility Study.....	43
Seed Transmission Study	44
Statistical Analyses.....	45
Results	47
PSbMV Infection Frequency	47
PSbMV Seed Transmission.....	50
PSbMV Risk Index.....	52
Discussion	54

Acknowledgements	59
Literature Cited	60
CHAPTER 2: EVALUATION OF BACTERICIDES FOR THE MANAGEMENT OF COMMON BACTERIAL BLIGHT IN DRY BEANS AND TOOLS TO ASSESS DISEASE SEVERITY	65
Abstract	65
Introduction	65
Materials and Methods	72
Site Description and Experimental Design.....	72
Product Applications and Field Evaluations	73
Statistical Analyses.....	76
Results	77
Discussion	82
Literature Cited	85
CHAPTER 3: EVALUATION OF THE EFFECT OF COMMON BACTERIAL BLIGHT ON TWO COMMON BEAN ARCHITECTURE TYPES AND THE VALIDATION OF YIELD PREDICTION TOOLS	90
Abstract	90
Introduction	91
Materials and Methods	95
Site Description and Experimental Design.....	95
Applications and Field Evaluations.....	96
Statistical Analyses.....	97
Results	98
Discussion	102
Literature Cited	106
APPENDIX A: DETAILED FOLIAR BACTERICIDE TRIAL RESULTS	111

APPENDIX B: SECONDARY DRY BEAN ARCHITECTURE TRIAL RESULTS.....124

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1.1. Componentets of the risk algorithm used to estimate grower risk when <i>Pea Seed-borne Mosaic Virus</i> (PSbMV) is present.....	46
1.2. LS Means of <i>Pea Seed-borne Mosaic Virus</i> (PSbMV) incidence in parent plants and transmission (%) from infected parent plant to progeny in 20 field pea cultivars.....	48
1.3. Symptoms of <i>Pea Seed-borne Mosaic Virus</i> (PSbMV) on 20 field pea cultivar mechanically inoculated under greenhouse conditions.....	49
1.4. Ratio of total seed weight, pods per plant, 100-seed weight and seeds per plant of field pea plants inoculated with <i>Pea Seed-borne Mosaic Virus</i> (PSbMV) and mock-inoculated plants.	52
1.5. Risk classification of 20 field pea cultivars to <i>Pea Seed-borne Mosaic Virus</i> (PSbMV) based on mechanically inoculated plants grown under greenhouse conditions.....	54
2.1. Products, rates, active ingredients and application timing (month/day) of cupric and biorational bactericides in the 2016 and 2017 common bacterial blight (CBB) management trials.	75
2.2. Area under the disease progress curve (AUDPC) for common bacterial blight (CBB) across treatments in dry bean trials conducted in Fargo 2017 and Oakes 2016 and 2017.....	78
2.3. Area under the Canopeo progress curve (AUCPC) of fractional green canopy cover (FGCC) across treatments in dry bean trials conducted in Fargo 2017 and Oakes 2016 and 2017.....	79
2.4. Area under the leaf area index (LAI) progress curve (AULPC) across treatments in dry bean trials conducted in Fargo 2017 and Oakes 2016 and 2017.	80
2.5. Yield (mT/ha ⁻¹) across treatments in dry bean trials conducted in Fargo 2017 and Oakes 2016 and 2017.....	81
2.6. Pearson’s correlation analyses of variables in dry bean trials conducted in Fargo 2017 and Oakes 2016 and 2017.....	82
3.1. Area under the disease progress curve (AUDPC), common bacterial blight (CBB) severity 14 days after inoculation (DAI), CBB severity 28 DAI and yield in dry bean field trials conducted in Fargo, North Dakota in 2017, and Oakes, North Dakota 2016 and 2017.....	100

3.2. Pearson’s correlation analysis of area under the disease progress curve (AUDPC), area under the Canopeo progress curve (AUCPC, area under the destructive leaf area index (LAI) progress curve (AUDLPC), area under the non-destructive LAI progress curve (AUNLPC) and yield in dry bean field trials conducted in Fargo, North Dakota in 2017, and Oakes, North Dakota in 2016 and 2017..... 101

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1.1. <i>Pea Seed-borne Mosaic Virus</i> PSbMV positive field pea seed exhibiting no symptoms (left) and characteristic symptoms (right) including seed coat cracking, scarring, water-soaked appearance and overall reduction in size.....	44
1.2. <i>Pea Seed-borne Mosaic Virus</i> (PSbMV) induced foliar symptoms in field pea including: leaf curling (left), mosaic (middle) and budding at internodes (right)	50
2.1. Common bacterial blight (CBB) symptoms on dry beans leaves and pods	67
2.2. Brown spot symptoms on foliar dry bean tissue.....	68
2.3. Halo blight symptoms on foliar dry bean tissue	69

LIST OF APPENDIX TABLES

<u>Table</u>	<u>Page</u>
A.1. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage and 14 days after inoculation (8/23) in Fargo, North Dakota in 2016.	111
A.2. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/25) and 14 days after inoculation (DAI) (8/8) and 28 DAI (8/25) in Oakes, North Dakota in 2016.	112
A.3. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/20) and 14 days after inoculation (8/6) in Prosper, North Dakota in 2016.	113
A.4. Fractional green canopy cover (FGCC) and non-destructive leaf area index (NDLAI) at dry bean R2 growth stage (8/9) and 14 days after inoculation (8/23) in Fargo, North Dakota in 2016.	114
A.5. Fractional green canopy cover (FGCC) and non-destructive leaf area index (NDLAI) at dry bean R2 growth stage (7/25), 14 days after inoculation (DAI) (8/8) and 28 DAI (8/25) in Oakes, North Dakota in 2016.	115
A.6. Fractional green canopy cover (FGCC) and non-destructive leaf area index (NDLAI) at dry bean R2 growth stage (7/20) and 14 days after inoculation (8/6) in Prosper, North Dakota in 2016.	116
A.7. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/17) and 14 days after inoculation (DAI) (8/17) in Fargo, North Dakota in 2017.	117
A.8. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/13), 14 days after inoculation (DAI) (7/27) and 28 DAI (8/10) in Oakes, North Dakota in 2017.	118
A.9. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/24), 14 days after inoculation (DAI) (8/8) and 28 DAI (8/21) in Prosper, North Dakota in 2017.	119
A.10. Fractional green canopy cover (FGCC) and non-destructive leaf area index (NDLAI) at dry bean R2 growth stage (7/17), 14 days after inoculation (DAI) (8/1) and 28 DAI (8/17) in Fargo, North Dakota in 2017.	120

A.11.	Fractional green canopy cover (FGCC) and non-destructive leaf area index (NDLAI) at dry bean R2 growth stage (7/13), 14 days after inoculation (DAI) (7/27) and 28 DAI (8/10) in Oakes, North Dakota in 2017.....	121
A.12.	Fractional green canopy cover (FGCC) and non-destructive leaf area index (NDLAI) at dry bean R2 growth stage (7/24), 14 days after inoculation (DAI) (8/8) and 28 DAI (8/21) in Prosper, North Dakota in 2017.	122
A.13.	Yield (mT/ha ⁻¹) in dry bean trials conducted across sites in 2016 and 2017.....	123
B.1.	Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (8/9) and 14 days after inoculation (8/23) in Fargo, North Dakota in 2016.	124
B.2.	Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/25), 14 days after inoculation (DAI) (8/8) and 28 DAI (8/25) in Oakes, North Dakota in 2016.	125
B.3.	Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/20) and 14 days after inoculation (8/6) in Prosper, North Dakota in 2016.	125
B.4.	Fractional green canopy cover (FGCC), non-destructive leaf area index (NDLAI) and destructive leaf area index (DLAI) at dry bean R2 growth stage (8/9) and 14 days after inoculation (8/23) in Fargo, North Dakota in 2016.	126
B.5.	Fractional green canopy cover (FGCC), non-destructive leaf area index (NDLAI) and destructive leaf area index (DLAI) at dry bean R2 growth stage (7/18), 14 days after inoculation (DAI) (8/1) and 28 DAI (8/15) in Oakes, North Dakota in 2016.	126
B.6.	Fractional green canopy cover (FGCC), non-destructive leaf area index (NDLAI) and destructive leaf area index (DLAI) at dry bean R2 growth stage (7/20) and 14 days after inoculation (8/6) in Prosper, North Dakota in 2016.	127
B.7.	Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/17), 14 days after inoculation (DAI) (8/1) and 28 DAI (8/17) in Fargo, North Dakota in 2017.....	128
B.8.	Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/13), 14 days after inoculation (DAI) (7/27) and 28 DAI (8/10) in Oakes, North Dakota in 2017.	129
B.9.	Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/24), 14 days after inoculation (DAI) (8/8) and 28 DAI (8/21) in Prosper, North Dakota in 2017.....	130

B.10.	Fractional green canopy cover (FGCC), non-destructive leaf area index (NDLAI) and destructive leaf area index (DLAI) at dry bean R2 growth stage (7/17), 14 days after inoculation (DAI) (8/1) and 28 DAI (8/17) in Fargo, North Dakota in 2017.....	131
B.11.	Fractional green canopy cover (FGCC), non-destructive leaf area index (NDLAI) and destructive leaf area index (DLAI) at dry bean R2 growth stage, 14 days after inoculation (DAI) and 28 DAI in Oakes, North Dakota in 2017.....	131
B.12.	Fractional green canopy cover (FGCC), non-destructive leaf area index (NDLAI) and destructive leaf area index (DLAI) at dry bean R2 growth stage (7/24), 14 days after inoculation (DAI) (8/8) and 28 DAI (8/21) in Prosper, North Dakota in 2017.....	132
B.13.	Yield (mT/ha ⁻¹) in dry bean trials conducted across sites in 2016 and 2017.....	132

PSBMV LITERATURE REVIEW

Dry Field Peas

Field pea (*Pisum Sativum* L.) is an important food source and forage which was initially cultivated and grown in Southwest Asia (Oelke et al., 1991). Since the domestication of peas, they have been used for human consumption as well as livestock feed. Peas provide a larger source of soluble carbohydrates than soymeal for some animals and serve as a good source of dietary protein and energy for humans (Pulse Australia 2009). Field peas were first brought to the American continents with colonists in the 1500s; however, winter type peas were not introduced to the United States until 1932 (McGee et al., 2017). Canada provides 60% of the world's field pea exports followed by the United States with 11%. France, Russia and Australia round out the top five world exporters of field peas. India is the world's largest importer of field peas, importing 41% followed by China, Bangladesh, Belgium, and Italy. In the United States, field pea production has grown annually about 8.5% between the years 1980-2012 (Janzen 2014). In 2017, 542,360 hectares of field peas were harvested in the US (USDA-NASS 2018). Montana is the leading producer of field pea in the US, with 193,440 hectares harvested last year, followed by North Dakota, with 165,921 hectares harvested (USDA NASS 2018).

Field peas are a cool season food and feed crop grown in semi-arid regions with average temperatures between 12 and 18°C. There are two main growth habits in field peas. The first is a "vining" field pea which has a normal leaf pattern where leaves are not replaced by tendrils. These peas have a vine of 0.9-1.8 meters long (Endres et al. 2016). Vining pea cultivars, in general, are better suited to outcompete weeds and appear to be more tolerant of excess moisture and heat stress than dwarf types (McKay et al., 2003). The "dwarf" type field pea has a semi-leafless leaf pattern where leaves have been replaced by tendrils. Dwarf type vines are much

shorter and only grow 0.6-1.2 meters long (Endres et al., 2016). This growth habit does not compete as well against weeds or tolerate environmental stress as well as vining field pea types; however, dwarf growth types are easier to harvest (McKay et al., 2003).

Growers have numerous incentives to raise dry field peas. Field peas have the potential to yield well, are highly marketable, and reduce input costs on fertilizers for growers over time. Field peas as a legume have the ability to fix atmospheric nitrogen, minimizing the need for supplemental nitrogen fertilizer. Field peas also help reduce the amount of fertilizer inputs for the next crop in the rotation by providing a 40 lb nitrogen/acre credit (Frazen, 2018). All crop residues contain nitrogen, however, it is not readily available for plant uptake until it has been decomposed by soil microbes. Field pea residue can be degraded by soil microbes more readily than other crop residues because it has a low carbon to nitrogen ratio (O'Leary and Connor 1997). When residues are degraded, available nitrogen is released and ready for uptake. In non-legume crops, available nitrogen can be immobilized in the soil due to high carbon to nitrogen ratio (O'Leary and Connor 1997). In addition to reducing fertilizer inputs, incorporating field peas into a crop rotation can be useful for the management of pathogens, weeds and insects.

Pea Seed-borne Mosaic Virus (PSbMV)

Potyviriidae is the largest virus family and contains six genera: *Potyvirus*, *Rymovirus*, *Bymovirus*, *Macluravirus*, *Ipomovirus* and *Tritimovirus*. The *Potyvirus* genus contains over 91 known species and 88 tentative species (Hull 2002). The particles are membrane bound with a genome made of repeating protein sequences of 30-47 kDa for a total of 9.7 kb in size (Hull 2002). PSbMV particles take the form of flexuous rods, 770 nm in length and 12 nm in width. PSbMV also has the ability to induce the production of inclusion bodies that are pinwheel in shape and aggregate in the cytoplasm of mesophyll cells (Wang et al., 1991).

PSbMV can infect a wide range of agronomic crops in the Fabaceae family such as field pea, lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), faba bean (*Vicia faba*), and pasture legumes such as alfalfa (*Medicago sativa*) and vetch (*Vicia spp.*) (Aftab 2006). PSbMV was first described in Czechoslovakia in 1966 as Pea leaf rolling virus. Within the next four years, the virus was also described around the world from Japan to Germany and the United States. The virus was given a unique name in each area based on symptoms observed including False pea leaf roll virus and Pea fizzle top virus. The one commonality among all isolates of the virus was its ability to be transmitted to the seed. PSbMV ability to transmit to the seed resulted in the rapid spread of PSbMV from continent to continent (Khetarpal 1987). In 1974, it was determined that viral isolates were serologically related between Japan and the United States and that many symptoms were similar. At that time, the universal name Pea seed-borne mosaic virus (PSbMV) was adopted (Mink et al., 1974).

PSbMV Symptomology

PSbMV causes a wide variety of symptoms on the seeds, pods and foliage. Symptomatic pea seeds often have characteristic off-color rings that resemble a tennis ball in pattern. Other symptoms include seed coat cracking, seed discoloration, shrunken seeds or seed abortion (Aftab 2006). The literature has consistently documented that the presence of symptoms on the seed coat of field peas is not an indicator of PSbMV infected within the embryo of the seed (Astier et al., 2007; Khetarpal and Maury 1987; Latham and Jones 2001).

A wide range of PSbMV symptoms can be observed on the foliage of the field pea plant including stunting, shortened internodes, and deformed terminal rosettes. The leaves of PSbMV-infected plants often have a mosaic discoloration, chlorosis and sometimes the leaf margins curl downwards. Pods produced by infected plants are often deformed or crescent-shaped. Shrunken

seeds or seed abortion causes these pod abnormalities (Hampton and Baggett 1970; Wunsch et al. 2014). The virus can also persist asymptotically in the plant, adding to the difficulty in detection. PSbMV infection should not be diagnosed based on symptoms alone given PSbMV infection symptomology is very similar to a number of abiotic stressors such as nutritional deficiencies, mechanical plant injury or herbicide injury, but rather through the use of lab diagnostic tools such as ELISA, rt-PCR and electron microscopy.

Economic Impact of PSbMV

The economic impact of PSbMV is of great concern to both growers and consumers. Shrunken seeds can significantly reduce test weight and yield. In addition, seed with a split coat, shrunken or with any other physical deformity is classified as defective and results in price reductions when peas are sold. Australian pea fields with 98% infection frequency displayed yield losses of 18 to 25% (Cou tts et al., 2009). In 2006 and 2007, PSbMV was a serious threat to pea production in South Australia, Victoria and New South Wales. Surveys were conducted to determine the number of field pea fields where PSbMV was present. In Victoria, 39% of fields were infected, 46% in South Australia and 54% in New South Wales. The frequency of virus infection within a field was also examined. In Victoria, PSbMV infection frequency levels ranged from 2 to 90%, fields in South Australia ranged from 1 to 74% and New South Wales ranged from 2 to 77% (Aftab and Freeman 2013).

Yield reductions due to PSbMV are determined by viral pathotype as well as host genotype. The PSbMV P1 and P4 pathotypes have been found to potentially reduce seed yield by up to 35% and 82%, respectively (Ali and Randles 1998). These yield losses were due to an overall reduction in seed size and quality. Field pea cultivars differ in response to PSbMV infection. In a field study in Manitoba, yield losses due to PSbMV infection were 11% and 36%

in cultivars Trapper and Century, respectively (Chiko and Zimmer 1978). Significantly different yield responses were observed across 34 field pea cultivars screened for a reaction to PSbMV in Australia (van Leur et al., 2013).

PSbMV Transmission

PSbMV is known to be transmitted by aphid vectors, mechanically, and via seed. Twenty-one species of aphids have been reported to transmit PSbMV (Khetarpal 1987). The most common and effective aphid vectors are the potato aphid (*Macrosiphum euphorbiae*), the green peach aphid (*Myzus persicae*), and the pea aphid (*Acyrtosiphon pisum*) (Khetarpal 1987). In pea production areas of North Dakota and Montana, the pea aphid is the vector of greatest concern. PSbMV is vectored by aphids in a non-persistent (stylet-borne) manner. Non-persistently transmitted viruses are not retained by chitin in the insect gut or within the internal tissue as is observed with semi-persistent and persistent viruses. Particles of non-persistently transmitted viruses remain on the stylet of the insect. PSbMV is capable of infecting a plant seconds after the aphid stylet punctures the plant epidermis (Dietzgen et al. 2016; Pirone and Harris 1977). Aphids often probe a large number of plants before finding a preferred host, which can result in PSbMV infection of a large number of plants in a short time (Pirone and Harris 1977).

Contact transmission of PSbMV from an infected, to a healthy neighboring plant has recently been documented. PSbMV transmission occurred when an infected and a healthy plant were intertwined together (Congdon et al. 2016b). Fan simulated wind generated ample canopy movement to cause leaves to abrade against each other, creating injury and exchange of plant sap, resulting in PSbMV transmission (Congdon et al. 2016b).

PSbMV is also spread from generation to generation through the seed. PSbMV is not transmitted by pollen, but rather directly from the maternal plant (Stevenson and Hagedorn 1973). In addition, the PSbMV virion directly invades pea embryos early in development and multiplies within the embryonic tissue, rather than a transmission route being provided by the male or female gametes (Wang 1992). PSbMV is thought to begin infecting the seed immediately following fertilization. The virus invades the ovule and moves to the space between the testa and the suspensor. The suspensor aids the embryo in development by providing nutritional support. The suspensor is the means by which PSbMV infects the embryo (Wang 1994). The window for the virus to pass through the suspensor is short. The embryonic suspensor undergoes a programmed degeneration early in embryo development (Wang and Maule 1994; Roberts et al. 2003). Multiple host genes have been found to affect the accumulation of PSbMV in the testa but do not stop the transmission to the embryo. The suspensor is critical to seed transmission efficiency. Therefore, it is critical to identify genes that prevent the movement of PSbMV virions through the suspensor (Wang 1994).

Virus transmission is also contingent on virus pathotype and host cultivar. Seed transmission from plants mechanically inoculated under greenhouse conditions with the P1 and P4 pathotypes ranged from 0 to 55% and 0 to 31%, respectively (Ali and Randles 1998), PSbMV seed transmission rates in 25 cultivars exhibited a wide variation, ranging from 2 to 49% (Wang et al. 1993), and seed transmission rates of PSbMV isolate W-1 (P4 pathotype) in 10 field pea cultivars ranged from 5 to 35% (Coutts et al. 2008). Across seed lots of three cultivars, the smallest seeds possessed the greatest PSbMV infection levels; however, seed infection levels (i.e. seed transmission) were highly variable across cultivars, ranging from 1 to 32% (Congdon et al. 2015).

PSbMV Pathotyping

Four pathotypes, P1, P2/L1, P3 and P4, of PSbMV have been well documented and characterized. The P2 pathotype, formerly called PSbMV-L or L1, infects lentils (*Lens culinaris* L.) but is not known to infect most US pea cultivars (Kasimor 1997). The P3 pathotype primarily infects broad bean and originated from Nepal (Lundsgaard 1981). Field pea is the primary host for pathotypes P1 and P4. The P1 pathotype is prevalent in Europe, North America, Australia, New Zealand and Pakistan. The P4 pathotype has been found throughout North America, Australia and Pakistan (Safarova 2008). Proposed new pathotypes, U1 and U2, were detected in Pakistan and differed in reaction to the host differential set; however, they have yet to be reported anywhere else, nor have they been characterized (Ali and Randles 1997; Torok and Randles 2007). Pathotyping can be conducted using molecular and traditional methods. Traditional methods involve inoculating a host differential set with purified virus isolates (Ali and Randles 1997). Reverse transcriptase-PCR (RT-PCR) assays have been developed to differentiate between P1 and P4 pathotypes based on amplicon sizes (Kohnen et al. 1992, 1995).

PSbMV Detection

Pea plants infected with PSbMV display a number of symptoms characteristic of the virus; however, it is often difficult to differentiate some symptoms from those caused by other viruses, or abnormalities caused by environmental stress. Enzyme-linked immunosorbent assays are most commonly utilized for PSbMV testing. Commercial double antibody sandwich (DAS) ELISA kits are available for the detection of PSbMV. RT-PCR assays are also available to reliably detect PSbMV (Safarova et al. 2014; van de Vlugt et al. 1999).

PSbMV Management

PSbMV is spread primarily through infected seed, but secondary spread occurs through an aphid vector or by mechanical means. Utilizing an integrated pest management (IPM) approach is the most effective method of managing PSbMV in the field and raising healthy field peas. The primary cultural method to reduce the risk of introducing PSbMV into a field is planting virus-free pea seed. Currently, seed testing for PSbMV is not required for certification in the US. In Australia, a threshold of <0.5% PSbMV in a seed lot is recommended with no yield losses anticipated. In a seed production operation, a threshold of <0.1% PSbMV infection is recommended (Coutts et al., 2009). Removal of alternative hosts and volunteers, and isolating production can reduce the risk of the re-introduction of PSbMV.

Resistance to PSbMV is dependent upon the host genotype as well as the viral pathotype. PSbMV host resistance is qualitatively inherited and recessive (Gao et al. 2004a). Host resistance genes include *sbm1*, *sbm1^l*, *sbm2* and *sbm3* which each confer resistance against a specific pathotype of PSbMV. The recessive gene *sbm1*, has been identified as the recessive allele eIF4e, a eukaryotic translation initiation factor. The recessive allele of eIF4e prevents cell to cell movement and replication of PSbMV. The *sbm1* gene specifically confers resistance to P1 and P4 pathotypes. *sbm4* was reported to confer resistance to the P4 pathotype and was thought to be tightly linked to *sbm1*; however, more recent research indicates that they are alleles of the same gene (Gao et al. 2004b; Provvidenti and Alconero 1988). The allele *sbm1^l* confers resistance to the P1 and P2 pathotypes while *sbm2* and *sbm3* each independently confer resistance to the P2 pathotype (Congdon et al. 2016a; Gao et al. 2004b; Makkouk et al. 2014; Provvidenti and Alconero 1988). Nine additional alleles of the eIF4E have been identified and confer resistance against the P1 pathotype; however, at this time, it is unknown whether these alleles offer any

resistance against any other PSbMV pathotypes (Konecna et al. 2014). Partial resistance to PSbMV has been observed and documented in field peas; however, this mechanism is not understood (Congdon et al. 2016a; Coutts et al. 2008; van Leur et al. 2013). It has been proposed to be polygenically controlled. Cultivars with partial resistance exhibit reduced susceptibility to PSbMV, visual symptoms and yield losses (Coutts et al. 2008; Hampton 1980; van Leur et al. 2013).

Secondary PSbMV spread occurs via aphid vectors. In general, insecticides are relatively ineffective in managing non-persistently transmitted viruses due to rapid virus transmission. Some classes of insecticides agitate aphids before they expire, causing an increase in aphid movement, feeding and spread of PSbMV (Aapola et al. 1974; Thackray et al. 2000). The application of insecticides also reduces beneficial aphid predators allowing aphids to reproduce rapidly following insecticide application (Knodel et al. 2013).

Summary

Field pea production is an important industry in North Dakota; however, success of this industry is threatened by a number of diseases. Recently, PSbMV was identified in North Dakota. PSbMV in other areas has resulted in substantial yield losses and the lack of phytosanitary guidelines has resulted in this virus being disseminated throughout the world. In Australia, very low acceptable levels of PSbMV have been established in seed; however, in North Dakota, state certified seed is not screened for PSbMV. Susceptibility, seed transmission frequency and yield losses are dependent on host genotype and PSbMV pathotype. Therefore, it would be prudent to evaluate the pathotype of the virus found in North Dakota, as well as examine the response of field pea cultivars commonly grown in this area to the virus. This work

would begin the ground work necessary to establish economic thresholds for PSbMV in seed lots grown in North Dakota.

Literature Cited

- Aapola, A. A., Knesek, J. E., and Mink, G. I. 1974. The influence of inoculation procedure on the host range of pea-seed-borne mosaic virus. *Phytopathol.* 64:1003-1006.
- Aftab, M., Freeman, A. J., and Leur, J. 2007. Virus diseases in South Australian pulse crops 2005-2006. Proceedings 16th Biennial Australasian Plant Pathology Society Conference. Adelaide, Australia. p 197.
- Aftab, M. and Freeman, A. 2013. Temperate Pulse Viruses: Pea Seed-borne Mosaic Virus (PSbMV). Dept. of Environ. and Primary Industries.
- Ali, A. and Randles, J. W. 1997. Early season survey of pea viruses in Pakistan and the detection of two new pathotypes of pea seedborne mosaic potyvirus. *Plant Dis.* 81:343-347.
- Ali, A., and Randles, J. W. 1998. The effects of two pathotypes of pea seed-borne mosaic virus on the morphology and yield of pea. *Aus. Plnt. Pathol.* 27:226-233.
- Astier, S., Albouy, J., Maury, Y., Robaglia, C., and Lecog, H. 2007. Principles of plant virology. page166. Direct invasion of the embryo. Astier, S. eds, Science Publishers, Enfield, NH, USA
- Chiko, A. W. and Zimmer, R. C. 1978. Effect of pea seed-borne mosaic virus on two cultivars of field pea grown in Manitoba. *Can J Plant Sci.* 58:1073-1080.
- Congdon, B., Coutts, B., Renton, M. and Jones, R. 2015. Pea seed-borne mosaic virus: occurrence and management in field pea crops in Western Australia. Grain Research and Development Corporation. Grains Research Update.

- Congdon, B. S., Renton, M., Banovic, M. and Jones, R. A. C. 2016a. *Pea seed-borne mosaic virus* in field pea: widespread infection, genetic diversity and resistance gene effectiveness. *Plant Dis.* 100:2475-2482.
- Congdon, B. S., Coutts, B. A., Renton, M., and Jones, R. A. C. 2016b. Pea seed-borne mosaic virus: stability and wind-mediated contact transmission in field pea. *Plant Dis.* 100:953-958.
- Coutts, B. A., Prince, R. T., and Jones, R. A. C. 2008. Further studies on *Pea seed-borne mosaic virus* in cool-season crop legumes: responses to infection and seed quality defects. *Aust J Agr Res.* 59:1130-1145.
- Coutts, B. A., Prince, R. T., and Jones, R. A. C. 2009. Quantifying effects of seedborne inoculum on virus spread, yield losses, and seed infection in the Pea seed-borne mosaic virus-field pea pathosystem. *Phytopathol* 99:1156-1167.
- Danci, O., Ziegler, A., Torrance, L., Gasemi S., and Danci, M. 2009. Potyviridae Family-short review. *J. of Hort. Forest and Biotech.* 13:421-425.
- Dietzgen R. G., Mann, K. S., and Johnson, K. N. 2016. Plant virus-insect interactions: Current and potential future research directions. *Viruses* 11:303-324.
- Endres, G., Forster, S., Kandel, H., Pasche, J., Wunsch, M., Knodel, J., and Hellevang K. 2016. Field Pea Production. North Dakota State University Extension Service. A1166.
- Franzen, D. W. 2018. North Dakota Fertilizer Recommendation Tables and Equations. North Dakota State University Extension Service. SF882.
- Gao, Z., Johansen, E., Evers, S., Thomas, C. L., Ellis, N., and Maule, A. 2004a. The potyvirus recessive resistance gene, *sbm1*, identifies a novel role for translation initiation factor eIF4E in cell-to-cell trafficking. *The Plant J.* 40:376-385.

- Gao, Z., Eysers, S., Thomas, C., Ellis, N., and Maule, A. 2004b. Identification of markers tightly linked to sbm recessive genes for resistance to *Pea seed-borne mosaic virus*. *Theor Appl Genet.* 109:488-494
- Hampton, R. O. and Baggett, J. R. 1970. Host effects and diagnostic symptoms of pea fizzletop disease. *Plant Dis. Rep.* 54:355-358.
- Hampton, R. O. 1980. Pea seed borne mosaic symptom variation among *Pisum* plant introduction accessions: Expressions and variation in symptom expression among different genotypes of *Pisum* and pathological implications. *Pisum Newsl.* 12:29-30.
- Hull, R. 2002. Matthews' Plant Virology. Pages 13-45 Nomenclature and classification of plant viruses Hull, R. eds, Academic Press, San Diego, CA, USA
- Janzen, J. P., Brester, G. W., and Smith, V. H. 2014. Field peas: Trends in production, trade, and price. Agricultural Marketing Policy Center. Retrieved from:
<http://www.ampc.montana.edu/briefings/briefing57-peasebruary2014.pdf>
- Kasimor, K. and Baggett, J. R. 1997. Pea cultivar susceptibility and inheritance of resistance to the lentil strain (Pathotype P2) of pea seedborne mosaic virus. *J. Amer. Soc. Hort. Sci.* 122:325-328.
- Khetarpal, R. K. and Maury, Y. 1987. Pea seed-borne mosaic virus: A review. *Agronomic.* 7: 215-224.
- Knesek, J. E., Mink, G. I., and Hampton, R. O. 1974. Purification and properties of *pea seed-borne mosaic virus*. *Phytopathol* 64:1076-1081.
- Knodel, J. J., Beauzay, P., and Boetel, M. 2013. 2014 Field Crop Insect Management Guide. Retrieved from: <http://www.ag.ndsu.edu/extensionentomology/field-crops-insect-pests/Documents/nd-field-crop-insect-mgt.-guide/e-1143-2014>

- Kohnen, P. D., Dougherty, W. G., and Hampton, R., O. 1992. Detection of pea seedborne mosaic potyvirus by sequence specific enzymatic amplification. *J. of Vir. Meth.* 37:253-258.
- Kohnen, P. D., Johansen, I. E., and Hampton, R. O. 1995. Characterization and molecular detection of the P4 pathotype of pea seedborne mosaic potyvirus. *Phytopathol* 85:789-793.
- Konecna, E., Safarova, D., Navratil, M., Hanacek, P., Coyne, C. et al.. 2014. Geographical gradient of the eIF4E alleles conferring resistance to potyviruses in pea (*Pisum*) germplasm *PLoS ONE* 9(3): e90394. DOI: 10.1371/journal.pone.0090394
- Latham, L. J., Joesn, R. A. C. 2001. Alfalfa mosaic and pea seed-borne mosaic viruses in cool season crop, annual pasture, and forage legumes: susceptibility, sensitivity,, and seed transmission. *Aust J Agric Res.* 52:771-790.
- Lundsgaard, T. 1981. *Pea seedborne mosaic virus* isolated from broad bean (*Vicia faba* L.) in Denmark. *Acta Agriculturae Scan.* 31:116-122
- Makkouk, K. M., Kumari, S. G., van Leur, J. A. G., and Jones, R. A. C. 2014. Control of Plant Virus Diseases in Cool-Season Grain Legume Crops. *Adv Virus Res.* 90:207-253
- McGee, R. J., Eisenbrade S., Neson, H., and Schillinger, W. 2017. Re-inventing Austrian winter pea towards developing food quality winter peas. *Crops and Soils.* 50:4-46.
- McKay, K., Schatz, B., and Endres, G. 2003. Field pea production. North Dakota Cooperative Extension Service Publication A-1166.
- Mink, G. I., Inouye, T., Hampton, R. O., and Knesek, J. E. 1974. Relationships among isolates of pea seed-borne mosaic virus from the United States and Japan. *Phytopathol* 64:569-570.

- Oelke, E. A., Oplinger, E. S., Hanson, C. V., Davis, D. W., Putnam, D. H., Fuller, E. I., and Rosen, C. J. 1991. Dry Field Pea. Alternative Field Crops Manual. University of Wisconsin-Extension and University of Minnesota Extension.
- O'Leary, G. J., and Connor D. J. 1997. Stubble retention and tillage in a semi-arid environment: 3. Response of wheat. *Field Crops Res.* 54:39-50
- Pirone T., and Harris, K., 1977. Nonpersistent transmission of plant viruses by aphids. *Ann. Rev. Phytopathol* 15:55-73.
- Plaster, E. J. 1992. Soil science and management. Albany (NY): Delmar Publishers
- Provvidenti, R., and Alconero, R. 1988. Inheritance of resistance to a lentil strain of *Pea seed-borne mosaic virus* in *Pisum sativum*. *J Hered.* 79:45-47
- Pulse Australia. 2009. Field Pea. Retrieved from: <http://www.pulseaus.com.au/Fieldpea.aspx>
- Roberts, I. M., Wang, D., Thomas, C. L., and Maule, A. J. 2003. Pea seed-borne mosaic virus seed transmission exploits novel symplastic pathways to infect the pea embryo and is, in part, dependent upon chance. *Protoplasma* 222:31-43.
- Safarova, D., Navratil, M., Petrusova, J., Pokorny, R., and Piakova, Z. 2008. Genetic and biological diversity of the pea seed-borne mosaic virus isolates occurring in Czech Republic. *Acta Virologica.* 52:53-57.
- Safarova, D., Brazda, P., and Navratil, M. 2014. Effect of artificial dsRNA on infection of pea plants by pea seed-borne mosaic virus. *Czech J Genet Plant Breed.* 50:105-108.
- Stevenson, W. R., and Hagedorn, D. J. 1973. Further studies on seed transmission of pea seedborne mosaic virus in *Pisum sativum*. *Plant Dis Rep.* 57:248-252.

- Thackray, D. J., Jones, R. A. C., Bwyne, A. M., and Coutts, B. A. 2000. Further studies on the effects of insecticides on aphid vector numbers and spread of cucumber mosaic virus in narrow-leaved lupins (*Lupines angustifolius*). *Crop Prot.* 19:121-139.
- Torok, V. A., and Randles, J. W. 2007. Discriminating between isolates of PSbMV using nucleotide sequence polymorphisms in the HC-Pro coding region. *Plant Dis.* 91:490-496
- USDA-NASS Quick Stats. 2018. United States Department of Agriculture National Agricultural Statistic Service. https://www.nass.usda.gov/Quick_Stats/
- van der Vlugt, R. A. C., Steffens, P., Cuperus, C., Barg, E., Lesemann, D. E., Bos, L., and Vetten H. J. 1999. Further evidence that shallot yellow stripe virus (SYSV) is a distinct potyvirus and reidentification of welsh onion yellow stripe virus as a SYSV strain. *Phytopathol* 89:148-155.
- van Leur, J., Kumari, S. G., Aftab, M., Leonforte, A., and Moore, S. 2013. Virus resistance of Australian pea (*Pisum sativum*) cultivars. *New Zeal J Crop Hort.* 41:2:86-101
- Wang, D., Woods, R. D., Swaby, A. G., and Cockbain, A. J. 1991. Detection of pea seed-borne mosaic virus virion and pinwheel inclusion body proteins in leaf and seed tissues of pea (*Pisum sativum* L) by immunogold labelling. *Agronomie* 11:387-394
- Wang, D., Hayes, I. M., and Maule, A. J. 1992. Procedures for the efficient purification of pea seed-borne mosaic virus and its genomic RNA. *J. of Vir. Meth.* 36:223-230.
- Wang, D., Woods, R. D., Cockbain, A. J., Maule, A. J., and Biddle, A. J. 1993. The susceptibility of pea cultivars to pea seed-borne mosaic virus infection and virus seed transmission in the UK. *Plant Pathol.* 42:42-47.

Wang, D., and Maule A. 1994. A model for seed transmission of a plant virus: Genetic and structural analyses of pea embryo invasion by pea seed-borne mosaic virus. *The Plant Cell* 6:777-787.

Wunsch, M., Pasche, J., Knodel, J., McPhee, K., Markell, S., Chapara, V., and Pederson, S. 2014. Pea seed-borne mosaic virus (PSbMV) in Field Peas and Lentils. *Plant Disease Management NDSU Extension Service*.

CBB LITERATURE REVIEW

Dry Bean Production

Phaseolus vulgaris (L), the common dry bean, is one of the most globally important grain legumes cultivated due to its high nutritional value. In the United States dry bean is an important commodity; in 2017, the US exported 230,000 metric tons of dry beans. North Dakota is currently the leading producer of common beans in the United States with 258,311 hectares harvested in 2017. Michigan ranks second with 89,198 hectares, followed by Minnesota (62,686), Idaho (24,888), and Washington (8,822) hectares harvested (USDA-NASS).

Origin

The dry bean is comprised of two main gene pools comprising two main centers of domestication, Middle America and Andean, which includes Mexico, Central America, Colombia, Ecuador, Northern Peru, and the Southern Andes. The Andean genepool has been further broken down into Central and Northern Andean groups (Rendon-Anaya et al., 2017; Singh et al., 1991a). Each of the genepools contains several races, characterized by morphological, agronomic, and molecular differences (Singh et al. 1991b). The Andean group contains the races Peru, Neuva Granada and Chile (Beebe et al. 2001). The race Neuva Granada contains market classes light and dark red kidneys, large reds, white kidney, and cranberry (Gepts et al. 1998). The four races Durango, Jalisco, Mesoamerican, and Guatemala comprise the Middle American genepool (Beebe et al. 2001). The Mesoamerican race can be divided into the market classes: pinto, great northern, small red, pink, navy, small white, and black beans (Mensack et al. 2010). In North Dakota, approximately 90% of dry bean production is comprised of market classes from the Middle American gene pool, pinto, navy, and black beans (Kandel 2013).

Growth Habits

Dry beans can have either a determinate or indeterminate growth habit (Kandel 2013). Determinate beans are characterized by ceasing stem elongation with the formation of terminal flowers, creating a bush-like appearance (Kandel 2013). Determinate dry beans have a large, thick stem with minimal vining. Indeterminate dry beans continue to flower and fill pods under favorable environmental conditions. Indeterminate beans typically grow horizontal to the ground creating a vine-like appearance (Kandel 2013). Determinate and indeterminate types can be further divided into four architectural growth habits. Type I includes determinate bush beans, Type II includes indeterminate upright short vine beans, Type III includes prostrate indeterminate vining beans, and Type IV are indeterminate beans with strong vining tendencies (Kandel 2013; Venette and Lamey 1998). Sub-types exist within dry bean architecture types depending on the environment the dry beans are grown in. As an example, Type II beans are typically upright; however, under certain environmental conditions they may appear to be more prostrate and resemble Type IIb. US common dry bean market classes are mainly architecture Type I, II or III (Singh, 1981). Type IV architecture type beans are typically wild common beans (*Phaseolus vulgaris* L.), snap beans grown for fresh production i.e. pole beans, lima bean (*Phaseolus lunatus* L.) and runner beans (*Phaseolus coccineus* L.). Greater yields have been observed with indeterminate beans than with the determinate bush types likely due to an increase in light distribution within the leaf canopy (Fageria and Santos 2008; Tanaka and Fujita 1979). Prostrate (Type III) beans may produce more leaves per plant but upright (Type II) beans typically have significantly larger leaves (Trindale et al., 2010). Type II beans, which produce more leaf area, are more tolerant of phosphorous deficiencies in the soil than Type I or IV (Trindale et al. 2010). When dry beans are competing with weeds for sunlight, the opposite

phenomena has been observed. Upright Type II navy beans have been reported to have higher yields when compared to prostrate Type III navy beans under high weed pressure (Blackshaw et al. 1999). The taller plants capture more sunlight and eventually outcompete weeds.

Cultivars

Cultivar Stampede (Reg. No. CV-292, PI 654382) is a pinto bean released in 2010 by the North Dakota Agricultural Experiment Station. Stampede has a Type IIb growth habit (upright, short vine), a strong resistance to lodging and matures in approximately 96 days. In 2017, dry bean growers were surveyed in the Northharvest region and Stampede made up 1% of all the dry beans planted in that region (Knodel 2018). Cultivar Maverick (Reg. No CV-142, PI 595894) is a pinto bean released by the North Dakota Agricultural Experiment Station in 1996. Maverick has a Type IIIa growth habit and matures in approximately 95 days. Type IIIa indicates it is semi-prostrate, with fluctuating growth habit depending on growing conditions. Maverick's popularity has dwindled, making up only 0.2% of all the dry beans planted in the Northharvest region in 2017 (Knodel 2018). Cultivar Medicine Hat (XP08550813) is a pinto bean released by Seminis seed. Medicine Hat is a type IIb (upright, short vine) growth habit that matures in 88 to 90 days. In the 2017 Northharvest dry bean grower survey, Medicine Hat made up 0.2% of all dry beans planted in that region (Knodel 2018). Cultivar Othello (Reg. No CV-121, PI578268) was released by the USDA-ARS in 1986. Othello is a Type IIIa prostrate indeterminate vine that matures in 70 to 92 days. In the 2017 Northharvest dry bean grower survey no acres were reported seeded to Othello (Knodel 2018).

Bacterial Blight of Dry Beans

Common bacterial blight (CBB), brown spot and halo blight are most commonly observed bacterial diseases on dry beans in North Dakota, individually or as a complex.

Environmental conditions, presence of inoculum, and cultivar susceptibility influence disease severity. These three bacterial diseases can be distinguished visually by foliar symptoms; however, they share similar disease cycles. The primary sources of inoculum for these bacterial pathogens are infected seed and infested plant debris. The bacteria can persist on the seed in two ways, on the outer seed coat or contained within the embryo. The bacteria are transmitted to seedlings via seed directly through the vascular system, or when the seed coat comes in contact with the emerging cotyledons (Akhavan et al., 2013). *Xap*, *Xff*, *Psp* and *Pss* persist epiphytically until favorable environmental conditions are presented (Belete and Bastas 2017; Gent et al. 2005; Schwartz et al. 2005) Bacterial infection occurs through natural openings such as stomata and hydathodes, or through wounds. Wounds are generated by wind driven rain and soil, hail, leaves abrading against each other or injury facilitated by equipment moving through the field. The time between initial and secondary infection can be as short as two weeks given favorable environmental conditions (Schwartz et al. 2005). When CBB, halo blight or brown spot disease severity is high, infected leaves may die, but remain attached to the plant, ultimately functioning as a source of secondary inoculum. Secondary spread and infection of the bacteria are facilitated by wind, rain splash, and water droplets that serve as a vessel for movement of bacterium to neighboring plants, new leaf tissue and pods, as well as generate wounds for the bacteria to enter the plant (Schwartz et al. 2005). In cotton, rain splash combined with wind was capable of spreading *Xanthomonas campestris* pv. *malvacearum* up to 5.5 meters from the inoculum source (Faulwetter 1917). Brown spot severity has been correlated with the intensity of the water droplets (Hirano et al. 1995). Irrigation of dry beans also favors secondary spread of the bacterium and if irrigation water is re-used, it can also contribute to the secondary spread. When halo blight, CBB, and brown spot progress, they can become systemic within the xylem of the

plant (Goodwin 1992; Zaumeyer and Thomas 1957). When systemic infection occurs, water soaked lesions can be observed on the stem and eventually turn necrotic (Muedi et al. 2015; Schwartz et al. 2005). Systemic bacterial infection also contributes to bacterial levels in the seed, with the bacteria entering the seed through the funiculus (Goodwin 1992; Zaumeyer and Thomas 1957). Seed infection with all three bacterial diseases in the complex occurs in the same manner and symptoms are virtually indistinguishable visually. Water soaked lesions appear on the pods which eventually become necrotic and sunken. Infected seeds can appear discolored, shriveled, with diminished vigor and lower rates of germination (Schwartz et al., 2005).

Bacterial Blight Complex

Halo Blight

Halo blight, caused by the Gram negative bacterium *Pseudomonas syringae* pv. *phaseolicola* (*Psp*), can be devastating to dry bean production worldwide. Halo blight has a wide host range including azuki bean, lima bean, mung bean, runner bean, soybean and tepary bean. Even low levels of infected seed can cause a severe halo blight epidemic when weather conditions are favorable (Webster et al. 1983). Halo blight is often considered a cool season disease, favored by a temperature range from 16 to 24°C and relative humidity greater than 95% (Schwartz et al., 2005). These conducive environmental conditions are more likely to occur at higher latitudes and altitudes (Fourie et al. 1998; Guven et al. 2004). Halo blight has been documented to cause up to a 45% yield loss (Asensio-S.-Manzanera et al. 2006; Félix-Gastélum et al. 2016; Singh and Schwartz 2010). As halo blight progresses, *Psp* releases non-host specific phaseolotoxin which inhibits OCTase activity in the plant, resulting in the formation of a chlorotic/light green halo around a small pin point necrotic lesion (Lopez-Lopez et al. 2004). The size of the halo varies depending on environmental conditions and host genotype. In the most

advanced stages, the disease can become systemic, causing yellowing and necrosis of new foliage.

Bacterial Brown Spot

Brown spot, caused by *Pseudomonas syringae* pv. *syringae* (*Pss*) has been reported throughout the United States, Canada, Africa, and Brazil (Hangwani et al. 2015; Harveson et al. 2007; Schwartz et al. 2005). The bacterium has a broad host range including field pea, faba beans, soybeans, Kudzu, cowpea and lima beans (Harveson et al. 2007). Optimal environmental conditions include temperatures that range between 28 to 32°C and high relative humidity (Harveson et al. 2007). Brown spot is characterized by small circular necrotic lesions that form on leaves. These lesions typically have a dark brown margin and narrow chlorotic halo. When compared to halo blight, the chlorotic halo produced by brown spot is narrower, and light green to yellow (Schwartz et al. 2005). As brown spot progresses, lesions coalesce and the center of the lesions may become necrotic and fall out.

Common Bacterial Blight

Common bacterial blight, caused by the Gram positive bacteria *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) (syn. *Xanthomonas campestris* pv. *phaseoli*) and *Xanthomonas fuscans* subsp. *fuscans* (*Xff*) (syn. *Xanthomonas phaseoli* var. *fuscans*), affects dry bean production globally. Yield losses in excess of 40% due to CBB in dry beans have been documented (Gillard et al. 2009; Opio et al. 1996; Serracin et al. 1991). *Xap* and *Xff* infection results in very similar symptoms. *Xff* isolates from Africa were more aggressive than *Xap* isolates; however, this has not been observed with North American isolates (Bett and Baninza 2014; Mutlu et al. 2008). *Xap* and *Xff* prevalence differ based on the market class of dry bean they are infecting. *Xap* is most commonly associated with large seeded Andean beans whereas, *Xff* is more commonly observed

infecting Andean and Middle American beans (Duncan et al. 2011). In a survey conducted in 2005 and 2006 in central Wisconsin, 98% of the CBB in dark red kidney beans was caused by *Xap* (Duncan et al. 2011). CBB is most destructive when environmental conditions include temperatures that range between 28 to 32°C and high humidity (Schwartz et al. 2005). CBB lesions, much like other bacterial diseases, initially appear as water soaked regions on the leaf tissue. Typically, lesions are observed along leaf margins and interveinal tissue (Schwartz et al. 2005). These lesions quickly expand, coalesce, and eventually turn necrotic with chlorosis around the margin.

Management

Implementing integrated pest management (IPM) is the most effective method of controlling and limiting the spread and proliferation of the bacterial pathogens that make up the bacterial blight complex on dry beans. In general, reducing the primary source of inoculum is the biggest priority. Implementing a two year crop rotation with crops that are not susceptible, or do not harbor the bacterial pathogens epiphytically reduces the amount of bacteria infested residue (Schwartz et al., 2005). In addition, incorporating infested plant residue into the soil also reduces the amount of inoculum, which decreases the rate of infection. Removal of weeds and volunteer bean plants that harbor epiphytic bacterial populations from the primary field or nearby fields will also reduce the amount of inoculum in the field (Gilbertson et al. 1990; Schwartz et al. 2005). Planting certified seed helps to prevent or delay the introduction of the bacteria into the field (Bailey et al., 2003). Limiting irrigation can reduce bacterial disease by preventing the introduction or promoting the spread of bacterial pathogens. Another method for the management of bacterial blight complex involves the incorporation of cultivars with partial resistance into the crop rotation. The utilization of genetic resistance where the bacterial blight

complex is endemic is one of the most effective tools in reducing yield and seed quality losses (Singh and Munoz 1999). Dry bean resistance to CBB, halo blight and brown spot is quantitatively inherited and challenging to incorporate into dry bean lines (Jung et al 2003; Singh and Munoz 1999; Tock et al. 2017; Webster et al. 1980). Under moderate to high CBB pressure, resistant cultivars had a 23% yield advantage over susceptible dry bean cultivars (Gillard et al. 2009). Chemical methods also have been utilized in IPM programs for the management of CBB in dry beans. Streptomycin can be used as seed treatment to minimize surface-borne inoculum; however, it is not widely utilized given the cost and limited efficacy. Copper based foliar bactericides can be used preventively on dry beans, to protect against the infection of *Xap*, *Xff*, *Pss* and *Psp*. In North Dakota and Minnesota in 2017, Copper hydroxide (Champ, Nufarm, Burr Ridge, IL) foliar bactericide, was sprayed on 3.4% of the acres of dry beans surveyed in the Northharvest region, making it among the top ten products, based on acres sprayed (Knodel 2018). Foliar copper products have been relatively inconsistent for the management of CBB; however, these products have shown benefits in limiting the spread of the pathogen (Harveson 2009). Chemical management of foliar brown spot and halo blight has been more successful than has been observed for CBB (Schwartz 2011).

Copper Products

Copper products registered for application on dry beans for the management of bacterial diseases are all members of the M1 FRAC group and all function as a preventive application applied every 7 to 14 days. Kocide 3000 is manufactured by Dupont (Wilmington, DE) with an active ingredient of copper hydroxide (46.1% a.i.). Kocide 3000 is labeled for the management of bacterial pathogens in a wide variety of field crops, vegetables, nuts, and fruits. Mastercop is manufactured by ADAMA Agricultural Solutions USA (Raleigh, NC) with an active ingredient

of copper sulfate pentahydrate (21.46% a.i.). Mastercop is labeled for use in citrus, vegetables, trees, small fruits, vines and field crops with activity on a number of bacterial and fungal organisms. Badge SC is manufactured by Isagro USA (Morrisville, NC) with two active ingredients: copper oxychloride (16.81% a.i.) and copper hydroxide (15.36% a.i.). Badge SC is marketed for use in citrus, field crops, trees, vegetables, vines and ornamentals. In dry beans, Badge SC is labeled for managing anthracnose, brown spot, halo blight, CBB, Cercospora leaf spot and downy mildew. ET-F recently has been labeled with Earth Science Laboratories (Ithaca, NY) with an active of copper sulfate pentahydrate (19.8% a.i.). ET-F is labeled for use in citrus, field crops, small fruits, trees, vegetables and vines. In dry beans, ET-F is only labeled for the management of HB, CBB and BS.

Surface Sanitizers

At this time, no literature has been published on the efficacy of surface sanitizers for the management of the bacterial blight complex in dry beans. Surface sanitizers have been utilized in other host:pathogen systems successfully such as the use of peroxyacetic acid for the management of bacterial wilt caused by *Ralstonia pseudosolanacearum* in tomato (Hong et al. 2018). Goldshield is a surface antimicrobial marketed by AP Goldshield LLC (El Paso, TX) with an active ingredient of 3-(trimethoxysilyl) propyl dimethyl octadecyl ammonium chloride (5% a. i.). The product is not currently marketed for agricultural use, but research is being conducted on numerous crop systems. SaniDate 12.0 and Oxidate 2.0 are sanitizers marketed by BioSafe Systems LLC (East Hartford, CT) with two active ingredients. SaniDate 12.0, hydrogen peroxide (18.5% a.i.) and peroxyacetic acid (12.0% a.i.), was developed for use in treatment of commercial, agricultural and horticultural water systems. SaniDate 12.0 is labeled for foliar, drench and chemigation operations for the management of a range of fungi and bacteria,

including *Xanthomonads* and *Pseudomonads*. Oxidate 2.0 has two active ingredients, hydrogen dioxide (27% a.i.) and peroxyacetic acid (2% a.i.). Oxidate 2.0 is marketed for the treatment of commercial agricultural and horticultural water systems as well as for seed treatments, soil drench and foliar applications. Oxidate 2.0 is labeled for the management of a range of fungi, oomycetes and bacteria. In dry beans, Oxidate 2.0 is labeled for the management of anthracnose, bacterial blights, botrytis, powdery mildew, rhizoctonia, rust, and white mold.

Growth Promoters

No literature has been published on the efficacy of growth promoters in dry beans for the management of brown spot, halo blight and CBB. Both of the products discussed below are natural products and do not require an EPA registration to be applied to crops. Both of these products are designed to improve the mobility of sugars and nutrients within the plant. By improving these functions, increased photosynthesis, root mass and higher BRIX (measurement of a solid i.e. amino acid proteins, minerals sugars in plant sap) levels could be anticipated. WakeUp Summer, comprised of plant derived oils and alcohols in a base of colloidal micelles, is a plant health promoter marketed by Renewable Farming LLC (Cedar Falls, IA). eA300 is a plant health promoter marketed by EcoSolv Technologies LLC (Chesapeake, VA). eA300, much like WakeUp Summer, is comprised of alkanolamines, amino acids, nonionic surfactants, fatty acids in a base of colloidal micelles.

Crop Growth Assessment

Leaf Area Index

Leaf area index (LAI) is defined as the measurement of the area of leaf surface per area of ground covered (Gallegos and Shibata 1989). The dry matter accumulation in a dry bean plant is intrinsically related to photosynthetic rates as well as light interception in the canopy

(Monteith 1977). LAI is designed to quantify the plant canopy, and has been utilized by researchers to predict yield, quantify defoliation, and assess water stress. LAI was significantly correlated to yield in plants with varying levels of water stress and accurately described water stress levels (Gallegos and Shibata 1989). LAI was strongly correlated with yield in corn and was used to generate several yield prediction models (Baez-Gonzalez et al. 2005). LAI also was used in the generation of a yield prediction model in wheat; however, researchers found that as a stand-alone, it did not accurately predict yield. However, when added to a pre-existing wheat yield prediction model, LAI improved model accuracy (Dente et al. 2008).

LAI can be measured in two ways. The planimetric technique assesses tissue in a destructive manner. Leaf tissue is physically removed from the plant and total leaf surface area is measured. Once the total leaf surface is calculated for a plant, LAI is determined by dividing the total area of ground that plant was covering based on stand establishment and row spacing (Jonckheere et al., 2004). LI-3100 C Area Meter manufactured by LI-COR Inc (Lincoln, NE) has been used to assess destructive LAI in soybeans and dry beans (Malone et al. 2002).

Gravimetric techniques used to assess LAI are non-destructive. Handheld devices, such as AccuPAR leaf Ceptometer (Decagon Devices; Pullman, WA), the Sunfleck Ceptometer (Decagon Devices; Pullman, WA) and the LAI-2000 Plant Canopy Analyzer (LI-COR, Lincoln, NE), measure the amount of light scattered throughout the canopy and derives an LAI measurement based on the quantity of light transmitted (Jonckheere et al., 2004). This method of assessing LAI is far less time consuming and expensive; however, the accuracy of some handheld devices in estimating LAI is inconsistent. The LAI-2000 Plant Canopy Analyzer (LI-COR, Lincoln, NE) is utilized in dry beans to calculate LAI. LAI-2000 Plant Canopy LAI outputs were highly correlated to LAI calculated using the central leaflet method (de Jesus et al.

2001). However, in soybeans, the LAI-2000 Plant Canopy analyzer was found to overestimate LAI 83% of the time when compared to destructive measurements (Malone et al. 2002). A possible explanation for this overestimation is the equipment lacks the ability to distinguish, pods, petioles and stem tissue and therefore includes them all in the LAI estimate (Malone et al. 2002). The Sunfleck Ceptometer has been used to assess LAI in dry beans; however, the LAI measurements did not correlate with yield (Amador-Ramirez et al. 2007). In corn, a gravimetric tool, the AccuPAR leaf Ceptometer, consistently overestimated LAI compared to destructive methods (Willhelm et al., 2000). In cotton, AccuPAR was observed to consistently overestimate LAI when compared to destructive methods (Tewolde et al., 2005).

Fractional Green Canopy Cover

Fractional green canopy cover (FGCC) is a measurement of percentage live “green” vegetation in an image and can be utilized to estimate canopy development. FGCC can be calculated rapidly and affordably through the use of Canopeo, a free smart phone application (Oklahoma State University App Center) (Patrignani and Ochsner 2015). FGCC has been utilized to assess ground cover, defoliation, plant senescence and disease severity in agricultural crops, turf and trees. FGCC was utilized in the estimation of green and senescent tissue in the soybean canopy (Purcell 2000), defoliation in cotton (Alchanatis et al. 2000) and forest ground cover (Korhonen et al., 2006). In turfgrass, FGCC was utilized to estimate ground cover chlorosis (Karcher and Richardson 2003; Richardson et al. 2001). Canopeo was used in potato to assess *Verticillium* wilt, which can cause rapid loss of leaf tissue consequently resulting in a loss of ground cover (Yellareddygari and Gudmestad 2017). FGCC correlations with NDVI and LAI have been observed in wheat, corn, and triticale (Carlson and Ripley 1997; Lati et al. 2011; Nielsen et al. 2012). While LAI and FGCC may correlate with each other, they are not truly

independent; therefore, the incorporation of both of these parameters into a yield prediction model would be ill advised (Carlson and Ripley 1997; Nielson et al. 2012).

Summary

Dry bean production in North Dakota is an extremely important industry. CBB, halo blight and brown spot have been reported throughout North Dakota; however, current management practices are insufficient, with little to no chemical products to effectively manage this complex. Surface sanitizers, new copper products and plant growth promoters have the potential to aid in the management of CBB; however, their efficacy is unknown or not publicly available. Furthermore, growers have begun to transition to upright dry bean architecture types to simplify harvest and potentially escape foliar diseases, particularly white mold. Little is known about the yield response of the two architecture groups under CBB pressure. Crop growth assessment tools have been used to predict yield but their potential to assess CBB disease severity is unknown. The use of these tools could provide a non-biased, rapid way to assess CBB disease severity in the field and potentially predict dry bean yield as well.

Literature Cited

- Alchanatis, V., Navon, A., Glazer, I., and Levski, S. 2000. An image analysis system for measuring insect feeding effects caused by biopesticides. *J Agric Engng Res.* 77:289-296
- Amador-Ramirez, M. D., Acosta-Diaz, E., Medina-Garcia, G., and Gutierrez-Luna, R. 2007. An empirical model to predict yield of rainfed dry bean with multi-year data. *Rev Fitotec Mex.* 30:311-319
- Asensio-S.-Manzanera, M. C., Asensio, C., and Singh, S. P. 2006. Gamete selection for resistance to common and halo bacterial blights in dry bean intergene pool populations. *Crop Sci.* 46, 131–135.

- Baez-Gonzalez, A. D., Kiniry, J. R., Maas, S. J., Tiscareno, M., L., Macias, J. C., Mendoza, J. L., Richardson, C. W., Salinas, J. G., and Manjarrez, J. R. 2005. Large-area maize yield forecasting using leaf area index based yield model. *Agron J.* 97:418-425
- 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.
- Beebe, S. E., Rengifo, J. A., Gaitan, E., and Tohme, J. 2001. Diversity and origin of Andean landraces of common bean. *Crop Sci.* 41:854-862.
- Belete, T., and Bestes, K. K. 2017. Common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) of beans with special focus on Ethiopian condition. *J Plant Pathol Microbiol.* 8:403.
- Bett, K., and Banniza, S. 2014. Population study of *Xanthomonas* spp. From bean growing regions of Canada and response of bean cultivars to pathogen inoculation. *Can J Plant Pathol.* 36:341-353.
- Blackshaw, R. E., Muendel, H. H., and Saindon, G. 1999. Canopy architecture, row spacing and plant density effects on yield of dry bean (*Phaseolus vulgaris*) in the absence and presence of hairy nightshade (*Solanum sarrachoides*). *Can J Plant Sci.* 79:663-669
- Carlson, T. N., and Ripley, D. A. 1997. On the relation between NDVI, fractional vegetation cover, and leaf area index. *Remote Sens. Environ.* 62:241-252.
- de Jesus, W. C., do Vale, F. X. R., Coelho, R. R., Hau, B., Zambolim, L., Costa, L., C., and Filho, A., B. 2001. Effects of angular leaf spot and rust on yield loss of *Phaseolus vulgaris*. *Phytopathol* 91:1045-1053

- 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. *Phytopathol* 101:425-435
- Fageria, N. K., and Santos, B. A. 2008. Yield Physiology of Dry Bean. *J of Plant Nutr.* 31:983-1004
- Faulwetter, R. C. 1917. Dissemination of the angular leaf spot of cotton. *J Agric Res.* 8:457-475.
- 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.
- Fourie, D. 1998. Characterization of halo blight races on dry beans in South Africa. *Plant Dis.* 82:307-310
- Gallegos, J. A. A., and Shibata, J. K. 1989. Effect of water stress on growth and yield of indeterminate dry-bean (*Phaseolus vulgaris*) cultivars. *Field Crop Res.* 20:81-83
- Gent, D. H. Lang, J. M. and Schwartz, H. F. 2005. Epiphytic survival of *Xanthomonas axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* on leguminous hosts and onion. *Plant Dis.* 89:558-564.
- Gepts, P., Kmiecik, K., Pereira, P., and Bliss, F., A. 1988. Dissemination pathways of common bean (*Phaseolus vulgaris*, Fabaceae) deduced from phaseolin electrophoretic variability. I. The Americas *Econ Bot.* 42:73-85.
- Gilbertson, R. L., Rand, R. E., and Hagedorn, D. J. 1990. Survival of *Xanthomonas campestris* pv. *phaseoli* and pectolytic strains of *X. campestris* in bean debris. *Plant Dis.* 74:322-327

- Gillard, C. L., Connor, R. L., Howard, R. J., Pauls, K. P., Shaw, L., and Taran, B. 2009. The performance of dry bean cultivars with and without common bacterial blight resistance in field studies across Canada. *Can J Plant Sci.* 89:405-410.
- Goodwin, P. I. 1992. Effect of common bacterial blight on leaf photosynthesis of bean. *Can J Plant Path.* 14:203-206.
- Güven, K., Jones, J. B., Mornol, M. T., and Dickstein, E. R. 2004. Phenotypic and genetic diversity among *Pseudomonas syringae* pv. *phaseolicola*. *J Phytopath.* 152:658-666
- Harveson R. M., and Schwartz, H. F. 2007. Bacterial diseases of dry edible beans in the central high plains. *Plant Health Progress.* doi:10.1094/PHP-2007-0125-01-DG.
- Harveson, R. M. 2009. Common bacterial blight of dry beans in Nebraska. NebGuide. University of Nebraska Extension Publication. G1956.
- Hirano, S. S., Rouse, D. I., Clayton, M. K., and Upper, C. D., 1995. *Pseudomonas syringae* pv. *syringae* and bacterial brown spot of snap bean: A study of epiphytic phytopathogenic bacteria and associated disease. *Plant Dis.* 79:1085-1093.
- Hong, J. K., Jang, S. J., Lee, Y. H., Jo, Y. S., Yun, J. G., Jo, H., Park, C., and Kim, H. J. 2018. Reduced bacterial wilt in tomato plants by bactericidal peroxyacetic acid mixture treatment. *Plant Pathol J.* 34:78-84.
- Jonckheere, I., Fleck, S., Nackaerts, K., Muys, B., Coppin, P., Weiss, M., Baret, F. 2004. Review of methods for in situ leaf area index determination Part I. Theories, sensors and hemispherical photography. *Agric For Meteorol.* 121:19-35
- Jung, G., Ariyaratne, 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

- Kandel, H. 2013. Dry bean production guide. North Dakota State University Extension Service. A1133.
- Karcher, D. E., and Richardson, M. D. 2003. Quantifying turfgrass color using digital image analysis. *Crop Sci.* 43:943–951
- Korhonen, L. K., T., Rautiainen, M., and Stenberg, P. 2006. Estimation of forest canopy cover: A comparison of field measurement techniques. *Silva Fenn.* 40:577–588.
- 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
- Lati, R. N., Filin, L., and Eizenberg, H. 2011. Robust methods for measurement of leaf-cover area and biomass from image data. *Weed Sci.* 59:276–284
- Lopez-Lopez, K., Hernandez-Flores, J. L., Cruz-Aguilar, M., and Alvarez-Morales, A. 2004. In *Pseudomonas syringae* pv. *phaseolicola*, Expression of the *argK* Gene, Encoding the phaseolotoxin-resistant ornithine carbamoyltransferase, is regulated indirectly by temperature and directly by a precursor resembling carbamoylphosphate. *J Bacteriol.* 186:146-153
- Malone, S., Herbert, A. D., and Holshouser, D. L. 2002. Evaluation of the LAI-2000 Plant Canopy Analyzer to Estimate Leaf Area in Manually Defoliated Soybean. *Agron. J.* 94:1012-1019.
- Mensack, M. M., Fitzgerald, V., K., Ryan, E. P., Lewis, M. R., Thompson, H. J., and Brick, M. A. 2010. Evaluation of diversity among common beans (*Phaseolus vulgaris* L.) from two centers of domestication using ‘omics’ technologies. *BMC Genomics* 11:686

- Muedi, H. T. H., Fourie, D., and McLaren, N. W. 2015. Distribution and severity of bacterial brown spot on dry beans in South Africa: An update. *S Afr J Sci.* 111:1-6
- 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* pv. *fuscans* strains for bean genotypes with common blight resistance. *Plant Dis.* 92:546-554.
- Nielsen, D. C., Midceli-Garcia, J. J., and Lyon, D. J. 2012. Canopy cover and leaf area index relationships for wheat, triticale, and corn. *Agron. J.* 104:1569-1573
- 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
- Patrignani, A., and Ochsner, T., E. 2015. Canopeo: A powerful new tool for measuring fractional green canopy cover. *Agron. J.* 107:2312-2320.
- Purcell, L. C. 2000. Soybean canopy coverage and light interception measurements using digital imagery. *Crop Sci.* 40:834–837
- Rendon-Anaya, M., Montero-Vargas, J. M., Saburido-Alvarez, S., Vlasova, A., Capella-Gutierrez, S., Ordaz-Ortiz, J. J.,...Herrera-Estrella, A. 2017. Genomic history of the origin and domestication of common bean unveils its closest sister species. *Genome Biol.* 18:60
- Richardson, M. D., Karcher, D. E., and Purcell, L. C. 2001. Quantifying turfgrass cover using digital image analysis. *Crop Sci.* 41:1884–1888
- 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

- Schwartz, H. F. 2011. Bacterial disease of Beans. Colorado State University Extension. Fact Sheet No. 2.913
- Serracin, J., Young, R. A., Rosas, J. C., and Cáceres, J. 1991. Daños causados por *Xanthomonas campestris* pv. *phaseoli* y su efecto en el rendimiento del frijol común (habichuela: *Phaseolus vulgaris*). J. Agric. Univ. P.R. 75: 353–361
- Singh, S. P., Gutierrez, J. A., Moina, A., Urrea, C., and Gepts, P. 1991a. Genetic diversity in cultivated common bean: II. Marker-based analysis of morphological and agronomic traits. Crop Sci. 31:23-29
- Singh, S. P., Gepts, P., and Debouck, D. G. 1991b. Races of common bean (*Phaseolus vulgaris*, Fabaceae). Econ Bot. 45:379-396
- Singh, S. P., and Muñoz, C. J. 1999. Resistance to common bacterial blight among *Phaseolus* species and common bean improvement. Crop Sci. 39:80–89
- Singh, S. P., and Schwartz, H. F. 2010. Breeding common bean for resistance to diseases: a review. Crop Sci. 50, 2199–2223
- Tanaka, A., and Fujita, K. 1979. Growth, photosynthesis and yield components in relation to grain yield of the field bean. J of the Faculty of Agriculture Hokkaido University. 59:145-238.
- Tewolde, H., Sistani, K. R., Rowe, D. E., Adeli, A., and Tsegaye, T. 2005. Estimating cotton leaf area index nondestructively with a light sensor. Agron. J. 97:1158-1163
- Tock, A., Fouire, D., Walley, P., Holub, E. B., Soler, A., Cichy, K. A., ... Miklas, P., N. 2017. Genome-Wide linkage and association mapping of halo blight resistance in common bean to race 6 of the globally important bacterial pathogen. Front. Plant Sci. 8:1170

- Trindale, R. S., Araujo, A. P., and Teixeira, M., G. 2010. Leaf area of common bean genotypes during early pod filling as related to plant adaptation to limited phosphorus supply. R Bras Ci Solo. 34:115-124
- USDA-NASS Quick Stats. 2018. United States Department of Agriculture National Agricultural
- 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
- Webster, D. M., Atkin, J. D., and Cross, J. E. 1983. Bacterial blights of snap beans and their control. Plant Dis. 67:935-940
- Willhelm, W. W., Ruwe, K., and Schlemmer, M. R. 2000 Comparison of three leaf area index meters in a corn canopy. Crop Sci. 40:1179-1183
- Yellareddygar, S. K. R., and Gudmestad, N. C. 2017. Bland-Altman comparison of two methods for assessing severity of Verticillium wilt of potato. Crop Protec. 101:68-75.
- Zaumeyer, W. J., and Thomas, H. R., 1957. Bacterial diseases of major importance, p. 65-88. In: W. J. Zaumeyer and H. R. Thomas (eds.) A monographic study of bean diseases and methods for their control. U.S. Dept. Agr., Washington, D.C.

CHAPTER 1: PEA SEED-BORNE MOSAIC VIRUS (PSbMV) RISK ANALYSIS OF FIELD PEA BASED ON SUSCEPTIBILITY AND SEED TRANSMISSION

Abstract

Pea seed-borne mosaic virus (PSbMV), a non-persistently aphid-transmitted potyvirus, has been reported in field pea (*Pisum sativum* L.) growing regions worldwide. In 2014, PSbMV was identified in field peas in North Dakota. Host susceptibility and yield losses attributed to PSbMV infection are influenced by the viral pathotype and host genotype. Isolate ND14-1, recovered from North Dakota infected seed and presumptively identified as pathotype 4 (P4), was mechanically inoculated onto 20 field pea cultivars under greenhouse conditions. PSbMV susceptibility, yield losses, symptom expression, and PSbMV seed transmission rates were assessed by cultivar. A risk assessment was developed based on cultivar susceptibility, yield reduction, and PSbMV seed transmission. Risk factors were weighted based on perceived importance to commercial field pea producers. Three cultivars were classified as low risk, seven cultivars were classified as intermediate risk and ten cultivars were classified as high risk. Two of the low risk cultivars, Aragorn and Cruiser were confirmed to be resistant to PSbMV. Cultivar Arcadia was susceptible to PSbMV infection with mild expression of symptoms, but classified as low risk based on a low seed transmission rate and reduced reductions in seed weight and number. This risk assessment could prove a useful tool for growers in field pea cultivar selection where PSbMV is prevalent.

Introduction

Pea seed-borne mosaic virus (PSbMV) was first reported in the United States in 1969 (Mink et al., 1969). Since that time, PSbMV has been confirmed in California, Idaho, Washington, Maryland, New York, Oregon, Vermont, Wisconsin, and North Dakota (EPPO

2014; Beck et al., 2018). The virus is a member of the Potyviridae family and is comprised of positive sense single stranded RNA (Knesek et al., 1974) inside a flexuous rod with an average size of 770 x 12 nm (Mink et al., 1974). PSbMV primarily infects members of the Fabaceae family such as field pea (*Pisum sativum* L.), lentil (*Lens culinaris* M.), chickpea (*Cicer arietinum* L.), and alfalfa (*Medicago sativa* L.) (Aapola et al. 1974). Plant symptoms include downward leaf curling, mosaic, budding and shoot development at nodes, shortening of internodes and delayed plant senescence; however, the virus can be present without expression of characteristic symptoms (Hampton and Baggett 1970). PSbMV can persist asymptotically in the seed, or symptoms such as seed size reduction, seed-coat scarring and cracking may be observed and result in reduced market value of the crop. PSbMV can be transmitted via seed or in a non-persistent manner by aphids (Hampton and Mink 1975). Aphids travel from plant to plant, stylet probing until a suitable host is found. This can result in rapid transmission of the virus to many plants in a very short period (Pirone and Harris 1977). Mechanical transmission of PSbMV via pea leaves rubbing against each other generating injury and exchanging plant sap has been demonstrated in the greenhouse before tendrils are formed and could contribute to an expansion in initial crop infection before secondary aphid transmission occurs (Congdon et al. 2016b). In Australia, a 0.5% seed-borne PSbMV threshold was established at which economic yield losses could be expected when aphids were present; however if peas are being raised for certified seed production a threshold of 0.1% is recommended (Coutts et al. 2009).

PSbMV pathotype, host cultivar, and timing of infection all play an important role in the host susceptibility, yield losses attributed to PSbMV infection and seed transmission of the virus (Ali and Randles 1998). Currently, four main PSbMV pathotypes (P1, P2/L1, P3, and P4) have been characterized (Ali and Randles 1997). P1 and P4 are predominantly found in field pea

(Alconero et al. 1986). Both P1 and P4 were identified among the Australian isolates based on sequences of the HC-Pro Coding region of PSbMV. P1 and P4 were also identified in the US via reactions on a host differential set, partial nucleotide sequence comparison, and nucleotide sequence polymorphisms (Ali and Randles 1998; Torok and Randles 2007). Pakistani PSbMV isolates have been pathotyped as P1, P4, U1, and U2 (Torok and Randles 2007). U1 and U2 differed in reaction to a host differential set, but have yet to be reported elsewhere or characterized further (Ali and Randles 1997; Torok and Randles 2007). The pathotype P2/L1, more commonly referred to as L1, lacks pathogenicity in field pea but can infect lentil (Hampton 1980). In 1997, P2/L1 was identified in seed lots in Pullman, Washington and later pathotyped as P2/L1 via indirect and direct double-sandwich ELISAs by using pathotype specific antigens (Kasimor et al. 1997; Alconero et al. 1986). P3 infects faba bean (*Vicia faba* L.) but can infect some field pea lines and was first described in Denmark 1981 and later in Nepal and Western Australia (Hjulsager et al. 2002; Lundsgaard 1981; Torok and Randles 2007).

Susceptibility to PSbMV is contingent on the presence of the host resistance genes *sbm1*, *sbm1^l*, *sbm2* and *sbm3*. The *sbm1* gene, identified as the recessive allele *elf4E* eukaryotic translation initiation factor, prevents cell to cell movement and replication of PSbMV (Gao et al. 2004a). The *sbm1* gene confers resistance to the P1 and P4 PSbMV pathotypes. The *sbm4* gene was originally reported to confer resistance to the P4 pathotype and be tightly linked to *sbm1*; however, recent research has indicated both are alleles of the same gene (Gao et al., 2004b; Provvidenti and Alconero 1988). The allele *sbm1^l* confers resistance to both P1 and P2/L1 pathotypes while *sbm2* and *sbm3* independently confer resistance to P2/L1 of PSbMV (Congdon et al. 2016a; Gao et al. 2004b; Makkouk et al. 2014; Provvidenti and Alconero 1988). Recently, nine additional alleles of the *elf4E* have been identified with resistance to P1; however, at this

time it is unknown if they confer resistance to other PSbMV pathotypes (Konecna et al., 2014). Partial resistance to PSbMV also has been documented in some field pea cultivars (Congdon et al. 2016a; Coutts et al. 2008; van Leur et al. 2013). Partial resistance is thought to be polygenically controlled and results in reduced PSbMV susceptibility, reduction in visual symptoms, and tolerance to yield losses (Coutts et al., 2008; Hampton 1980; van Leur et al. 2013).

PSbMV seed transmission is heavily dependent on cultivar, pathotype and timing of infection. The virus is transmitted to seed only when infection occurs before fertilization (Wang and Maule 1994). PSbMV virus particles from the maternal plant directly invade the pea embryos early in development and multiply within embryonic tissue (Wang and Maule 1994; Wang et al. 1992). In a greenhouse assay employing mechanical inoculations, cultivar Dundale seed transmission of the P4 pathotype ranged from 0 to 31%, whereas the transmission of the P1 pathotype ranged from 0 to 55% (Ali and Randles 1998). A second greenhouse study examined the response of 10 field pea cultivars to mechanical inoculation with PSbMV isolate W-1. Seed transmission across cultivars varied from 5 to 35% (Coutts et al. 2008).

Yield losses can be affected by pathotype as well as cultivar. In a greenhouse study conducted using cultivar Dunbar in southern Australia, yield losses differed between P1 and P4. Yield losses attributed to infection by P4 were as high as 82% whereas losses due to PSbMV P1 were upward to 35% (Ali and Randles 1998). Yield losses due to PSbMV infection were a result of a reduction in seed size and quality. Seeds weighed less, were shrunken, and had wrinkled seed coats (Ali and Randles 1998). Cultivars also differ in response to PSbMV. In a field study in Manitoba, yield losses due to PSbMV infection were 11% and 36% in cultivars Trapper and Century, respectively (Chiko and Zimmer 1978). It has been speculated that some of the same

genes that confer partial resistance to PSbMV also could offer tolerance. Field pea cultivars exhibiting tolerance were highly susceptible to PSbMV; however, no yield losses occurred (Coutts et al. 2008; Hampton 1980; van Leur et al. 2013).

The observed differences in the effect of infection timing, PSbMV pathotype and field pea cultivar make estimating the risk of losses to PSbMV difficult. In peanuts (*Arachis hypogaea* L.), risk assessment has been used to evaluate parameters such as cultivar, planting date and plant populations, among others, to assess the risk of *tomato spotted wilt virus* (TSWV) infection (Brown et al. 2005). Empirical models have been developed to forecast PSbMV incidence and yield losses in a growing season; however, these models are strictly based on aphid populations and growing conditions in Mediterranean-type conditions (Congdon et al. 2017). Currently, a PSbMV risk assessment tool does not exist.

PSbMV was first identified in field pea seed grown in North Dakota in 2014, but nothing is known about the reaction of cultivars grown in the state to the pathogen population (Beck et al. 2018). The objectives of this research were to determine the reaction of field pea cultivars to PSbMV, evaluate the seed transmission rate and develop a PSbMV risk analysis model for field pea cultivars commonly grown in North Dakota. Cultivar response to PSbMV was gauged in harvest elements including individual seed weight, and the number of pods and seeds per plant. In addition to assessing susceptibility and response of each cultivar to PSbMV infection, seed transmission rates and symptom expression were also evaluated.

Materials Methods

Virus Purification

PSbMV isolate ND14-1 was derived from infected field pea seed grown in Carrington, North Dakota in 2014. Infected seed was planted and grown under greenhouse conditions. Five

weeks after planting, the youngest plant tissue was collected and tested for PSbMV. Double Antibody Sandwich-Enzyme Linked Immunosorbent Assays (DAS-ELISA) (AC Diagnostics, Catalog #V036-K1) were conducted following manufacturer's protocol. A positive ELISA reading was characterized as an optical density of two time greater than the negative control (EPPO, 2015). The youngest tissue of PSbMV positive plants was collected, crushed and homogenized in a 0.01 M Potassium Phosphate Buffer, pH 7.4 at a 1:10 (tissue: buffer) ratio. Carborundum (0.1 grams Silicon Carbide /5 mL of crude sap) served as an abrasive to create plant injury. The crude sap was inoculated onto four-week-old cultivar Ginny field pea. Traditional virus purification methods involve the use of a local lesion host, for PSbMV this is typically *Chenopodium quinoa* (Ali-Khan and Zimmer 1979). PSbMV from a single plant was inoculated onto *C. quinoa* four weeks after planting using plant sap as described above. Lesions developed on inoculated leaves; however, the virus did not remain localized. Positive DAS-ELISA results were obtained from the newest tissue on the *C. quinoa* plants three weeks after inoculation, indicating systemic movement. Due to the systemic nature on *C. quinoa*, a serial dilution method was used to purify the isolate. All serial dilution inoculations were performed on cv. Ginny field pea plants. Serial 10-fold dilutions from 1:10 to 1:100,000 were made from homogenized virus-infected leaf tissue from a six-week old plant and inoculated onto four-week old plants. Two weeks following inoculation, plants were screened with DAS-ELISA. The youngest plant tissue was collected from positive PSbMV plants inoculated with largest successful dilution and used to create another set of serial dilutions from 1:10 to 1:100,000. This sap was inoculated onto four-week old plants. Two weeks following inoculation, plants again were screened with the DAS-ELISA. This process was performed two additional times and tissue from the newest plant growth was collected and inoculated onto four-week old Ginny field peas

to maintain the virus for future inoculations. Pathotype of the PSbMV isolate ND14-1 used in greenhouse evaluations was determined to be most similar to pathotype 4 via sequencing.

Cultivar Susceptibility Study

Ten green-seeded field pea cultivars ('CDC Striker', 'Bluemoon', 'K2', 'Viper', 'Ginny', 'Daytona', 'Greenwood', 'Arcadia', 'Cruiser' and 'Aragorn') and 10 yellow-seeded ('Bridger', 'SW Midas', 'AC Agassiz', 'Nette 2010', 'Vegas', 'Spider', 'Salamanca', 'Hyline', 'DS Admiral' and 'CDC Treasure'), commonly grown in North Dakota were screened for their reaction to PSbMV isolate ND14-1. The experiment was conducted in a randomized split plot design with five biological replicates and performed three times. Fifty seeds of each cultivar were planted in the greenhouse into PRO-MIX FLX growing mix (Premier Horticulture Inc., Canada) and maintained at $20\pm 2^{\circ}\text{C}$. Three weeks after planting, two leaves from the newest growth of each plant were bulked in groups of ten and screened with DAS-ELISA to ensure they were PSbMV-free. Two weeks after screening, plants were inoculated with a PSbMV crude sap as described above. Six plants per biological replicate of each cultivar were inoculated with PSbMV and four plants were mock-inoculated with sterile 0.01M potassium phosphate buffer and carborundum (0.1 grams/5 mL of buffer). Ten minutes following inoculations, plants were rinsed with tap water to remove any excess carborundum. Eight weeks after planting, two leaves of the newest growth were collected from each plant and tested with DAS-ELISA. Eleven weeks after planting, plants again were screened with DAS-ELISA and each plant was evaluated for symptoms of PSbMV, including leaf rolling, mosaic, stunting, and budding at internodes. Plants were harvested 15 weeks after planting. Seed weight and symptoms and the number of seeds and

Pods were recorded from all plants. Seeds were sorted based on presence or absence of visual PSbMV-infection symptoms (Figure 1.1).

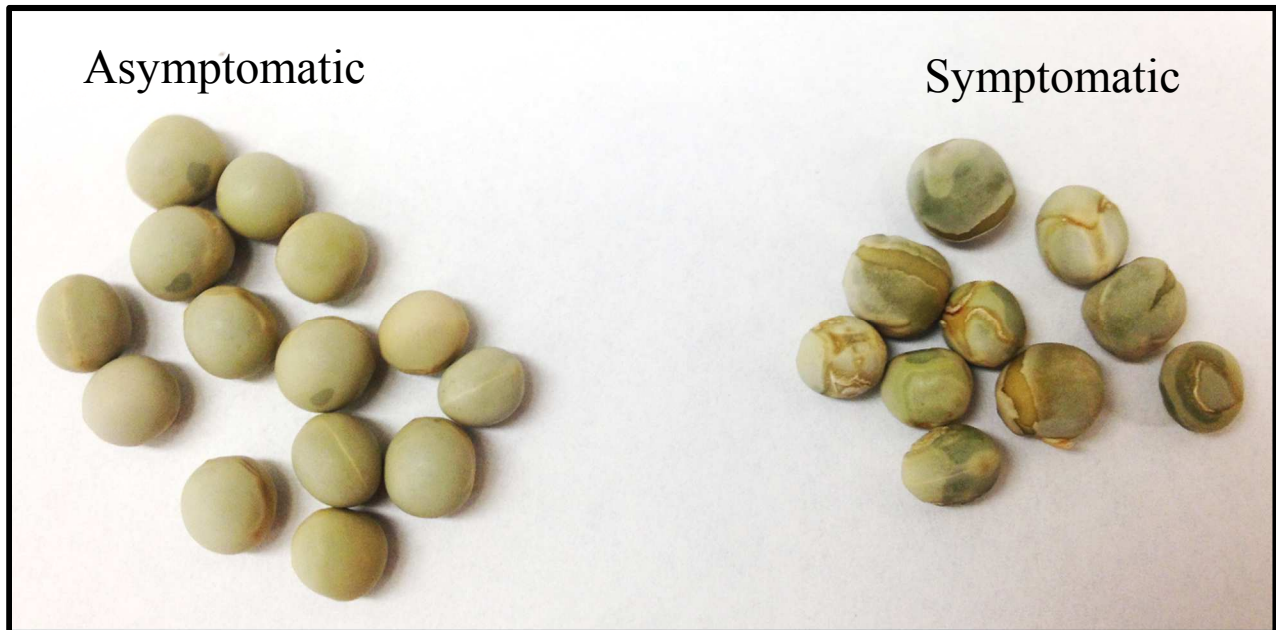


Figure 1.1. *Pea Seed-borne Mosaic Virus* PSbMV positive field pea seed exhibiting no symptoms (left) and characteristic symptoms (right) including seed coat cracking, scarring, water-soaked appearance and overall reduction in size.

Seed Transmission Study

For each of the four cultivar susceptibility trials, progeny seed grown from five infected pea plants (five biological replicates) from each cultivar was used in the seed transmission study. Ten symptomatic and ten asymptomatic seeds from each selected PSbMV positive plant were grown under greenhouse conditions as described above. Five weeks after planting, two leaves from the newest growth were collected from each individual plant and tested with DAS-ELISA. PSbMV infection frequency was recorded for individual plants. Cultivars were classified as highly resistant (no infection), resistant (1 to 10% infection), moderately resistant (11 to 20%), moderately susceptible (21 to 30%), susceptible (31 to 40%), or highly susceptible (>40%)

depending on whether PSbMV incidence based solely on the transmission rate of PSbMV from infected to progeny plants as previously established (Bashir et al. 2005).

Statistical Analyses

Analyses of data from the cultivar susceptibility and seed transmission studies were conducted using SAS version 9.4 (SAS Institute, Cary, NC). The Shapiro-Wilk test in the univariate procedure was used to test the data of each trial for normality. To compare the response of each cultivar to PSbMV, plant infection frequency values from the 11-week sampling date and the number of pods and seeds, seed weight and frequencies of PSbMV-symptomatic and asymptomatic seeds produced per PSbMV-inoculated and mock-inoculated plants were established. Data from these variables were expressed on a per plant basis with exception of seed weight that was expressed on a 100-seed base. In preparation for analyses, the rank procedure of SAS was used to rank the data in ascending order by trial and replicate. A combined analysis of variance was conducted on number of pods and seeds, seed weight, and yield using the minimum variance quadratic unbiased estimation method (mivque0) of the mixed procedure to compare the reaction of cultivars to PSbMV. For the analyses, cultivars were considered fixed effects. The least squares means of plant infection frequency and PSbMV seed transmission were separated through a post hoc analysis and fisher's test which provides a least significant difference ($\alpha = 0.05$) across cultivars. To further examine whether seed symptomology is an indicator of virus presence within each cultivar, least squares means of PSbMV seed transmission within symptomatic and asymptomatic seeds were conducted and a chi-squared test was used to distinguish significant differences between symptomatic and asymptomatic seeds ($\alpha = 0.05$).

To assess inherent risk assumed by growing field pea cultivars when PSbMV is present, a weighted scale from 1 to 9 was developed to evaluate the parameters (PSbMV infection frequency, yield and seed transmission rate) that contribute to the risk. Yield loss, calculated as percentage ratios of difference between yield from mock-inoculated plants and PSbMV-inoculated plants, was weighted at 50%; rate of transmission of PSbMV from confirmed positive plant to progeny plants through seed at 30%; and PSbMV infection frequency via mechanical inoculation at 20% (Table 1.1). These weights were designated based on the perceived importance of the three variables to field pea production economics. Within each variable, four increasing scale levels (1, 3, 6 and 9) were used to classify cultivar risk. The scale value assigned to each variable by cultivar was multiplied by the weight of the variable to give a weighted scale value. The three weighted scale values assigned for each cultivar were summed to give a risk value ranging from 1 to 9. Values 1 to 3 indicate a low risk, 4 to 6 intermediate risk, and 7 to 9 a high risk (Table 1.1).

Table 1.1. Components of the risk algorithm used to estimate grower risk when *Pea Seed-borne Mosaic Virus* (PSbMV) is present

Risk variables ^a	Reaction to PSbMV	Level	Contribution to risk index ^b
Infection incidence	<15%	1	0.2
	16-30%	3	0.6
	31-50%	6	1.2
	>50%	9	1.8
Yield loss	<15%	1	0.5
	16-25%	3	1.5
	26-50%	6	3
	>50%	9	4.5
Seed transmission	<10%	1	0.3
	11-20%	3	0.9
	21-30%	6	1.8
	>30%	9	2.7

^a Weighted variables, Incidence=20%, Seed transmission=30%, Yield loss=50%

^b Contribution to risk index is equivalent to the level multiplied by the variable weight

Results

PSbMV Infection Incidence

Significant ($P < .0001$) differences among cultivars were observed in infection frequencies via mechanical inoculation. Cultivars Aragorn and Cruiser had PSbMV infection frequencies of 0.5% and 0% respectively and no expression of virus symptoms (Tables 1.2 and 1.3). Infection level in these two cultivars was significantly lower than all other cultivars and were classified as resistant based on previously described criteria (Bashir et al. 2005). PSbMV infection frequency for cultivar Arcadia was 37% and infected plants displayed foliar symptoms of leaf curling and budding. This cultivar was infected significantly less frequently than all cultivars except Aragorn and Cruiser; however, it was classified as susceptible under the previously described parameters (Bashir et al. 2005). All other cultivars had median PSbMV infection frequencies greater than 60% and were classified as highly susceptible (Table 1.2). All highly susceptible cultivars displayed a range of visible symptoms of PSbMV infection including leaf curling, stunting, budding, mosaic, and internode shortening; however, no symptoms were observed on highly susceptible cultivars CDC Striker and Spider (Figure 1.2) (Table 1.3). Across all the highly susceptible cultivars exhibiting symptoms, downward leaf curling occurred on all infected plants. Budding was the second most prevalent symptom expressed by infected plants followed by stunting and mosaic. Leaf mosaic symptoms were only observed on the cvs Ginny, Greenwood, SW Midas, and Hyline but were not displayed by every plant.

Table 1.2. LS Means of *Pea Seed-borne Mosaic Virus* (PSbMV) incidence in parent plants and transmission (%) from infected parent plant to progeny in 20 field pea cultivars.

Cultivar	PSbMV incidence (%) ^a		Response ^b	Seed transmission (%) ^d				
				Total	Asymptomatic ^c	Symptomatic		
Aragorn	0.5	g	HR	-	-	-	-	-
Arcadia	37.0	f	S	8.4	jk	8.3		8.5
Bluemoon	70.8	cde	HS	10.2	hij	6.5	*	15.0
CDC Striker	67.5	e	HS	19.7	def	21.8		16.4
Cruiser	0.0	g	HR	-	-	-		-
Daytona	71.4	cde	HS	23.1	bcd	16.7	*	30.5
Ginny	87.2	a	HS	12.4	ghij	12.6		12.3
Greenwood	73.0	bcde	HS	15.5	efgh	16.2		14.7
K2	79.9	abc	HS	9.7	ij	8.4		11.3
Viper	82.4	ab	HS	14.7	fghi	17.9		11.2
AC Agassiz	76.6	bcd	HS	4.1	k	3.9		4.2
Bridger	82.5	ab	HS	19.3	def	15.2	*	25.8
CDC	73.8	bcde		32.3	a			
Treasure			HS			26.4	*	36.8
DS Admiral	72.8	cde	HS	16.6	efg	17.2		15.8
Hyline	72.0	cde	HS	20.7	de	16.0	*	25.7
Nette 2010	75.2	bcde	HS	22.1	cd	21.1		23.3
Salamanca	77.1	bcd	HS	26.7	bc	17.4	*	40.6
Spider	65.2	e	HS	12.4	ghij	11.6		13.9
SW Midas	64.7	e	HS	27.7	ab	25.5		30.0
Vegas	70.3	cde	HS	20.6	de	17.8		25.0
Pr<F	P<.0001			P<.0001				
CV	62.9			215.6				

Cultivars sharing at least one letter in common in the same column are not significantly different based on Fisher's least significant difference test ($\alpha=0.05$).

^a Parent plant PSbMV incidence produced via mechanical inoculation of seedlings.

^b Cultivars were classified as HR=Highly resistant (0%), R=Resistant (1-10%), MR=Moderately Resistant (11-20%), MS=Moderately Susceptible (21-30%), S=Susceptible (31-40%), or HS=Highly Susceptible (>40%) based on PSbMV incidence in parent plants (Bashir et al. 2005).

^c Seed transmission evaluated using seeds collected from PSbMV-positive plants.

^d Chi Square test used to evaluate differences between PSbMV seed transmission in asymptomatic and symptomatic seed, *significant at $\alpha=0.05$.

Table 1.3. Symptoms of *Pea Seed-borne Mosaic Virus* (PSbMV) on 20 field pea cultivars mechanically inoculated under greenhouse conditions.

Cultivar	Seed type	Symptoms			
		Leaf curling ^b	Budding ^c	Stunting ^d	Mosaic ^e
Aragorn	Green				
Arcadia	Green	x	x		
Bluemoon	Green	x	x		
CDC Striker	Green				
Cruiser	Green				
Daytona	Green	x		x	
Ginny	Green	x			x
Greenwood	Green	x	x		x
K2	Green	x			
Viper	Green	x	x		
AC Agassiz	Yellow	x			
Bridger	Yellow	x		x	
CDC Treasure	Yellow	x			
DS Admiral	Yellow	x			
Hylene	Yellow	x			x
Nette 2010	Yellow	x	x		
Salamanca	Yellow	x	x	x	
Spider	Yellow				
SW Midas	Yellow	x			x
Vegas	Yellow	x	x	x	

^a Plants were mechanically inoculated 5 weeks after planting. Symptom ratings were taken 3 weeks after inoculation.

^b Downward curling of leaves

^c Buds formed at the terminal growth points

^d Diminished plant height and shortened internodes

^e Mosaic leaf pattern on leaves (green and yellow flecking)



Figure 1.2. *Pea Seed-borne Mosaic Virus* (PSbMV) induced foliar symptoms of field pea including: leaf curling (left), mosaic (middle) and budding at internodes (right).

PSbMV Seed Transmission

The rates of PSbMV transmission through seed varied significantly by cultivar ($P < .0001$) (Table 1.2). Seed transmission rates ranged between 4 and 28%. Susceptible cv. Arcadia had a lower rate of PSbMV seed transmission than most highly susceptible cultivars with exception of AC Agassiz. Across all cultivars, when seeds were harvested from PSbMV positive plants, 16% of plants grown from asymptomatic seeds were positive for PSbMV, whereas 20% of plants grown from symptomatic seeds were PSbMV positive. Significant higher transmission rates from symptomatic vs. asymptomatic seed were observed only in cvs. Bluemoon, Daytona, Bridger, CDC Treasure, Hyline and Salamanca (Table 1.2). Seed transmission was higher from asymptomatic seed of Ginny, Greenwood, Viper and DS Admiral; however, this difference was not significant.

When comparing infected and mock-inoculated plants within each cultivar, significant differences were observed in the impact PSbMV had on the total seed weight number of pods,

seeds per plant and in the weight of the individual seeds (Table 1.4). Arcadia was the only to have significantly higher total seed weight, and produce more pods and seeds per plant in the presence of PSbMV than in its absence. PSbMV-infected plants from highly susceptible cultivars produced significantly lower total seed weight, between 0.3 and 0.6-fold, than the corresponding PSbMV-free plants (Table 1.4). PSbMV infected plants of cvs CDC Treasure, Hyline, and Salamanca produced significantly fewer pods than mock-inoculated plants. The 100-seed weight from infected plants of 11 cultivars was significantly less than PSbMV-free plants. Among these highly susceptible 11, total seed weight, pods and seeds per plant did not depart significantly from one in cvs CDC Striker, Daytona, AC Agassiz, DS Admiral, and Nette 2010 . While all harvest components were lower in infected plants when compared to PSbMV-free plants of cultivars Greenwood and Spider, no significant differences in the ratios were observed based on the sign test.

Table 1.4. Ratio of total seed weight, pods per plant, 100-seed weight and seeds per plant of field pea plants inoculated with *Pea Seed-borne Mosaic Virus* (PSbMV) and mock-inoculated plants.

Cultivar	Seed color	Ratio (mock-inoculated/PSbMV-inoculated) ^a			
		Total seed weight	Pods per plant	100-seed weight	Seeds per plant
Arcadia	Green	1.62 *	2.36 **	0.87 **	1.86 *
Bluemoon	Green	0.39 **	1.43	0.63 **	0.57 *
CDC Striker	Green	0.51	1.27	0.84 *	0.73
Daytona	Green	0.56	0.88	0.80 *	0.61
Ginny	Green	0.38 **	0.56	0.78 **	0.58
Greenwood	Green	0.53	0.65	0.95	0.52
K2	Green	0.39 **	0.80	0.97	0.40 **
Viper	Green	0.30 *	1.13	0.74	0.45
AC Agassiz	Yellow	0.60	0.76	0.85 **	0.67
Bridger	Yellow	0.31 **	0.45	0.96 *	0.35 **
CDC Treasure	Yellow	0.36 **	0.63 *	0.70	0.50 **
DS Admiral	Yellow	0.65	0.60	0.92 **	0.67
Hyline	Yellow	0.30 *	0.49 *	0.84 **	0.34 *
Nette 2010	Yellow	0.82	1.19	0.82 *	0.94
Salamanca	Yellow	0.54 **	0.67 *	0.85 *	0.60 *
Spider	Yellow	0.55	0.99	0.92	0.73
SW Midas	Yellow	0.47	0.56	0.88	0.44 *
Vegas	Yellow	0.62 **	0.84	0.82	0.73

Statistical significances produced by sign test on the difference of PSbMV- and mock-inoculated medians per variety. * = significant at $\alpha \leq 0.05$; ** = significance at $P \leq 0.01$.

^a Ratios produced using medians. A value >1 signifies an increase, a value <1 signifies a decrease.

PSbMV Risk Index

The results from the seed transmission, total seed weight reduction and infection frequency via mechanical inoculation were used in the development of the risk analysis of all 20 field pea cultivars (Table 1.5). Distinctions among high, intermediate and low risk were made across cultivars based on the risk algorithm (Table 1.1). Cultivars Aragorn, Arcadia, and Cruiser, were identified as low risk. These cultivars had low infection frequencies when mechanically inoculated under greenhouse conditions. Arcadia displayed very low transmission rates to progeny through seed and no reductions in seed size or weight. The rate of seed transmission

could not be evaluated for Cruiser and Aragorn due to the lack of infected plants. Cultivars CDC Striker, Ginny, Greenwood, AC Agassiz, DS Admiral, Nette 2010 and Salamanca were rated at intermediate risk. Infection incidence in these cultivars was greater than 65%. The intermediate ranking was based on low seed weight loss or seed transmission, depending on cultivar. Viral transmission rates through seed ranged from 4% to 27%, and yield losses ranged from 18% to 62%. The remaining 10 cultivars were ranked as high risk based on high infection incidence (>56%), yield losses (>44%) and seed transmission rates (>10%).

Table 1.5. Risk classification of 20 field pea cultivars to *Pea Seed-borne Mosaic Virus* (PSbMV) based on mechanically inoculated plants grown under greenhouse conditions.

Cultivar	Incidence (%)	Total seed weight reductions (%)	Seed transmission (%)	Score ^a	Risk ^b
Aragorn	0	0	0	1	Low
Arcadia	37	0	8	2	Low
Bluemoon	71	61	10	7	High
CDC Striker	68	49	20	6	Intermediate
Cruiser	0	0	0	1	Low
Daytona	71	44	23	7	High
Ginny	87	62	12	6	Intermediate
Greenwood	73	47	16	6	Intermediate
K2	80	61	10	7	High
Viper	82	70	15	7	High
AC Agassiz	77	40	4	5	Intermediate
Bridger	83	69	19	7	High
CDC Treasure	74	64	32	8	High
DS Admiral	73	35	17	5	Intermediate
Hyline	72	70	21	8	High
SW Midas	75	53	22	8	High
Nette 2010	77	18	27	5	Intermediate
Salamanca	65	46	12	6	Intermediate
Spider	65	45	28	7	High
Vegas	70	38	21	7	High

^aScore is based on the level multiplied by the weight (Described in Table 1.1) for incidence, total seed weight loss, and seed transmission which are tallied to give a total score.

^bRisk values coincide with the score and can range from 1 to 9. Values 1 to 3 indicate a low risk, 4 to 6 intermediate risk, and 7 to 9 a high risk

Discussion

This work determined the response of 20 field pea cultivars to PSbMV infection including yield and seed transmission rates and developed a risk assessment analysis to classify these cultivars. Cultivars Cruiser and Aragorn were resistant to the PSbMV isolate ND14-1. This resistant response is likely a result of the incorporation of the *sbm1* resistance gene, which

provides resistance to the P1 and P4 pathotype (Adrian Russel and Kurt Braunwart ProGene Plant Research personal communication; Gao et al. 2004a). PSbMV infection frequencies of 0.5% and 0% were observed in Aragorn and Cruiser, respectively, via mechanical inoculation under greenhouse conditions. Successful infection has been demonstrated previously with resistant cultivars using aggressive mechanical inoculations (Congdon 2017). Alternatively, seed may not have been genetically homogeneous. Incidence of PSbMV in Arcadia was 37%, significantly lower than all other cultivars, with the exception of resistant Cruiser and Aragorn. While Arcadia was classified as susceptible using the pre-existing scale (Bashir et al. 2005), the significantly lower PSbMV infection rate indicates that a classification of moderately susceptible or moderately resistant may be more accurate. Partial resistance to PSbMV has been previously documented; however, no research has been performed to identify the genes or understand the mechanism of partial PSbMV resistance in this pathosystem (Coutts et al. 2008; Hampton 1980; van Leur et al. 2013).

The genes responsible for partial resistance also have been proposed to convey tolerance to PSbMV (Coutts et al. 2008; Hampton 1980; van Leur et al. 2013). The cultivar Nette 2010 may possess tolerance to PSbMV, given the relatively low seed weight reductions observed. Viral tolerance could be a result of recovery from virus-like symptoms (Bengyell et al. 2015). Recovery of the plant could be genetically based, as has been observed with Sweet Potato Feathery Mottle Virus (SPFMV), a potyvirus that causes Sweet Potato Virus Disease. In a biparental cross between a SPFMV resistant sweet potato lines and four maternal lines, some progeny exhibited varying levels of virus recovery from/tolerance to SPFMV (Gasura 2008; Gasura et al., 2009). Plants have been shown to recover from virus infection using RNA silencing to stop the replication of ssRNA by targeting the replicative form of the virus (Hull et

al. 2002). Recovery manifested in a reduced viral titre, likely a result of a basal immune response resulting in a reduction in viral replication (Bengyell et al., 2015). A similar situation has been observed in Potato Virus Y (PVY) titre reduction associated with host genotype (Bengyell et al., 2015).

Before a universal name was adopted for PSbMV in 1977, it was known by a number of names including Pea Leaf Rolling Virus, False Pea Leaf Roll Virus and Pea Fizzle Top Virus (Kvicala and Musil 1967; Thottappilly and Schmutterl 1968; Mink et al. 1969). Leaf rolling described in some of the former names of PSbMV, was the most prevalent symptom expressed by infection of PSbMV ND14-1 on the cultivars screened in this study. Given the manifestation of several distinct symptoms observed as a result of PSbMV infection, it would appear that symptomology observed is heavily dependent upon the pathotype of the virus and the cultivar. These deviations in symptomology due to pathotype of the virus and the host cultivar is what contributed to the large expanse of time that occurred between characterization of the virus across the world and the realization that scientists were all characterizing the same pathogen.

Infection by PSbMV isolate ND14-1 resulted in seed weight reductions in most cultivars. Total seed weight reductions due to PSbMV were attributed to reductions in the individual seed weight rather than a reduction in seed quantity. In contrast to reductions in seed weight, diseased plants of some cultivars displayed increases in seed quantity and potentially could be due to delayed plant maturity caused by PSbMV infection. Delaying plant maturity results in the pea plant continuing to flower, develop pods and produce more seeds. In the results reported here generated under greenhouse conditions, all seeds produced, regardless of size, were included in yield calculations. Under commercial field conditions, yield losses due to PSbMV would likely be greater. Field peas raised in the upper Midwest are often desiccated or harvested when the

majority of the field reaches maturity, if plant maturity is not uniform. These practices do not allow for diseased plants to continue to flower and produce seed. In addition, shrunken seeds from diseased plants can easily fall through the sieves of combine and be eliminated as waste. It is possible that cultivars exhibiting signs of tolerance in the greenhouse may not be observed as tolerant in the field. Nette 2010 for example had minimal seed weight reductions; however, there was a significant drop in 100-seed weight under PSbMV infection. This diminished 100-seed weight may be the result of small seeds that would easily sieve out the back of a combine in commercial production. Diseased seeds often have cracked seed coats, scarring, and discoloration, which can result in dockage and a reduction in payouts to the growers.

PSbMV transmission to progeny plants through seed is affected by a number of variables including cultivar, infection timing, and viral pathotype. The twenty cultivars in this study were inoculated with the same PSbMV isolate and inoculation occurred at the same time and allowed for PSbMV seed transmission. With these variables constant, variation in cultivar could be measured. The low seed transmission rate of PSbMV in cultivars Arcadia, AC Agassiz, Bluemoon and K2 also could be a result of the host genetics (Wang and Maule 1994). A combination of reduced virus titres due to virus-infected plant recovery, and a barrier in the suspensor of the seed may be responsible for the significantly lower seed transmission rates of PSbMV (Wang and Maule 1994; Bengyell et al. 2015). Suspensor's primary function is to provide a pathway for nutrition and growth regulators to embryo of the seed as well as attach it to the seed coat of the seed (Schwartz et al. 1997). The mechanism of tolerance to PSbMV and reduction in seed transmission rates of PSbMV have yet to be studied.

The presence of PSbMV seed symptoms did not prove to be an indicator of whether the seed contained the virus in most cultivars. On average across all cultivars, 16% of asymptomatic

seeds contained PSbMV whereas symptomatic seeds contained 20%. Given this information, culling symptomatic seed would not be an effective method to eliminating PSbMV from a seed lot since PSbMV can persist asymptotically in the seed. Conversely, this is the first report to our knowledge to examine PSbMV seed transmission rates in numerous cultivars and observe significantly different seed transmission rates across cultivars. As indicated for infection frequency, cultivar appears to play a role in this aspect of the disease as well.

This risk assessment concept was applied to the field pea:PSbMV pathosystem to develop a preliminary risk assessment encompassing infection frequency, seed transmission, and seed weight across all cultivars to efficiently summarize the risk associated with growing the particular field pea cultivar when PSbMV is present. In the risk analysis, seed weight is most heavily weighted (50%), followed by seed transmission (30%) and cultivar susceptibility (20%) based on perceived importance of these factors to commercial field pea growers. This risk analysis could be customized for an operation raising certified seed. These modifications would involve increasing the weight of seed transmission and susceptibility and reducing the risk analysis scale weight of seed weight.

Cultivars Bluemoon, Daytona, K2, Viper, Bridger, CDC Treasure, Hyline, SW Midas, Spider and Vegas were all classified as high risk due to the high seed weight losses, transmission to progeny plants through seed and susceptibility. When growing these cultivars, care should be taken to ensure seed stock planted is PSbMV free and should not be planted in an area where PSbMV historically has been identified. Cultivars CDC Striker, Ginny, Greenwood, AC Agassiz, DS Admiral, Nette 2010 and Salamanca were classified as intermediate risk but were all highly susceptible to PSbMV, with smaller seed weight reductions and diminished seed transmission rates. Cultivar Nette 2010 had small seed weight losses, despite high susceptibility to PSbMV

infection, which is characteristic of a cultivar exhibiting disease tolerance. Care should be taken when planting a PSbMV tolerant cultivar as it can serve as a reservoir for the virus and endanger neighboring field pea fields. Cultivars Aragorn and Cruiser both were resistant to PSbMV ND14-1 and should likewise be resistant to any other P1 or P4 pathotypes of PSbMV.

This is the first study that has examined the effect of a specific pathovar of PSbMV on numerous cultivars and closely analyzed several plant, seed and transmission parameters by cultivar. Furthermore, a risk assessment of PSbMV in specific cultivars had not been developed. Field pea cultivars were confirmed with resistance to PSbMV isolate ND14-1 and one cultivar was identified with partial resistance and tolerance. Cultivar plays a significant role in yield losses due to PSbMV infection and seed transmission of the virus. The susceptibility of a cultivar to PSbMV, yield losses due to PSbMV infection and the seed transmission of the virus all contribute to the risk of growing field peas when the virus is present. The risk analysis of each cultivar can be an important tool for growers when making planting decisions or by breeders when releasing field pea varieties.

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Literature Cited

- Aapola, A. A., Knesek, J. E., and Mink, G. I. 1974. The influence of inoculation procedure on the host range of *pea seed-borne mosaic virus*. *Phytopathol* 64:1003-1006.
- Ali, A., and Randles, J. W. 1997. Early season survey of pea viruses in Pakistan and the detection of two new pathotypes of *pea seed-borne mosaic* potyvirus. *Plant Dis.* 81:343-347.
- Ali, A., and Randles, J. W. 1998. The effects of two pathotypes of *pea seed-borne mosaic virus* on the morphology and yield of pea. *Australas Plant Pathol.* 27:226-233.
- Ali-Khan, S. T., and Zimmer, R. C. 1979. Screening of field pea breeding lines for *Pea Seed-borne Mosaic Virus*. *Can J Plant Sci.* 59:171-175.
- Alconero, R., Provvidenti, R., and Gonsalves, D. 1986. Three pea seedborne mosaic virus pathotypes from pea and lentil germ plasm. *Plant Dis.* 70:783-786.
- Bashir, M., Azeem, T., Ali, A., and Ijaz, M. 2005. Variation in lentil germplasm for reaction to virus infection. *Pakistan J Bot.* 37:53-60.
- Beck, A. L., Simons K. J., and Pasche, J. S. 2018. Occurrence of Pea seed-borne mosaic virus in Field peas in North Dakota. *Plant Dis.* 102:1466.
- Bengyella, L., Waikhom S. D., Allie, F., and Rey, C. 2015. Virus tolerance, and recovery from viral induced-symptoms in plants are associated with transcriptome reprogramming. *Plant Molec. Biol.* 89:243-252.
- Brown, S. L., Culbreath, A. K., Todd, J. W., Gorbett, D. W., Baldwin, J. A., and Beasley, J. P. 2005. Development of a method of risk assessment to facilitate integrated management of spotted wilt of peanut. *Plant Dis.* 89:348-356.

- Chiko, A. W. and Zimmer, R. C. 1978. Effect of pea seed-borne mosaic virus on two cultivars of field pea grown in Manitoba. *Can J Plant Sci.* 58:1073-1080.
- Congdon, B. S. 2017. Understanding, forecasting and managing Pea seed-borne mosaic virus in field pea (Published Dissertation). University of Western Australia, Australia.
- Congdon, B. S., Coutts, B. A., Jones, R. A. C., and Renton, M. 2017. Forecasting model for *Pea seed-borne mosaic virus* epidemics in field pea crops in a Mediterranean-type environment. *Virus Res.* 241:163-171.
- Congdon, B. S., Renton, M., Banovic, M., and Jones, R. A. C. 2016a. *Pea seed-borne mosaic virus* in field pea: widespread infection, genetic diversity and resistance gene effectiveness. *Plant Dis.* 100:2475-2482.
- Congdon, B. S., Coutts, B. A., Renton, M., and Jones, R. A. C. 2016b. Pea seed-borne mosaic virus: stability and wind-mediated contact transmission in field pea. *Plant Dis.* 100:953-958.
- Coutts, B. A., Prince, R. T., and Jones, R. A. C. 2008. Further studies on *Pea seed-borne mosaic virus* in cool-season crop legumes: responses to infection and seed quality defects. *Aust J Agr Res.* 59:1130-1145.
- Coutts, B. A., Prince, R. T., and Jones, R. A. C. 2009. Quantifying effects of seedborne inoculum on virus spread, yield losses, and seed infection in the Pea seed-borne mosaic virus-field pea pathosystem. *Phytopathol* 99:1156-1167.
- EPPO, 2014. PQR database. Paris, France: European and Mediterranean Plant Protection Organization. <http://www.eppo.int/DATABASES/pqr/pqr.htm>
- EPPO, 2015. PM 7/125 (1) ELISA tests for viruses. *OEPP/EPPO Bulletin.* 45(3):445-449.

- Gao, Z., Johansen, E., Eyers, S., Thomas, C. L., Ellis, N., and Maule, A. 2004a. The potyvirus recessive resistance gene, *sbm1*, identifies a novel role for translation initiation factor eIF4E in cell-to-cell trafficking. *The Plant J.* 40:376-385.
- Gao, Z., Eyers, S., Thomas, C., Ellis, N. and Maule, A. 2004b. Identification of markers tightly linked to *sbm* recessive genes for resistance to *Pea seed-borne mosaic virus*. *Theor Appl Genet.* 109:488-494.
- Gasura, E. 2008. Mechanisms associated with sweet potato virus disease resistance in Ugandan sweet potato genotypes. (Published Masters Thesis) Makerere University. Kampala, Uganda.
- Gasura, E., Mashingaidze, A. B., and Mukasa, S. B. 2009. Occurrence, prevalence and implications of sweet potato recovery from sweetpotato virus disease in Uganda. *African Crop Science Conference Proceedings* 9:601-608.
- Hampton, R. O., and Baggett, J. R. 1970. Host effects and diagnostic symptoms of pea fizzle top disease. *Plant Dis Rep.* 54:355-358.
- Hampton, R. O., and Mink, G. I. 1975. *Pea seed-borne mosaic virus*. CMI/AAB Descriptions of Plant Viruses. #146.
- Hampton, R. O. 1980. Pea seed borne mosaic symptom variation among *Pisum* plant introduction accessions: Expressions and variation in symptom expression among different genotypes of *Pisum* and pathological implications. *Pisum Newsl.* 12:29-30.
- Hjulsager, C. K., Lund, O. S., and Johansen, E. I. 2002. A new pathotype of *Pea seedborne mosaic virus* explained by properties of the P3-6k1 and viral genome-linked protein (VPg)-coding regions. *Mol Plant Microbe In.* 15:169-171.

- Kasimor, K., Baggett, J. R., and Hampton, R. O. 1997. Pea cultivar susceptibility and inheritance of resistance to the lentil strain (Pathotype P2) of *Pea seedborne mosaic virus*. J Amer Soc Hort Sci. 122:325-328.
- Knesek, J. E., Mink, G. I., and Hampton, R. O. 1974. Purification and properties of *pea seed-borne mosaic virus*. Phytopathol 64:1076-1081.
- Konecna, E., Safarova, D., Navratil, M., Hanacek, P., Coyne, C. et al., 2014. Geographical gradient of the eIF4E alleles conferring resistance to potyviruses in pea (*Pisum*) germplasm PLoS ONE 9(3): e90394. DOI: 10.1371/journal.pone.0090394.
- Kvicala B. A., and Musil, M. 1967. Transmission of pea leaf rolling virus by aphids. Biologia (Bratislava). 22:10-16.
- Lundsgaard, T. 1981. *Pea seedborne mosaic virus* isolated from broad bean (*Vicia faba* L.) in Denmark. Acta Agriculturae Scan. 31:116-122.
- Makkouk, K. M., Kumari, S. G., van Leur, J. A. G., and Jones, R. A. C. 2014. Control of Plant Virus Diseases in Cool-Season Grain Legume Crops. Adv Virus Res. 90:207-253.
- Mink, G. I., Kraft, J., Knesek J., Jafrić, A. 1969. A seed-borne virus of pea. Phytopathol 59:1342-1343.
- Mink, G. I., Inouye, T., Hampton, R. O., and Knesek, J. E. 1974. Relationships among isolates of *pea seed-borne mosaic virus* from the United States and Japan. Phytopathol 64:569-570.
- Pirone, T., and Harris, K., 1977. Nonpersistent transmission of plant viruses by aphids. Ann. Rev. Phytopathol 15:55-73.
- Provvidenti, R., and Alconero, R. 1988. Inheritance of resistance to a lentil strain of *Pea seed-borne mosaic virus* in *Pisum sativum*. J Hered. 79:45-47.

- Safarova, D., Brazda, P., and Navratil, M. 2014. Effect of artificial dsRNA on infection of pea plants by Pea seed-borne mosaic virus. *Czech J Genet Plant Breed.* 50:105-108.
- Schwartz, B. W., Vernon, D. M., and Meinke, D. W. 1997. Development of the Suspensor: Differentiation, Communication, and Programmed Cell Death During Plant Embryogenesis. In: Larkins B.A., Vasil I.K. (eds) *Cellular and Molecular Biology of Plant Seed Development. Advances in Cellular and Molecular Biology of Plants*, vol 4. Springer, Dordrecht.
- Thottappilly, G. E., and Schmutter H. 1968. Zur Kenntnis eines mechanisch Samen-, Pilz- and Insektenubertragbaren neuen Virus der Erbse. *Z. Pflanzenkr. Pflanzen pathol. Pflanzensch.* 75:1-8.
- Torok, V. A., and Randles, J. W. 2007. Discriminating between isolates of PSbMV using nucleotide sequence polymorphisms in the HC-Pro coding region. *Plant Dis.* 91:490-496.
- van Leur, J., Kumari, S., G., Aftab, M., Leonforte, A., and Moore, S. 2013. Virus resistance of Australian pea (*Pisum sativum*) cultivars. *New Zeal J Crop Hort.* 41:2:86-101.
- Wang, D. and Maule, A. J. 1994. A model for seed transmission of a plant virus: genetic and structural analyses of pea embryo invasion by *pea seed-borne mosaic virus*. *Plant Cell.* 6:777-787.
- Wang, D. Hayes, I. M., and Maule, A. J. 1992. Procedures for the efficient purification of *pea seed-borne mosaic virus* and its genomic RNA. *J Virol Methods.* 36:223-230.

CHAPTER 2: EVALUATION OF BACTERICIDES FOR THE MANAGEMENT OF COMMON BACTERIAL BLIGHT IN DRY BEANS AND TOOLS TO ASSESS DISEASE SEVERITY

Abstract

Dry edible beans are an important industry in the United States. In 2017, North Dakota led US production with approximately 34% of total US dry edible bean production. Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), is an important bacterial disease reported in over 75% of North Dakota fields from 2012 to 2017. Yield losses due to CBB in dry beans have been reported in excess of 40% under favorable conditions. Copper bactericide products are used for the management of CBB in dry beans; however, efficacy has been inconsistent. In this study, eleven products including copper bactericides, plant growth promoters, and anti-microbial sanitizers were evaluated for the management of CBB in dry beans. Select copper products and antimicrobials consistently reduced CBB disease severity; however, significant yield responses were not observed. Fractional green canopy cover (FGCC) and leaf area index (LAI) were not correlated to CBB disease severity or yield.

Introduction

North Dakota contributes approximately 34% to US dry edible bean production annually (USDA-NASS 2018). Dry bean yields in North Dakota are compromised by several diseases, including those caused by bacterial pathogens that typically present as a complex. Common bacterial blight (CBB) is caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) (syn. *Xanthomonas campestris* pv. *phaseoli*) and *Xanthomonas fuscans* subsp. *fuscans* (*Xff*) (syn. *Xanthomonas phaseoli* var. *fuscans*) which infect foliar tissues of the dry bean and eventually the pods and seeds (Bett and Banniza, 2014; Gillard et al., 2009). In a survey conducted in Central

Wisconsin in 2005 and 2006, 98% of CBB in dark red kidney beans was caused by *Xap* (Duncan et al., 2011). While no extensive surveys have been conducted to evaluate the frequency of these CBB-causing pathogens in North Dakota, the evaluation of occasional field samples indicated that *Xff* is present at a low frequency when compared to *Xap* (Lamppa, personal communication). In 2017, dry bean growers surveyed in the Northharvest region (Eastern North Dakota and Minnesota) reported CBB as the second most important disease (Knodel et al., 2017). Yield losses up to 40% have been reported due to *Xap* infection, but were dependent upon disease severity, environment, timing of infection and dry bean cultivar (Opio et al., 1996; Tafera, 2006). Brown spot, caused by *Pseudomonas syringae* pv. *syringae* (*Pss*), and halo blight, caused by *Pseudomonas syringae* pv. *phaseolicola* (*Psp*) are less frequently observed in North Dakota and elsewhere in the US, but can be important when the environment is conducive.

CBB is characterized by water soaked-lesions that turn necrotic and are surrounded by chlorotic tissue. Lesions expand and coalesce, leading to a loss in photosynthetic leaf area and ground cover. Infected pods display round, water-soaked lesions that eventually become a dark red brown and are sunken in appearance. Infected seeds can be either asymptomatic or have slight discoloration, brown spots and be shrunken (Schwartz et al., 2005) (Figure 2.1). Symptoms of brown spot consist of small necrotic lesions that coalesce as disease progresses (Figure 2.2). Symptoms of halo blight include small necrotic pinpoint lesions with large, light green chlorotic rings (halo) resulting from phaseolotoxin, a chlorosis-inducing phytotoxin produced by the bacterium (Bender et al., 1999) (Figure 2.3). *Pss* and *Psp* also cause pod and seed infections under favorable conditions and are similar in appearance to those caused by *Xap* (Goodwin, 1992; Schwartz et al., 2005).



Figure 2.1. Common bacterial blight (CBB) symptoms on dry beans leaves and pods



Figure 2.2. Brown spot symptoms on foliar dry bean tissue



Figure 2.3. Halo blight symptoms on foliar dry bean tissue

Primary sources of bacterial inoculum include infected plant debris or seed. *Xap*, *Xff*, *Pss*, and *Psp* also have the ability to persist epiphytically on the surface of bean leaves (Belete and Bastas, 2017; Gent et al., 2005; Schwartz et al., 2005). These bacterial pathogens enter the plant through injuries from wind, rain, or hail and through natural openings on the plant. CBB and brown spot are favored by warm temperatures ranging from 28 to 32°C. In contrast, halo blight is favored by cooler temperatures ranging from 16 to 24°C (Schwartz et al., 2005). Secondary spread occurs via wind, rain splash, and water droplets that can carry the bacteria to neighboring

plants (Bett and Banniza, 2014; Harveson, 2009). In cotton, rain splash combined with wind, spread *Xanthomonas campestris* pv. *malvacearum* up to 5.5 meters from the inoculum source (Faulwetter, 1917). *Xap*, *Xff*, *Pss*, and *Psp* can persist systemically within the xylem of the plant allowing for seed infection through the funiculus. In addition, infection of the seed can occur when the developing seed is exposed to the bacterium through the pod wall (Goodwin 1992; Zaumeyer and Thomas, 1957). While some yield loss is attributed to pod infection, most is attributed to the large, coalescing lesions caused by bacterial infection, resulting in premature defoliation (Goodwin, 1992; Osdaghi et al., 2009).

Management practices for CBB, halo blight, and brown spot include host resistance, cultural and chemical methods. Some cultural methods include a three year crop rotation, planting certified disease free seed and not re-using irrigation water. At this time, the most effective tool for growers to utilize for the management of CBB is the use of cultivars with partial resistance. Under moderate to high CBB pressure, resistant cultivars have on average displayed a 23% yield advantage over susceptible dry bean cultivars (Gillard et al., 2009). Streptomycin is labelled as a seed treatment for bacterial blight, but is not commonly used. Foliar applications of copper products have been used to manage CBB; however, the level of control has been inconsistent (Harveson, 2009). Foliar applications of copper products have been demonstrated to be more efficacious for the management of halo blight and brown spot than for CBB (Schwartz 2011).

Plant traits such as fractional green canopy cover (FGCC) and leaf area index (LAI) have been used to assess plant health. FGCC measures the percentage of green vegetation in an image and has been used to quantify defoliation in cotton (Alchanatis et al., 2000). FGCC can be measured using Canopeo, a free smartphone application (Patrignani and Ochsner, 2015).

Canopeo FGCC was directly compared to visual disease severity ratings of *Verticillium* wilt of potato (Yellareddygari and Gudmestad, 2017). *Verticillium* wilt in potato, much like CBB in dry beans, can result in senescence of leaf tissue and reduction in ground cover.

LAI is defined as the leaf surface area per area of ground cover and is used to quantify foliar biomass, which directly relates to the photosynthetic capacity of the plant. In dry beans, LAI correlated with yield under varying levels of water stress, as drought has a significant impact on LAI and plant biomass (Gallegos and Shibata, 1989). Yield prediction models in corn have been successfully developed based on LAI readings (Baez-Gonzalez et al., 2005). In wheat, LAI was not accurate as a stand-alone variable for the prediction of yield, but improved the accuracy of a crop yield prediction model when it was incorporated (Dente et al., 2008). LAI can be measured using direct or indirect methods. Direct, or destructive, LAI measurement involves removing all the leaf tissue from plants and scanning with a planimeter to measure the total leaf surface. Indirect LAI is based on the amount of light transmitted and scattered within the canopy, and does not require the destruction of the plant canopy and is measured by a hand held device (Jonckheere et al., 2004). FGCC and LAI values are not independent measurements and care should be taken when used in conjunction to model yield (Carlson et al. 1997; Nielson et al. 2012).

Copper-based bactericides have been used preventively for the management of brown spot and halo blight; however, the management of CBB through the use of cupric bactericides has been relatively inconsistent (Schwartz et al., 2011). Little is known about the efficacy of other anti-microbial, new copper based and plant-health stimulating products in managing CBB. Copper hydroxide, copper sulfate pentahydrate and oxychloride are bactericides used for the management of CBB, halo blight and brown spot and are classified in Fungicide Resistance

Action Committee (FRAC) group M1. M1 bactericides Kocide 3000 (Copper hydroxide 46.1 % a.i.; DuPont, Wilmington, DE), MasterCop (Copper sulfate pentahydrate 21.46% a.i.; ADAMA Agricultural Solutions, Raleigh, NC), Badge SC (Copper oxychloride 16.81% a.i. and Copper hydroxide 15.36% a.i.; Isagro, Morrisville, NC) and ET-F (Copper sulfate pentahydrate 19.8% a.i.; Earth Science Laboratories, Ithaca, NY) are labeled for the management of CBB, halo blight and brown spot. Goldshield (3-(trimethoxysilyl) propyl dimethyl octadecyl ammonium chloride 5% a.i.; AP Goldshield LLC, El Paso, TX), Sanidate 12.0 (Hydrogen peroxide 18.5% and Peroxyacetic acid 12.0% a.i.; BioSafe Systems LLC, East Hartford, CT) and Oxidate 2.0 (Hydrogen dioxide 27% a.i and Peroxyacetic acid 2.0%.; BioSafe Systems LLC, East Hartford, CT) have anti-microbial properties. Sanidate 12.0 and Oxidate 2.0 are labeled for the treatment of fruit and vegetables during processing to prevent the growth of micro-organisms that result in spoilage. WakeUp Summer (plant derived oils, alcohols, and colloidal micelles, a.i. concentration undisclosed; Renewable Farming LLC, Cedar Falls, IA) and eA300 (Alkanolamines, amino acids, nonionic surfactants, fatty acids and colloidal micelles, a.i. concentration undisclosed; EcoSoly Technologies LLC, Chesapeake, VA) promote plant growth and vigor. The objectives of this research were to evaluate the efficacy of cupric and biorational bactericides for the management of the bacterial blight complex in dry beans and to compare methods for evaluating foliar bacterial blight disease severity and determine if LAI and FGCC were directly related to yield.

Materials and Methods

Site Description and Experimental Design

Field trials were conducted in Oakes, ND in 2016 and 2017 and in Fargo, ND in 2017. All studies were performed using a randomized complete block design (RCBD) with four

replicates and twelve treatments, including a non-treated control. Pinto bean cultivar Stampede was seeded at all site-years. The target seeding rate was 220,000 seeds per hectare at all trial sites. In Fargo, beans were planted in 4 rows per plot with 38.1 cm row spacing and 4.58 m long with a drill planter on May 30, 2017. The 2017 Oakes trial was seeded with 4 rows per plot with 76.2 cm row spacing with a drill planter on June 6. The 2016 Oakes trial was seeded with 2 rows per plot 76.2 cm row spacing with a drill planter on May 17. All trials were planted within the time-frame for commercial bean planting in ND. Oakes trials were conducted with supplemental overhead irrigation. The trial conducted in Fargo did not receive supplemental water. All management practices were performed following commercial production standards for the region.

Product Applications and Field Evaluations

In all trials at 70 to 90% bloom, 80 grit abrasive garnet sand was applied to all sides of the dry bean rows, 0.6 meters above the canopy, with a 30 lb portable air sand blaster powered by an air compressor. Small, water soaked lesions could be visualized on the underside of dry bean leaves following sandblasting. All plants were drenched with a 1×10^8 cell suspension of *Xap* at approximately 20 gallons per acre within two hours of sandblasting. Sandblasting and inoculations were conducted in the evening when canopy humidity was high. In Oakes 2016 (July 22), relative humidity was 75% with an air temperature of 30.5°C at sandblasting, followed by an average relative humidity of 79% and an air temperature of 26.1°C for the next 24 hours. In Oakes 2017 (July 11), relative humidity was 59% with an average air temperature of 21.7°C, followed by an average relative humidity of 74% with an air temperature of 18.3°C in the next 24 h. In Fargo 2017 (July 14), relative humidity was 54% with an air temperature of 26.7°C at the time of inoculation, followed by an average relative humidity of 68% and an air temperature

of 21.7°C in the next 24 h. Forty-eight hours following inoculation, bactericidal products were applied to dry beans at 90% bloom (Table 2.1). Early product applications were applied at V3-V4 stage and again at 90% bloom. Products were applied using a backpack sprayer at 93.5 L ha⁻¹ with CO₂ propellant.

Table 2.1. Products, rates, active ingredients and application timing (month/day) of cupric and biorational bactericides in the 2016 and 2017 common bacterial blight (CBB) management trials.

Product Type	Product ^a	Active Ingredient	Rate	Crop Stage	Application Timing ^b					
					Oakes 2016		Fargo 2017		Oakes 2017	
Plant growth promoters	WakeUp Summer	Plant derived oils and alcohols	5 OZ/A	V3-V4	6/29	7/25	6/29	7/17	6/29	7/13
	eA300	Plant derived oils and alcohols	2.5 OZ/A							
Copper-based	Kocide 3000	Copper hydroxide	1.24 LB/A							
	MasterCop	Copper sulfate pentahydrate	1 PT/A							
	Badge SC	Copper hydroxide + Oxychloride	2 PT/A							
	ET-F	Copper sulfate pentahydrate	19.2 OZ/A							
Surface sterilizers	Goldshield	3-(trimethoxysilyl) propyl dimethyl octadecyl ammonium chloride	20%	90% Bloom	7/25	8/8	7/17	8/1	7/13	7/27
	SaniDate 12.0	Hydrogen peroxide (18.5%) + Peroxyacetic acid (12%)	2.56%							
	Oxidate 2.0	Hydrogen dioxide (27%) + Peroxyacetic acid (2%)	0.5%							
	OxiDate 2.0	Hydrogen dioxide (27%) + Peroxyacetic acid (2%)	1%							

^aGoldshield was applied only in trials conducted in 2016, ET-F was applied only in trials conducted in 2017.

^bEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. Late applications of Wake Up Summer and eA300 were conducted at 90% bloom and 14 days later. All other applications were conducted at 90% bloom and 14 days later.

Plant populations were determined by counting of the number of emerged bean plants in 3 meters in the center two rows of the plot when plants were at the V1-V2 crop stage. Visual evaluations of disease severity were conducted for CBB, halo blight, and brown spot just prior to inoculation at 70 to 90% bloom, 14, and 28 days later. Four individual plants were selected arbitrarily within each plot and disease severity was appraised as a percent leaf area affected on each individual plant and assessed at equal time intervals across all site-years. In addition to a visual disease assessment, Canopeo (OSU Plant and Soil Sciences and OSU App Center, Stillwater, OK, USA) and AccuPAR LP-80 (Decagon Devices Inc., Pullman, WA, USA) were used to assess plant health of FGCC and LAI, respectively. Four readings from Canopeo and AccuPAR LP-80 were taken in each plot simultaneously with visual disease ratings at 70-90% bloom, 14, and 28 days later. Canopeo measurements were taken 0.6 meters above the plant canopy. AccuPAR LP-80 measurements were taken 0.6 meters above the canopy and at the ground level to estimate LAI. Plants were harvested at maturity and seed yield was measured.

Statistical Analysis

All analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC). Area under the disease progress curve (AUDPC) was calculated from CBB disease severity across three evaluation dates in all site-years. FGCC and LAI across evaluation dates were reported as area under the Canopeo progress curve (AUCPC) and area under the LAI progress curve (AULPC). An analysis of variance (ANOVA) was used to compare AUDPC, AUCPC, AULPC and yield across treatments. The Waller-Duncan K-ratio t-tests was used to distinguish differences across treatments. Pearson's Correlation Analysis was used to compare AUDPC, AUCPC, AULPC and yield.

Results

No significant differences in plant establishment were observed in any site-year. In 2016, brown spot was observed in Oakes, with an average disease severity of 29.3% (data not shown). Less than 1% halo blight and brown spot was observed in the other trials (data not shown). Significant differences in visual disease severity, as measured by AUDPC, were observed across foliar treatments for all trials ($P < .0001$) (Table 2.2). Moderate disease severity was observed in the Fargo (26.7%) and Oakes (21.9%) in the 2017 trial when compared to the trial conducted in Oakes in 2016 (40%) (data not shown). In the trial conducted in Oakes in 2016 all treatments, with the exception of the early application of WakeUp Summer, resulted in significantly lower disease severity than the non-treated control (Table 2.2). The application of 1% Oxidate 2.0 resulted in the lowest disease severity, not statistically different than Kocide 3000 and 0.5% Oxidate 2.0. In Fargo 2017, applications of Kocide 3000, MasterCop, Badge SC, ET-F, Sanidate 12.0 and 1% Oxidate 2.0 significantly reduced disease severity compared to the non-treated control as measured by AUDPC. The application of ET-F resulted in the lowest disease severity. Copper products Kocide 3000 and MasterCop, and surface sanitizer Sanidate 12.0 performed similar to ET-F in this trial ($P < .0001$). In Oakes 2017, the application of Kocide 3000, MasterCop, and Sanidate 12.0 resulted in significant reductions in disease severity compared to the non-treated ($P < .0001$). However, disease severity in these treatments were not statistically different from Wakeup Summer, Badge SC, ET-F or Oxidate 2.0 at either rate (Table 2.2).

Table 2.2. Area under the disease progress curve (AUDPC) for common bacterial blight (CBB) across treatments in dry bean trials conducted in Fargo 2017 and Oakes 2016 and 2017.

Treatment ^a	2016		2017			
	Oakes		Fargo		Oakes	
Non-treated	420.6	a	616.9	ab	397.7	ab
WakeUp Summer (Early)	387.5	ab	607.3	ab	484.8	a
eA300 (Early)	373.8	b	722.3	a	399.9	ab
WakeUp Summer	385.4	b	560.9	abc	297.1	bcd
eA300	338.9	cde	500.5	bc	466.8	a
Kocide 3000	322.5	ef	295.3	de	217.4	d
MasterCop	331.6	de	277.4	de	210.9	d
Badge SC	376.6	b	393.8	cd	327.7	bcd
Goldshield	370.8	bc	-	-	-	-
ET-F	-	-	115.1	e	313.7	bcd
Sanidate 12.0	360.6	bcd	307.6	de	258.6	cd
Oxidate 2.0 (0.5%)	318.9	ef	439.3	bcd	370.1	abc
Oxidate 2.0 (1%)	295.0	f	310.2	d	326.8	bcd
Pr>F	<.0001		<.0001		<.0001	
CV	6.66		60.99		51.07	

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

^bCultivars sharing at least one letter in common in the same column are not significantly different based on Waller-Duncan K-ratio t-tests ($\alpha=0.05$).

The application of Kocide 3000 yielded the greatest FGCC AUCPC, as measured using Canopeo, across all treatments in both trials conducted in Oakes (Table 2.3). However, the FGCC was not significantly different than the non-treated in either of these trials. Only the early application of WakeUp Summer resulted in significantly lower AUCPC than the non-treated in the Oakes trial conducted in 2017 (Table 2.3). All other treatments were statistically similar to

the non-treated control. The application of 1% Oxidate 2.0 resulted in the highest AUCPC in the Fargo trial, but no significant differences were observed across treatments.

Table 2.3. Area under the Canopeo progress curve (AUCPC) of fractional green canopy cover (FGCC) across treatments in dry bean trials conducted in Fargo 2017 and Oakes 2016 and 2017.

Treatment ^a	2016		2017			
	Oakes		Fargo		Oakes	
Non-treated	2210.2	a	2048.5	a	2460.2	ab
WakeUp Summer (Early)	2167.3	a	2066.1	a	2344.2	c
eA300 (Early)	2200.8	a	2012.4	a	2424.2	bc
WakeUp Summer	2113.5	a	2006.7	a	2438.4	b
eA300	2168.9	a	2034.3	a	2413.1	bc
Kocide 3000	2251.2	a	2067.1	a	2549.6	a
MasterCop	2230.2	a	2072.3	a	2428.2	bc
Badge SC	2211.7	a	2070.7	a	2466.2	ab
Goldshield	2178.4	a				
ET-F	-		2073.8	a	2441.7	b
Sanidate 12.0	2213.9	a	2041.0	a	2448.6	b
Oxidate 2.0 (0.5%)	2146.9	a	1966.5	a	2472.2	ab
Oxidate 2.0 (1%)	2223.4	a	2266.5	a	2450.6	b
Pr>F	0.2772		0.4554		0.0210	
CV	6.44		14.17		5.29	

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

^bCultivars sharing at least one letter in common in the same column are not significantly different based on Waller-Duncan K-ratio t-tests ($\alpha=0.05$).

No differences were observed in LAI or yield in any trials (Tables 2.4 and 2.5). The application of all products numerically increased AULPC compared to the non-treated in the Oakes 2016 trial (Table 2.4). All treatments, except MasterCop increased AULPC in the Fargo trial conducted in 2017. The application of eA300, Kocide 3000, MasterCop, ET-F, Sanidate 12.0 and 1% Oxidate 2.0 resulted in numerically increased AULPC in the Oakes trial conducted in 2017. The application of Kocide 3000, Sanidate 12.0, and ET-F most consistently resulted in

positive yield responses (Table 2.5). ET-F performed best across both trials in 2017, increasing yield by 0.13 and 0.45 mT ha⁻¹ in Fargo and Oakes, respectively.

Table 2.4. Area under the leaf area index (LAI) progress curve (AULPC) across treatments in dry bean trials conducted in Fargo 2017 and Oakes 2016 and 2017.

Treatment ^a	2016		2017			
	Oakes		Fargo		Oakes	
Non-treated	76.9	a	61.3	a	98.8	a
WakeUp Summer (Early)	79.1	a	61.5	a	95.9	a
eA300 (Early)	86.4	a	65.2	a	96.6	a
WakeUp Summer	85.3	a	63.8	a	94.6	a
eA300	82.9	a	68.3	a	103.9	a
Kocide 3000	84.1	a	67.4	a	101.5	a
MasterCop	90.4	a	59.1	a	100.8	a
Badge SC	86.2	a	62.5	a	98.3	a
Goldshield	78.0	a	-		-	
ET-F	-		64.0	a	104.1	a
Sanidate 12.0	85.5	a	62.3	a	103.1	a
Oxidate 2.0 (0.5%)	81.3	a	60.5	a	91.7	a
Oxidate 2.0 (1%)	85.3	a	64.2	a	103.3	a
Pr>F	0.1985		0.2940		0.2056	
CV	16.24		15.61		14.11	

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

^bCultivars sharing at least one letter in common in the same column are not significantly different based on Waller-Duncan K-ratio t-tests ($\alpha=0.05$).

Table 2.5. Yield (mT/ha⁻¹) across treatments in dry bean trials conducted in Fargo 2017 and Oakes 2016 and 2017.

Treatment ^a	2016		2017			
	Oakes	Fargo	Oakes	Fargo		
Non-treated	3.31	a	1.92	a	2.51	a
WakeUp Summer (Early)	3.61	a	1.90	a	2.78	a
eA300 (Early)	3.54	a	1.74	a	2.81	a
WakeUp Summer	3.51	a	1.78	a	2.86	a
eA300	3.56	a	1.92	a	2.61	a
Kocide 3000	3.65	a	1.93	a	2.96	a
MasterCop	3.44	a	2.01	a	2.49	a
Badge SC	3.31	a	1.90	a	2.62	a
Goldshield	3.50	a	-		-	
ET-F	-		2.05	a	2.84	a
Sanidate 12.0	3.57	a	2.02	a	2.80	a
Oxidate 2.0 (0.5%)	3.38	a	1.86	a	2.61	a
Oxidate 2.0 (1%)	3.40	a	1.86	a	2.77	a
Pr>F	0.9616		0.7691		0.8482	
CV	10.69		11.81		14.67	

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

^bCultivars sharing at least one letter in common in the same column are not significantly different based on Waller-Duncan K-ratio t-tests ($\alpha=0.05$).

Significant correlations were identified across data parameters evaluated in all three trials (Table 2.6). In the trial conducted in Oakes in 2016, AULPC was significantly correlated with AUDPC ($R^2 = 0.43$; $P = 0.001$) and AUCPC ($R^2 = 0.19$; $P = 0.01$). In the Fargo trial, significant linear relationships were observed between yield and AUDPC ($R^2 = -0.53$; $P = 0.0005$) and AUCPC ($R^2 = 0.37$; $P = 0.01$). In the Oakes 2017 trial, a significant linear relationship was observed between AUDPC and AUCPC ($R^2 = -0.29$; $P < .0001$) (Table 2.6).

Table 2.6. Pearson's correlation analyses of variables in dry bean trials conducted in Fargo 2017 and Oakes 2016 and 2017.

Trial	Variable	AUCPC	AULPC	Yield
Oakes 2016	AUDPC	0.24	0.43**	-0.21
	AUCPC		0.19*	0.11
	AULPC			0.22
Fargo 2017	AUDPC	-0.13	0.02	-0.53**
	AUCPC		0.12	0.37*
	AULPC			0.24
Oakes 2017	AUDPC	-0.29***	-0.08	-0.03
	AUCPC		-0.01	-0.23
	AULPC			0.01

Statistically significant correlation *0.05 < P > 0.01; **0.01 < P > 0.0001; ***P < .0001

Discussion

A wide range of CBB disease severity was observed across site-years from relative low (20%) to high (61%). Several copper bactericides and surface sanitizers were successful in significantly reducing CBB disease severity when compared to the non-treated control. Growth promoting products were unsuccessful in significantly reducing CBB disease severity and did not consistently result in a positive yield response. Application of select copper products and surface sanitizers significantly reduced CBB disease pressure and consistently resulted in a positive yield response; however, the level of control was not great enough to elicit significant yield response. In dry beans, yield accumulates over time, and has been linked to the genetics of the plant, vigor and environment (Araujo and Teixeira, 2008; Fageria and Santos, 2008). It is possible that the infection timing of *Xap* in the field may play a pivotal role in determining if yield losses will occur. It has been demonstrated in field peas (*Pisum sativum* L.) that the infection timing of *Pseudomonas syringae* pv. *lisi* and *Pseudomonas syringae* pv. *syringae* plays a critical role in the amount of yield loss due to infection by the pathogen (Roberts, 1993). Field peas inoculated at the reproductive stage suffered a yield loss of 24%; however, when peas were inoculated at a

vegetative stage, an average yield loss of 47% was observed (Roberts, 1993). At this time, no research has been performed to assess the impact of infection timing of *Xap* on dry bean yield. In North Dakota, favorable conditions for *Xap* infection typically occurs mid-July and August, when dry beans are in the reproductive stage. These experiments were conducted to closely mimic this grower scenario, with inoculations occurring at 70 to 90% bloom.

Xap and *Xff* inoculum in dry beans comes from infected plant debris and infected seed. Reductions in disease severity did not result in significant yield increases; however, they may reduce the amount of inoculum in the field, subsequently reducing disease pressure the next cropping season. Evaluations of bacterial blight populations on seed harvested from these trials is ongoing. Reducing inoculum on debris and on seed may aid in reducing disease severity over time.

Two indirect methods to measure plant health attributes were evaluated as part of this research. Canopeo discerned significant differences in the percent green canopy across treatments in only of the trial conducted in Oakes in 2017. Additionally, a significant negative correlation was observed in the Oakes 2017 trial between visual disease severity, as measured by AUDPC, and FGCC as measured by AUCPC. *Xap* infection and CBB development is favored by higher humidity, which is greater in the lower portion of the plant canopy. CBB symptoms were first observed on leaves in the lower canopy, with infection of the newest growth occurring later in the season. Canopeo assesses the FGCC, diseased plant tissue within the canopy is masked by healthy tissue in the upper canopy, which likely contributed to the lack of significant differences across treatments. Canopeo may be better suited to estimate disease severity when CBB is observable in the upper canopy or for pathogens that cause rapid defoliation or plant senescence. Verticillium wilt in potatoes causes defoliation of the potato plant, making Canopeo a more

appropriate tool for assessing disease severity compared to CBB, where defoliation begins in the lower canopy and is undetectable unless the canopy is parted (Yellareddygari and Gudmestad, 2017). Canopeo likely was able to detect these differences due to the CBB symptoms in the upper plant canopy. Consistent correlations were not observed between AUCPC and yield, therefore Canopeo should not solely be used to predict yield of dry beans under CBB disease pressure. Ultimately, based on the results of these trials, disease epidemiology plays an important role in the accuracy of Canopeo as a disease prediction tool.

The AccuPAR LP80 also did not detect significant differences in LAI among treatments in these trials. Non-destructive LAI measures the amount of light transmitted through the plant canopy. While bacterial blight reduces the amount of photosynthetic leaf area, it does not necessarily reduce the total leaf area. Bacterial infection does not typically result in defoliation until disease severity is high, and that defoliation typically occurs in the lower canopy. Non-destructive LAI assessments may be more applicable under higher disease pressure, or in diseases where defoliation occurs earlier in disease development. In 2017, no correlations were observed between LAI and visual disease severity, FGCC, and yield; however, in Oakes 2016 significant correlations were observed between LAI and disease severity. As a standalone, based on the results from these trials, the AccuPAR LP-80 was unsuccessful in assessing CBB disease severity or yield as indicated by the lack of correlations. It is possible that LAI measurement using non-destructive methods may be less accurate than destructive methods. Non-destructive methods of assessing LAI cannot distinguish between leaves and other plant matter (i.e. stems and pods) and could also contribute to inaccuracy issues. In a study designed to build a model to predict yield of rainfed dry beans, LAI measured using a Sunfleck Ceptometer (Decagon; Pullman, WA, USA) did not correlate with yield and could not be used to predict yield (Amador-

Ramirez et al., 2007). LAI measured at a specific developmental stage is not a good indicator of yield since yield potential is something that accumulates over time (Amador-Ramirez et al., 2007). In this study, LAI was only measured after flowering; however, it is likely that some of the yield potential is accumulated in the vegetative stages in addition to after flowering.

To our knowledge, this is the first study that has examined the efficacy of alternative bactericidal products for the management of *Xap* in dry beans under non-irrigated/chemigated conditions. Furthermore, Canopeo and AccuPAR LP-80 had not been assessed as prediction tools for CBB disease severity and yield in dry beans. While some success was found with select copper products to significantly reduce CBB disease levels, none of the products provided a level of suppression to consistently result in a significantly positive yield response. Canopeo and AccuPAR LP-80 were not consistently correlated with CBB disease severity; however, these tools may be more successful in assessing the reaction between other host:pathosystems if the epidemiology fits with the specifications of both of these tools.

Literature Cited

- Amador-Ramirez, M. D., Acosta-Diaz, E., Medina-Garcia, G., and Guitierrez-Luna, R. 2007. An Empirical Model to Predict Yield Rainfed Dry Bean with Multi-Year Data. *Rev Fitotec Mex.* 30:311-319.
- Alchanatis, V., Navon, A., Glazer, I., and Levski, S. 2000. An image analysis system for measuring insect feeding effects caused by biopesticides. *J Agric Engng Res.* 77:289-296.
- Baez-Gonzalez, A. D., Kiniry, J. R., Maas, S. J., Tiscareno, M. L., Macias, J. C., Mendoza, J. L., Richardson, C. W., Salinas, J. G., and Manjarrez, J. R. 2005. Large-Area Maize Yield Forecasting Using Leaf Area Index Based Yield Model. *Agron J.* 97:418-425

- Beattie G. A., and Lindow S. E. 1995. The secret life of foliar bacterial pathogens on leaves. *Annu Rev Phytopathol* 33:145-172.
- Belete, T., and Bestes, K. K. 2017. Common Bacterial Blight (*Xanthomonas axonopodis* pv. *phaseoli*) of Beans with Special Focus on Ethiopian Condition. *J Plant Pathol Microbiol.* 8:403.
- Bender, C. L., Alarcon-Chaidez, F., and Gross, D. C. 1999. *Pseudomonas syringae* phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. *Microbiol Mol Biol Rev.* 63:266-292.
- Bett, K., and Banniza, S. 2014. Population study of *Xanthomonas* spp. From bean growing regions of Canada and response of bean cultivars to pathogen inoculation. *Can J Plant Pathol.* 36:341-353.
- Carlson, T. N., and Ripley, D. A. 1997. On the relation between NDVI, fractional vegetation cover, and leaf area index. *Remote Sens Environ.* 62:241-252.
- Cervantes, C., and Guitierrez-Corona, F. 1994. Copper resistance mechanisms in bacteria and fungi. *FEMS Microbiol Rev.* 14:121-137.
- Claflin, L. E., Vidaver, A. K., and Sasser, M. 1987. MXP, a semi-selective medium for *Xanthomonas campestris* pv. *phaseoli*. *Phytopathol* 77:730-734.
- Dente, L., Satalino, G., Mattia, F., and Rinaldi, M. 2008. Assimilation of leaf area index derived from ASAR and MERIS data into CERES-Wheat model to map wheat yield. *Remote Sens Environ.* 112:1395-1407.
- 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. *Phytopathol* 101:425-435.

- Faulwetter, R. C. 1917. Dissemination of the angular leaf spot of cotton. *J Agric Res.* 8:457-475.
- Gallegos, J. A., and Shibata, J. K. 1989. Effect of water stress on growth and yield of indeterminate dry-bean (*Phaseolus vulgaris*) cultivars. *Field Crops Res.* 20:81-93.
- Gent, D. H., Lang, J. M., and Schwartz H. F. 2005. Epiphytic survival of *Xanthomonas axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* on leguminous hosts and onion. *Plant Dis.* 89:558-564.
- Gillard, C. L., Connor, R. L., Howard, R. J., Pauls, K. P., Shaw, L., and Taran, B. 2009. The performance of dry bean cultivars with and without common bacterial blight resistance in field studies across Canada. *Can J Plant Sci.* 89:405-410.
- Goodwin, P. I. 1992. Effect of common bacterial blight on leaf photosynthesis of bean. *Can J Plant Path.* 14:203-206.
- Grimault, V., Olivier, V., Rolland, M., Darrasse, A., and Jacques, M. 2014. Detection of *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* on *Phaseolus vulgaris* (bean) seed. *International Rules for Seed Testing*, 7-021-2.
- Gutierrez-Rodrigues, M., Escalante-Estrada, J., Rodriguez-Gonzalez, M., and Reynolds, M. P. 2006. Canopy Reflectance Indices and its Relationship with Yield in Common bean Plants (*Phaseolus vulgaris* L.) with Phosphorous Supply. *Int J Agr Biol.* 2:203-207
- Harveson, R. M. 2009. Common bacterial blight of dry beans in Nebraska. *NebGuide*. University of Nebraska Extension Publication. G1956.
- He, Y. 2010. Improved seed health tests for *Xanthomonas axonopodis* pv. *phaseoli* in common bean. *Graduate Theses and Dissertations*. Iowa State University, Ames. 11565.

- Jonckheere, I., Fleck, S. Nackaerts, K., Muys, B. Coppin, P., Weiss, M., and Baret, F. 2004. Review of methods for in situ leaf area index determination Part I. Theories, sensors and hemispherical photography. *Agr Forest Meteorol.* 121:19-35
- 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.
- 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* pv. *fuscans* strains for bean genotypes with common blight resistance. *Plant Dis.* 92:546-554.
- Nielsen, D. C., Midceli-Garcia, J. J. and Lyon, D. J. 2012. Canopy cover and leaf area index relationships for wheat, triticale, and corn. *Agron. J.* 104:1569-1573.
- 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.
- Osdaghi, E., Alizadeh, A., Shams-Bakhsh, M., and Reza Lak, M. 2009. Evaluation of common bean lines for their reaction to the common bacterial blight pathogen. *Phytopathol Mediterr.* 48:461-468
- Patrignani, A., and Ochsner, T. E. 2015. Canopeo: A powerful new tool for measuring fractional green canopy cover. *Agron J.* 107:2312-2320.
- 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.
- Schwartz, H. F. 2011. Bacterial disease of beans. Colorado State University Extension. 2.913

Tafera, T. 2006. Effect of common bacterial blight severity on common bean yield. *Trop Sci.* 46:41-44.

USDA-NASS Quick Stats. 2018. United States Department of Agriculture National Agricultural Statistic Service. https://www.nass.usda.gov/Quick_Stats/

Yellareddygari, S. K. R., and Gudmestad, N. C. 2017. Bland-Altman comparison of two methods for assessing severity of *Verticillium* wilt of potato. *Crop Prot.* 101:68-75.

Zaumeyer, W. J., and Thomas, H. R. 1957. Bacterial diseases of major importance, p. 65-88. In: W. J. Zaumeyer and H. R. Thomas (eds.) *A monographic study of bean diseases and methods for their control.* U.S. Dept. Agr., Washington, D.C

CHAPTER 3: EVALUATION OF THE EFFECT OF COMMON BACTERIAL BLIGHT ON TWO COMMON BEAN ARCHITECTURE TYPES AND THE VALIDATION OF YIELD PREDICTION TOOLS

Abstract

North Dakota is an important supplier of common bean, leading US production with 258,311 hectares harvested in 2017. Common bacterial blight (CBB) has been observed in more than 75% of North Dakota fields surveyed over the past five years. CBB has been reported to cause yield losses in excess of 40% under favorable conditions. Recently, a shift has been observed in North Dakota towards dry beans with an upright growth habit (Type II) rather than a prostrate growth habit (Type III). Four dry bean cultivars, Stampede and Medicine Hat (Type II) and Maverick and Othello (Type III), were examined for differential yield response to CBB, and several methods were evaluated for correlation to visual disease severity ratings and yield. No significant yield losses were observed when comparing two levels of CBB in any cultivar, even across disease severity levels from 0 to 46%. The two Type II dry bean cultivars produced significantly larger yields than did the two Type III cultivars evaluated in this study, despite having lower leaf area index (LAI) values. Fractional green canopy cover significantly correlated with yield and CBB severity in all trials and with non-destructive LAI in 2017 trials; however, it lacked the sensitivity to discern differences in disease severity that were discernable in visual assessments. LAI did not correlate with visual disease ratings or yield. Results from this research provide valuable information concerning expected yield losses in Type II and Type III cultivars and the utility of CBB evaluation methods.

Introduction

Common bean (*Phaseolus vulgaris* L.) production is an important industry in the United States with 230,000 metric tons exported in 2017. North Dakota is the leading producer of common beans in the United States with 258,311 hectares harvested in 2017. Michigan ranks second with 89,198 hectares harvested followed by Minnesota (62,686) Idaho (24,888) and Washington (8,822) (USDA-NASS). Numerous market classes are produced in North Dakota; however, approximately 90% are pinto beans (Kandel, 2013).

Common beans have been categorized into four growth types. Type I includes determinate bush beans, Type II includes indeterminate, upright short vine beans, Type III includes indeterminate, prostrate vine beans and Type IV includes beans with an indeterminate, prostrate, strong vining growth habit. US common bean market classes are either architecture Type I, II or III (Singh, 1981). Type IV architecture type beans are typically wild common beans (*Phaseolus vulgaris* L.), pole bean, lima bean (*Phaseolus lunatus* L.) and runner bean (*Phaseolus coccineus* L.). A letter is used, under some circumstances, to provide further information about the growth type of a genotype. For example, growth Type IIb indicates the genotype is typically upright, but displays a prostrate growth type under certain environmental conditions. Increased yields in upright (Type II) bean cultivars has been attributed to taller plants capturing more sunlight and competing with weeds (Blackshaw et al., 1999). Prostrate (Type III) common bean cultivars, on average, produce more leaves than upright common beans; however, upright cultivars produce larger leaves (Trindale et al., 2010). Type III, prostrate common beans have exhibited the largest yield potential; however, they are generally more susceptible to diseases, due to limited airflow through the dense canopy structure (Kelly and Adams, 1987).

Foliar bacterial infections in common beans have the potential to cause substantial yield losses due to lesion development and premature defoliation when disease severity is high (Goodwin, 1992; Osdaghi et al., 2009). Common bacterial blight (CBB), brown spot and halo blight are the three major bacterial diseases observed on common beans (Schwartz et al., 2005). CBB is caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) (syn. *Xanthomonas campestris* pv. *phaseoli*) and *Xanthomonas fuscans* subsp. *fuscans* (*Xff*) (syn. *Xanthomonas phaseoli* var. *fuscans*). CBB symptoms begin as water soaked lesions. As the disease progresses, lesions expand and coalesce, become necrotic and develop a chlorotic halo. Symptoms also can be observed on the pods and seeds. *Xap* or *Xff* infected pods exhibit round, water soaked lesions that become sunken and necrotic. Bacteria can be observed oozing from the lesions under periods of high humidity. Infected seeds can become discolored and shrunken or the bacterium can persist on the seed asymptotically (Schwartz et al., 2005). Symptoms of brown spot, caused by *Pseudomonas syringae* pv. *syringae* (*Pss*), include small, circular lesions sometimes surrounded by a small chlorotic ring. These lesions coalesce between the leaf veins, forming necrotic strips as the disease progresses. Halo blight, caused by *Pseudomonas syringae* pv. *phaseolicola* (*Psp*), is characterized by small, pinpoint necrotic lesions with large, light green halo. Seed and pod symptoms of brown spot and halo blight are similar to CBB (Schwartz et al., 2005).

CBB, brown spot and halo blight have two primary sources of inoculum, seed and infected plant residue. *Xap*, *Xff*, *Psp*, and *Pss* possess the ability to persist epiphytically on the leaf surface of resistant and susceptible common bean cultivars. Under favorable conditions, the bacterium enters the plant via wounds or natural openings (Belete and Bastas, 2017; Gent et al., 2005; Schwartz et al., 2005). Most commonly, plant injuries are caused by wind driven soil, heavy rains, and hail. Secondary spread is facilitated by wind, rain splash and water droplets that

function as vessels for the movement of bacteria to neighboring plants, new leaf tissue and pods (Bett and Banniza, 2014; Harveson, 2009). Air temperature plays an important role in the proliferation of the bacterial pathogens. *Psp* is favored by cool temperatures ranging from 16 to 24°C. Conversely, *Pss*, *Xap* and *Xff* are favored by warmer conditions ranging from 28 to 32°C (Schwartz et al 2005). Bacterial seed infection in common beans occurs in two manners. Infection of the pods can result in the seed being exposed to the bacterium; however, it can also persist systemically within the xylem tissue; allowing for infection of the seed through the funiculus (Goodwin, 1992; Zaumeyer and Thomas, 1957).

Integrated pest management (IPM) is critical in the management of CBB, brown spot and halo blight including planting certified seed, incorporating a three year crop rotation to non-hosts of the bacteria and utilizing cultivars with partial resistance (Harveson, 2009). Chemical methods are available for the suppression of *Xap*, *Xff*, *Pss*, and *Psp*; however, their usage and success has been limited. Streptomycin seed treatments are registered to reduce bacteria on the seed coat but does not affect bacteria contained within the seed (Harveson, 2009).

Leaf area index (LAI) and fractional green canopy cover (FGCC) have been utilized by researchers to predict yield, quantify defoliation and assess disease severity. An LAI measurement is the area of a leaf surface are per area of ground cover (Gallegos and Shibata 1989). LAI can be assessed using non-destructive (gravimetric) or destructive (planimetric) techniques. Destructive LAI is determined by physically removing all leaves from a plant and measuring the total leaf surface area through the use of a planimeter such as the LI-3100 C Area Meter (LI-COR Inc, Lincoln, NE). The total leaf area is divided by the total area of ground covered by that plant based on the seeding rate/stand counts and row spacing (Jonckheere et al., 2004; Tewolde et al., 2005). Non-destructive LAI employs the use of a handheld device to

measure light scattered through the canopy and derives a LAI value (Jonckheere et al., 2004). LAI was used to evaluate water stress in common beans, where LAI and biomass were found to be significantly correlated to yield (Gallegos and Shibata, 1989). LAI has been used to generate yield prediction models for corn and cotton (Baez-Gonzalez et al., 2005; Su et al. 2015). In wheat, LAI was not effective as a standalone variable to predict yield, but when incorporated into the pre-existing yield model, it improved the accuracy (Dente et al., 2008). LAI was also strongly associated with green normalized difference vegetation index (GNDVI) (Gutierrez-Rodriguez et al., 2006).

FGCC is a measurement of the percentage of green vegetation. Green vegetation is a direct representation of the photosynthetic capacity of the plant and intrinsically tied to yield. FGCC has been utilized to determine the effect of numerous pests on plants and to assess rate of plant establishment. In cotton, FGCC was successfully used to assess the defoliation of cotton leaves due to feeding of lepidopterous larvae (Alchanatis et al., 2000). FGCC also was used to measure ground cover in a forest of scotch pine and norway spruce, but large variances rendered it ineffective for modeling purposes (Korhonen et al., 2006). In soybeans, new digital software which assesses FGCC was used successfully for the approximation of green and senescing tissue in the canopy and groundcover (Purcell, 2000). FGCC also was highly correlated with visual ratings of turf grass groundcover and senescing tissue (Karcher and Richardson, 2003; Richardson et al. 2001). FGCC correlated to Verticillium wilt in potato in separate trials (Yellareddygar and Gudmestad, 2017). Verticillium wilt in potato can result in defoliation and loss of ground cover. FGCC correlated with NDVI and LAI and observed in wheat, corn and triticale (Carlson and Ripley, 1997; Lati et al., 2011; Nielson et al., 2012). However, FGCC and

LAI are not independent and care should be taken when incorporating both into a yield prediction model (Carlson and Ripley, 1997; Nielson et al., 2012).

In recent years, US common bean growers have transitioned from raising prostrate cultivars to upright cultivars due to a reduction in time and expenses at harvest. At this time, no research has been done to assess if the yield response to bacterial blight differs by common bean architecture type. The objectives of this research were to determine the effect of bacterial blight on yield across plant architecture types and determine if crop health assessment tools are effective for extrapolating bacterial blight disease severity and predicting yield in common beans. To complete these objectives, four common bean cultivars, two Type II and two Type III, were evaluated under field conditions using visual bacterial blight ratings, destructive LAI, non-destructive LAI, and FGCC as tools for evaluating bacterial blight disease severity and their relationship to yield.

Materials and Methods

Site Description and Experimental Design

Field trials were conducted in Oakes, Fargo and Perham North Dakota in 2016 and 2017, near traditional common bean producing regions of the state. All trials were conducted as a split plot arrangement with six replicates. The main effect was the common bean cultivar. Four pinto bean cultivars. Stampede (IIb), Medicine Hat (IIb), Maverick (IIIa), and Othello (IIIa) were planted at all site-years. The sub-plot effect was disease level, non-inoculated/control and inoculated with *Xap*. Soybeans, a non-host of *Xap*, were seeded between each plot and replicate in both directions to minimize interplot interference. In Fargo, beans were seeded at a rate of 220,000 seeds per hectare with 4 rows per plot \times 38.1 cm row spacing on May 30, 2017. Trials conducted in Oakes were seeded with 2 and 4 rows per plot with 76.2 cm row spacing on May

17, 2016 and June 6, 2017, respectively. All trials were planted with a drill planter. Supplemental overhead irrigation was applied to trials conducted in Oakes. No supplemental irrigation was utilized in Fargo. All management practices were performed under commercial production standards for pinto beans.

Applications and Field Evaluations

In 2016 and 2017, the bactericide Kocide 3000 (copper hydroxide, 227.6 grams/ha DuPont, Wilmington, DE) was applied at 70 to 90% bloom, alternated every 7 days with Oxidate (hydrogen peroxide (27%) and peroxyacetic acid (2%), 1% V/V; BioSafe Systems LLC, East Hartford, CT) for six weeks in the non-inoculated/control plots. Products were applied using a backpack sprayer at 93.5 L ha⁻¹ using CO₂ propellant. In all trials, 80 grit abrasive garnet sand was applied to common beans in the non-treated/inoculated plots with a 30 lb portable air sand blaster to create small abrasions that could be visualized on the underside of the leaves at 70 to 90% bloom. Within two hours after sandblasting, plants were drenched with approximately 187 L ha⁻¹ of a 1×10⁸ cell suspension of *Xap* isolate ND15-1 collected in North Dakota in 2015. Sand blasting and inoculations were conducted in the evening when canopy humidity was high. Based on data from North Dakota Agricultural Weather Network (NDAWN) stations at each site, relative humidity was 75% with an air temperature of 30.5°C at sandblasting, in the trial conducted in Oakes in 2016. Average relative humidity of 79% and an air temperature of 26°C were recorded in the next 24 hours. In Oakes 2017, at sand blasting, relative humidity was 59% with an average air temperature of 22°C, followed by an average relative humidity of 74% with an air temperature of 18°C in the next 24 hours. In Fargo 2017, relative humidity was 54% with an air temperature of 27°C at the time of inoculation, followed by an average relative humidity of 68% and an air temperature of 22°C in the next 24 hours.

Visual assessments of CBB severity, halo blight and brown spot were conducted at 70 to 90% bloom (at inoculation), 14, and 28 days later. In addition to a visual assessment, Canopeo (OSU Plant and Soil Sciences and OSU App Center, Stillwater, OK, USA), LI-3100C Area Meter (LI-COR, Lincoln, NE, USA), and the AccuPAR LP-80 (Decagon Devices Inc., Pullman, WA, USA) were used to assess FGCC and LAI. Four readings from Canopeo and the AccuPAR LP-80 were taken in each plot simultaneously with disease severity ratings. Canopeo measurements were taken 0.61 meters above the plant canopy. For each LAI measurement using AccuPAR LP-80, an above canopy measurement with the light bar was taken at 0.61 meters, a below canopy measurement was taken with the bar was placed under the canopy on the ground. Destructive LAI was taken on 5 plants per plot. Leaves were removed, and total leaf area was calculated using LI-300C Area Meter. Destructive LAI was calculated as the sum of the leaf area of all leaves collected per plant divided by the space the plant occupied (determined based on plant stand and row spacing) (Tewolde et al., 2005). Harvest was conducted at plant maturity and yield was measured.

Statistical Analyses

All analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC). CBB disease severity at time points two and three, area under the disease progress curve (AUDPC), area under the canopeo/FGCC progress curve (AUCPC), area under the non-destructive LAI progress curve (AUNLPC), and area under the destructive LAI progress curve (AUDLPC), and yield were evaluated with an analysis of variance (ANOVA) and the Waller-Duncan K-ratio t-tests ($\alpha = 0.05$) was used to distinguish significant differences between treatment means within each cultivar. Levene's test of homogeneity was used to evaluate the variances between trials and architecture groups ($\alpha = 0.05$). When significant differences in variances were not observed,

the Waller-Duncan K-ratio t-test ($\alpha = 0.05$) was used to differentiate significant differences in means between the architecture groups. Spearman's correlation was used to evaluate if linear relationships existed among AUDPC, AUCPC, AUNLPC, AUDLPC, and yield and the change in CBB severity, AUDPC and yield.

Results

Trials were not combined due to non-homogeneous variances. Additionally, the 2016 trial was the only trial where cultivars could be grouped by architecture type for visual CBB severity; therefore, analyses were conducted individually for each trial, and across cultivars. No differences were observed in plant population in any trial (data not shown). CBB was observed visually in non-inoculated/control plots in all trials; however, significant differences in CBB progression (AUDPC) were observed between the non-inoculated/control and inoculated plots of each cultivar (Table 3.1). In the trial conducted in Fargo in 2017, average visual CBB severity in non-inoculated/control plots was 4.3% at 28 DAI compared to 33% in inoculated plots. Othello had the highest CBB severity (44.6%), followed by Medicine Hat (29.6%), Stampede (29.3%) and Maverick (28.8%). Visual CBB severity in Oakes 2017 was substantially lower than observed in the other two trials (Table 3.1). Average visual CBB severity was 8% in inoculated plots and 2% in non-inoculated/control plots 28 DAI. CBB severity was greatest in the upright cultivar Stampede (13.8%) followed by Medicine Hat (13.7%), Othello (11.8%) and Maverick (9.7%). In the trial conducted at Oakes in 2016, Othello was the most susceptible to CBB with an average disease severity of 48%. The second highest disease severity was observed on the prostrate cultivar Maverick with an average CBB disease severity of 44.2%. Substantially higher visual CBB severity was observed in non-inoculated/control plots in this trial. On average, CBB severity was 42% and 36% in inoculated and non-inoculated/control plots, respectively. No

significant differences in yield within cultivars were observed in any of the three trials (Table 3.1).

Table 3.1. Area under the disease progress curve (AUDPC), common bacterial blight (CBB) severity 14 days after inoculation (DAI), CBB severity 28 DAI and yield in dry bean field trials conducted in Fargo, North Dakota in 2017, and Oakes, North Dakota in 2016 and 2017

Trial	Architecture	Cultivar	Treatment	CBB severity		AUDPC	Yield			
				14 DAI	28 DAI					
Fargo 2017	Prostrate	Maverick	Inoculated	15.7	*	28.8	*	133.0	*	1317.8
			Control	1.4		3.3		35.3		1280.5
		Othello	Inoculated	36.5	*	44.6	*	274.5	*	1398.3
			Control	4.0		3.5		52.2		1311.3
	Upright	Medicine Hat	Inoculated	18.0	*	29.6	*	152.3	*	1103.7
			Control	1.6		4.9		37.9		1111.7
		Stampede	Inoculated	21.4	*	29.3	*	172.1	*	1358.3
			Control	1.6		5.5		40.5		1546.0
Oakes 2017	Prostrate	Maverick	Inoculated	3.6	*	9.7	*	28.8	*	4691.3
			Control	0.9		2.4		6.8		4587.0
		Othello	Inoculated	3.3	*	11.8	*	36.7	*	3142.0
			Control	0.0		0.8		7.8		3465.2
	Upright	Medicine Hat	Inoculated	8.0	*	13.7	*	60.1	*	4073.3
			Control	0.6		2.3		17.3		4237.7
		Stampede	Inoculated	3.6	*	13.8	*	27.0	*	4634.5
			Control	0.2		0.3		1.7		4829.8
Oakes 2016	Prostrate	Maverick	Inoculated	4.2		44.2	*	653.3	*	1333.8
			Control	2.0		36.7		561.2		1253.0
		Othello	Inoculated	9.3	*	48.0	*	764.2	*	1444.7
			Control	3.2		41.5		611.3		1496.5
	Upright	Medicine Hat	Inoculated	3.8		43.7		666.2	*	1265.8
			Control	2.7		40.3		604.3		1281.8
		Stampede	Inoculated	3.3	*	31.5	*	554.2	*	1503.3
			Control	1.2		26.0		460.8		1538.8

^aStatistical significance produced by t-test between inoculated and control (*) at $\alpha=0.05$

Pearson’s correlations revealed several significant relationships across parameters measured. In all three trials, CBB progression (AUDPC) was significantly correlated with Canopeo (AUCPC) (Table 3.2). CBB progression also was significantly correlated with yield in the trials conducted in Fargo in 2017 and Oakes in 2016, while Canopeo was significantly correlated to yield in all three trials. CBB progression was not correlated with LAI, either measured destructively, or using non-destructive methods. The non-destructive LAI method was correlated to Canopeo in both trials conducted in 2017 and yield in the trial conducted in Oakes in 2017 (Table 3.2). AUDLPC did not correlate with any other variable (Table 3.2).

Table 3.2. Pearson’s correlation analysis of area under the disease progress curve (AUDPC), area under the Canopeo progress curve (AUCPC), area under the destructive leaf area index (LAI) progress curve (AUDLPC), area under the non-destructive LAI progress curve (AUNLPC) and yield in dry bean field trials conducted in Fargo, North Dakota in 2017, and Oakes, North Dakota in 2016 and 2017.

Trial	Variable	AUCPC	AUDLPC	AUNLPC	Yield
Fargo 2017	AUDPC	-0.24**	-0.07	-0.04	-0.30*
	AUCPC		0.07	0.40***	0.44**
	AUDLPC			0.13	-0.05
	AUNLPC				0.28
Oakes 2017	AUDPC	-0.24**	0.05	-0.12	-0.22
	AUCPC		-0.05	0.51***	0.35*
	AUDLPC			-0.02	0.07
	AUNLPC				0.39*
Oakes 2016	AUDPC	-0.30*	-0.01	-0.24	-0.29*
	AUCPC		0.11	0.02	0.29*
	AUDLPC			0.07	0.08
	AUNLPC				-0.16

^aStatistically significant correlation *0.05<P>0.01; **0.01<P>0.001; ***P<.0001

Discussion

Four common bean cultivars from two architecture types were grown under low and high CBB disease pressure to determine if yield losses were affected by architecture type. Significant differences in CBB disease severity were observed between the inoculated and non-inoculated; however, this did not translate to significant differences in yield. Yield responses remained consistent and unaffected by the widely varying CBB severity observed across trials. Common bean yield accumulates over time and is intrinsically linked to plant genetics, vigor, and the environment (Araujo and Teixeira 2008; Fageria and Santos 2008). The lack of differences in yield in all three trials could be related to the infection timing of *Xap*. If infection had occurred earlier in the season, differences in yield may have been observed. It has been demonstrated that different yield losses could be anticipated in field peas (*Pisum sativum* L.) based on the infection timing of *Pseudomonas syringae* pv. *lisi* and *Pseudomonas syringae* pv. *syringae*, causal agents of bacterial blight (Roberts 1993). Field peas inoculated with these bacterial pathogens at the reproductive stage displayed a 24% reduction in yield, 47% reduction at the vegetative stage and a 71% yield reduction when inoculated at the vegetative and the reproductive stage (Roberts 1993). In previous trials examining the effect of CBB on dry bean yield, yield losses were observed when infection occurred 20 days after planting, but these losses were inconsistent across site years and locations (Gillard et al. 2009; Opio et al. 1996; Serracin et al. 1991). At this time, no research has been done to assess the effect of *Xap* inoculation timing on common bean yield. Inoculation timing in the trials described here was chosen to closely mimic when conditions are most favorable for *Xap* infection in North Dakota. The R2 stage of common bean development was chosen based on the corresponding environmental conditions, when high relative humidity and damaging thunderstorms are commonly observed. Significantly different

levels of CBB severity were successfully obtained across years and locations. However, despite significant differences in CBB severity across cultivars, no significant yield differences were observed. Two explanations are possible from these results. Infection earlier in the growing season may be necessary to realize yield losses due to *Xap* infection. Alternatively, cultivars evaluated in this study may exhibit tolerance to CBB. *Xap* is a ubiquitous pathogen and has been reported throughout the common bean growing regions in the United States. All of the cultivars examined in this study were developed in various locations in the United States and were almost certainly grown under CBB disease pressure. These cultivars may have inadvertently been selected for tolerance to *Xap* infection (pers. comm. Juan Osorno).

The 2017 trials were the only trials that could be grouped by architecture type in regards to AUDPC of CBB. Overall, AUDPC was greater in the prostrate architecture group than the upright; however, the differences was only significant in Oakes 2017. The Oakes 2017 trial had very low CBB disease severity and little difference between the non-inoculated and the inoculated treatment. When CBB pressure was greater, as illustrated in Oakes 2016 there were large variances in the reaction of the cultivars, thus they could not be combined by architecture group. The large discrepancies in reaction to CBB pressure is, therefore, the reaction of cultivars individually and not representative of the architecture group as a whole. Two of cultivars, one from each architecture group were consistently more susceptible to *Xap* infection than the other two cultivars. Medicine Hat and Othello were more susceptible to *Xap* infection whereas Stampede and Maverick consistently had lower disease severity than the aforementioned cultivars. These large differences between the cultivars susceptibility contributed to the large variances within each architecture group. Given these results, it would not be appropriate to conclude that one architecture type is more “susceptible” than the other to *Xap* infection but

rather dry bean cultivar selection should be based on individual cultivar susceptibility and performance rather than selection based on architecture type. Furthermore the yield response of both architecture groups to *Xap* infection was relatively unaffected with no significant differences observed from the non-inoculated control, leading to the conclusion that under CBB disease pressure, the upright dry bean architecture group is not more susceptible to yield losses than the prostrate dry bean architecture group.

Plant health assessment tools Canopeo (FGCC), LI-3100C Area Meter (non-destructive LAI) and AccuPAR LP-80 (destructive LAI) were evaluated to determine their potential as CBB assessment and yield prediction tools. Canopeo, which measures FGCC (reported here as AUCPC), was successful in the sense that it significantly correlated with visual CBB severity ratings in all trials. However, Canopeo lacked the sensitivity to detect significant differences in CBB severity between inoculated and non-inoculated plots based on visual ratings (data not shown). This could be due to CBB disease progression, which begins in the lower canopy and would not be discernable by FGCC. Given that CBB disease often slowly progresses through the canopy, FGCC may be better suited to estimate disease severity of a pathogen that causes a more rapid defoliation or plant senescence. One example is *Verticillium* wilt in potato, which can result in rapid defoliation and loss of ground cover and has been observed to correlate with FGCC (Yellareddygar and Gudmestad 2017). FGCC values were also observed to correlate with yield; however these correlations were low, and this parameter would likely not suffice alone in a yield prediction model.

Non-destructive LAI, measured by the AccuPAR LP-80, lacked the sensitivity to discern significant CBB severity differences between inoculated and non-inoculated subplots (data not shown) and did not correlate with visual CBB severity ratings. A significant correlation was

observed between non-destructive LAI and yield in the trial conducted in Oakes in 2017; however, this correlation was very low and was not observed in any of the other trials. Destructive LAI, measured by the LI-3100C Area Meter, much like non-destructive LAI, was unsuccessful in assessing CBB severity based on the lack of correlation between AUDLPC and visual CBB severity ratings. *Xap* infection, given the timing of infection likely did not cause defoliation before the natural senescence of the common bean plant; therefore, LAI was unable to discern differences between the CBB severity levels. As indicated with FGCC, LAI may be better suited to assess CBB severity and yield if infection occurred earlier in the season and results in defoliation before natural plant senescence or assess disease severity of a pathogen that can cause rapid defoliation and plant senescence. LAI was correlated to yield in common bean when water stress was evaluated across growth stages (Gallegos and Shibata 1989). While LAI could be correlated to dry bean yield, water stress can cause rapid leaf senescence and it is likely that the rapid leaf senescence impacted yield more so than the gradual CBB symptom development observed in the trials performed during this research. In another study, researchers sought to develop a dry bean yield prediction model based on LAI and were unsuccessful; however, these beans were not under any stress (Amador-Ramirez et al. 2007).

The plant traits FGCC and LAI varied when cultivars were group by architecture type. Across all trials, the prostrate architecture group possessed significantly greater FGCC than the upright architecture group. Interestingly LAI, measured in a destructive manner found a greater LAI in the upright architecture group compared to the prostrate architecture group. The difference was only significant under greater CBB disease pressure. In Oakes 2017, a significant difference was observed in FGCC via AUCPC yet CBB disease severity was low. The larger FGCC average in the prostrate architecture group compared to the upright, is likely a reflection

of the plant growth behavior which rapidly covers the ground rather than its response to CBB infection. The same trend of greater FGCC values in prostrate cultivars in Oakes 2017 that had 8% infection of *Xap* was observed in Oakes 2016 which had an average infection level of 70%. CBB infection did; however, cause defoliation in the lower canopy that was only discernable by measuring LAI and resulted in significant differences between architecture groups which was not observed in Oakes 2017 which had low CBB disease pressure. Given this trend, it was observed that the upright cultivars possess a greater LAI and may be better suited to tolerate defoliation due to CBB disease pressure.

To our knowledge, this is the first study to examine the effect of CBB on yield in different dry bean architecture types. Furthermore, Canopeo, AccuPAR LP-80 and the LI-3100 C had not been used to assess CBB severity and predict yield. No significant yield losses were observed with *Xap* infection occurring at the R2 stage of dry beans, despite significantly different CBB severity levels ranging from 3.5% to 45%. Dry bean yield of the four cultivars evaluated was not compromised by CBB levels observed in these trials, regardless of architecture type. These tools have been used successfully in evaluating other growth parameters. Canopeo had limited success in predicting yield; however, it lacked the sensitivity to detect subtle differences in CBB severity observed visually. AccuPAR LP-80 and LI-3100 C were unsuccessful at assessing CBB severity and predicting yield. Disease progression must be taken into consideration when relying on these tools for data collection.

Literature Cited

Alchanatis, V., Navon, A., Glazer, I., and Levski, S. 2000. An image analysis system for measuring insect feeding effects caused by biopesticides. *J Agric Engng Res.* 77:289-296

- Amador-Ramirez, M. D., Acosta-Diaz, E., Medina-Garcia, G., and Gutierrez-Luna, R. 2007. An empirical model to predict yield of rainfed dry bean with multi-year data. *Rev Fitotec Mex.* 30:311-319
- Araujo, A. P., and Teixeira, M. G. 2008. Relationships between grain yield and accumulation biomass, nitrogen, and phosphorus in common bean cultivars. *Brazilian J Soil Sci.* 32:1977-1986
- Baez-Gonzalez, A. D., Kiniry, J. R., Maas, S. J., Tiscareno, M. L., Macias, J. C., Mendoza, J. L., Richardson, C. W., Salinas, J. G., and Manjarrez, J. R. 2005. Large-area maize yield forecasting using leaf area index based yield model. *Agron J.* 97:418-425
- Belete, T., and Bestes, K. K. 2017. Common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) of beans with special focus on Ethiopian condition. *J Plant Pathol Microbiol.* 8:403.
- Bett, K., and Banniza, S. 2014. Population study of *Xanthomonas* spp. From bean growing regions of Canada and response of bean cultivars to pathogen inoculation. *Can J Plant Pathol.* 36:341-353.
- Carlson, T. N., and Ripley, D. A. 1997. On the relation between NDVI, fractional vegetation cover, and leaf area index. *Remote Sens Environ.* 62:241-252.
- Cervantes, C., and Guitierrez-Corona, F. 1994. Copper resistance mechanisms in bacteria and fungi. *FEMS Microbiol Rev.* 14:121-137.
- Dente, L., Satalino, G., Mattia, F., and Rinaldi, M. 2008. Assimilation of leaf area index derived from ASAR and MERIS data into CERES-Wheat model to map wheat yield. *Remote Sens Environ.* 112:1395-1407.

- Fageria, N. K., and Santos, B. A. 2008. Yield Physiology of Dry Bean. *J of Plant Nutrition*. 31:983-1004
- Gallegos, J. A. A., and Shibata, J. K. 1989. Effect of water stress on growth and yield of indeterminate dry-bean (*Phaseolus vulgaris*) cultivars. *Field Crops Res*. 20:81-83
- Gent, D. H., Lang, J. M., and Schwartz H. F. 2005. Epiphytic survival of *Xanthomonas axonopodis* pv. *allii* and *X. axonopodis* pv. *phaseoli* on leguminous hosts and onion. *Plant Dis*. 89:558-564.
- Gillard, C. L., Connor, R. L., Howard, R. J., Pauls, K. P., Shaw, L., and Taran, B. 2009. The performance of dry bean cultivars with and without common bacterial blight resistance in field studies across Canada. *Can J Plant Sci*. 89:405-410.
- Goodwin, P. I. 1992. Effect of common bacterial blight on leaf photosynthesis of bean. *Can J Plant Path*. 14:203-206.
- Gutierrez-Rodrigues, M., Escalante-Estrada, J., Rodriguez-Gonzalez, M., and Reynolds, M. P. 2006. Canopy reflectance indices and its relationship with yield in common bean plants (*Phaseolus vulgaris* L.) with phosphorous supply. *Int J of Agric Biol*. 2:203-207
- Harveson, R. M. 2009. Common bacterial blight of dry beans in Nebraska. *NebGuide*. University of Nebraska Extension Publication. G1956.
- Jonckheere, I., Fleck, S., Nackaerts, K., Muys, B., Coppin, P., Weiss, M., and Baret, F. 2004. Review of methods for in situ leaf area index determination Part I. Theories, sensors and hemispherical photography. *Agric For Meteorol*. 121:19-35
- Kandel, H. 2013. Common bean production guide. North Dakota State University Extension Service. A1133.

- Karcher, D. E., and Richardson, M. D. 2003. Quantifying turfgrass color using digital image analysis. *Crop Sci.* 43:943–951
- Korhonen, L. K. T., Rautiainen, M., and Stenberg, P. 2006. Estimation of forest canopy cover: A comparison of field measurement techniques. *Silva Fenn.* 40:577–588.
- Lati, R. N., Filin, S., and Eizenberg, H. 2011. Robust methods for measurement of leaf-cover area and biomass from image data. *Weed Sci.* 59:276–284
- Mensack, M. M., Fitzgerald, V. K., Ryan, E. P., Lewis, M. R., Thompson, H. J., and Brick, M. A. 2010. Evaluation of diversity among common beans (*Phaseolus vulgaris* L.) from two centers of domestication using ‘omics’ technologies. *BMC Genomics* 11:686
- Nielsen, D. C., Midceli-Garcia, J. J., and Lyon, D. J. 2012. Canopy cover and leaf area index relationships for wheat, triticale, and corn. *Agron. J.* 104:1569-1573.
- Osdaghi, E., Alizadeh, A., Shams-Bakhsh, M., and Reza Lak, M. 2009. Evaluation of common bean lines for their reaction to the common bacterial blight pathogen. *Phytopathol Mediterr.* 48:461-468
- Purcell, L. C. 2000. Soybean canopy coverage and light interception measurements using digital imagery. *Crop Sci.* 40:834-837.
- Richardson, M. D., Karcher, D. E., and Purcell, L. C. 2001. Quantifying turfgrass cover using digital image analysis. *Crop Sci.* 41:1884–1888.
- Roberts, S. J. 1993. Effect of bacterial blight (*Pseudomonas syringae* pv. *pisi*) on the growth and yield of single pea (*Pisum sativum*) plants under glasshouse conditions. *Plant Pathol.* 42:568–576
- 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

- Schwartz, H. F. 2011. Bacterial disease of beans. Colorado State University Extension. 2.913
- Su, L., Wang Q., Wang, C., and Shan, Y. 2015. Simulation models of leaf area index and yield for cotton grown with different soil conditioners. PLoS ONE 10(11): e0141835. doi:10.1371/journal.pone.0141835
- Tanaka, A., and Fujita, K. 1979. Growth, photosynthesis and yield components in relation to grain yield of the field bean. J of the Faculty of Agriculture Hokkaido University. 59:145-238.
- USDA-NASS Quick Stats. 2018. United States Department of Agriculture National Agricultural Statistic Service. https://www.nass.usda.gov/Quick_Stats/
- Voloudakis, A. E., Reignier, M. T., and Cooksey, D. A. 2005. Regulation of resistance to copper in *Xanthomonas axonopodis* pv. *vesicatoria*. Appl Environ Microbiol. 71:782-789
- Yellareddygari, S. K. R., and Gudmestad, N. C. 2017. Bland-Altman comparison of two methods for assessing severity of Verticillium wilt of potato. Crop Protect. 101:68-75.
- Zaumeyer, W. J., and Thomas, H. R. 1957. Bacterial diseases of major importance, p. 65-88. In: W. J. Zaumeyer and H. R. Thomas (eds.) A monographic study of bean diseases and methods for their control. U.S. Dept. Agr., Washington, D.C.

APPENDIX A: DETAILED FOLIAR BACTERICIDE TRIAL RESULTS

Table A.1. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (8/9) and 14 days after inoculation (8/23) in Fargo, North Dakota in 2016

Treatment	8/9/2016						8/23/2016					
	HB		CBB		BS		HB		CBB		BS	
Nontreated	0.0	a	1.8	a	1.3	a	0.0	a	39.0	a	4.8	a
Wakeup Summer (Early)	0.0	a	2.3	a	1.8	a	0.0	a	32.5	b	2.5	a
eA300 (Early)	0.0	a	2.5	a	1.3	a	0.0	a	29.5	bc	4.5	a
Kocide 3000	0.0	a	2.0	a	1.5	a	0.0	a	26.5	cd	4.5	a
MasterCop	0.0	a	2.5	a	1.5	a	0.0	a	28.0	cd	4.5	a
Badge SC	0.0	a	2.3	a	1.8	a	0.0	a	29.8	bc	4.3	a
Wakeup Summer	0.0	a	2.5	a	1.8	a	0.0	a	28.5	bcd	4.3	a
eA300	0.0	a	2.3	a	1.3	a	0.0	a	25.3	de	3.8	a
GoldShield	0.0	a	2.3	a	1.5	a	0.0	a	29.8	bc	4.3	a
Sanidate	0.0	a	1.8	a	1.8	a	0.0	a	24.5	de	4.8	a
Oxidate (2.0) 0.5%	0.0	a	2.0	a	1.8	a	0.0	a	25.3	de	4.0	a
Oxidate (2.0) 1%	0.0	a	2.0	a	1.5	a	0.0	a	21.5	e	4.3	a
Pr>F	.		0.570		0.935		.		<.0001		0.513	
CV	.		30.9		41.6		.		10.1		13.8	

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

Table A.2. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/25), 14 days after inoculation (DAI) (8/8) and 28 DAI (8/25) in Oakes, North Dakota in 2016

Treatment	7/25/2016			8/8/2016			8/25/2016		
	HB	CBB	BS	HB	CBB	BS	HB	CBB	BS
Nontreated	0.0 a	2.5 a	2.3 a	0.0 a	3.8 a	6.0 a	0.0 a	44.7 a	33.0 a
Wakeup Summer (Early)	0.0 a	2.8 a	1.3 a	0.0 a	2.3 abc	5.0 abc	0.0 a	44.8 ab	32.5 a
eA300 (Early)	0.0 a	3.3 a	1.5 a	0.0 a	3.0 ab	4.3 bcde	0.0 a	41.0 cd	31.0 a
Kocide 3000	0.0 a	2.5 a	1.3 a	0.0 a	1.3 cd	3.8 cde	0.0 a	38.3 ef	29.3 a
MasterCop	0.0 a	1.8 a	2.8 a	0.0 a	2.5 abc	3.3 e	0.0 a	37.8 ef	29.0 a
Badge SC	0.0 a	2.0 a	1.0 a	0.0 a	2.3 abc	4.3 bcde	0.0 a	44.0 b	32.8 abc
Wakeup Summer	0.0 a	2.5 a	2.0 a	0.0 a	3.0 ab	5.0 abc	0.0 a	43.3 cb	32.3 a
eA300	0.0 a	3.0 a	1.8 a	0.0 a	1.8 bcd	4.8 abcd	0.0 a	39.0 de	30.3 a
GoldShield	0.0 a	1.0 a	1.5 a	0.0 a	3.8 a	5.5 ab	0.0 a	41.3 cd	23.0 a
Sanidate	0.0 a	2.5 a	1.8 a	0.3 a	3.8 a	5.0 abc	0.0 a	38.5 ef	29.0 a
Oxidate (2.0) 0.5%	0.0 a	1.8 a	2.0 a	0.3 a	1.8 bcd	4.3 bcde	0.0 a	37.5 ef	21.5 a
Oxidate (2.0) 1%	0.0 a	2.0 a	1.5 a	0.5 a	0.5 d	3.5 de	0.0 a	36.5 f	28.0 a
Pr>F	.	0.508	0.446	-	0.006	0.007	.	<.0001	0.198
CV	.	55.5	56.2	-	48.2	20.7	.	3.9	21.0

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

Table A.3. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/20) and 14 days after inoculation (8/6) in Prosper, North Dakota in 2016

Treatment	7/20/2016						8/6/2016					
	HB		CBB		BS		HB		CBB		BS	
Nontreated	11.0	a	10.3	a	15.8	a	0.0	a	68.0	a	27.8	a
Wakeup Summer (Early)	9.0	a	9.0	a	15.3	a	0.0	a	64.3	ab	25.3	a
eA300 (Early)	8.3	a	10.8	a	16.0	a	0.0	a	60.5	b	24.5	a
Kocide 3000	9.5	a	8.8	a	15.8	a	0.0	a	53.8	c	21.8	a
MasterCop	9.8	a	10.5	a	13.8	a	0.0	a	61.5	b	22.3	a
Badge SC	10.3	a	9.0	a	15.5	a	0.0	a	63.5	ab	23.0	a
Wakeup Summer	9.8	a	10.3	a	14.8	a	0.0	a	63.3	ab	23.8	a
eA300	9.0	a	8.3	a	16.0	a	0.0	a	59.5	b	21.3	a
GoldShield	8.5	a	9.5	a	15.5	a	0.0	a	59.8	b	20.3	a
Sanidate	8.3	a	9.0	a	15.8	a	0.0	a	62.3	b	20.8	a
Oxidate (2.0) 0.5%	9.3	a	8.5	a	15.0	a	0.0	a	60.5	b	20.0	a
Oxidate (2.0) 1%	9.0	a	7.8	a	15.5	a	0.0	a	52.5	c	19.5	a
Pr>F	0.951		0.19		0.873		.		<.0001		0.1310	
CV	28.25		16.97		11.42		.		6.19		17.03	

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

Table A.4. Fractional green canopy cover (FGCC) and non-destructive leaf area index (NDLAI) at dry bean R2 growth stage (8/9) and 14 days after inoculation (8/23) in Fargo, North Dakota in 2016

Treatment	8/9/2016				8/23/2016			
	FGCC		NDLAI		FGCC		NDLAI	
Nontreated	92.2	a	3.0	a	38.8	a	1.2	a
Wakeup Summer (Early)	89.5	a	2.9	a	33.9	a	1.0	a
eA300 (Early)	90.9	a	2.7	a	27.2	a	0.9	a
Kocide 3000	90.8	a	3.1	a	32.0	a	1.1	a
MasterCop	90.3	a	2.8	a	32.2	a	1.0	a
Badge SC	90.9	a	2.9	a	35.2	a	1.3	a
Wakeup Summer	82.6	a	3.3	a	31.8	a	1.3	a
eA300	91.6	a	3.1	a	35.0	a	1.1	a
GoldShield	91.0	a	2.6	a	27.2	a	1.0	a
Sanidate	90.8	a	3.2	a	34.3	a	1.0	a
Oxidate (2.0) 0.5%	90.0	a	2.8	a	32.6	a	1.2	a
Oxidate (2.0) 1%	90.6	a	3.1	a	39.9	a	1.4	a
Pr>F	0.322		0.340		0.600		0.160	
CV	3.6		25.2		0.5		42.4	

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

Table A.5. Fractional green canopy cover (FGCC) and non-destructive leaf area index (NDLAI) at dry bean R2 growth stage (7/25), 14 days after inoculation (DAI) (8/8) and 28 DAI (8/25) in Oakes, North Dakota in 2016

Treatment	7/25/2016		8/8/2016		8/25/2016	
	FGCC	NDLAI	FGCC	NDLAI	FGCC	NDLAI
Nontreated	96.6	a 3.9	a 95.2	a 2.9	a 28.9	a 1.3
Wakeup Summer (Early)	96.8	a 4.5	a 95.5	a 2.7	a 29.6	a 1.4
eA300 (Early)	95.4	a 4.7	a 94.8	a 3.0	a 29.4	a 1.6
Kocide 3000	96.3	a 4.4	a 95.7	a 3.0	a 33.9	a 1.6
MasterCop	96.6	a 4.7	a 96.1	a 3.2	a 29.8	a 1.8
Badge SC	96.4	a 4.7	a 96.5	a 3.1	a 26.5	a 1.4
Wakeup Summer	96.8	a 4.5	a 95.9	a 2.9	a 29.2	a 1.8
eA300	96.2	a 4.4	a 94.9	a 3.1	a 23.8	a 1.4
GoldShield	95.5	a 3.7	a 95.5	a 3.0	a 24.6	a 1.6
Sanidate	96.1	a 4.2	a 95.2	a 3.1	a 29.7	a 1.8
Oxidate (2.0) 0.5%	96.3	a 4.5	a 94.2	a 2.9	a 22.1	a 1.3
Oxidate (2.0) 1%	96.7	a 4.6	a 95.2	a 3.1	a 30.5	a 1.5
Pr>F	0.394	0.220	0.300	0.820	0.400	0.040
CV	1.9	24.9	2.4	22.1	45	37.4

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

Table A.6. Fractional green canopy cover (FGCC) and non-destructive leaf area index (NDLAI) at dry bean R2 growth stage (7/20) and 14 days after inoculation (8/6) in Prosper, North Dakota in 2016

Treatment	7/20/2016				8/6/2016			
	FGCC		NDLAI		FGCC		NDLAI	
Nontreated	95.3	a	3.3	a	41.7	a	1.8	ab
Wakeup Summer (Early)	95.5	a	3.0	a	48.7	a	1.7	abc
eA300 (Early)	95.3	a	3.0	a	42.5	a	1.5	bcd
Kocide 3000	95.5	a	3.0	a	51.5	a	1.6	bcd
MasterCop	95.6	a	3.2	a	41.5	a	1.4	d
Badge SC	95.5	a	3.2	a	44.8	a	1.4	cd
Wakeup Summer	95.9	a	3.1	a	44.2	a	1.5	bcd
eA300	95.8	a	3.3	a	48.3	a	1.5	bcd
GoldShield	95.5	a	3.2	a	40.8	a	1.5	bcd
Sanidate	96.0	a	3.2	a	51.0	a	1.9	a
Oxidate (2.0) 0.5%	96.0	a	3.3	a	43.7	a	1.9	a
Oxidate (2.0) 1%	95.8	a	3.0	a	46.8	a	1.6	abcd
Pr>F	0.921		0.600		0.300		0.010	
CV	1.5		18.6		30		30.1	

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

Table A.7. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/17), 14 days after inoculation (DAI) (8/1) and 28 DAI (8/17) in Fargo, North Dakota in 2017

Treatment	7/17/2017			8/1/2017			8/17/2017		
	HB	CBB	BS	HB	CBB	BS	HB	CBB	BS
Nontreated	0.0 a	0.1 a	0.2 a	0.0 a	28.6 ab	0.0 a	0.0 a	30.9 b	0.0 a
Wakeup Summer (Early)	0.0 a	0.6 a	0.2 a	0.0 a	29.0 ab	0.0 a	0.0 a	28.2 bcd	0.0 a
eA300 (Early)	0.0 a	0.1 a	0.3 a	0.0 a	30.2 a	0.0 a	0.0 a	42.7 a	0.0 a
Kocide 3000	0.0 a	0.2 a	0.4 a	0.0 a	10.2 def	0.0 a	0.0 a	21.6 de	0.0 a
MasterCop	0.0 a	0.1 a	0.1 a	0.0 a	10.6 def	0.0 a	0.0 a	18.3 ef	0.0 a
Badge SC	0.0 a	0.4 a	0.1 a	0.0 a	17.3 cd	0.0 a	0.0 a	21.3 de	0.0 a
Wakeup Summer	0.0 a	0.1 a	0.3 a	0.0 a	24.8 abc	0.0 a	0.0 a	30.4 b	0.0 a
eA300	0.0 a	0.1 a	0.2 a	0.0 a	19.7 bcd	0.0 a	0.0 a	32.1 b	0.0 a
ET-F	0.0 a	0.3 a	0.0 a	0.0 a	3.9 f	0.0 a	0.0 a	12.4 f	0.0 a
Sanidate	0.0 a	0.0 a	0.0 a	0.0 a	7.0 ef	0.0 a	0.0 a	29.9 bc	0.0 a
Oxidate (2.0) 0.5%	0.0 a	0.3 a	0.1 a	0.0 a	15.7 cde	0.0 a	0.0 a	31.1 b	0.0 a
Oxidate (2.0) 1%	0.0 a	0.6 a	0.1 a	0.0 a	10.9 def	0.0 a	0.0 a	21.9 cde	0.0 a
Pr>F	.	0.222	0.919	.	<.0001	.	.	<.0001	.
CV	.	291.0	386.0	.	83.3	.	.	43.3	.

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

Table A.8. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/13), 14 days after inoculation (DAI) (7/27) and 28 DAI (8/10) in Oakes, North Dakota in 2017

Treatment	7/13/2017			7/27/2017			8/10/2018											
	HB	CBB	BS	HB	CBB	BS	HB	CBB	BS									
Nontreated	0.0	a	0.1	a	0.0	a	0.0	a	14.8	bc	0.0	a	0.0	a	27.3	ab	0.0	a
Wakeup Summer (Early)	0.0	a	0.2	a	0.0	a	0.0	a	19.9	ab	0.0	a	0.0	a	29.2	a	0.0	a
eA300 (Early)	0.0	a	0.3	a	0.0	a	0.0	a	14.4	bc	0.0	a	0.0	a	28.1	a	0.0	a
Kocide 3000	0.0	a	0.3	a	0.0	a	0.0	a	8.4	cd	0.0	a	0.0	a	13.9	e	0.0	a
MasterCop	0.0	a	0.3	a	0.0	a	0.0	a	7.1	d	0.4	a	0.0	a	15.6	de	0.0	a
Badge SC	0.0	a	0.3	a	0.0	a	0.0	a	13.6	bcd	0.0	a	0.0	a	19.4	cde	0.0	a
Wakeup Summer	0.0	a	0.2	a	0.0	a	0.0	a	10.7	cd	0.0	a	0.0	a	20.9	bcd	0.0	a
eA300	0.0	a	0.2	a	0.0	a	0.0	a	21.9	a	0.0	a	0.0	a	22.8	abc	0.0	a
ET-F	0.0	a	0.3	a	0.0	a	0.0	a	12.0	cd	0.0	a	0.0	a	20.6	cd	0.0	a
Sanidate	0.0	a	0.1	a	0.0	a	0.0	a	9.4	cd	0.0	a	0.0	a	18.0	cde	0.0	a
Oxidate (2.0) 0.5%	0.0	a	0.4	a	0.0	a	0.0	a	14.1	bc	0.0	a	0.0	a	24.3	abc	0.0	a
Oxidate (2.0) 1%	0.0	a	0.1	a	0.0	a	0.0	a	11.8	cd	0.0	a	0.0	a	22.9	abc	0.0	a
Pr>F	-		0.964		-		-		0.0002		-		-		<.0001		-	
CV	-		293.7		-		-		70.1		-		-		42.7		-	

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

Table A.9. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/24), 14 days after inoculation (DAI) (8/8) and 28 DAI (8/21) in Prosper, North Dakota in 2017

Treatment	7/24/2017			8/8/2017			8/21/2017											
	HB	CBB	BS	HB	CBB	BS	HB	CBB	BS									
Nontreated	1.5	a	0.3	a	0.3	a	0.0	a	62.5	a	0.5	cd	0.0	a	66.4	bcde	2.1	a
Wakeup Summer (Early)	1.1	a	1.1	a	0.8	a	0.0	a	57.0	a	0.9	bcd	0.0	a	62.8	e	0.7	a
eA300 (Early)	1.3	a	1.0	a	0.0	a	0.0	a	59.0	a	1.8	abcd	0.0	a	65.8	de	1.4	a
Kocide 3000	0.8	a	0.1	a	0.1	a	0.0	a	58.4	a	1.1	bcd	0.0	a	66.4	cde	1.4	a
MasterCop	1.3	a	0.1	a	1.0	a	0.0	a	50.8	a	3.3	a	0.0	a	68.6	abcd	1.0	a
Badge SC	1.4	a	0.4	a	0.4	a	0.0	a	58.0	a	1.0	bcd	0.0	a	66.4	abcd	1.3	a
Wakeup Summer	1.0	a	0.8	a	0.6	a	0.0	a	57.9	a	2.3	abc	0.0	a	70.1	abc	1.0	a
eA300	1.8	a	0.0	a	0.4	a	0.0	a	65.9	a	0.4	cd	0.0	a	66.1	bcde	1.6	a
ET-F	2.4	a	0.3	a	0.0	a	0.0	a	59.6	a	2.5	ab	0.0	a	67.1	abcd	1.3	a
Sanidate	2.4	a	0.0	a	0.3	a	0.0	a	62.9	a	1.6	abcd	0.0	a	70.7	ab	1.1	a
Oxidate (2.0) 0.5%	1.3	a	0.1	a	0.4	a	0.0	a	62.0	a	0.1	d	0.0	a	71.5	a	1.3	a
Oxidate (2.0) 1%	1.1	a	0.4	a	0.6	a	0.0	a	56.1	a	0.4	cd	0.0	a	70.1	ab	0.2	a
Pr>F	0.897		0.313		0.501		-		0.704		0.030		.		0.004		0.629	
CV	141.9		264.2		224.3		-		21.7		145.4		.		10.0		130.1	

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

Table A.10. Fractional green canopy cover (FGCC) and non-destructive leaf area index (NDLAI) at dry bean R2 stage (7/17), 14 days after inoculation (DAI) (8/1) and 28 DAI (8/17) in Fargo, North Dakota in 2017

Treatment	7/17/2017		8/1/2017		8/17/2017							
	FGCC	NDLAI	FGCC	NDLAI	FGCC	NDLAI						
Nontreated	66.4	a	1.7	a	70.2	a	1.9	a	85.8	ab	3.2	a
Wakeup Summer (Early)	63.4	a	1.8	a	74.2	a	2.0	a	83.3	abcd	3.1	a
eA300 (Early)	63.4	a	1.8	a	73.2	a	2.1	a	77.9	e	3.3	a
Kocide 3000	64.8	a	1.8	a	72.8	a	2.0	a	83.9	abcd	3.3	a
MasterCop	67.0	a	1.9	a	72.8	a	2.2	a	85.5	ab	3.5	a
Badge SC	65.6	a	1.9	a	72.5	a	2.3	a	83.9	abcd	3.2	a
Wakeup Summer eA300	64.8	a	1.7	a	70.7	a	1.9	a	79.7	de	2.9	a
ET-F	64.2	a	2.2	a	72.5	a	1.9	a	80.8	cde	3.0	a
Sanidate	64.2	a	1.7	a	72.3	a	1.9	a	87.5	a	3.5	a
Oxidate (2.0) 0.5%	66.5	a	1.7	a	69.8	a	2.0	a	85.4	abc	3.2	a
Oxidate (2.0) 1%	63.1	a	2.0	a	69.1	a	1.7	a	79.6	de	3.2	a
Pr>F	0.914		0.2104		0.932		0.194		0.901		0.177	
CV	11.9		27.3		13.2		24.5		8.2		20.0	

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

Table A.11. Fractional green canopy cover (FGCC) and non-destructive leaf area index (NDLAI) at dry bean R2 growth stage (7/13), 14 days after inoculation (DAI) (7/27) and 28 DAI (8/10) in Oakes, North Dakota in 2017

Treatment	7/13/2017		7/27/2017		8/10/2017							
	FGCC	NDLAI	FGCC	NDLAI	FGCC	NDLAI						
Nontreated	95.9	a	4.1	a	92.5	a	4.0	a	70.5	abc	2.1	a
Wakeup Summer (Early)	96.5	a	4.0	a	88.5	a	3.9	a	61.3	c	2.0	a
eA300 (Early)	96.2	a	4.1	a	92.1	a	3.9	a	65.8	bc	2.0	a
Kocide 3000	97.2	a	4.3	a	93.0	a	4.1	a	81.2	a	2.1	a
MasterCop	97.6	a	4.4	a	90.6	a	4.0	a	68.0	bc	2.1	a
Badge SC	96.7	a	4.4	a	91.0	a	4.0	a	73.7	abc	1.7	a
Wakeup Summer	97.2	a	3.8	a	91.5	a	4.0	a	68.1	bc	1.8	a
eA300	96.7	a	3.8	a	92.4	a	4.5	a	63.1	bc	2.1	a
ET-F	97.2	a	3.6	a	92.2		4.6	a	67.2	bc	2.0	a
Sanidate	97.4	a	4.3	a	91.9	a	4.3	a	68.7	bc	1.8	a
Oxidate (2.0) 0.5%	97.5	a	4.0	a	91.4	a	3.6	a	73.0	ab	1.9	a
Oxidate (2.0) 1%	96.5	a	3.9	a	92.4	a	4.3	a	68.8	bc	2.2	a
Pr>F	0.425		0.118		0.155		0.081		0.054		0.308	
CV	2.2		19.1		4.3		21.6		22.3		31.1	

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

Table A.12. Fractional green canopy cover (FGCC) and non-destructive leaf area index (NDLAI) at dry bean R2 growth stage (7/24), 14 days after inoculation (DAI) (8/8) and 28 DAI (8/21) in Prosper, North Dakota in 2017

Treatment	7/24/2017				8/8/2017				8/21/2017			
	FGCC		NDLAI		FGCC		NDLAI		FGCC		NDLAI	
Nontreated	58.3	bcd	1.0	a	61.3	d	1.6	a	63.2	a	1.5	a
Wakeup Summer (Early)	57.3	cd	0.9	a	66.2	abcd	1.6	a	65.0	a	1.8	a
eA300 (Early)	55.7	d	1.0	a	65.7	abcd	1.4	a	67.7	a	1.8	a
Kocide 3000	61.1	bcd	0.8	a	65.3	bcd	1.5	a	68.5	a	1.5	a
MasterCop	62.5	abc	1.0	a	66.4	abcd	1.9	a	62.9	a	1.7	a
Badge SC	62.0	abcd	0.9	a	70.4	abcd	1.3	a	68.0	a	1.5	a
Wakeup Summer	64.4	ab	1.1	a	67.5	abcd	1.9	a	63.9	a	1.5	a
eA300	64.1	ab	1.2	a	66.3	abcd	1.8	a	68.6	a	1.7	a
ET-F	61.1	bcd	1.0	a	69.7	ab	1.7	a	66.2	a	1.5	a
Sanidate	60.5	bcd	1.0	a	62.7	cd	1.7	a	64.4	a	1.4	a
Oxidate (2.0) 0.5%	64.4	ab	1.1	a	66.9	abcd	1.8	a	66.4	a	1.5	a
Oxidate(2.0) 1%	68.0	a	1.2	a	71.5	a	1.7	a	61.8	a	1.6	a
Pr>F	0.034		0.095		0.051		0.163		0.928		0.394	
CV	11.0		21.0		9.1		27.2		15.2		24.7	

^aEarly applications of WakeUp Summer and eA300 were conducted at V3-V4 stage and at 90% bloom. All other applications were conducted at 90% bloom and 14 days later.

Table A.13. Yield (mT/ha⁻¹) in dry bean trials conducted across sites in 2016 and 2017

Treatment	2016						2017				
	Fargo		Oakes		Prosper		Fargo	Oakes	Prosper		
Nontreated	2.7	a	4.3	a	1.1	a	1.9	a	2.5	a	-
Wakeup Summer (Early)	2.6	a	4.7	a	1.4	a	1.9	a	2.8	a	-
eA300 (Early)	2.7	a	4.6	a	1.0	a	1.7	a	2.8	a	-
Kocide 3000	3.0	a	4.8	a	1.1	a	1.9	a	3.0	a	-
MasterCop	2.9	a	4.5	a	1.2	a	2.0	a	2.5	a	-
Badge SC	3.2	a	4.3	a	1.1	a	1.9	a	2.6	a	-
Wakeup Summer	2.8	a	4.6	a	0.9	a	1.8	a	2.9	a	-
eA300	3.2	a	4.6	a	1.2	a	1.9	a	2.6	a	-
ET-F	3.0	a	4.6	a	1.1	a	2.1	a	2.8	a	-
Sanidate	3.0	a	4.6	a	1.3	a	2.0	a	2.8	a	-
Oxidate (2.0) 0.5%	3.4	a	4.4	a	1.1	a	1.9	a	2.6	a	-
Oxidate (2.0) 1%	2.6	a	4.4	a	1.1	a	1.9	a	2.8	a	-
Pr>F	0.876		0.962		0.501		0.769		0.848		-
CV	22.9		10.7		19.1		11.8		14.7		-

APPENDIX B: DRY BEAN ARCHITECTURE TRIAL RESULTS

Table B.1. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (8/9) and 14 days after inoculation (8/23) in Fargo, North Dakota in 2016

Architecture	Cultivar	Treatment	8/9/2016			8/23/2016		
			HB	CBB	BS	HB	CBB	BS
Prostrate	Maverick	Inoculated	0	3.2	3.2	0	33.8	2.5
		Control	0	3.5	3.5	0	14.0 *	3.2
	Othello	Inoculated	0	11.7	4.7	0	38.3	1.7
		Control	0	11.5	4.3	0	18.2 *	1.6
Upright	Medicine Hat	Inoculated	0	8.0	4.5	0	30.0	0
		Control	0	7.3	4.3	0	15.6 *	0
	Stampede	Inoculated	0	2.7	2.2	0	34.7	4.5
		Control	0	2.7	2.0	0	13.8 *	4.3

^aStatistical significance produced by t-test between inoculated and control (*) at $\alpha=0.05$

Table B.2. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/25), 14 days after inoculation (DAI) (8/8) and 28 DAI (8/25) in Oakes, North Dakota in 2016

Architecture	Cultivar	Treatment	7/25/2016			8/8/2016			8/25/2016		
			HB	CBB	BS	HB	CBB	BS	HB	CBB	BS
Prostrate	Maverick	Inoculated	0	2.3	2.5	0	4.2	4.3	0	44.2	28.8
		Control	0	5.3	1.3	0	2	4.7	0	36.7	* 26.5
	Othello	Inoculated	0	3.2	1.7	0	9.3	4.0	0	48	29.7
		Control	0	5.3	0.5	* 0	3.2	* 3.3	0	42	* 27 *
Upright	Medicine Hat	Inoculated	0	2.5	1.8	0	3.8	5.3	0	43.7	29.7
		Control	0	1.7	1.8	0	2.7	5.3	0	40.3	27 *
	Stampede	Inoculated	0	3.5	3.5	0	3.3	3.8	0	31.5	26.3
		Control	0	4.0	2.3	0	1.2	3.5	0	26.0	* 31.5 *

^aStatistical significance produced by t-test between inoculated and control (*) at $\alpha=0.05$

125

Table B.3. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/20) and 14 days after inoculation (8/6) in Prosper, North Dakota in 2016

Architecture	Cultivar	Treatment	7/20/2016			8/6/2016		
			HB	CBB	BS	HB	CBB	BS
Prostrate	Maverick	Inoculated	23.7	13.8	24.7	0	71.3	18.0
		Control	23.7	13.2	22.8	0	61.3	* 16.8
	Othello	Inoculated	22.7	15.0	24.5	0	70.2	22.2
		Control	24.8	14.5	23.0	0	61.5	* 22.3
Upright	Medicine Hat	Inoculated	24.2	15.0	27.0	0	73.8	19.7
		Control	23.3	14.2	28.3	0	69.7	18.5
	Stampede	Inoculated	13.8	13.2	16.2	0	64.0	18.0
		Control	14.7	12.2	14.7	0	60.3	17.3

^aStatistical significance produced by t-test between inoculated and control (*) at $\alpha=0.05$

Table B.4. Fractional green canopy cover (FGCC), non-destructive leaf area index (NDLAI) and destructive leaf area index (DLAI) at dry bean R2 growth stage (8/9) and 14 days after inoculation (8/23) in Fargo, North Dakota in 2016

Architecture	Cultivar	Treatment	8/9/2016			8/23/2016		
			FGCC	NDLAI	DLAI	FGCC	NDLAI	DLAI
Prostrate	Maverick	Inoculated	87.6	2.6	3.0	15.6	0.7	0.7
		Control	86.2	2.5	2.6	15.1	0.9 *	0.9 *
	Othello	Inoculated	86.9	2.6	2.6	2.3	0.4	0.4
		Control	88.2	2.8	2.4	4.4 *	0.5	0.8
Upright	Medicine Hat	Inoculated	86.4	2.2	2.6	3.3	0.4	0.4
		Control	87.1	2.4	3.0	3.3	0.3	0.6
	Stampede	Inoculated	91.0	2.8	3.0	35	1.4	1.6
		Control	89.7	3.1	3.2	32	1.2	1.9

^aStatistical significance produced by t-test between inoculated and control (*) at $\alpha=0.05$

Table B.5. Fractional green canopy cover (FGCC), non-destructive leaf area index (NDLAI) and destructive leaf area index (DLAI) at dry bean R2 growth stage (7/18), 14 days after inoculation (DAI) (8/1) and 28 DAI (8/15) in Oakes, North Dakota in 2016

Architecture	Cultivar	Treatment	7/18/2016			8/1/2016			8/15/2016		
			FGCC	NDLAI	DLAI	FGCC	NDLAI	DLAI	FGCC	NDLAI	DLAI
Prostrate	Maverick	Inoculated	96.5	4.3	4.1	95.8	3.3	4.8	28.7	0.7	1.6
		Control	96.0	4.7	3.1	94.4	3.9	4.4	37.7	1	2.4
	Othello	Inoculated	96.2	5.1	2.6	94.4	3.1	4.5	27.0	0.8	1.4
		Control	95.5	4.4	2.6	94.7	3.0	4.3	38.5 *	1	1.3
Upright	Medicine Hat	Inoculated	95.8	3.8	2.7	94.8	3.0	4.6	33.6	0.9	1.6
		Control	96.7	3.3	3.8 *	94.4	3.4	4.9	36.2	1.1	1.8
	Stampede	Inoculated	97.4	4.3	3.1	96.4	3.5	4.8	27.6	0.9	1.8
		Control	97.1	4.0	2.7	97.3	3.7	5.0	30.5	1.1	1.8

^aStatistical significance produced by t-test between inoculated and control (*) at $\alpha=0.05$

Table B.6. Fractional green canopy cover (FGCC), non-destructive leaf area index (NDLAI) and destructive leaf area index (DLAI) at dry bean R2 growth stage (7/20) and 14 days after inoculation (8/6) in Prosper, North Dakota in 2016

Architecture	Cultivar	Treatment	7/20/2016			8/6/2016		
			FGCC	NDLAI	DLAI	FGCC	NDLAI	DLAI
Prostrate	Maverick	Inoculated	95.0	2.8	3.4	24.2	0.8	0.6
		Control	95.9	2.8	3.7	31.6	0.9	1 *
	Othello	Inoculated	94.7	2.9	3.5	32.5	1	0.8
		Control	94.5	2.6	3.1	31.9	0.9	0.9
Upright	Medicine Hat	Inoculated	96.1	2.5	2.9	20.6	0.5	0.4
		Control	95.6	2.4	3.5 *	19.4	0.5	0.4
	Stampede	Inoculated	97.2	2.6	3.2	34.3	0.9	0.7
		Control	97.3	3	3.2	37.4	0.9	1.2

^aStatistical significance produced by t-test between inoculated and control (*) at $\alpha=0.05$

Table B.7. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/17), 14 days after inoculation (DAI) (8/1) and 28 DAI (8/17) in Fargo, North Dakota in 2017

Architecture	Cultivar	Treatment	7/17/2017			8/1/2017			8/17/2017		
			HB	CBB	BS	HB	CBB	BS	HB	CBB	BS
Prostrate	Maverick	Inoculated	0	0.4	0.5	0	15.7	0	0	28.8	0
		Control	0	0.6	0.8	0	1.4 *	0	0	3.3 *	0
	Othello	Inoculated	0	0.1	0.5	0	36.5	0	0	44.6	0
		Control	0	0.5	0.1	0	4.0 *	0	0	3.5 *	0
Upright	Medicine Hat	Inoculated	0	0.7	1.2	0	18.0	0	0	29.6	0
		Control	0	0.6	1	0	1.6 *	0	0	4.9 *	0
	Stampede	Inoculated	0	0.3	0.4	0	21.4	0	0	29.3	0
		Control	0	0.8	0.6	0	1.6 *	0	0	5.5 *	0

^aStatistical significance produced by t-test between inoculated and control (*) at $\alpha=0.05$

Table B.8. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/13), 14 days after inoculation (DAI) (7/27) and 28 DAI (8/10) in Oakes, North Dakota in 2017

Architecture	Cultivar	Treatment	7/13/2017			7/27/2017			8/10/2017		
			HB	CBB	BS	HB	CBB	BS	HB	CBB	BS
Prostrate	Maverick	Inoculated	0	0	0	0	3.1	0	0	5.9	0.2
		Control	0	0	0	0	0.5 *	0	0	1.7 *	0
	Othello	Inoculated	0	0	0	0	3.3	0	0	8.9	0
		Control	0	0	0	0	0.4 *	1.0	0	2.4 *	0.3
Upright	Medicine Hat	Inoculated	0	0	6.5	0	6.6	17.4	0	12	15.3
		Control	0	0	5.8	0	1.0 *	9.6 *	0	5.2 *	6.0 *
	Stampede	Inoculated	0	0	0	0	3.0	0	0	5.6	0
		Control	0	0	0	0	0.1 *	0	0	0.2 *	0

^aStatistical significance produced by t-test between inoculated and control (*) at $\alpha=0.05$

Table B.9. Average disease severity of halo blight (HB), common bacterial blight (CBB) and brown spot (BS) at dry bean R2 growth stage (7/24), 14 days after inoculation (DAI) (8/8) and 28 DAI (8/21) in Prosper, North Dakota in 2017

Architecture	Cultivar	Treatment	7/24/2017			8/8/2017			8/21/2017		
			HB	CBB	BS	HB	CBB	BS	HB	CBB	BS
Prostrate	Maverick	Inoculated	1.0	0.7	1.8	0	26.7	15.6	0	36.7	4.1
		Control	0.9	1.0	1.4	0	24.7	14.9	0	32.8	* 4.5
	Othello	Inoculated	1.0	0.7	1.8	0	31.4	14.1	0	48.6	5.3
		Control	0.6	1.0	1.5	0	24.8	* 14.5	0	28.9	* 5.4
Upright	Medicine Hat	Inoculated	1.9	1.3	1.5	0	25.6	12.4	0	28.0	5.4
		Control	1.8	1.4	2.4	0	21.7	13.4	0	19.8	* 3.2 *
	Stampede	Inoculated	2.3	0.4	0.9	0	30.3	9.1	0	35.0	5.6
		Control	2.6	0.8	1.2	0	17.1	* 9.5	0	32.9	4.7

^aStatistical significance produced by t-test between inoculated and control (*) at $\alpha=0.05$

Table B.10. Fractional green canopy cover (FGCC), non-destructive leaf area index (NDLAI) and destructive leaf area index (DLAI) at dry bean R2 growth stage (7/17), 14 days after inoculation (DAI) (8/1) and 28 DAI (8/17) in Fargo, North Dakota in 2017

Architecture	Cultivar	Treatment	7/17/2017			8/1/2017			8/17/2017		
			FGCC	NDLAI	DLAI	FGCC	NDLAI	DLAI	FGCC	NDLAI	DLAI
Prostrate	Maverick	Inoculated	73.7	1.9	13.0	86.3	2.6	22.3	81.3	2.2	10.6
		Control	71.4	1.6 *	13.7	81.0	2.4	20.6	85.4 *	2.5	10.8
	Othello	Inoculated	72.1	1.6	14.4	85.1	2.7	19.1	67.8	1.7	7.9
		Control	77 *	1.6	13.6	91.6 *	2.8	20.1	82.7 *	1.8	9.3
Upright	Medicine Hat	Inoculated	73.9	1.4	12.4	84.0	2.3	17.5	68.4	1.7	7.2
		Control	78.5 *	1.5	11.4	83.1	2.3	15.9	78.0 *	1.7	7.4
	Stampede	Inoculated	72.1	1.5	13.0	81.1	2.2	22.5	81.0	2.6	9.8
		Control	74.8	1.4	14.1	79.9	2.5	23.9	85.3	2.7	10.0

^aStatistical significance produced by t-test between inoculated and control (*) at $\alpha=0.05$

131

Table B.11. Fractional green canopy cover (FGCC), non-destructive leaf area index (NDLAI) and destructive leaf area index (DLAI) at dry bean R2 growth stage (7/13), 14 days after inoculation (DAI) (7/27) and 28 DAI (8/10) in Oakes, North Dakota in 2017

Architecture	Cultivar	Treatment	7/13/2017			7/27/2017			8/10/2017		
			FGCC	NDLAI	DLAI	FGCC	NDLAI	DLAI	FGCC	NDLAI	DLAI
Prostrate	Maverick	Inoculated	96.5	4.4	26.6	95.2	5.4	26.0	93.6	3.2	22.3
		Control	96.7	4.3	17.2 *	95.2	5.7	17.6 *	94.1	3.6 *	16.7 *
	Othello	Inoculated	96.6	4.2	18.6	94.4	5.2	24.4	87.4	3.0	19.2
		Control	94.5	3.3 *	15.6	94.2	4.9	17.2 *	92.4 *	2.8	17.9
Upright	Medicine Hat	Inoculated	95.4	3.4	25.9	91.6	4.2	30.7	82.6	2.4	22.5
		Control	95.2	3.3	18.7 *	89.7	4.0	21.2 *	86.3	2.4	18.5
	Stampede	Inoculated	95.8	3.6	17.7	95.4	5.3	21.0	94.2	2.8	16.5
		Control	96.3	3.4	22.7 *	94.7	5.4	19.3	92.8	3.0	21.2 *

^aStatistical significance produced by t-test between inoculated and control (*) at $\alpha=0.05$

Table B.12. Fractional green canopy cover (FGCC), non-destructive leaf area index (NDLAI) and destructive leaf area index (DLAI) at dry bean R2 growth stage (7/24), 14 days after inoculation (DAI) (8/8) and 28 DAI (8/21) in Prosper, North Dakota in 2017

Architecture	Cultivar	Treatment	7/24/2017			8/8/2017			8/21/2017		
			FGCC	NDLAI	DLAI	FGCC	NDLAI	DLAI	FGCC	NDLAI	DLAI
Prostrate	Maverick	Inoculated	61.5	1.2	7.8	69.2	1.4	12.3	67.1	1.6	9.8
		Control	63.5	1.3	7.9	66.1	1.5	11.4	62.5	1.2	*
	Othello	Inoculated	66.8	1.3	9.5	68.8	1.3	10.9	63.6	1.2	9.1
		Control	61.7	1.2	7.6	* 64.7	1.2	10.9	64.6	1.2	10.4
Upright	Medicine Hat	Inoculated	58.9	1.0	6	64.5	1.5	8.8	63.8	1.5	8.2
		Control	57.9	1.0	6.3	61.7	1.4	8.8	63.3	1.3	6.8
	Stampede	Inoculated	64.4	1.3	7.8	68.8	1.6	10.2	66.1	1.5	8.4
		Control	63.5	1.2	9.1	68.8	1.4	11.1	61.8	1.3	* 9.0

^aStatistical significance produced by t-test between inoculated and control (*) at $\alpha=0.05$

Table B.13. Yield (mT/ha⁻¹) in dry bean trials conducted across sites in 2016 and 2017

Architecture	Cultivar	Treatment	2016			2017		
			Fargo	Oakes	Prosper	Fargo	Oakes	Prosper ^a
Prostrate	Maverick	Inoculated	1.3	1.5	1.0	1.5	5.3	-
		Control	1.4	1.4	1.1	1.4	5.1	-
	Othello	Inoculated	1.0	1.4	0.9	1.6	3.5	-
		Control	0.9	1.4	1.0	1.5	3.9	-
Upright	Medicine Hat	Inoculated	1.3	1.6	1.0	1.2	4.6	-
		Control	1.4	1.7	1.1	1.3	4.8	-
	Stampede	Inoculated	1.9	1.7	1.2	1.5	5.2	-
		Control	2.2	1.7	1.3	1.7	5.4	-

^aProsper trial was compromised due to Dicamba drift; therefore, was not harvested