

**Microwave Radiation as an Alternative Control for Seed-borne Diseases in  
Dry Bean**

**by**

**Allison P. Friesen**

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## ABSTRACT

### MICROWAVE RADIATION AS AN ALTERNATIVE CONTROL FOR SEED-BORNE DISEASES IN DRY BEAN

Allison P. Friesen  
University of Guelph, 2014

Advisory Committee:  
Chris L. Gillard  
Darren E. Robinson  
Robert L. Conner

Controlling the seed-borne pathogens *Xanthomonas axonopodis* pv. *phaseoli*, *Pseudomonas syringae* pv. *phaseolicola*, and *Colletotrichum lindemuthianum* is difficult in Canadian dry bean production. Laboratory and field studies conducted in 2012-2013 evaluated microwave radiation as an alternative control for these pathogens. In the laboratory, seed germination and vigour decreased by <10% and 25%, respectively, between 40-60 s of microwave exposure. Microwave radiation of seed infected with *C. lindemuthianum* resulted in a 0.10-0.14-% s<sup>-1</sup> decrease in disease incidence, but no similar response was observed with bacterial diseases. Field studies evaluated the effect of microwave radiation and chemical treatment (pyraclostrobin + fluxapyroxad + metalaxyl, thiamethoxam + fludioxonil + metalaxyl-M + azoxystrobin or copper hydroxide 53.8%) on seed health and disease control. Microwave treatment decreased emergence <9%, but did not consistently improve the other parameters. Chemical treatment decreased disease symptoms, but did not provide season long control. Combining microwave and chemical treatments provided no additional disease control or economic benefit.

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## TABLE OF ACRONYMS

AUDPC	- Area under the disease progress curve
BBCH	- Biologische Bundesanstalt, Bundessortenamt and Chemische Industrie scale
CBB	- Common bacterial blight
CFIA	- Canadian Food Inspection Agency
DAP	- Days after planting
DCT	- Diazinon + captan + thiophanate-methyl
EMR	- Electromagnetic radiation
EPS	- Extracellular polysaccharides
HSW	- Hundred seed weight
IC	- Infected control
IPM	- Integrated pest management
MC	- Moisture content
MER	- Maximum exposure rate
MOA	- Mode of action
MSRP	- Manufacturer's suggested retail price
NIC	- Non-infected control
OAC	- Ontario Agricultural College
PDA	- Potato dextrose agar
PDI	- Pod destruction index
PFM	- Pyraclostrobin + fluxapyroxad + metalaxyl
Psp	- <i>Pseudomonas syringae</i> pv. <i>phaseolicola</i>
RCBD	- Randomized complete block design
RH	- Relative humidity
ROI	- Return on investment
ST (fungus)	- Pyraclostrobin + fluxapyroxad + metalaxyl and thiamethoxam + fludioxonil + metalaxyl –M + azoxystrobin
ST (blights)	- Pyraclostrobin + fluxapyroxad + metalaxyl and copper hydroxide 53.8%
TFMA	- Thiamethoxam + fludioxonil + metalaxyl –M + azoxystrobin
WAP	- Weeks after planting
Xap	- <i>Xanthomonas axonopodis</i> pv. <i>phaseoli</i>

# CHAPTER ONE

## Literature Review and Research Objectives

### 1.1 Introduction to Dry Beans

#### 1.1.1 History

*Phaseolus vulgaris* L., also known as dry bean, belongs to the family Fabaceae and is an important legume crop grown worldwide for human consumption. Although it prefers temperate zones and the tropics, dry bean has adapted to numerous climatic conditions and is grown over a large geographical area (Chase, 1987). The widespread production of dry bean has been attributed to its similar protein characteristics to the more expensive red meat, which makes dry bean a cheaper staple food in many countries' diets (Wright, 2007; Health Canada, 2012). Dry bean has a high dietary fibre and folate content along with its high protein level (15-25%), which allows it to serve as an important dietary source of these nutrients compared to other legume crops (Tosh and Yada, 2010).

The origin of cultivated common bean stems from two specific regions, Middle America and the Andes, and it is a domesticated form of the wild-growing vine-like ancestor (van Schoonhoven and Voysest, 1991). The Mesoamerican lines (e.g. navy and black market classes) originated in Mexico and Central America and typically have a smaller seed size than the Andean lines (Voysest and Dessert, 1991). The larger seed classes (e.g. cranberry and kidney beans) are Andean and originated on the western side of South America in Ecuador and Peru. The domestication of both of these gene pools has led to the distribution of Mesoamerican and Andean types to other growing regions (van Schoonhoven and Voysest, 1991). Although both lines are now worldwide, certain classes still predominate in their specific area of development,

like black beans in Latin America and white beans in Africa (van Schoonhoven and Voysest, 1991).

### **1.1.2 Development**

Although domestication of dry bean has led to the worldwide distribution of classes from both ancestries, human selection pressure has also affected the process significantly, as seen with traits such as growth habit (van Schoonhoven and Voysest, 1991). In dry bean there are four growth habits (I-IV) classified based on their growing pattern, plant structure and flowering period (Kelly, 2001). Type I, also known as determinate or bush type, are upright, have limited ability to produce vines and have a relatively short flowering period (Voysest and Dessert, 1991). The short nature and upright style of Type I make them easy to harvest and desirable for commercial production (van Schoonhoven and Voysest, 1991; Kelly, 2001). Types II-IV are indeterminate, which possess a vegetative meristem that enables increased branch production and an extended flowering period compared to Type I (Kelly, 2001). The Type IV growth habit is the least favourable for commercial production as it has a high tendency to vine and intertwine with neighbouring plants. Type II and III are intermediates of Type I and IV and are commonly used in commercial production.

Although the vegetative growth pattern varies in dry bean, all types reproduce through self-pollination (Ibarra-Perez et al., 1997). Cross pollination has been observed in dry bean, however, the range in frequency was large (0-78%) and environmental factors were shown to have a strong influence on its frequency (Ibarra-Perez et al., 1997). Insect vectors, such as bumblebees (*Bombus* spp.) and bees (*Apis* spp.) are known to cross pollinate dry bean (Ibarra-Perez et al., 1999).

Pollination begins at the point of initial flowering; this growth stage can be determined and monitored using the Biologische Bundesanstalt, Bundessortenamt and Chemische Industrie (BBCH) scale (Hess et al., 2008). The BBCH scale is commonly used in research to separate the vegetative and reproductive stages of plant development by numerical values, zero to twenty-nine for stages of vegetative growth and fifty-one to eighty-nine for stages of reproductive growth.

### **1.1.3 Market Classes**

Dry beans can be classified based into market classes as well as by growth habit. In Canada, several market classes are grown across the major bean production areas of Manitoba and Ontario, as well as niche areas in Saskatchewan, Alberta, and Quebec (Goodwin, 2003). Commonly occurring market classes for these areas are navy, black, pinto, cranberry, great northern, as well as light red, dark red and white kidney beans (Goodwin, 2003; Kelly et al., 2009). The navy, black and pinto bean classes are of the Mesoamerican ancestry and are commonly grown in all bean producing areas of Canada, except Alberta (Goodwin, 2003; Mamidi et al., 2011). The remaining classes, usually referred to as the coloured beans, originate from the Andean ancestry and are grown across all the Canadian bean producing regions (Goodwin, 2003; Mamidi et al., 2011).

In Ontario the navy, coloured, and Japanese (Kintoki and Otebo) classes are commonly grown. The more common coloured classes grown in Ontario are cranberry and kidney classes. The Japanese classes consist of otebo beans (*Phaseolus spp.*) and adzuki beans (*Vigna angularis* (Willd.) Ohwi & H. Ohashi) and are primarily used for confectionary purposes (Kelly et al., 2009). The Japanese classes are specific to Ontario and not produced in Manitoba, where navy, black and a variety of coloured beans are more commonly grown.

#### **1.1.4 Production**

Worldwide approximately 20 million tonnes of dry beans are produced annually (FAOSTAT, 2012). The majority of production occurs in developing countries in Asia, Africa, and South America as well as some developed countries in Oceania, Europe, and North America (Gepts et al., 2008). Asia and the Americas contribute approximately 81% of bean production each year and include the top five countries for seed and food grade production (FAOSTAT, 2012). The individual countries that contribute the most to the total average bean production globally in the last five years are India (16.1%), Brazil (15.8%), Myanmar (13.7%), China (7.5%), USA (5.8%), and Mexico (5.2%) (FAOSTAT, 2012).

Canada contributes 1.4 % of the total bean production worldwide and is responsible for an average production of 250 thousand (K) tonnes annually over the last ten years (Stats Canada, 2012; FAOSTAT, 2012). Of the dry beans grown in Canada, navy beans make up 38% while coloured beans make up the remainder. Ontario is the largest producer in Canada and accounts for approximately 42% of bean production in the past ten years (Stats Canada, 2012).

Despite the low percentage that Canada contributes to the total bean production worldwide, dry bean serves as an important export commodity. Canada is one of the top five countries for dry bean export and on average Canada exports almost all of its tonnage to the USA and Europe annually (Agriculture and Agri-Food Canada, 2012; FAOSTAT, 2012). Other major exporting countries are Myanmar (1.09 M tonnes), China (860 K tonnes), USA (345 K tonnes), and Argentina (240 K tonnes) (FAOSTAT, 2012). In the last ten years the majority of exports from these countries go to India and the European Union, who have annually imported over 500 K tonnes and 1.8 M tonnes, respectively (FAOSTAT, 2012).

## **1.2 Seed-Borne Pathogens**

### **1.2.1 Infection**

Seed-borne pathogens commonly occur in dry bean seed and can cause significant crop and economic loss (Agarwal, 1997). Seed-borne pathogens can be transmitted as an infection (carried internally) or as an infestation (carried passively) on the seed coat (Maude, 1996). Once infected, all seed parts can be colonized; however some pathogens are restricted to specific areas of the seed, like bacterial blights, which occur in the embryo, pericarp or seed coat, but not the endosperm or perisperm (Agarwal, 1997; Singh, 2004). The location of the pathogen also depends on factors such as pathogen species, the mode and timing of infection, environmental conditions, host type and developmental stage, as well as crop management practices (Singh, 2004). Due to the location of some pathogens the seed can appear asymptomatic, causing problems for the production of clean seed, as the pathogens can be transmitted from seed to seedling to adult plant (Maude, 1996; Agarwal, 1997).

The transmission of a pathogen from an infected seed to a seedling can occur systemically or non-systemically depending on the pathogen (Maude, 1996; Agarwal, 1997; Singh, 2004). Systemic seed transmission is caused when infected seed results in the systemic spread of the disease at germination, whereas non-systemic transmission occurs as pre- or post-emergence infection (Agarwal, 1997). Both types of transmission can result in high levels of seed infection that produce large amounts of inoculum (Agarwal, 1997). These seedlings can cause secondary infections throughout the crop resulting in reductions in yield and seed quality (Agarwal, 1997).

### **1.2.2 Control & Regulation**

Integrated pest management (IPM) is key to control seed-borne pathogens in dry bean (Agarwal, 1997). Integrated pest management systems utilize numerous methods to achieve



control; such as the use of resistant cultivars, disease-free seed, chemical treatments, eradication treatments, and alterations of cultural practices (Maude, 1996; Agarwal, 1997; McGee, 1997).

The use of resistant cultivars is the most effective management strategy for disease control, however, the availability of such cultivars varies for the numerous seed-borne pathogens that affect dry bean (Singh and Muñoz, 1999). When resistant cultivars are not available the use of disease-free seed is the next most important strategy, as infected seed is usually the primary inoculum source for most seed-borne pathogens (McGee, 1997; Bailey et al., 2003).

Disease-free dry bean seed is difficult to maintain in certain production areas, such as Ontario and Manitoba, due to favorable environmental conditions for disease. Therefore, seed is imported from areas like Idaho, where humidity levels are low and do not favour the buildup of disease (Coyne and Schuster, 1974a; Scott and Michaels, 1992). Other management strategies like avoidance and exclusion also help to maintain disease-free seed by preventing the spread of seed-borne pathogens to areas where they are not yet present (Siddiqui and Vidhyasekaran, 1990; Swings and Civerolo, 1993; Maude, 1996; Agarwal, 1997). These strategies are implemented and supported by legislative and international regulations (e.g. European Plant Protection Organization OEPP/EPPU), which prevent the movement of infected seed based on biological concerns for pests that pose potential risks to countries or areas in which that pest does not currently occur (Agarwal, 1997; McGee, 1997). In order to meet these regulations, seed certification standards are set for seed health testing to determine and detect incidences of seed infection and to prevent the further spread of the pathogen (McGee, 1997).

If disease-free seed is not available, eradication practices can be utilized to eliminate or reduce seed-borne infections (McGee, 1995; Maude, 1996; Agarwal, 1997). Eradication can be achieved through the application of chemical, physical and biological seed treatments (McGee,

1995). The use of chemical pesticides is the most common practice as they may control deep-seated infections and provide seedling protection for weeks after planting (McGee, 1995). The use of diazinon, captan, and thiophanate-methyl (DCT) has been the conventional standard seed treatment since 1978 (Edgington and MacNeill, 1978), however new chemistries like metalaxyl, azoxystrobin and fludioxonil are now the industry standard (McGee, 1995; MAFRI, 2012; Gillard and Ranatunga, 2013). Various foliar pesticides are also effective in preventing and reducing the spread of infection. Foliar pesticide efficacy varies with the pathogen species and may require numerous applications for effective disease control throughout the growing season (Garrett and Schwartz, 1998).

Physical and biological treatments are not commonly used in dry bean even though they can be effective in the removal of pathogens as well. Physical treatments such as hot water, hot air, and solar heat have been used, however, most of these are ineffective for disease control in dry bean (Grondeau et al., 1994; McGee, 1995; Agarwal, 1997). The use of biologicals such as saprophytic bacteria (*Pseudomonas* spp., *Bacillus* sp. and *Erwinia herbicola* [*Pantoea agglomerans* (Beijerinck 1888) comb. nov.]) have also been tested for control of bacterial pathogens. Although they showed some promise, they were not able to control the pathogens effectively (Arsenijevic et al., 1998).

Finally, alterations to cultural practices can also aid in disease management strategies by reducing the amount of initial inoculum available (Maude, 1996; Agarwal, 1997). Crop rotation is highly effective in reducing inoculum build up by using non-host crops, which allows for the decomposition of infected materials between the production of successive crops (Chase, 1987; Maude, 1996; Bailey et al., 2000). The management of crop residues via incorporation, burning, or removal also helps to decrease the viability and amount of inoculum for the upcoming seasons

(Chase, 1987; Bailey et al., 2000). Altering planting dates can aid in reducing crop losses by avoiding key pathogen growth stages; for example, in tropical countries planting later can help to avoid the rainy season and can decrease the amount of disease spread via rain splash (Maude, 1996).

Integrated pest management practices are the most effective method to manage pathogens in dry bean production (Peshin and Dhawan, 2009). However, even with the utilization of all available management strategies, challenges still exist for many of the seed-borne pathogens affecting dry bean production. Three diseases in particular, common bacterial blight, halo blight and anthracnose, can significantly affect dry bean yield and seed quality and gaps exist in the management practices for each.

### **1.3 Common Bacterial Blight**

#### **1.3.1 Development & Symptomology**

*Xanthomonas axonopodis* pv. *phaseoli* (Smith) Vauterin et al. (Xap) (syn. *X. campestris* pv. *phaseoli* (Smith) Dye), the causal organism responsible for common bacterial blight (CBB) in dry beans, occurs worldwide and can impact yield and seed quality (Coyne and Schuster, 1974b; Saettler, 1989a). This gram negative hemibiotrophic bacteria is from the Proteobacteria family and can be identified in culture by its yellow pigmentation (xanthomonadin) and convex, round, mucoid colony formation (Hall, 1991).

The Xap bacterium thrives in warm climates with high humidity where the availability of free water and temperatures of 28-32°C favor rapid disease development (Singh and Muñoz, 1999). Under favourable conditions, the pathogen can multiply on the plant surface and infect the foliage and pods of bean via passive movement through natural openings, such as stomata and hydathodes, or wounds (Swings and Civerolo, 1993; Singh and Muñoz, 1999). Upon entry, the

bacteria multiply rapidly inside the sub-stomatal cavity forming microcolonies on top of the mesophyll cells (Swings and Civerolo, 1993; Goodwin et al., 1995). The colonies become surrounded by extracellular polysaccharides (EPS) that fill in the intercellular space creating water soaked lesions (Swings and Civerolo, 1993). The water soaking of the leaves usually occurs four to ten days after infection and later develops into larger necrotic lesions surrounded by a small chlorotic zone, giving the host tissue a burnt appearance (Hall, 1991; Gillard et al., 2009). Pod symptoms appear similar to leaf lesions initially and later develop into circular lesions with a purple-brown margin and water soaked center (Hall, 1991; Swings and Civerolo, 1993). Extensive bacterial multiplication can cause the extrusion of bacterial colonies from the stomata of severely infected plants throughout the growing season (Swings and Civerolo, 1993). These extrusions act as a source for secondary infection by increasing the amount of inoculum available for spread via rain, wind or aerosols (Hall, 1991).

External symptoms of infection are easily identifiable; however Xap can spread systemically within the host as well (Weller and Saettler, 1980; Aggour et al., 1989). The systemic movement of this pathogen allows for the colonization of plant tissues in the absence of visible symptoms, known as a latent infection (Bozzano-Saguier, 1993; Goodwin et al., 1995). Infected seed is identifiable by visible butter-yellow to brown discolouration of the seed which may also be shrivelled in appearance. However, latent infections can go unnoticed at first and later produce symptoms after planting, which can lead to the unintentional spread of the pathogen (Chase, 1987; Swings and Civerolo, 1993; Singh and Muñoz, 1999).

### **1.3.2 Vectors**

Infected seed is the primary mode of transmission for Xap and is highly efficient, as seed lots over fifteen years old have been shown to still have viable latent populations (Schuster and

Sayre, 1967). The pathogen's ability to survive on seed for long periods of time may be due to the presence of dried EPS, which help to prevent the desiccation of the bacterial cells (Leach et al., 1957). The preservation of the bacterial cells by EPS is important for its survival from season to season as Xap forms no spores or other resting structures and therefore relies on passive dispersal for dissemination (Leach et al., 1957; Wilson and Lilly, 1965).

Other sources of initial inoculum have been found in infected debris and soil where crop rotation and tillage practices are minimal (Schuster, 1967; Saettler, 1989a). The Xap pathogen has been shown to survive for up to seven months in Wisconsin on dry crop debris allowing the disease to survive from season to season (Saettler, 1989a; Gilbertson et al., 1990). Weeds, such as lamb's-quarters (*Chenopodium album* (L.)) and redroot pigweed (*Amaranthus retroflexus* (L.)), can act as hosts for Xap allowing it to live epiphytically until favourable conditions return (Cafati and Saettler, 1980; Chase, 1987; Saettler, 1989a). While living epiphytically on these hosts, Xap can also be spread via aerosols, or wind and rain dispersal (Hirano and Upper, 1983). Other dispersal methods include irrigation water, animal and human movement through infested fields as well as on volunteer seedlings (Chase, 1987).

### **1.3.3 Control Measures**

In order to reduce the spread and destruction of Xap within and between seasons the previously mentioned IPM strategies can be effective. The application of antibiotics and bactericides on seed and foliage are a popular method for control of CBB in dry bean (Howard et al., 2000). However, few foliar treatments are highly effective and the use of antibiotics are now prohibited in Canada due to concerns regarding the buildup of antibiotic resistance (Swings and Civerolo, 1993; Howard et al., 2000). Prior to their ban, antibiotics such as streptomycin and oxytetracycline were commonly used and the most effective control methods available (Taylor

and Dudley, 1977; Howard et al., 2000). To replace antibiotic treatments, numerous studies on alternative seed and foliar treatments have been conducted with products such as copper sulfate mixtures, zinc based compounds, as well as microbials (Howard et al., 2000). Studies have demonstrated that all treatments are comparable to streptomycin application, however, each product was unable to control CBB effectively (Howard et al., 2000).

Due to the lack of control with the above bactericides, the need for resistant cultivars is important for the management and control of Xap (Gillard et al., 2009). Natural resistance to CBB in dry bean has been identified in beans, however the resistant loci is tightly linked to a gene for late maturity and is not widely used (Coyne et al., 1973). However, there are close relatives to *P. vulgaris* that do possess more accessible resistance genes, such as tepary bean (*Phaseolus acutifolius* A. Gray) and scarlet runner bean (*Phaseolus coccineus* L.) (Parker, 1985). *Phaseolus vulgaris* has been interspecifically crossed with its close relatives to genetically engineer new resistant *P. vulgaris* cultivars (Parker, 1985). The cultivar OAC Rex was the first CBB-resistant navy cultivar registered in Canada and displays resistance in both pods and foliage (Michaels et al., 2006). Cultivar OAC Rex reduces disease symptoms and allows the plant to remain healthier and produce higher yields than non-resistant cultivars under high disease pressure (Tar'an et al., 2001; Gillard et al., 2009). Research continues on breeding of new resistant lines for other navy bean cultivars as well as other market classes.

#### **1.3.4 Yield Reductions**

The development and use of resistant cultivars in combination with the other control methods mentioned above, is very important in controlling CBB, as it can severely affect dry bean yields and seed quality. Most treatments are not highly effective in controlling CBB but still impact yield, as studies have shown that for every 1% increase in CBB severity on leaves there is an

average 10 kg ha<sup>-1</sup> loss in yield for Mexican-142, Awash-1, and brown speckled bean cultivars (Tefera, 2006). Yield losses caused by CBB can vary based on the influence of environmental conditions, crop growth stage, available moisture and cultivar type (Singh and Muñoz, 1999). Research has shown that yield losses can range between 10-45% over the range of environments for dry bean production around the world (Saettler, 1989a; Tefera, 2006; Gillard et al., 2009). These losses can be devastating for dry bean producers and there is a clear incentive for the development of more effective controls and resistant cultivars.

## **1.4 Halo Blight**

### **1.4.1 Development & Symptomology**

*Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al. (Psp), the causal agent of halo blight in dry beans, is another Proteobacteria that causes damage to foliage, stems, pods, and seed worldwide (Chase, 1987; Taylor et al., 1996). In culture, Psp appears quite similar to Xap as it forms round, white to cream, mucoid, convex colonies (Chase, 1987; Arnold et al., 2011). However Psp is easily distinguishable from Xap on iron-deficient media, as *Pseudomonas* spp. produce the siderophore pyoverdine, a fluorescent yellow-green pigment that is visible under ultra-violet light (Chase, 1987). Another distinguishing feature is that Psp prefers cooler temperatures (18-22°C) and most commonly occurs in temperate areas (Chase, 1987). This biotrophic bacterium can survive epiphytically and as a facultative saprophyte in the phyllosphere on hosts such as *Phaseolus* spp., mung bean (*Vigna radiat* (L.) R. Wilczek), azuki beans (*Vigna angularis* (Willd.) Ohwi & H. Ohashi)), and soybean (*Glycine max* (L.) Merr.) (Chase, 1987; Arnold et al., 2011).

*Pseudomonas* spp. invade host tissues through natural openings, such as stomata, hydathodes or wounds during periods of high humidity or when free moisture is available (Chase, 1987).

Initial infection usually occurs on the lower leaf surface and symptoms appear similar to those of CBB (Chase, 1987). The pathogens can be differentiated later in infection as the halo blight infection foci do not enlarge or become necrotic like Xap infections, instead they develop a distinctive yellow-green chlorotic ‘halo’ around the small infection foci (Chase, 1987; Arnold et al., 2011). This chlorosis is due to the production of phaseolotoxin, a non-specific phytotoxin, which is released into the extracellular space of the host cells (Mitchell and Bielecki, 1977; Chase, 1987; Arnold et al., 2011). Phaseolotoxin causes the accumulation of ornithine and the breakdown of chlorophyll, which results in the formation of the chlorotic halo surrounding the infection foci (Mitchell and Bielecki, 1977).

Although foliar symptoms are expressed mainly as a result of phaseolotoxin, the bacteria can also move to the pods and cause water-soaked red to brown lesions to develop (Chase, 1987; Agrios, 2004). These lesions may affect the pod and pod suture, which can result in the discolouration and shrivelling of the developing seed (Chase, 1987). Infected seed commonly have a buttery-yellow discolouration that appear similar to that seen with CBB (Arnold et al., 2011).

#### **1.4.2 Vectors**

Seed infected with Psp occurs worldwide and can cause severe infection under conducive environments at infection rates as low as 1% (Taylor et al., 1979b; Arnold et al., 2011). Infected seed is the primary inoculum source for Psp and is monitored vigorously to prevent its spread to other dry bean production areas (Chase, 1987; Maude, 1996; Agarwal, 1997). Under optimal conditions, a bacterial ooze can develop seven to ten days after infection on both foliar and pod lesions (Chase, 1987; Arnold et al., 2011). The bacterial ooze acts as a source of secondary inoculum that can spread the disease to healthy plants by leaf contact, wind, and splashing of rain



or irrigation water (Taylor et al., 1979b; Chase, 1987). The spread of Psp between seasons occurs through its survival on infested plant residue or seed, which can be a concern in warmer climates (Taylor et al., 1979b; Chase, 1987; Arnold et al., 2011).

### **1.4.3 Control Measures**

In order to control the spread of halo blight the same IPM strategies mentioned earlier for CBB should be utilized (Chase, 1987; Maude, 1996). The seed and foliar treatments recommended for CBB are also used in controlling halo blight, however, as with CBB, they are not highly effective. The use of disease free seed or resistant cultivars are the most successful control practices when available (McGee, 1997; Bailey et al., 2000). Unfortunately, obtaining disease free seed is not always possible if environmental conditions are favourable for the pathogen. In addition, few resistant cultivars are currently available for Psp.

The lack of available resistant cultivars is due to the variance in genes required for leaf and pod resistance, which makes breeding for total plant resistance difficult, as with CBB (Coyne and Schuster, 1974b). The genetic variability of Psp also makes breeding for resistance difficult (Bozkurt and Soyly, 2011). Currently there are nine identified races of Psp, five (race 1, 2, 5, 6, and 7) of which occur worldwide with race 6 being the most predominant (Taylor et al., 1996; Bozkurt and Soyly, 2011). The races were identified based on their interactions with eight differential bean cultivars. Cultivars such as Red Mexican U13 and Tendergreen were among the first recognized sources of resistance based on their hypersensitive response to races 1 and 3, respectively (Taylor et al., 1996). Several other resistant cultivars have been documented over time, but none are resistant to all nine races of Psp (Arnold et al., 2011; Bozkurt and Soyly, 2011). Although breeding for total halo blight resistance is a difficult process, Psp is considered

a model organism for studying hypersensitive responses and research continues for a cultivar that can decrease the impact of this devastating disease (Arnold et al., 2011).

#### **1.4.4 Yield Reductions**

The effect of halo blight on dry beans is most notable early in the season when increased seedling death arises due to high disease pressure (Saettler, 1989b; Hall, 1991). Infected seedlings that do survive produce the distinctive chlorosis, which can cause premature leaf drop and result in substantial crop defoliation throughout the season (Saettler, 1989b). Yield losses of up to 43% have been reported due to halo blight infection, with an even greater economic loss once seed quality is taken into account (Saettler and Potter, 1970; Arnold et al., 2011).

### **1.5 Anthracnose**

#### **1.5.1 Development & Symptomology**

The causal agent of anthracnose in dry beans is *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara, a hemibiotrophic fungus that thrives under moderate, damp conditions (Holliday et al., 1971; Chase, 1987). *Colletotrichum lindemuthianum*, the anamorph of *Glomerella lindemuthianum* Shear, was first described on dry bean in France in 1843 and is currently found in bean production areas worldwide (Schwartz and Corrales, 1989; Martínez-Pacheco et al., 2009). *Colletotrichum lindemuthianum* can be identified in culture by its slow growing, compact, grey to black hyphae and salmon coloured conidia (Holliday, 1980; Prusky et al., 2000).

The fungus causes the greatest damage in tropical and subtropical regions as it thrives under humid conditions with optimal temperatures between 17-25°C (Chase, 1987; Schwartz and Corrales, 1989). However, it can tolerate cooler temperatures as well, which enables the fungus to cause damage in temperate regions where rainfall is frequent and relative humidity is high

(Holliday et al., 1971). The presence of free moisture is required for numerous stages of the infection process, especially the germination phase where the presence of moisture can cause conidia to germinate six to nine hours after contacting the plant surface (Chase, 1987).

After germination, *C. lindemuthianum* releases a gelatinous substance to attach itself to the hosts cuticle and then forms an appressoria with an infection peg, which allows the fungus to penetrate the host cuticle via mechanical pressure created by the accumulation of melanin (Chase, 1987; Martínez-Pacheco et al., 2009). Once inside the host, the infective hyphae continue to grow asymptotically for two to four days, after which the fungus will switch from the biotrophic to necrotrophic stage and begin releasing cell wall degrading enzymes (Agrios, 2004; Martínez-Pacheco et al., 2009). The degradation of the cells causes lesions that initially appear water-soaked and later darken. These lesions are usually first seen on the lower leaf surface and eventually move to the stems and pods of infected plants (Holliday, 1980; Chase, 1987). Leaf lesions usually form near the petioles on veins and are elongate, angular and brick red to purple in colour (Chase, 1987). The pod lesions can initially appear similar to those of CBB, but are easily differentiated a few days after infection due to their sunken nature, raised black rim and cluster of pink conidia exuded from the lesion (Holliday, 1980; Chase, 1987).

On young pods, severe infection may cause the pods to shrivel and die, while older pods can survive, but the seed and seed coat of developing seeds are infected (Holliday et al., 1971; Chase, 1987). Infected seed is often discoloured, shrivelled and covered with yellow-brown to black lesions. When planted, this seed can infect emerging cotyledons, which in turn develop lesions that appear small and dark brown to black in colour (Chase, 1987; Conner et al., 2009). Conidia from these lesions can be spread to the developing hypocotyl, where elongated rust coloured lesions can occur. In severe seedling infections, hypocotyl rot and damping off can occur

(Mohammed and Sangchote, 2007). If the seedling survives, the conidia produced from these lesions can move up the plant through leaf contact or rain splash, causing secondary infections, which increases the amount of inoculum available (Tu, 1981; Schwartz and Corrales, 1989; Fininsa and Tefera, 2002).

### **1.5.2 Vectors**

The use of disease-free seed is important to prevent early season infection, which can result in larger yield losses through a potential increase in secondary inoculum later in the growing season (Chase, 1987; Chang, 2001). When disease-free seed is replaced with healthy looking common seed, high infection rates can still occur as anthracnose can cause latent seed infections (Chang, 2001; Conner et al., 2006a). If seed with latent infections are planted the conidia produced from infected seed can be locally disseminated to healthy plants by leaf contact and splashing from rain or irrigation water throughout the growing season (Tu, 1981; Chase, 1987).

*Colletotrichum lindemuthianum* can also survive in infested soil and crop residues for up to twenty-two months and has occasionally been reported to develop sclerotia (Dillard and Cobb, 1993). The fungus can spread over long distances through dispersal of conidia on infected debris and driving rains, which have been shown to spread the disease over distances of 4.5 m (Tu, 1981; Fininsa and Tefera, 2002). However, the primary source of inoculum over long distances is the movement of infected seed, so the use of disease free seed and resistant cultivars is recommended (Chase, 1987; Conner et al., 2006a).

### **1.5.3 Control Measures**

Integrated pest management strategies for controlling anthracnose include cultural controls (tillage, crop rotation, planting alterations), chemical controls and genetic resistance to help reduce disease incidence and severity as well as the survival of the fungus between seasons

(Maude, 1996). It is recommended that crop rotations with a minimum of two years between susceptible crops be used to reduce inoculum build up and allow for the proper degradation of infected debris by soil-borne microorganisms (Holliday, 1980; Ntahimpera et al., 1997). Increased tillage should be practiced to bury debris, since the fungus has been reported to have an inoculum potential 20% higher for a chisel plow, where the debris remains close to the soil surface, than for a moldboard plow which buries the debris (Ntahimpera et al., 1997; Fininsa and Tefera, 2002). The removal or burning of debris and alternative hosts also aids in reducing potential hosts for inoculum build up (Chase, 1987; Maude, 1996). Cultural alterations are also practiced within cropping seasons to decrease infection spread. Increased row spacing allows for cultivation and the removal of weeds which increases air flow in the canopy, which can decrease leaf wetness, making the environment less conducive for the pathogen (Chase, 1987; Hall and Nasser, 1996). It is highly recommended to avoid cultivation during periods of leaf wetness or high humidity, as the pathogen can be transferred onto machinery and spread throughout the crop and to other fields (Tu, 1988; Norman and Strandberg, 1997; McMullen and Lamey, 2009). Irrigation practices should also be closely monitored as they can increase the amount of free water available to the pathogen and cause further spread by splashing of spores or mycelia (Tu, 1981; Chase, 1987).

When cultural practices are not sufficient to control the spread of anthracnose, chemical controls are commonly used as a secondary measure of protection (Tu, 1996). Chemical treatments for the control of anthracnose can be applied as seed treatments and foliar fungicides. The use of chemical seed treatments like DCT, fludioxonil, and azoxystrobin have been reported to reduce disease incidence and severity early in the season resulting in higher yield (Tu, 1996; Gillard and Ranatunga, 2013). The foliar fungicides, pyraclostrobin and azoxystrobin, are

commonly used for the management of anthracnose by preventing disease progression and the production of secondary inoculum throughout the season (Gillard et al., 2012b). In the past benomyl based chemicals were effective in disease control, but were discontinued due to health concerns (Health Canada, 2009). Altogether, foliar fungicides are effective in increasing yields, but they are costly and require very specific application timings for the best control (Bailey et al., 2000; Conner et al., 2004; McMullen and Lamey, 2009; Gillard et al., 2012a). Therefore, more efficient disease management practices are required, such as the development of resistant cultivars.

The use of resistant cultivars is the most efficient method of control for anthracnose and has had considerable success with cultivars such as dark red kidney varieties Montcalm and Michigan Red (Dongfang et al., 2008). However, *C. lindemuthianum* exhibits a very high degree of variability and many races have been identified, which can lessen the durability of many resistant cultivars (Melotto et al., 2000; Dongfang et al., 2008). The occurrence of new races can lead to further problems, as they can be easily introduced into new geographic areas through infected seed (Melotto et al., 2000). Common races that have been introduced to Canada over the years are alpha, alpha Brazil, delta, epsilon, and kappa (Tu et al., 1984; Tu, 1988; Tu, 1994; Dongfang et al., 2008). These races have been identified with a Greek lettering system, but this has been replaced with a binary number system (Pastor-Corrales, 1991; Kelly and Vallejo, 2004). The use of the new binary system is based on the differential reactions to races of twelve differential host cultivars, which are assigned a binary value of between 1 and 2048 (Pastor-Corrales, 1991; Kelly and Vallejo, 2004). The most recently discovered type, race 73, was reported in 2001 in Manitoba and later in Ontario in 2003 (del Rio et al., 2003). This race is now the predominant race in major Canadian dry bean growing areas and is a concern, as resistant

cultivars effective against the older races (delta, 23 and alpha-Brazil, 89) have little to no resistance to race 73 (Dongfang et al., 2008).

In the past, breeding programs used monogenic resistance as the focus and the pathogen was able to quickly overcome the resistant genes by developing new races (Melotto et al., 2000). The new races displayed genetic variability for virulence as well as changes in morphology, vitamin requirements and phenolic metabolism, making breeding for resistance against these new races more difficult (Holliday et al., 1971). Today breeding programs are focusing on the use of multiple resistance genes pyramided into a single cultivar to avoid the rapid breakdown of resistance (Melotto et al., 2000; Kelly and Vallejo, 2004; Dongfang et al., 2008). This type of breeding can extend the durability of resistant varieties, as seen with cultivar G2333, which carries the resistant genes *Co-4<sup>2</sup>*, *Co-5* and *Co-7* and is currently resistant to all known races (Dongfang et al., 2008). Research continues on molecular genetic detection of lines with multigenic resistance and development of resistance cultivars that focus on a more durable approach.

#### **1.5.4 Yield Reductions**

In the absence of suitable control measures, anthracnose has been shown to substantially reduce seed yield and quality (Conner et al., 2006a). In tropical and subtropical regions, yield losses of up to 95% have been reported following severe infections under favorable conditions for anthracnose (Mohammed and Sangchote, 2007). In more temperate areas like Manitoba, yield losses of 15-30% have been reported with infections as low as 7% (Conner et al., 2004). The high incidence of seed discoloration associated with infected seed also impacts consumer acceptance, which further impacts crop value (Pynenburg et al., 2011; Gillard et al., 2012a). This pathogen is a large threat in dry bean production areas as it is a seed-borne pathogen that can

cause significant yield losses and can form new races rapidly. Therefore, it is an important pathogen that requires further research for new control options.

## **1.6 Thermotherapy Treatment**

### **1.6.1 Introduction**

Controlling pathogens, such as those that cause CBB, halo blight, and anthracnose can be difficult due to their seed-borne nature. The primary control measure currently recommended is the use of disease free seed (Janse and Wenneker, 2002); however, production of this seed is difficult in most dry bean growing areas of Canada due to high humidity. Cultural, chemical, and genetic controls are utilized by growers to reduce disease pressure in conducive environments (Grondeau et al., 1994; Janse and Wenneker, 2002). However, poor or inconsistent control with these methods and the growing concerns with the hazards and costs of chemical control has led to many studies that utilize alternative control methods (McGee, 1995; Tinivella et al., 2005). A potential area for alternate control is the use of thermotherapy.

Thermotherapy is a century-old method defined as the application of heat to plant propagation materials or plant parts using specific temperature-time regimes to damage or kill the pathogen without causing significant harm to the host (Baker, 1962; Grondeau et al., 1994).

Thermotherapy is also considered an option for organic growers for seed disinfection and an alternative to the more commonly used chemical controls (Tinivella et al., 2005). Numerous studies have demonstrated that various thermotherapy treatments can be effective in reducing or eliminating seed-borne pathogens in vegetables (Shiomi, 1992; Jahn et al., 2006), cereals (Forsberg, 2004) and legumes (Grondeau et al., 1994).

Studies using thermotherapy have shown that if sufficiently high temperatures are reached, microbes on or more importantly within the seed can be killed. (Baldi et al., 1981; Grondeau et



al., 1994; McGee, 1995). The ability to destroy microbes within the seed is something that cannot be achieved with most chemical treatments. The application of heat for seed treatment has been conducted in various forms, including hot water, hot dry air, aerated steam, hot oil soak, microwave radiation and several other derivations of these heat treatments (Baker, 1962; Doornik, 1992; Cavalcante and Muchovej, 1993; Grondeau et al., 1994; Grum et al., 1998; Forsberg, 2004). The first three are the most commonly used forms of thermotherapy with hot water treatments being the longest used. The first recorded hot water treatment was in 1887 by Jensen for the control of loose smut in cereals (Baldi et al., 1981; Grondeau et al., 1994).

Water is an ideal treatment source as it can penetrate plant tissues, has a high capacity for thermic change and therefore requires low temperatures and short exposure time (Baldi et al., 1981). However, there are many disadvantages associated with this treatment, such as injury to the seed itself through damage to the seed coat, invasion of saprophytic microorganisms during drying and its adverse effects on germination (Baldi et al., 1981; Grondeau et al., 1994). Dry heat has been used as an alternative to hot water, as it causes less injury to the host and does not require prolonged drying after treatment (Baldi et al., 1981; Grondeau et al., 1994). Yet the application of dry heat requires increased exposure time and temperatures compared to hot water and may require rehydration for germination to occur (Grondeau et al., 1994). Aerated steam, on the other hand, is generally the most efficacious of the three as it is able to penetrate the outer layers of the seed like hot water, without the adverse effects on seed health and germination, and requires shorter exposure times than dry heat (Baldi et al., 1981; Grondeau et al., 1994). The application of aerated steam, however, requires expensive and complex equipment. (Baldi et al., 1981; Grondeau et al., 1994). Hot oil soaks have been successful in eliminating seed-borne pathogens without the adverse effects of hot water soaks, but it is not suitable for large scale

production due to its cost (Sinclair, 1993; Grondeau et al., 1994). The effect of microwave radiation will be discussed in detail later.

The application of the aforementioned treatments, as well as the less common derivations of them, are simple in principle, but their successful use is very dependent on seed type (Grondeau et al., 1994; Clear et al., 2002). The optimal temperature and time exposure required for each treatment varies for different crops and sometimes even for different cultivars within a crop (Grondeau et al., 1994; Clear et al., 2002). The differences in the susceptibility to heat among seed cultivars and species may be due to seed age, moisture content, vigour, dormancy period and seed size (Baker, 1962; Grondeau et al., 1994). Therefore, preliminary testing of each crop and cultivar is required to determine the optimal temperature-time regime as well as the thermotherapy treatment type required for effective control of the pathogen without harming the host (Grondeau et al., 1994; McGee, 1995).

### **1.6.2 Thermotherapy in Various Crops**

Thermotherapy has been used for pathogen eradication from seeds for a large range of plant species, from soybeans (*Glycine max* (L.) Merr.) to garden poppies (Zinnen and Sinclair, 1982; Shiomi, 1992). For each plant-pathogen interaction the efficacy of thermotherapy varies based on seed type, cultivar, treatment type, exposure time, temperature, and pathogen type (Baker, 1962; Shiomi, 1992). Based on these factors, the impact of thermotherapy on the control of pathogens and seed germination varies. Studies on these differential interactions have been conducted on both bacterial and fungal plant pathogens (Baker, 1962; Baldi et al., 1981; Doornik, 1992).

Bacterial pathogens, such as *Xanthomonas* spp. and *Pseudomonas* spp., cause bacterial diseases on a wide range of plant species (Grondeau et al., 1994). Their ability to cause serious disease outbreaks, when present in as few as one in five thousand seeds, enables these pathogens

to have detrimental effects on crop yield (Grondeau et al., 1994). Thermotherapy has been able to satisfactorily control these diseases in numerous crops. Black rot in *Brassica oleracea* caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson was eliminated using hot air treatment at 75°C for 7 days, but adverse effects on germination were observed if seed was not pre-dried prior to heat exposure (Shiomi, 1992). Hot water soaks were recommended at 50-54°C for 5-30 minutes for controlling bacterial diseases in *Solanum lycopersicum* L., *Daucus carota* subsp. *sativus* var. *atrorubens* Alef., and *Brassicaceae* spp. with minimal effect on seed germination (Chupp and Sherf, 1960; Janse and Wenneker, 2002). This same type of treatment was also recommended for control of fungal diseases of *Allium cepa* L., *Pisum sativum* L., and *Solanum tuberosum* L. (Chupp and Sherf, 1960; Janse and Wenneker, 2002).

Thermotherapy is effective in controlling the fungal species *Fusarium graminearum* Schwabe in barley (*Hordeum vulgare* L.) and wheat (*Triticum* spp.) seed using dry air at 60°C for 21 and 15 days, respectively, and *Fusarium culmorum* (W.G. Smith) Sacc. in winter wheat using dry air at 60-65°C for 30 min (Hoersten, 1996; Clear et al., 2002). The efficacy of a hot water treatment on *F. culmorum* in winter wheat has also been tested and caused the destruction of the pathogen between 48-55°C without resulting in heat damage to the seed (Hoersten, 1996). The use of aerated steam was evaluated to treat large batches of cereal crops, using a process of heat and moisture transfer that was specific to individual seed lots, but was unable to effectively eradicate deep seated pathogens (Forsberg, 2004). Thermotherapy for the control of fungi has been particularly successful in cereal crops, as well as in several vegetable crops, poppies, and soybean. Various temperature-time combinations were utilized for optimal disease control, while maintaining high seed germination rates (Zinnen and Sinclair, 1982; Grondeau et al., 1994; Jahn et al., 2006). Treatments such as a hot water soak, a hot oil soak, a carbon tetrachloride soak, a

polyethylene glycol soak and an electron treatment are a few of the treatments currently used for various fungal diseases (Grondeau et al., 1994; McGee, 1995).

The control of *Colletotrichum* spp. using thermotherapy has been observed in lupins (*Lupinus angustifolius* L.) and *Anemone* spp. corms using hot air and hot water treatments, respectively (Doornik, 1992; Thomas and Adcock, 2004). The pathogen *Colletotrichum acutatum* J. H. Simmonds, which causes leaf curl and necrosis in poppy, is almost completely suppressed using a hot water treatment at 50°C for 1 h or 47.5°C for 1.5 h (Doornik, 1992). This treatment had a slight effect on corm germination, but this problem could be rectified if corms were stored for four days in moist vermiculite at 20°C between treatment and planting. The use of a dry heat treatment to control *C. acutatum* was also tested, however results were inconsistent (Doornik, 1992). Dry heat was successful for treating *Lupinus* spp. seed infected with *Colletotrichum lupini* (Bonden) Nivenberg, Feilert Hagedorn comb. nov. (Thomas and Adcock, 2004). Various exposure times and temperatures were successful in reducing *C. lupini*, however the only times that germination was not affected was at 60°C for seven days or 65°C for four days (Thomas and Adcock, 2004). Variable results were seen in different seed lots.

### **1.6.3 Thermotherapy in Dry Beans**

Although there have been many successful uses of thermotherapy on numerous crops, there has been limited success in dry bean. The control of CBB and halo blight has been limited due to issues with seed germination and little research has been conducted on the control of anthracnose (Grondeau et al., 1994). The use of thermotherapy on large seeded legumes such as dry bean, to control fungal and bacterial pathogens is difficult due to the similar heat tolerance ranges of the pathogen and the host seed (Grondeau et al., 1994; Hoersten, 1996; Reddy et al., 1998). In an experiment on *P. vulgaris*, temperatures above 90°C were lethal for dry bean seed, while

temperatures between 60-80°C for various time periods did not affect germination (Baldi et al., 1981). Therefore, a pathogen must be sensitive to a specific temperature-time regime that does not result in a significant loss in germination before satisfactory control of the pathogen is obtained.

Dry bean seed can also be adversely affected by the presence of increased moisture, as was seen with hot water treatments. When immersed in hot water, the seed quickly imbibes water, causing the seed to swell and slough off its seed coat (Sinclair, 1993). The use of vegetable oil was recommended in place of water to eliminate the sloughing of seed coats and the imbibing of water (Sinclair, 1993; McGee, 1995). The concept of soaking dry beans in vegetable oil was utilized in a study in Ethiopia and CBB was reduced by 60% when seed was soaked at 65°C for seven minutes in one out of two years of the study, however, no significant advantages for disease development, yield, or seed quality were noted in that year (Fininsa and Tefera, 2001). The use of low seed moisture is another property that decreases the adverse effects of thermotherapy treatments on dry beans. Moisture content has been shown to be closely correlated with seed germination and low moisture can minimize the adverse effects of thermotherapy on germination (McGee, 1995). The need to establish inoculum transmission thresholds is also required for more pathogens to enable more practical disease control in the field. *Pseudomonas syringae* pv. *phaseolicola* is one of the few pathogens for which such information is available, where the presence of 12 infected seeds per acre can cause a severe epidemic (Walker and Patel, 1964; McGee, 1995).

In reviewing the use of thermotherapy, several experiments utilizing hot water or hot air alone, have been found to not sufficiently control CBB or halo blight in bean seed under varying temperature time regimes (Grondeau et al., 1994). Other studies using techniques such as

electrotherapy and electrostatic treatment to control viruses and increase seed health in dry bean have also demonstrated poorer efficacy than desired (Morar et al., 1999; Hormozi-Nejad et al., 2011). Therefore, the use of a two-step process using dry heat was tested for treating both the seed and the meristem tissue of plants infected with CBB and halo blight (Grum et al., 1998). This method was successful in eradicating the pathogens from the seed as well as the meristem tissues; however, this is not practical at a commercial level (Grum et al., 1998).

The success of thermotherapy for controlling or eradicating seed-borne diseases of dry bean is still lacking. Even though several studies concerning CBB and halo blight have been conducted, few have had success on a large scale (Grondeau et al., 1994). The dearth of information on the control of dry bean anthracnose using thermotherapy is also surprising, as success has been noted for other crops infected by *Colletotrichum* spp. (Doornik, 1992; Thomas and Adcock, 2004). Therefore an investigation into a more promising thermotherapy technique like microwave radiation is required for these seed-borne diseases.

#### **1.6.4 Microwave Radiation**

Microwave radiation has been utilized for control of pests in food processing, crop storage, and seed production (Spilde, 1989; Bouraoui et al., 1993; Cunha et al., 1993; Adu and Otten, 1996). Microwave radiation is believed to use heat as the lethal mode of action for controlling pathogens (Grondeau et al., 1994; Reddy et al., 1998). It has been suggested that radiation may disrupt the microbial cells directly, although this has not been confirmed (Copson, 1975; Hankin and Sands, 1977; Yoshida and Kajimoto, 1988). Although the mode of action is unclear, microwave radiation shows promise as an alternative to other thermotherapy methods. Benefits such as earlier germination and increased vigour have been noted in previous studies (Van Assche and Leuven, 1987; Van Biervliet, 1987; Spilde, 1989; Tylkowska et al., 2010).

Another beneficial characteristic of microwave radiation is the short exposure time required for treatment, due to its ability to rapidly generate heat (Adu et al., 1995). Thermal energy in microwave radiation is produced through dielectric heating (Copson, 1975). Dielectric heating is the process in which high-frequency alternating electromagnetic radiation (EMR), 300 MHz-300 GHz, heats a dielectric material using two major mechanisms, namely dipole rotation and ionic polarization (Bouraoui et al., 1993). Dielectric materials are materials that can become polarized and are able to store electric energy when exposed to EMR (Adu et al., 1995). These materials tend to act as insulators, where charges do not flow through the material, but rather are stored and rearranged to align with the rapidly changing electric field (Copson, 1975). It is through the rearrangement of the dipoles which causes friction to produce heat and is then transferred between molecules, warming the material thoroughly (Bouraoui et al., 1993).

The production of heat through dielectric heating is highly dependent on the dielectric permittivities of the materials involved (Nelson, 1996; Jiao et al., 2011). Permittivity is defined as how an electric field affects, and is affected by, a dielectric material and is expressed as a complex quantity with a real and an imaginary part (Nelson, 1996). The real part is associated with the materials capability to store electric energy and the imaginary part is associated with the dissipation of electric energy, which is converted to heat energy in the material. Permittivity is a function of the materials moisture content (MC), temperature, bulk density, and the frequency of the electric field applied (Nelson, 1996; Berbert et al., 2002; Jiao et al., 2011). Relative permittivity was positively correlated with MC of dry bean seed at various frequencies (Berbert et al., 2002). The imaginary part of permittivity, also referred to as dielectric loss or heat production, was also positively correlated with MC (Berbert et al., 2002). This can be a deterrent to utilizing microwave radiation for the treatment of seed-borne pathogens, as overheating of

seed can have adverse effects on seed germination (Berbert et al., 2002; Han, 2010). In order to utilize heat treatments like microwave radiation, specific time-temperature regimes, similar to those used for hot water treatments, are still required to sufficiently harm/kill the pathogen without overheating the seed itself (Grondeau et al., 1994).

Numerous studies have tried to determine the optimum time-temperature regimes for microwave radiation on various seed types to eliminate pathogens and to improve seed germination and vigour. The use of microwave radiation of true seed of cassava (*Manihot esculenta* Crantz) infected with *Xanthomonas campestris* pv. *manihotis*, *Fusarium* spp., *Cladosporium* spp., and *Colletotrichum* spp. was shown to control these pathogens when temperatures reached 77 °C after 120 seconds of exposure at 1400 W, 2450 MHz (Lozano et al., 1986) . The use of various power settings, exposure times, and seed moistures were tested on wheat seed to control *F. graminearum* Schwabe. Infection was reduced from 36% to 7% , while seed quality, (85% germination and 80% vigour) was maintained (Reddy et al., 1998).

The use of microwave radiation on winter wheat has been compared to the traditional hot water and hot air treatments for the eradication *F. culmorum* (W.G. Smith) Sacc. (Hoersten, 1996). In this comparison, microwave treatment alone was the only treatment that did not eradicate the pathogen completely (Hoersten, 1996). However, with the addition of steam to the microwave treatment, the pathogen was controlled at lower temperatures than hot water and this process was three times faster than the hot air treatment in a closed system (Hoersten, 1996). Microwave radiation has also successfully eradicated *Erwinia carotovora* var. *carotovora* (Jones) Dye from tobacco (*Nicotiana tabacum* L.) seed after twenty minutes of microwave radiation (Hankin and Sands, 1977). This success led to the testing of other seed types, but after two minutes of irradiation, seed germination was reduced to 10% in cabbage and to 0% in bean



seed (Hankin and Sands, 1977). From this, a relationship between seed size and microwave tolerance was formed (Hankin and Sands, 1977). It appeared that large seeds are unable to tolerate long exposure times due to their inability to radiate heat away from seed during treatment.

Expanding on the previous research on microwave radiation, the tolerance of navy bean was further tested with exposures at three intensities for various time periods (Spilde, 1989). It was determined that when seed was exposed to temperatures between 48-52°C, germination increased significantly (6-10%). However at temperatures over 68°C significant seed damage occurred (Spilde, 1989). Increases in microwave power, from 32 to 500 W, also resulted in significant decreases in germination when temperatures rose above 32°C. Further tests on the use of microwave radiation on dry bean was conducted on seed infected with *Alternaria alternata* (Fr.) Keissl., *Fusarium* spp. and *Penicillium* spp. (Tylkowska et al., 2010). When exposed to 650 W, 2450 MHz, dry bean seeds were able to tolerate 120 seconds of microwave exposure with no adverse effects on germination or vigour (Tylkowska et al., 2010). Microwave radiation significantly decreased the presence of *Penicillium* spp., but was unable to control *A. alternata* and *Fusarium* spp. (Tylkowska et al., 2010). Other studies have shown contrary results for *Fusarium* spp. (Lozano et al., 1986; Reddy et al., 1998). The difference in results may be attributed to differences in methodology and location of the inoculum in the seed (Tylkowska et al., 2010).

The placement and morphology of the inoculum may play a large role in the efficacy of microwave radiation on seed-borne pathogens (Cavalcante and Muchovej, 1993; Tylkowska et al., 2010). It has been demonstrated that fungal spores react differently to microwave radiation based on cell composition (Cavalcante and Muchovej, 1993). Pathogens that produce hyaline

single-celled spores, like *C. lindemuthianum*, are believed to be more sensitive to microwave radiation than those that produce multi-celled or dark pigmented spores, (*Fusarium oxysporum* Schlecht and *Bipolaris sorokiniana* (Sacc.) Shoemaker, respectively) (Tylkowska et al., 2010). Previous research on *C. lindemuthianum* and Psp in dry bean showed no control as the inoculum was applied artificially and did not produce disease symptoms even on the control treatments (Van Biervliet, 1987). Further research into this topic is required to confirm the implications on the control of anthracnose and the effect of pathogen placement on the seed.

For dry beans, microwave treatment appears to have less of an adverse impact on seed germination and vigour than other thermotherapy methods. Previous studies on the control of seed-borne disease indicate that microwave radiation is capable of reducing or eliminating pathogens if a suitable temperature-time regime can be developed for the host-pathogen relationship.

## **1.7 Chemical Treatment**

### **1.7.1 Introduction**

Chemical treatments for the control of seed-borne diseases in dry bean are commonly utilized when 'disease free' seed has latent infections or is unavailable. Despite concerns regarding the hazards of chemical control, pesticides are currently one of the most effective management strategies available for the control of seed-borne diseases (McGee, 1995). Several pesticides provide broad spectrum control and can affect pathogens in multiple ways (Agrios, 2004). Other pesticides provide a narrow spectrum of control on a specific metabolic site or enzyme that is affected directly (Agrios, 2004). Both types of pesticides can be applied as seed or foliar treatments.

Seed treatments protect seeds from pathogens in, on or around the seed and aid in germination and seedling growth. Foliar pesticides are applied after emergence and are used to suppress disease symptoms that have developed from infected seed or secondary infection (Conner et al., 2004). Currently there are few pesticides that are highly effective for the control of CBB and halo blight, while there is little diversity in mode of action of pesticides currently recommended for the control of anthracnose in dry bean. However, several pesticides have been reported to suppress disease symptoms and reduce losses in yield and seed quality when applied as seed treatments, foliarly, or in combination (Gillard et al., 2012a).

### **1.7.2 Seed Treatment**

Seed treatments are utilized for many soil- and seed-borne diseases. Numerous seed treatments are currently available to protect dry bean seed from a broad range of pathogens, such as those that cause seed rot, pre- and post-emergent damping off, and root rot (MAFRI, 2012). However, the spectrum and efficacy of seed treatments is limited for the control of CBB, halo blight, and anthracnose (Howard et al., 2000; Gillard et al., 2012a).

The use of seed treatments to control bacterial blights in dry bean over the last three decades has mainly consisted of the use of antibiotics such as streptomycin or copper-based products (Howard et al., 2000). The use of agricultural streptomycin sulfate was a standard seed treatment on dry beans in the late 1980's to the late 1990's for seed imported into Canada from the US (Howard et al., 2000). Streptomycin is effective in suppressing bacterial growth through the inhibition of protein synthesis in the bacterial ribosomes, eventually causing cell death (MAFRI, 2012). However streptomycin was not fully registered in Canada as a seed treatment and eventually the importation of seed treated with antibiotics was banned due to antibiotic resistance concerns (Howard et al., 2000). Kasugamycin was another antibiotic tested in the late 1970's and

was effective in reducing bacterial infection by up to 98%, but was never registered for use in Canada (Taylor and Dudley, 1977; Taylor et al., 1979b).

Copper based products have been commonly used since the early 1970's and are available in various formulations, such as copper sulphate ( $\text{CuSO}_4$ ), cupric hydroxide, tribasic copper sulphate, and copper oxychloride (Howard et al., 2000).  $\text{CuSO}_4$  was as effective as streptomycin treatment, but both products were only able to suppress the disease (Howard et al., 2000). Currently registered copper based seed treatments consist of between 35-50% copper hydroxide and can suppress bacterial growth when copper is in a "free" or "ionic" state (Ritchie, 2004; MAFRI, 2012). The "ionic" state of copper is very reactive and allows it to bind and kill bacteria at multiple contact sites (Ritchie, 2004). The reactivity of copper is ideal for suppressing bacteria; however, it can have phytotoxic effects on the host plant if the pH of the water in contact with the copper is very acidic. Other inorganic compounds like zinc based products, seed coat polymers, and micronutrient based products have been tested, but they were generally ineffective for the control of bacterial blights in dry bean (Howard et al., 2000).

The use of fungicide seed treatments for the control of seed-borne anthracnose in dry beans was implemented over thirty-five years ago when the use of DCT (diazinon, captan, and thiophanate-methyl) was first introduced to Ontario (Edgington and MacNeill, 1978). The active ingredient, thiophanate-methyl, is able to control the pathogen *C. lindemuthianum* by binding to tubulin, which then inhibits the fungus from completing mitosis and cell division (Clemons and Sisler, 1971; Tu, 1996). Although DCT is effective in controlling anthracnose, it has slowly been phased out in the last ten years due to lack of control of soil pathogens (Conner et al., 2004).

To replace DCT, products like Vitaflo 280 (carbathiin and thiram, Bayer CropScience, Guelph, ON), Apron Maxx (metalaxyl-M and fludioxonil, Syngenta Crop Production Inc.,

Guelph, ON) and FMA (fludioxonil, metalaxyl-M, and azoxystrobin, Syngenta Crop Production Inc., Guelph, ON) have been tested (Gillard and Ranatunga, 2013). Vitaflo 280, which affects pathogen respiration and has multisite contact effects on anthracnose, decreased leaf infection similarly to DCT and improved plant emergence under low disease pressure, however control under high disease pressure was insufficient (Matus et al., 2004; MAFRI, 2012). When Apron Maxx, a signal transduction inhibiting fungicide, was compared with DCT, DCT provided superior disease control on both leaf and pod tissue (Gillard et al., 2012a). The FMA treatment was the most comparable to DCT for anthracnose control and has currently become the dominant product in the market place, based on efficacy and ease of use (Gillard and Ranatunga, 2013). The FMA product controls anthracnose through the inhibition of signal transduction (fludioxonil) and respiration (azoxystrobin) in the fungal cells (MAFRI, 2012). However, there are growing concerns about applying strobilurins such as azoxystrobin, as seed treatments, since they are currently used for foliar treatment. With continuous use, this could lead to a buildup in insensitivity of the pathogen to this fungicide group (Gillard and Ranatunga, 2013).

### **1.7.3 Foliar Treatment**

Foliar pesticides are important for the season long control of CBB, halo blight and anthracnose when disease develops on infected plants, as well as for the control of secondary infections. Pesticides increase seed quality and yield when applied at the early- to mid-flowering stage. Despite continued research on application timing, few foliar pesticides completely control these seed-borne diseases (Garrett and Schwartz, 1998; Conner et al., 2004).

For the foliar control of bacterial blights in dry bean, only streptomycin and copper products are effective. Since streptomycin is banned in Canada, copper products are primarily used for foliar applications (Howard et al., 2000). These products have the same formulation as in the

seed treatments and require continuous application throughout flowering to suppress disease. Under certain conditions they have little effect on pod infection and no clear benefit for yield (Weller and Saettler, 1976; Saettler, 1989a). Other inorganic products like potassium and sulphate have been tested, but they have demonstrated poor efficacy in the control of bacterial blights in dry bean (Weller and Saettler, 1976; Schwartz and Pastor-Corrales, 1989).

Foliar control of anthracnose in dry beans is currently limited to the strobilurin fungicide group, including azoxystrobin and pyraclostrobin (Bartlett et al., 2002). Strobilurins control anthracnose through the inhibition of respiration in fungal cells, and can increase yields by 15-60% (Oliveira, 2003; Conner et al., 2004; Gillard et al., 2012b). The sequential application of pyraclostrobin at 40 and 80% bloom has been the most effective timing for decreasing seed discolouration and leaf and pod disease severity, resulting in increased yields (Conner et al., 2004). Azoxystrobin also reduces disease severity and seed discolouration, however not as effectively as pyraclostrobin (Pynenburg et al., 2011; Gillard and Ranatunga, 2013).

Overall, copper and strobilurin foliar treatments for bacterial blights and anthracnose, respectively, have been shown to decrease disease severity, but not eradicate the disease completely. The use of these modes of action (MOA) as a foliar treatment is of concern as the same MOA are being utilized in seed treatments as well (Pynenburg et al., 2011; Gillard et al., 2012a). The overlap of these MOA's in seed and foliar treatment raises risks of the buildup of pesticide resistance in these pathogens, which could eventually leave the few available control options ineffective.

## **1.8 Economics**

### **1.8.1 Dry Bean Prices & Yield**

The overall cost of production must be considered for disease management in a dry bean crop. Input costs such as fertilizer, herbicides, and pesticides need to be evaluated based on the economic return a grower may expect in a typical growing season. Factors such as price and expected yields will also play a large part in this decision. The export demand for dry bean is high in Canada, which provides good pricing opportunities for growers (FAOSTAT, 2012).

The demand for dry bean production and increased prices for competing crops (corn, soybean, wheat and canola) is positive for growers and is reflected in the increased average wholesale prices for dry beans over the last ten years. Navy beans have averaged \$30 per hundred weight (cwt) in the last ten years with 2012 prices increasing to \$38 per cwt (Barkley, J., pers. comm., Agricorp, 2013). For all market classes, the wholesale price for dry beans in the last ten years has increased. The increased profit potential in dry bean impacts the level of inputs growers will consider in order to obtain a higher yield potential and quality for their crop.

### **1.8.2 Net Yield & Economic Return**

Pesticides and other management strategies are needed to maintain a healthy crop to maximize profit potential in dry bean. The control of diseases in dry beans plays a large role in crop health, yield and seed quality, which can be seen with the yield increases of up to 32% in anthracnose infected fields and 43% in CBB and halo blight infected fields when chemical or thermotherapy treatments were used to control these seed-borne diseases (Saettler, 1989a; Conner et al., 2004; Arnold et al., 2011; Gillard et al., 2012a; Gillard et al., 2012b). However, the economic returns from these treatments need to be taken into account when making pest management decisions, especially under low disease pressure.

Factors such as pathogen type, disease pressure, and crop tolerance can influence the economic return obtained from a particular input. The application of seed treatments is an input that is usually recommended for all dry bean seed for protection against seed- and soil-borne organisms, whereas foliar chemical treatments are not always necessary. Foliar treatments are more dependent on environmental conditions and require further investigation before application. Economic returns can be increased when continuous field scouting, monitoring of environmental conditions, and assessment of the economic threshold are carried out in order to determine what chemical controls are required and the timing of application (Pedigo, 1998). However, the economic thresholds for many pathogens are still unknown, which makes management decision difficult for many diseases.

To determine the effect an input can have on a dry bean crop the following formula can be used to determine the return on investment (ROI) (Gillard and Ranatunga, 2013):

$$\text{ROI} = (((\text{Seed Yield} - \text{Dockage} - 2(\text{Pick})) * \text{Price kg}^{-1}) - \text{Pesticide Cost} - \text{Pesticide Application Cost})$$

This equation does not account for some production costs, such as cultivation, fertilizer, seeding materials and equipment, or harvesting equipment, as these are considered fixed costs. It does take into account moisture, dockage and seed pick, which are used to adjust net yield (Pynenburg et al., 2011) Moisture is adjusted to 18% for net yield using the moisture conversion formula:

$$\frac{100 - \text{wet moisture}}{100 - \text{dry moisture}} = \frac{\text{dry weight}}{\text{wet weight}}$$

Dockage includes material or seed below the standard quality and split seed (Canadian Grains Commission, 2010). Seed pick is the estimated percentage of infected or discoloured seed in a representative sample. Seed pick is removed twice to account for the weight of discoloured seed and the cost for removing infected seed. Once dock and pick are removed, net yield is converted



to  $\text{kg ha}^{-1}$  and multiplied by the market price, which is estimated based on crop wholesale values for that season (Gillard and Ranatunga, 2013). After calculating the crop value, the variable costs of the pesticide, surfactant and pesticide application are subtracted in order to determine economic return.

### **1.8.3 Pesticide & Pesticide Application Costs**

The cost of pesticides and their application to control pathogens is an important factor for determining the economic return in dry bean production. In calculating the return on investment, the cost of the pesticide as well as the required surfactants and application costs must be considered. The cost of the pesticides and surfactants are determined using the manufacturer suggested retail price (MSRP), which for foliar treatments like pyraclostrobin or azoxystrobin can range from \$43.24 to \$52.74 per hectare, respectively (Wright, H., pers. comm., Agricorp, 2013). The MSRP for seed treatments such as DCT or FMA, which increase yield by an average of 25%, can cost \$61.87  $\text{ha}^{-1}$  (Gillard and Ranatunga, 2013; Wright, H., pers. comm., Agricorp, 2013). The application costs are estimated using averages from custom framework rates and average around \$25 per hectare for ground application (OMAFRA, 2011; Gillard and Ranatunga, 2013). Therefore, when selecting management strategies and input requirement, the demand, wholesale price and overall return on investment needs to be considered in order to grow and maintain a profitable dry bean crop.

## **1.9 Research Proposal**

### **1.9.1 Hypothesis**

Dry bean production in Canada is most common in the provinces of Ontario and Manitoba; however the environmental conditions in both provinces are highly conducive to the spread of seed-borne diseases, which can lower yields and seed quality. The use of a chemical treatment as

both seed and foliar treatments is common for the control of seed-borne diseases in dry beans. However, the overlapping use of copper based pesticides and strobilurin fungicides on bacterial blights and anthracnose, respectively, as a seed treatment and a foliar treatment raises concerns due to the buildup of resistance of the pathogens to the pesticides. Alternative types of seed treatment, like thermotherapy, have been reported to reduce decrease disease pressure in numerous crops and we hypothesize that microwave radiation can reduce or eliminate seed-borne pathogens from dry bean without causing harm to the seed itself.

### **1.9.2 Objective & Justification**

The objective of this study is to determine if microwave radiation can reduce or eliminate seed-borne diseases in dry beans without affecting seed germination and vigour. The efficacy of microwave treatment will be tested with and without the use of chemical treatment to determine if there is increased control when treatments are applied in combination. The economic benefit of the microwave treatment will also be evaluated as well as the practicality of this type of treatment for seed producers. If this treatment is successful it could provide a new pillar of control for seed-borne diseases and an alternative for organic growers.

## CHAPTER TWO

### **Effect of microwave radiation on dry bean seed infected with *Xanthomonas axonopodis* pv. *phaseoli* with and without the use of chemical seed treatment**

#### **2.1 Abstract**

Common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) is a seed-borne pathogen that is difficult to control in dry bean (*Phaseolus vulgaris* L.). Laboratory and field studies were conducted over a two-year period to determine the effect of microwave radiation on navy (cv. Navigator and Envoy) and pinto (cv. AC Ole) bean. Laboratory tests resulted in a 12-25% decrease in germination following 50-60 s of radiation, while less than a 10% loss was observed between 0-40 s. Pathogen viability was also tested, however the incidence of pathogen infection was low and no correlation was observed between exposure time and the incidence of colonization. In field studies conducted at Morden, MB (2012) and Ridgetown and Exeter, ON (2012-2013) microwave radiation and two chemical seed treatments (copper hydroxide 53.8% and pyraclostrobin + fluxapyroxad + metalaxyl) were evaluated for their effect on emergence, disease infection, seed pick, yield and return on investment. The application of microwave treatment decreased emergence up to 7%, but did not impact the other parameters measured. Chemical treatment alone or in combination with microwave treatment also did not affect emergence, disease incidence, yield, seed pick, or return on investment.

#### **2.2 Introduction**

*Xanthomonas axonopodis* pv. *phaseoli* (Smith) Vauterin et al. (Xap) (syn. *X. campestris* pv. *phaseoli* (Smith) Dye) is the causal agent of common bacterial blight (CBB) in dry bean (*Phaseolus vulgaris* L.). Common bacterial blight is easily spread from infected seed and is the

number one foliar disease of dry bean in Canada (Bailey et al., 2003). Symptoms first appear as water soaked spots on the leaves, which develop into necrotic lesions surrounded by a chlorotic border (Hall, 1991). Infection can move to the pods in environments with high humidity and temperature. Pod lesions initially appear water soaked and become slightly sunken with a brick-red border (Schwartz et al., 2005). Severe pod lesions can infect the developing seed and cause shrivelling and a butter-yellow discolouration (Swings and Civerolo, 1993).

Infected seed is the primary inoculum source for CBB and the use of disease-free seed is an important management strategy to control its spread (Bailey et al., 2003; Schwartz et al., 2005). In Canada, seed production areas experience frequent rainfall and high humidity, which typically results in infected seed lots (Gillard et al., 2009). Therefore, seed has to be obtained from production areas with less conducive environments for foliar disease, such as Idaho. The transportation of seed from these production areas can be costly, particularly for eastern Canadian growers (Coyne and Schuster, 1974a). Other strategies have been developed to reduce CBB, such as the use of cultural (Hall and Nasser, 1996), chemical (Fininsa, 2003), antibiotic (Howard et al., 2000), and genetic controls (Michaels et al., 2006). Despite these practices, CBB is often not completely controlled. The development of resistant cultivars is ongoing and the use of cultivars, such as OAC Rex, has become a primary method of control for CBB. The development of resistant cultivars continues in navy bean and other market classes. Developing such cultivars, however, can be difficult as resistance to CBB is a quantitative trait and is affected by multiple genes, traits, and environmental conditions (St. Clair, 2010).

The use of bactericides applied foliarly and as seed treatments was used to control CBB in Canada, but the use of antibiotics is now prohibited (Howard et al., 2000). Copper hydroxide ( $\text{Cu}(\text{OH})_2$ ) has become the current industry standard for controlling numerous bacterial blights.

However, this product is more efficacious as a foliar spray than as a seed treatment (Fininsa and Tefera, 2001). Alternative seed treatment methods have also been investigated in order to control CBB, including the use of thermotherapy (Grondeau et al., 1994).

Thermotherapy has been used to effectively manage various pathogens in many cropping systems through the use of hot liquid soaks (either oil or water), hot dry air, and steam treatment (Sinclair, 1993; Grondeau et al., 1994; McGee, 1995; Forsberg, 2004). These thermotherapy treatments have been less successful in dry bean, due to seed health issues (Spilde, 1989; Sinclair, 1993). The use of microwave radiation, however, has been shown to have minimal effect on dry bean seed germination and vigour (Tylkowska et al., 2010). Microwave radiation has also controlled numerous bacterial and fungal pathogens in tobacco (*Nicotiana tabacum* ‘Consolidated L.’), *Triticum* spp., cassava true seed (*Manihot esculenta* Crantz), and soybean (*Glycine max* L.) (Hankin and Sands, 1977; Cavalcante and Muchovej, 1993; Han, 2010; Knox et al., 2013). In dry bean, the presence of the fungal pathogen *Penicillium* spp. decreased after short intervals of radiation (Tylkowska et al., 2010), but its effect on bacterial pathogens was not tested.

Microwave radiation may be a low cost addition or an alternative to current chemical treatments, to reduce or eliminate seed-borne pathogens, without affecting seed germination. In order to determine the benefit of such a treatment, laboratory and field studies were conducted to evaluate the efficacy of microwave radiation (Tylkowska et al., 2010) using seed naturally infected with Xap for both navy and pinto seed lots. These seed classes were chosen as they are common in dry bean production areas of Manitoba and Ontario and few CBB resistant cultivars are available. The studies were organized to determine the effect of microwave radiation on seed germination and vigour as well as its effect on Xap viability in the laboratory and field.

## **2.3 Materials & Methods**

### **2.3.1 Laboratory Study**

Thermotherapy studies were conducted over a two-year period (2012-2013) using a randomized complete block design (RCBD) to determine the effect of microwave radiation on dry bean seed naturally infected with Xap in navy (cv. Navigator in 2012 and cv. Envoy in 2013) and pinto (cv. AC Ole) bean. Seed was obtained from field trials in 2011 in Morden, MB and 2012 in Exeter, ON for the 2012 and 2013 laboratory studies, respectively. Seed was collected from plots with foliar disease ratings of 15-25% to ensure high disease pressure. Seed moisture was assessed using a Fisher Scientific Isotemp Forced Air Oven (120 V, 60 Hz, 1800 w, 15.5 A) to dry down seed to a uniform moisture. For the seed used in navy bean and the 2012 pinto bean experiments, the seed moisture ranged between 7.1-8.6% moisture, while the seed for the 2013 pinto bean experiments was 10.3%. A microwave oven (1100W 2450 MHz, General Electric Co., Fairfield, Connecticut, U.S.) was used to treat four replicates of each seed lot with ten microwave exposure times ranging from 0-90 s, in 10 s increments. To determine the temperature change due to microwave radiation, a 450-500 ml beaker containing 200 ml of water was placed in the center of the microwave oven on a paper plate and the water temperature was measured before and after the microwave treatment. Each plate contained 150 seeds, which were evenly spread around the beaker in a single layer.

To determine the effect of microwave radiation on germination, 100 seeds from each experimental unit were planted into 50 WG 60 Wellpak silica sand (B.P. Dust Control, Walton, ON) in 21.6 cm x 13.0 cm x 10.2 cm plastic clam lid containers (Par-Pak Ltd., Brampton, ON) . The containers were incubated for 6-7 days in a germination chamber set at 25°C with a 12L:12D photoperiod and a relative humidity (RH) above 60%. To evaluate germination, the Canadian

Food Inspection Agency's (CFIA) methods and procedures for testing seed were used (CFIA, 2011). At the end of the study, the total dry weight of germinated plant matter was recorded from each container as an assessment of plant vigour.

In 2012, an experiment with four replicates was conducted to evaluate seed colonization through plating on potato dextrose agar (PDA) following different lengths of exposure to microwave radiation. Before plating, the seed was surface-sterilized in a 10% sodium hypochlorite solution for 2 min. A total of 50 seeds were distributed evenly over ten 100 x 15 mm petri plates (Fisher Scientific Company, Ottawa, ON) and left to germinate for a minimum of 7 d at room temperature. Seed was then evaluated for percentage of seed with visible bacterial colonization and the percent germination.

### **2.3.2 Field Study**

Over a two-year period, two field trials per site were conducted in Morden, MB (2012) and Exeter and Ridgetown, ON (2012- 2013). At all sites, a randomized complete block design (RCBD) with four replications was used to arrange a total of ten treatments (Table 2.1). Seed lots from the laboratory studies were used for the field studies, except for the Exeter navy bean trial in 2012. At that site, the cv. Envoy was used, due to lack of infected Navigator seed. Disease-free seed for the non-infected controls (NICs) was obtained from the Archer Daniels Midland Company (New Plymouth, Idaho) for cv. Navigator, Gentec Seeds Inc. (Twin Falls, Idaho) for cv. Envoy and disease-free seed increase plots in Morden for pinto bean.

From the germination results in the laboratory studies, a non-linear regression was used to determine a maximum exposure rate (MER), a point in time in which a less than 10% loss in germination was noted. This loss was considered an acceptable loss for infected seed, as there are few treatments currently available that effectively control Xap. The full MER was set as the

1x Microwave treatment and half the MER was used for the ½x Microwave treatment. These microwave treatments were assessed alone and in combination with two chemical treatments; pyraclostrobin + fluxapyroxad + metalaxyl (PFM) (BASF Canada, Mississauga, ON), at 14.0 g a.i. per 100 kg of seed and a 53.8% copper hydroxide treatment or Cu(OH)<sub>2</sub> (Kocide 2000, E.I. du Pont Canada Company, Mississauga, ON), at 30.4 g a.i. per 100 kg of seed. To reduce treatment interference from soil-borne pathogens all treatments included a metalaxyl-M (Bayer CropScience, Guelph, ON) treatment at a rate of 4.0 g a.i. per 100 kg of seed.

At each location, a navy and pinto trial were planted at a seeding rate of 20 seeds m<sup>-1</sup> of row. In Morden, trials were seeded 2 June 2012 with an eight row cone-seeder with 30 cm spacing. Each plot consisted of four rows that were trimmed to 5 m in length. Plots were separated by a border of four rows of soybean (*Glycine max* (L.) Merr.) at a rate of 50 seeds m<sup>-1</sup> of row. In Exeter, plots were seeded 30 May 2012 and 19 June 2013 with a six row cone-seeder with 38 cm spacing. The navy and pinto trials had 4 and 3 rows, respectively, that were 6 m long. In the pinto bean experiment, only three rows were seeded due to limitations of available seed. In Ridgetown, plots were planted 5 June 2012 and 6 June 2013 using a five row cone-seeder with 43 cm spacing. Plots consisted of three rows that were 6 m long. For all of the Ontario sites, plots were trimmed to 5 m in length and the middle rows on the cone-seeders were used while the outer rows were left empty to allow room for evaluation.

Emergence counts were taken at 7, 10, 14 and 21 days after planting (DAP). Emergence was evaluated as a percentage of seedlings in a 4 m row length from the center row based on the seeding rate of 20 seeds m<sup>-1</sup> of row. Foliar disease ratings were conducted three times throughout the season at 4, 6 and 8 weeks after planting (WAP). Ratings of the incidence of leaf infection were taken on ten randomly selected plants per plot once the incidence of leaf symptoms



exceeded 3% in the infected control (IC). Plants were evaluated using disease severity scale of 1-9 for leaf infection (1 equalled 0% infection and 9 >25% infection) (Corrales and van Schoonhoven, 1987). For severity, a 0-5 scale was used where 0 equalled no lesion and 5 equalled 50-100% of area of leaf covered by the lesion (Mutlu et al., 2005). At the Ontario trial sites, ratings were recorded as a percentage of area of leaf tissue covered with CBB lesions and converted to the 1-9 scale afterward. The initial Ontario percentage ratings were used to analyze the data using the area under the disease progress curve (AUDPC). The AUDPC took the modified form of:

$$\text{AUDPC} = \left( \frac{R_1 + R_2}{2} \right) (t_2 - t_1) + \left( \frac{R_2 + R_3}{2} \right) (t_3 - t_2)$$

where  $R_1$  to  $R_3$  were ratings at times  $t_1$ ,  $t_2$ , and  $t_3$  (Wilcoxson et al., 1975). Ratings of the percentage of pod infection were taken when lesions became visible in the IC. At early flowering, all of the plots at Exeter (1 July 2012 and 15 July 2013) and Ridgetown (10 July 2012 and 15 July 2013) were sprayed with pyraclostrobin (BASF Canada, Mississauga, ON) at a rate of 100.0 g a.i. ha<sup>-1</sup> to minimize the establishment of fungal diseases, such as anthracnose. Additional applications were made every two weeks until maturity to minimize other foliar disease infection. The NIC treatments at Exeter were sprayed every two weeks from July to September with copper hydroxide 50% (Parasol® WG, Nufarm Agriculture Inc., Calgary, AB) at 1.3 kg ha<sup>-1</sup> in an effort to maintain a disease-free environment for this treatment.

In Morden, plots were harvested using a Wintersteiger NurseryMaster plot combine (Wintersteiger Ag, Ried im Innkreis, Austria). In Exeter, an Almaco SPC 40-2 (Almaco, Nevada, IA) small-plot combine was used for harvest. In Ridgetown, all plots were cut using an Echo SRM-260 power trimmer (Kioritz Crop., London, ON) and threshed using a stationary Almaco thresher (Almaco, Nevada, IA). Seed moisture was determined using a Motomco 919 Moisture

Meter (Dickey-John Corp., Patterson, NJ) in order to adjust plot weight to a standard storage moisture of 18%. Using a 10/64 x 3/4 slotted screen, seed was cleaned to remove foreign material or split/undersized seed, which is referred to as dockage (Canadian Grains Commission, 2010). Hundred seed weight (HSW) and seed pick were recorded once dockage was removed. Seed pick was assessed on a random sample of 100 seeds and recorded as the estimated percentage of seed in a sample visibly discoloured due to CBB infection. Every effort was made to mimic the methods used by the Ontario dry bean processors in the grading of beans for seed pick.

Later, seed yield in  $\text{kg ha}^{-1}$  and return on investment (ROI) were calculated for each treatment. The ROI was calculated using the modified equation below (Gillard and Ranatunga, 2013):

$$\text{ROI} = ((\text{Seed Yield} - \text{Dockage} - 2(\text{Pick})) * \text{Seed Price } \text{kg}^{-1}) - \text{Cost of Seed Treatment}$$

The formula accounted for seed moisture, dockage and pick to calculate seed yield, the market price of the seed and the cost of the seed treatments. This equation did not account for pesticide application costs, but in this study the cost of application for the seed treatments was considered negligible. The value for pick was doubled, firstly to account for the loss due to poor seed quality and secondly for the costs of removal of infected seed. The seed prices used were based on average crop insurance values from 2012-2013 (Agricorp, 2013) and were set at \$0.84 per kg and \$0.73 per kg for navy and pinto beans, respectively. The manufacturer's suggested retail price (MSRP) was used to calculate the cost of  $\text{Cu}(\text{OH})_2$ . To make the treatments comparable to the market place, the MSRP for PFM was calculated based on the current industry standard seed treatment for dry bean, thiamethoxam + fludioxonil + metalaxyl-M + azoxystrobin, as no price had been set for PFM at the time. The cost of microwave treatment was calculated based on a cost for electricity of 12.54¢  $\text{kWh}^{-1}$  in accordance with the Ontario Energy Board's average cost

kWh<sup>-1</sup>. Due to differences in seeding density at the three trial sites, the cost of seed treatment varied slightly for each site (Table 2.1). The 1x treatment cost for microwave was divided by two for the cost of ½x Microwave treatment for all the trials.

### 2.3.3 Statistical Analysis

Statistical analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC, U.S.A) for all trials. To meet the assumptions of normality, data were transformed when necessary based on the highest Shapiro-Wilk's statistic and outliers were removed using Lund's test for outliers (Rotondi and Koval, 2009). For all analyses a Type I error rate of  $\alpha= 0.05$  was used and the models were assumed to be additive and linear when appropriate.

In laboratory studies, PROC MIXED was used to run an analysis of variance (ANOVA) where the fixed effect was time, while replicate and year were the random effects. The percentage of germination and vigour were analyzed using PROC NLIN in the form of a dosage response nonlinear regression (Bowley, 2008):

$$Y = C + \frac{D - C}{1 + \exp[ b (\log(X) - \log(I_{50}))]}$$

where  $X$  represented microwave radiation time,  $I_{50}$  represented the microwave radiation time where a 50% response was seen and  $C$  and  $D$  represented the upper and lower limits of the percentage germination response ( $Y$ ), respectively. Data for percentage of germination were arcsine square root transformed for the navy beans in all years.

In the field studies, PROC MIXED was used to perform an ANOVA where treatment was the fixed effect and environment, environment nested within replicate, environment x treatment and environment x year were the random effects. Preplanned contrasts were compared for all the ratings. All treatments were compared to the IC to determine the treatment effect on emergence as well as the treatment efficacy in controlling CBB. The NIC and IC control were compared to

estimate the impact of the disease. To analyse the effect of prolonged microwave treatment 1x Microwave and ½x Microwave treatments were compared. The PFM treatment was compared to a standard (Cu(OH)<sub>2</sub>) for the efficacy of CBB control in the field. Microwave and chemical treatment applied alone and in combination were also compared to determine if there was any increase in emergence or control of CBB. Emergence for all sites was arcsine square root transformed for data analysis. To determine the correlation between yield and AUDPC, ROI and yield, and ROI and AUDPC, linear regressions were performed using PROC GLM.

## **2.4 Results & Discussion**

### **2.4.1 Laboratory Study**

Seed germination, vigour, and pathogen viability were not affected by short intervals of microwave radiation (<40 s) on navy or pinto bean seed naturally infected with Xap (Figure 2.1). A dramatic decrease in germination (12-25%) was observed between 40-60 s. A greater decrease in germination was observed for the 2013 pinto beans compared to the 2012 pinto beans and the navy beans for both years. This was likely due to the higher moisture content (MC) of the pinto bean seed lot in 2013. Microwave radiation uses dielectric heating to align dipole water molecules through the use of high-frequency electromagnetic radiation (Bouraoui et al., 1993). Therefore, when more water was present in the dielectric material (i.e., the seed), greater friction from the realignment of the molecules occurred, increasing the amount of heat produced within the material (Bouraoui et al., 1993). The 2013 pinto bean seed had a lower tolerance to microwave radiation due to the increased seed temperature, which caused seed mortality at a lower exposure time (Appendix A) (Knox et al., 2013). At microwave exposure times over 60 s the effect of increased seed temperature continued, resulting in decreased seed germination (Figure 2.1) due to increased seed mortality. Plant vigour, assessed as total dry weight of

germinated plant matter, also decreased rapidly over 60 s of exposure, while in the 0-60 s exposure range a decrease up to only 19% was measured (Figure 2.2). For incidence of Xap colonization, there was no correlation between microwave exposure and Xap viability, as there was a low incidence of colonization at all exposure times (data not shown).

Following the laboratory tests, microwave radiation showed some promise as an alternate seed treatment for Xap, due to minor effects on seed germination and vigour, despite a lack of difference in pathogen incidence. To further evaluate the control of Xap with microwave radiation, field studies were conducted using a MER determined from non-linear regression performed on the data for each seed lot from the laboratory experiments. For field studies, a MER of 60 s was set for the 2012 pinto beans and navy bean (both years) and 50 s for 2013 pinto bean (Figure 2.1).

#### **2.4.2 Field Study**

##### *Weather Conditions*

Weather conditions in both 2012 and 2013 were conducive for CBB development. During early emergence in 2012, cooler conditions were experienced at the Morden trial sites than at the Ontario sites, resulting in delayed emergence in the pinto bean class. However, warmer conditions in the following weeks evened out the emergence patterns. Hail events occurred at the Exeter trial site on 21 June 2012 and the Ridgetown trial site on 17 June 2013. Hail damage incurred by the seedlings did not severely impact emergence ratings, but did cause noticeable leaf wounds. The differences in disease incidence, however, did not appear to be significantly affected. At the Ridgetown trials sites in 2012, increased heat and humidity during flowering and pod formation resulted in high disease pressure at the early foliar ratings.

### *Emergence*

The NIC had 7- 9% lower emergence than the IC for the majority of the trials (Table 2.2). This result was unexpected, as infected seed usually has lower seed germination compared to non-infected seed. Lower emergence may have been related to the origin of the seed lot, as the NIC were obtained from a different environment than the IC and the other treatments. Factors that may have contributed to the decreased emergence include seed viability, size or seed coat thickness (Lush and Wien, 1980; Souza and Marcos-Filho, 2001), storage conditions (Spilde, 1989; TeKrony and Egli, 1991), mechanical damage (Moore, 1972) or various environmental factors during seed production. Lower emergence was also observed for cv. Envoy, which was used in place of cv. Navigator in the 2012 Exeter navy trial site. This location was analyzed separately due to the lower percentage of emergence (data not shown). The lower emergence observed for the Envoy seed at Exeter in 2012 was likely due to poorer germination of the seed lot, as well as a MER that was too high for the MC of the seed, as emergence was lowest in the 1x Microwave treatment. Neither assumption was confirmed in the laboratory, as a preliminary germination test was not performed. Lower emergence was also observed at 10 DAP in the 2012 Morden pinto bean trial. Emergence for this trial was likely delayed due to the cold, wet conditions during the first WAP. At 14 and 21 DAP, no differences were seen between the pinto trial sites and therefore the data were combined for analysis.

The application of chemical treatments did not increase emergence compared to the IC (Table 2.2). However, a loss of up to 7% was observed in several contrast comparisons when microwave treatment was applied. This loss was most notable in several pinto bean trials where the IC and PFM + Cu(OH)<sub>2</sub> (ST) and ½x Microwave treatments had increased emergence compared to the microwave treatment. In navy bean, differences in treatments with chemical

components only occurred 14 DAP where ST + Microwave decreased emergence compared to the IC. The combination of chemical and microwave treatment did not affect emergence compared to microwave or chemical treatment alone, except between PFM and PFM + Microwave treatment in the pinto bean. Increased emergence has been associated with PFM (W. R. Barton, personal communication, BASF Canada, Mississauga, ON); however, this was not observed in the other PFM treatments and a substantial number of field observations did not support this claim. Finally, up to a 7% loss in emergence was observed when comparing 1x Microwave treatment to ½x Microwave treatment at all trial sites. This decrease was likely due to the increased seed temperature from prolonged microwave exposure. Even though increased microwave exposure lowered emergence in all trials, the loss was still below the 10% standard set for the MER in this study.

#### *Disease Incidence*

Incidence of leaf infection collected using a 1-9 scale indicated few differences in CBB control (Table 2.3). At 4 WAP, the 2012 Ridgetown navy bean trial was analysed separately because of higher initial disease symptoms which was likely due to high humidity and heat. The NIC had the lowest disease incidence in navy bean for both leaf (4 and 8 WAP) and pod infection (Table 2.3). Low disease incidence was expected in the NIC; however infection was still quite evident, particularly in the pinto bean. The presence of disease in the NIC may have been due to bacterial infection on the seed, which was not visible at planting, as disease plating was not conducted on the disease-free lots prior to seeding.

The use of microwave and chemical treatments had little impact on incidence of CBB. At 4 WAP, no differences in leaf infection were detected in pinto bean, but minor differences were seen in four of the five navy bean trial sites (Table 2.3) where the 1x Microwave had a lower

disease incidence than the ½x Microwave. This may be attributed to increased microwave exposure time; however, disease symptoms were not reduced in the microwave treatments in navy bean at Ridgetown or the pinto beans or in the later ratings, suggesting the microwave treatment had little effect overall. When comparing microwave treatment to Cu(OH)<sub>2</sub> + Microwave treatment, greater disease control was observed in the Cu(OH)<sub>2</sub> + Microwave treatment. The addition of Cu(OH)<sub>2</sub> likely provided the increased control seen in the Cu(OH)<sub>2</sub> + Microwave treatment in the navy beans. The effect of Cu(OH)<sub>2</sub> was also observed at 8 WAP in navy bean when compared to PFM, indicating potential for more prolonged disease control using Cu(OH)<sub>2</sub>. This result was expected, as PFM has less activity on CBB and more activity on other plant pathogens (Barton, W. R., pers. comm., BASF Canada, Mississauga, ON). However, the benefits observed with Cu(OH)<sub>2</sub> had limited value, since the treatment was not significantly better than the IC.

The microwave and chemical treatments did not reduce pod infection (Table 2.3), since the only difference observed was between the NIC and IC in navy bean. The lower disease symptoms in the NIC check are likely due to the use of disease-free seed and frequent oversprays with Cu(OH)<sub>2</sub> to promote a disease-free environment. The effect of Cu(OH)<sub>2</sub> as a seed treatment is not as well studied as a foliar application (Fininsa, 2003) and the results of this study suggests Cu(OH)<sub>2</sub> is quite ineffective as a seed treatment in reducing CBB symptoms.

#### *Yield & Seed Pick*

There were no differences in yield for any of the contrast comparisons, including the NIC and IC (Table 2.4). This was likely due to the similar emergence and disease pressure patterns observed across all treatments. In linear regressions of leaf AUDPC and yield, there was a negative linear relationship between disease progression and yield based on data from the



Ontario trials only (Figure 2.3). Increased disease pressure lowered yield by 2.91-2.92 kg ha<sup>-1</sup> in navy bean and 5.24 kg ha<sup>-1</sup> in pinto bean (Figure 2.3); however the correlation between yield and disease was low ( $R^2 = 0.07-0.20$ ). The low correlation between disease incidence and yield indicates the lack of differences observed in yield contrasts (Table 2.4).

No differences between treatments were observed for seed pick (Table 2.4), even when trials were analyzed separately to adjust for sites with increased seed pick (Morden 2012 navy bean and Ridgetown 2013 pinto bean). The effect on seed pick and final yield was not as obvious for CBB as it can be with other seed-borne diseases like anthracnose, which can be more destructive to the pods and seed (Schwartz et al., 2005). However, the lack of differences noted between the microwave and chemical treatments compared to the IC for both yield and seed pick likely indicate the poor efficacy of these treatments. This corresponds with the lack of difference for disease incidence ratings (Table 2.3), for the microwave and chemical treatments compared to the IC.

#### *Return on Investment*

Contrast comparisons showed no differences in ROI (Table 2.5). The relationship between ROI and yield was strong, as observed in a linear regression in Figure 2.4 ( $R^2 = 0.92-0.97$ ). For every kg ha<sup>-1</sup> of yield increase, ROI increased \$0.79 and \$0.59 ha<sup>-1</sup> in navy and pinto bean, respectively. The absence of differences in the contrasts (Table 2.5) was not surprising as there were no differences in yield, which has a large influence on ROI. Any benefit resulting from differences between the costs of microwave and chemical treatments were not apparent. The uniform disease pressure observed between treatments resulted in a minimal impact on the ROI. From a linear regression on the Ontario trials, an increase of up to \$3.17 ha<sup>-1</sup> and \$2.52 ha<sup>-1</sup> was observed for navy and pinto bean, respectively, when leaf disease pressure was low (Figure 2.5).

The relationship between ROI and AUDPC was poor ( $R^2 = 0.10-0.14$ ) however, and did little to clarify the lack of differences in contrast comparisons for ROI (Table 2.5).

Overall, the results from these studies showed that the use of short intervals of microwave radiation had little negative effect on seed germination and emergence, but also had little positive effect on disease incidence of Xap infected seed. The use of chemical treatment applied alone and in combination with a microwave treatment did not impact seed emergence or CBB control in the field compared to the IC. The lack of disease control observed from both treatments was reflected in the lack of differences in yield and pick. Despite the fact that  $\text{Cu}(\text{OH})_2$  and microwave treatments were less costly than the PFM treatment, no differences were seen in ROI. Although previous laboratory studies have indicated the effectiveness of microwave treatment on seed-borne pathogens (Tylkowska et al., 2010), the use of microwave treatment as an alternative seed treatment for control of Xap was not supported in these studies. Further research on the efficacy of PFM and  $\text{Cu}(\text{OH})_2$  is necessary to determine if these treatments are beneficial for CBB control as seed treatments.

**Table 2.1. Treatments and treatment costs allocated for the dry bean common bacterial blight seed treatment experiments in 2012 and 2013**

Treatments	Chemical Rate (a.i. g 100 kg <sup>-1</sup> )	Microwave Time (s)		Treatment Cost (\$ ha <sup>-1</sup> )								
				Morden		Ridgetown			Exeter			
		Navy & Pinto 2012	Pinto 2013	Navy	Pinto	Navy	Pinto 2012	Pinto 2013	Navy	Pinto 2012	Pinto 2013	
1 Non-infected control	-	-	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2 Infected control	-	-	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3 PFM <sup>z</sup>	14.0	-	-	77.33	77.33	53.96	53.96	53.96	61.87	61.87	61.87	61.87
4 Cu(OH) <sub>2</sub> <sup>y</sup>	30.4	-	-	1.47	1.47	1.02	1.02	1.02	1.17	1.17	1.17	1.17
5 1/2x Microwave	-	30.0	25.0	1.74	2.32	1.22	1.62	1.35	1.39	1.86	1.55	1.55
6 1 x Microwave	-	60.0	50.0	3.48	4.64	2.43	3.24	2.70	2.79	3.72	3.10	3.10
7 1/2x Microwave + PFM	14.0	30.0	25.0	79.07	79.65	55.18	55.58	55.31	63.26	63.73	63.42	63.42
8 1 x Microwave + PFM	14.0	60.0	50.0	80.81	81.97	56.39	57.20	56.66	64.66	65.59	64.97	64.97
9 1/2x Microwave + Cu(OH) <sub>2</sub>	30.4	30.0	25.0	3.21	3.79	2.24	0.64	2.37	2.56	3.03	2.72	2.72
10 1x Microwave + Cu(OH) <sub>2</sub>	30.4	60.0	50.0	4.95	6.11	3.45	4.26	3.72	3.96	4.89	4.27	4.27

<sup>z</sup>PFM= pyraclostrobin + fluxapyroxad + metalaxyl

<sup>y</sup>Cu(OH)<sub>2</sub>= Copper Hyrdoxide 53.8%

**Table 2.2. Contrasts comparing percentage of emergence of navy and pinto beans for various seed treatments to control common bacterial blight at Morden, MB and Ridgetown and Exeter, ON in 2012-2013**

Treatment comparison	Emergence <sup>z</sup> (%)						
	10 DAP			14 DAP		21 DAP	
	Navy <sup>y</sup>	Pinto	Pinto <sup>x</sup>	Navy <sup>y</sup>	Pinto	Navy <sup>y</sup>	Pinto
IC <sup>w</sup> vs NIC	96 vs 87 **	95 vs 88 **	79 vs 70	97 vs 89 **	95 vs 87 **	97 vs 90	96 vs 91 **
IC vs ST	96 vs 94	95 vs 95	79 vs 75	97 vs 95	95 vs 95	97 vs 95	96 vs 96
IC vs Microwave	96 vs 95	95 vs 94	79 vs 74	97 vs 94	95 vs 92 *	97 vs 94	96 vs 93 *
IC vs ST + Microwave	96 vs 92	95 vs 94	79 vs 76	97 vs 92 *	95 vs 93	97 vs 92	96 vs 94 *
PFM vs Cu(OH) <sub>2</sub>	94 vs 94	97 vs 93	76 vs 73	95 vs 95	96 vs 93	95 vs 96	97 vs 94
PFM vs PFM + Microwave	94 vs 94	97 vs 93 *	76 vs 72	95 vs 94	96 vs 93 *	95 vs 94	97 vs 93 **
Cu(OH) <sub>2</sub> vs Cu(OH) <sub>2</sub> + Microwave	94 vs 90	93 vs 94	73 vs 80	95 vs 90	93 vs 93	96 vs 91	94 vs 94
ST vs Microwave	94 vs 95	95 vs 94	75 vs 74	95 vs 94	95 vs 92	95 vs 94	96 vs 93 *
ST vs ST + Microwave	94 vs 92	95 vs 94	75 vs 76	95 vs 92	95 vs 93	95 vs 92	96 vs 94 *
<sup>1</sup> / <sub>2</sub> x Microwave vs 1x Microwave	98 vs 91 *	97 vs 91 **	74 vs 73	97 vs 90 *	95 vs 90 *	97 vs 91	96 vs 89 **
Microwave vs PFM + Microwave	95 vs 94	94 vs 93	74 vs 72	94 vs 94	92 vs 93	94 vs 94	93 vs 93
Microwave vs Cu(OH) <sub>2</sub> + Microwave	95 vs 90	94 vs 94	74 vs 80	94 vs 90	92 vs 93	94 vs 91	93 vs 94
Microwave vs ST + Microwave	95 vs 92	94 vs 94	74 vs 76	94 vs 92	92 vs 93	94 vs 92	93 vs 94

<sup>z</sup>Emergence was arcsine square root transformed for data analysis to satisfy the assumptions of analysis and back-transformed estimates are presented.

<sup>y</sup>Exeter 2012 trial was excluded from analysis due to low germination in seed lot.

<sup>x</sup>Morden 2012 trial presented separately to meet the assumptions of normality.

<sup>w</sup>IC= infected control; NIC= non-infected control; Microwave= <sup>1</sup>/<sub>2</sub>x and 1x Microwave rates; ST= PFM and Cu(OH)<sub>2</sub>; PFM= pyraclostrobin + fluxapyroxad + metalaxyl; Cu(OH)<sub>2</sub>= Copper Hydroxide 53.8%.

\*,\*\* Denotes significance at  $P < 0.05$  and  $P < 0.01$ , respectively, based on orthogonal contrasts.

**Table 2.3. Contrasts comparing percentage of leaf and pod infection on navy and pinto beans for various seed treatments to control common bacterial blight at Morden, MB and Ridgetown and Exeter, ON in 2012-2013**

Treatment comparison	Leaf Infection <sup>z</sup>								Pod Infection (%)	
	4 WAP			6 WAP		8 WAP		10 WAP		
	Navy	Navy <sup>y</sup>	Pinto	Navy	Pinto	Navy	Pinto	Navy	Pinto	
IC <sup>x</sup> vs NIC	2.2 vs 1.4 **	5.7 vs 4.5	3.2 vs 2.9	3.7 vs 2.4	3.9 vs 3.9	5.5 vs 4.6 *	6.0 vs 5.8	1.6 vs 0.9 **	1.1 vs 1.0	
IC vs ST	2.2 vs 2.0	5.7 vs 5.4	3.2 vs 2.9	3.7 vs 3.2	3.9 vs 3.6	5.5 vs 5.2	6.0 vs 5.4	1.6 vs 1.4	1.1 vs 0.9	
IC vs Microwave	2.2 vs 2.1	5.7 vs 5.7	3.2 vs 3.1	3.7 vs 3.6	3.9 vs 4.1	5.5 vs 5.3	6.0 vs 5.7	1.6 vs 1.5	1.1 vs 0.9	
IC vs ST + Microwave	2.2 vs 1.9	5.7 vs 5.5	3.2 vs 3.0	3.7 vs 3.1	3.9 vs 3.7	5.5 vs 5.2	6.0 vs 5.5	1.6 vs 1.5	1.1 vs 1.0	
PFM vs Cu(OH) <sub>2</sub>	2.2 vs 1.9	5.8 vs 5.0	3.0 vs 2.9	3.8 vs 2.6	3.6 vs 3.6	5.6 vs 4.8 *	5.3 vs 5.5	1.5 vs 1.3	0.8 vs 1.1	
PFM vs PFM + Microwave	2.2 vs 2.0	5.8 vs 5.7	3.0 vs 3.1	3.8 vs 3.3	3.6 vs 3.8	5.6 vs 5.3	5.3 vs 5.6	1.5 vs 1.5	0.8 vs 1.0	
Cu(OH) <sub>2</sub> vs Cu(OH) <sub>2</sub> + Microwave	1.9 vs 1.7	5.0 vs 5.3	2.9 vs 2.8	2.6 vs 2.8	3.6 vs 3.6	4.8 vs 5.2	5.5 vs 5.5	1.3 vs 1.5	1.1 vs 0.9	
ST vs Microwave	2.0 vs 2.1	5.4 vs 5.7	2.9 vs 3.1	3.2 vs 3.6	3.6 vs 4.1	5.2 vs 5.3	5.4 vs 5.7	1.4 vs 1.5	0.9 vs 0.9	
ST vs ST + Microwave	2.0 vs 1.9	5.4 vs 5.5	2.9 vs 3.0	3.2 vs 3.1	3.6 vs 3.7	5.2 vs 5.2	5.4 vs 5.5	1.4 vs 1.5	0.9 vs 1.0	
<sup>1</sup> / <sub>2</sub> x Microwave vs 1x Microwave	2.4 vs 1.9 *	5.5 vs 6.0	3.1 vs 3.1	3.8 vs 3.4	3.9 vs 4.2	5.5 vs 5.1	5.8 vs 5.7	1.6 vs 1.5	0.8 vs 1.0	
Microwave vs PFM + Microwave	2.1 vs 2.0	5.7 vs 5.7	3.1 vs 3.1	3.6 vs 3.3	4.1 vs 3.8	5.3 vs 5.3	5.7 vs 5.6	1.5 vs 1.5	0.9 vs 1.0	
Microwave vs Cu(OH) <sub>2</sub> + Microwave	2.1 vs 1.7 *	5.7 vs 5.3	3.1 vs 2.8	3.6 vs 2.8	4.1 vs 3.6	5.3 vs 5.2	5.7 vs 5.5	1.5 vs 1.5	0.9 vs 0.9	
Microwave vs ST + Microwave	2.1 vs 1.9	5.7 vs 5.5	3.1 vs 3.0	3.6 vs 3.1	4.1 vs 3.7	5.3 vs 5.2	5.7 vs 5.5	1.5 vs 1.5	0.9 vs 1.0	

<sup>z</sup>Data was collected using a 1-9 scale to evaluate percentage of leaf infection where 1= No visible lesions, 3= 2% of leaf area, 5= 5% of leaf area, 7= 10% of leaf area , and 9= 25% or more of leaf area.

<sup>y</sup>Ridgetown 2012 trial presented separately to meet the assumptions of normality.

<sup>x</sup>IC= infected control; NIC= non-infected control; Microwave= <sup>1</sup>/<sub>2</sub>x and 1x Microwave rates; ST= PFM and Cu(OH)<sub>2</sub>; PFM= pyraclostrobin + fluxapyroxad + metalaxyl; Cu(OH)<sub>2</sub>= Copper Hydroxide 53.8%.

\*,\*\* Denotes significance at  $P < 0.05$  and  $P < 0.01$ , respectively, based on orthogonal contrasts.

**Table 2.4. Contrasts comparing yield and percentage of seed pick on navy and pinto beans for various seed treatments to control common bacterial blight at Morden, MB and Ridgetown and Exeter, ON in 2012-2013**

Treatment comparison	Yield (kg ha <sup>-1</sup> )		Seed Pick (%)			
	Navy	Pinto	Navy	Navy <sup>z</sup>	Pinto	Pinto <sup>y</sup>
IC <sup>x</sup> vs NIC	3946 vs 4064	4223 vs 4382	4 vs 3	6 vs 3	5 vs 5	5 vs 3
IC vs ST	3946 vs 3808	4223 vs 4232	4 vs 4	6 vs 9	5 vs 5	5 vs 4
IC vs Microwave	3946 vs 3895	4223 vs 4179	4 vs 5	6 vs 6	5 vs 5	5 vs 4
IC vs ST + Microwave	3946 vs 3835	4223 vs 4288	4 vs 4	6 vs 7	5 vs 5	5 vs 4
PFM vs Cu(OH) <sub>2</sub>	3714 vs 3903	4199 vs 4264	4 vs 4	8 vs 10	4 vs 5	5 vs 4
PFM vs PFM + Microwave	3714 vs 3862	4199 vs 4205	4 vs 4	8 vs 6	4 vs 5	5 vs 3
Cu(OH) <sub>2</sub> vs Cu(OH) <sub>2</sub> + Microwave	3903 vs 3808	4264 vs 4371	4 vs 4	10 vs 7	5 vs 6	4 vs 4
ST vs Microwave	3808 vs 3895	4232 vs 4179	4 vs 5	9 vs 6	5 vs 5	4 vs 4
ST vs ST + Microwave	3808 vs 3835	4232 vs 4288	4 vs 4	9 vs 7	5 vs 5	4 vs 4
<sup>1</sup> / <sub>2</sub> x Microwave vs 1x Microwave	3776 vs 4013	4280 vs 4077	5 vs 4	8 vs 5	5 vs 5	4 vs 3
Microwave vs PFM + Microwave	3895 vs 3862	4179 vs 4205	5 vs 4	6 vs 6	5 vs 5	4 vs 3
Microwave vs Cu(OH) <sub>2</sub> + Microwave	3895 vs 3808	4179 vs 4371	5 vs 4	6 vs 7	5 vs 6	4 vs 4
Microwave vs ST + Microwave	3895 vs 3835	4179 vs 4288	5 vs 4	6 vs 7	5 vs 5	4 vs 4

<sup>z</sup>Ridgetown 2013 trial presented separately to satisfy the assumptions of normality.

<sup>y</sup>Morden 2012 trial presented separately to meet the assumptions of normality.

<sup>x</sup>IC= infected control; NIC= non-infected control; Microwave= <sup>1</sup>/<sub>2</sub>x and 1x Microwave rates; ST= PFM and Cu(OH)<sub>2</sub>; PFM= pyraclostrobin + fluxapyroxad + metalaxyl; Cu(OH)<sub>2</sub>= Copper Hydroxide 53.8%.

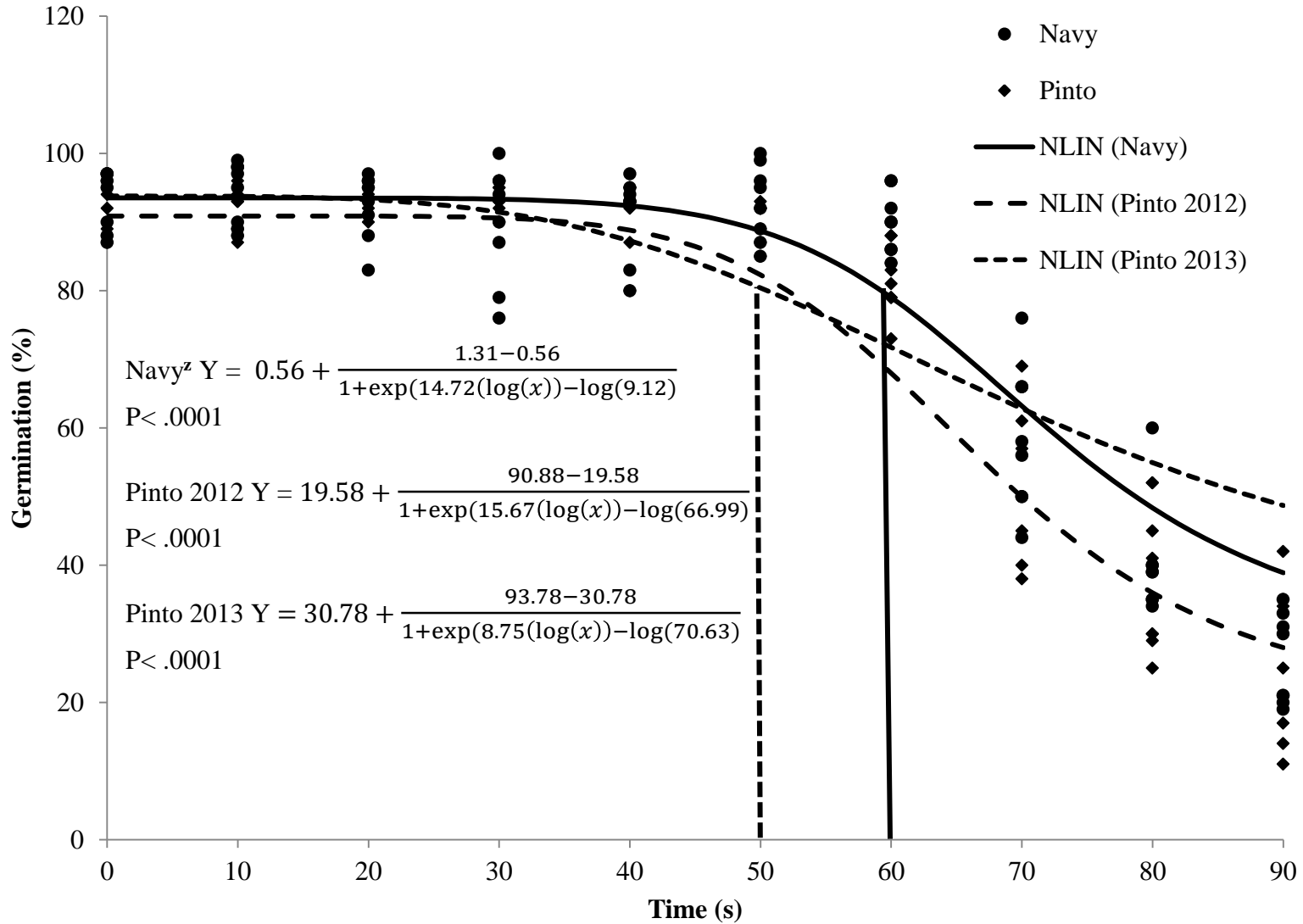
\*,\*\* Denotes significance at  $P < 0.05$  and  $P < 0.01$ , respectively, based on orthogonal contrasts.

**Table 2.5. Contrasts comparing return on investment (ROI) on navy and pinto beans for various seed treatments to control common bacterial blight at Morden, MB and Ridgeway and Exeter, ON in 2012-2013**

Treatment comparison	ROI (\$ ha <sup>-1</sup> )	
	Navy	Pinto
IC <sup>x</sup> vs NIC	3150 vs 3281	2800 vs 2965
IC vs ST	3150 vs 2979	2800 vs 2812
IC vs Microwave	3150 vs 3063	2800 vs 2796
IC vs ST + Microwave	3150 vs 3028	2800 vs 2794
PFM vs Cu(OH) <sub>2</sub>	2860 vs 3098	2759 vs 2865
PFM vs PFM + Microwave	2860 vs 3020	2759 vs 2740
Cu(OH) <sub>2</sub> vs Cu(OH) <sub>2</sub> + Microwave	3098 vs 3037	2865 vs 2847
ST vs Microwave	2979 vs 3063	2812 vs 2796
ST vs ST + Microwave	2979 vs 3028	2812 vs 2794
<sup>1</sup> / <sub>2</sub> x Microwave vs 1x Microwave	2944 vs 3182	2854 vs 2739
Microwave vs PFM + Microwave	3063 vs 3020	2796 vs 2740
Microwave vs Cu(OH) <sub>2</sub> + Microwave	3063 vs 3037	2796 vs 2847
Microwave vs ST + Microwave	3063 vs 3028	2796 vs 2794

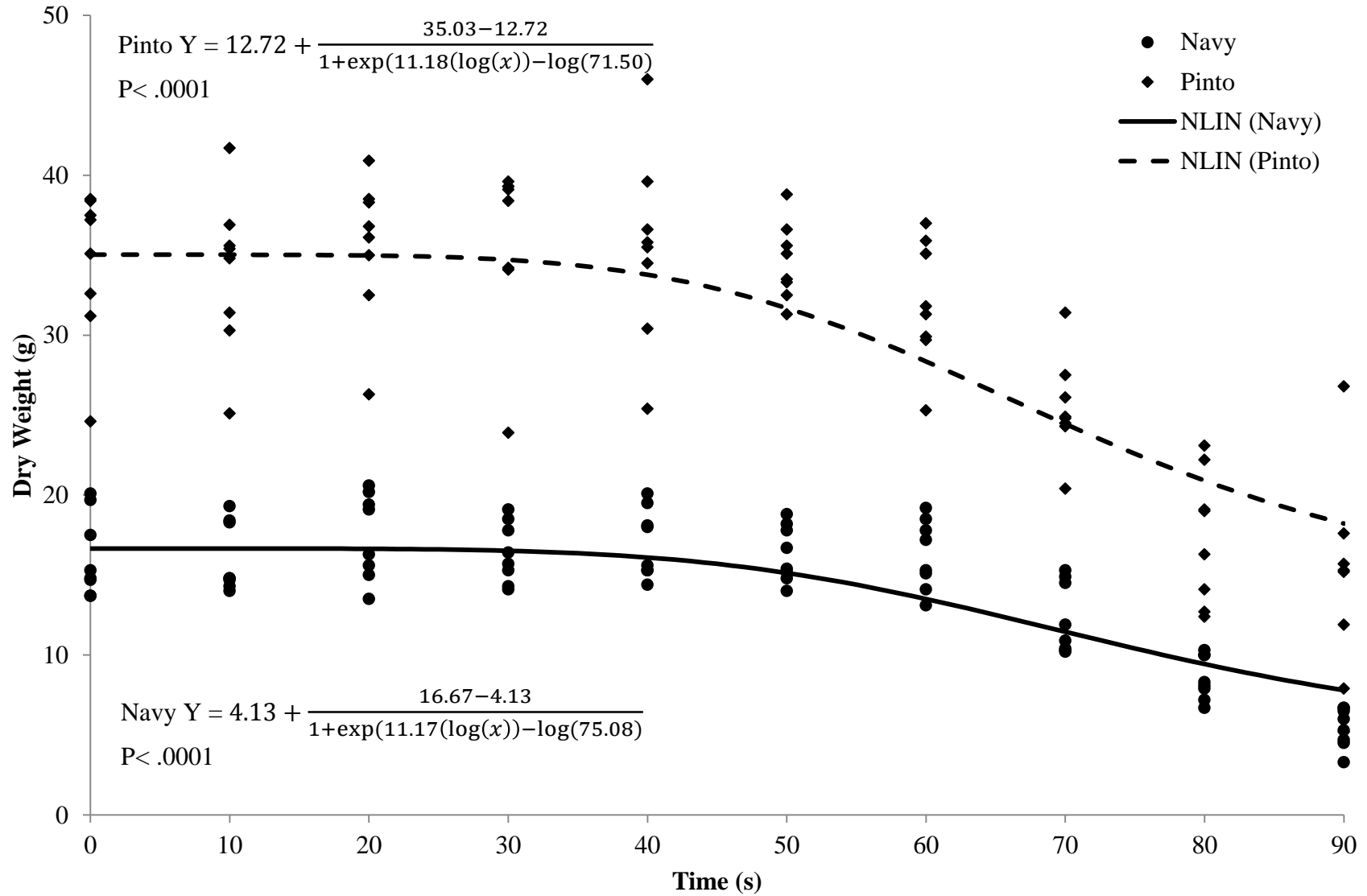
<sup>x</sup>IC= infected control; NIC= non-infected control; Microwave= <sup>1</sup>/<sub>2</sub>x and 1x Microwave rates; ST= PFM and Cu(OH)<sub>2</sub>; PFM= pyraclostrobin + fluxapyroxad + metalaxyl; Cu(OH)<sub>2</sub>= Copper Hydroxide 53.8%.

\*,\*\* Denotes significance at  $P < 0.05$  and  $P < 0.01$ , respectively, based on orthogonal contrasts.

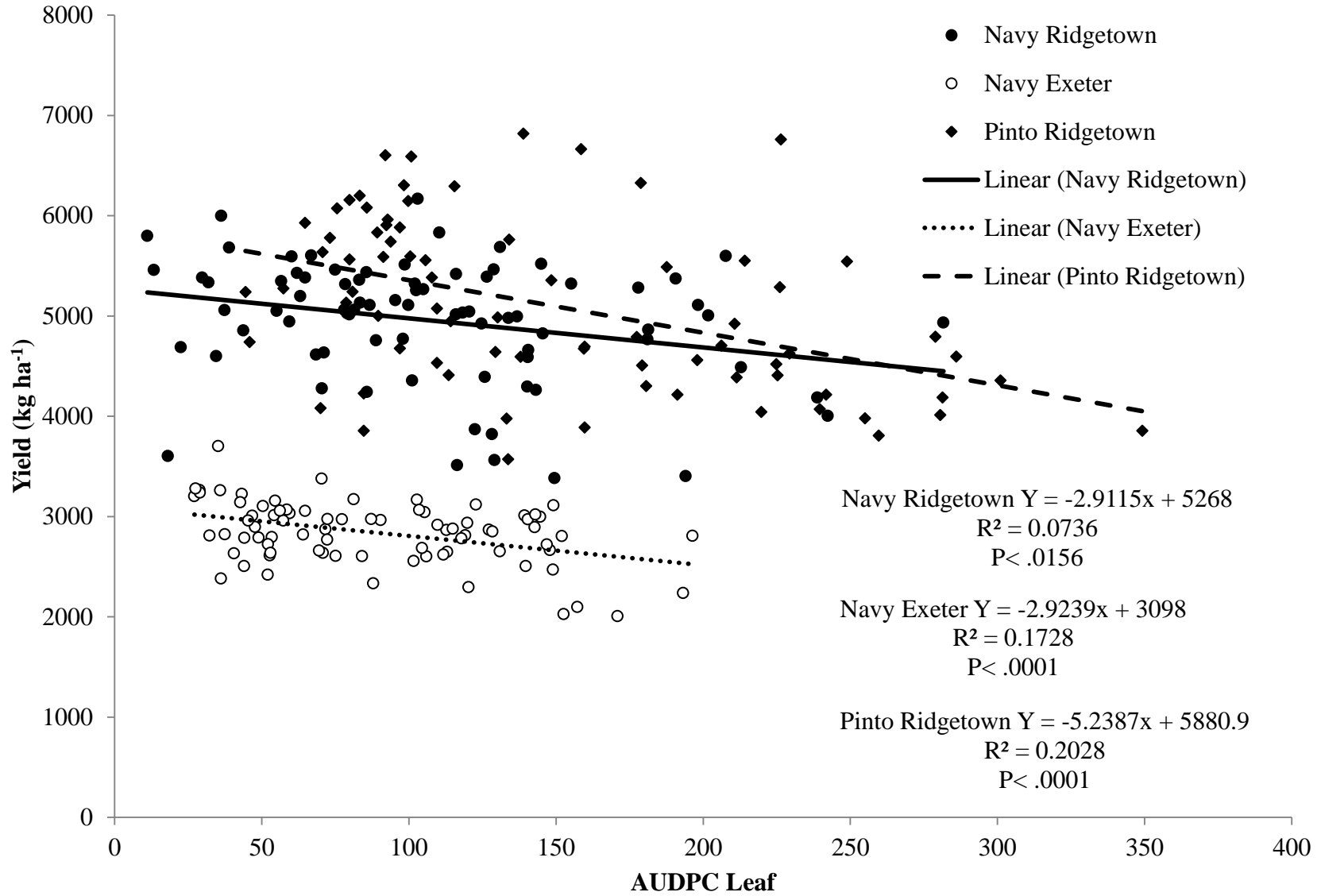


**Figure 2.1.** Nonlinear regressions (NLIN) of percentage of germination of navy and pinto bean seed incubated at 25°C for 7 d in a germination chamber after various microwave radiation treatments in 2012 and 2013. Navy data was arcsine square root transformed for data analysis to meet the assumptions of normality and back-transformed estimates are presented.

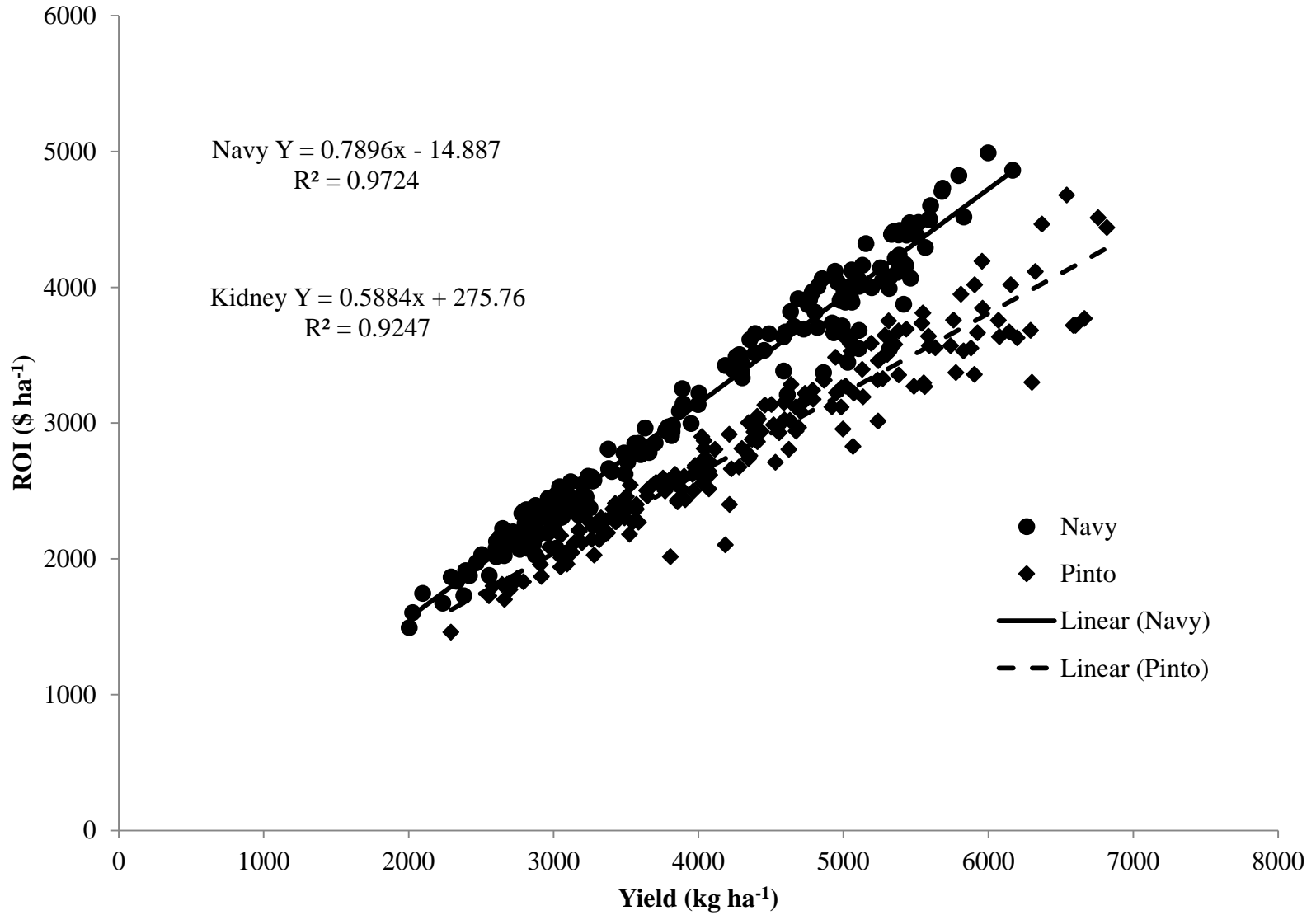




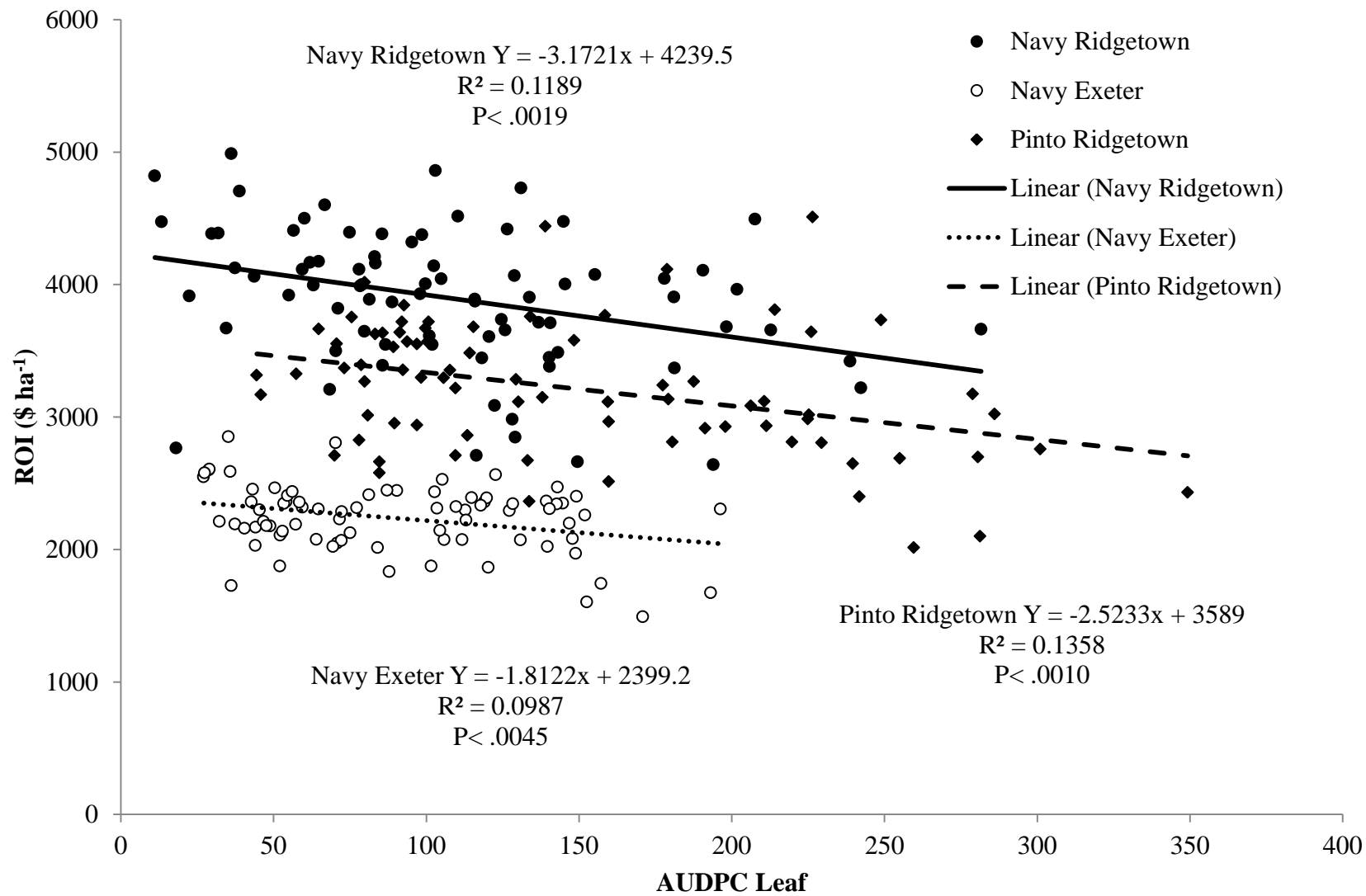
**Figure 2.2.** Nonlinear regressions (NLIN) of plant vigour (dry weights of germinated material) from navy and pinto bean seed incubated at 25°C for 7 d in a germination chamber after various microwave radiation treatments in 2012 and 2013.



**Figure 2.3.** Linear regression of area under the disease progress curve (AUDPC) for leaf infection and yield for navy and pinto bean seed treatment study for common bacterial blight control in Ridgetown and Exeter, ON in 2012 and 2013.



**Figure 2.4.** Linear regression of return on investment (ROI) and yield for navy and pinto bean seed treatment study for common bacterial blight control in Morden, MB and Ridgetown and Exeter, ON in 2012 and 2013.



**Figure 2.5.** Linear regression of area under the disease progress curve (AUDPC) for leaf infection and return on investment (ROI) for navy and pinto bean seed treatment study for common bacterial blight control in Ridgetown and Exeter, ON in 2012 and 2013.

## CHAPTER THREE

### Effect of microwave radiation on dry bean seed infected with *Pseudomonas syringae* pv. *phaseolicola* with and without the use of chemical seed treatment

#### 3.1 Abstract

Halo blight, caused by *Pseudomonas syringae* pv. *phaseolicola*, is a seed-borne disease in dry bean (*Phaseolus vulgaris* L.) that lowers seed quality and yield. Over two years, laboratory and field studies were conducted to evaluate the effect of microwave radiation on two market classes: navy (cv. Envoy) and white kidney bean (cv. GTS 402). In the laboratory, seed germination and vigour decreased up to 15% after 40 s of microwave exposure, while for 0-30 s less than a 7% decrease was observed. Disease plating showed no correlation between pathogen colonization of the seed and microwave radiation, as incidence of pathogen colonization was low across all exposure times. In field trials in Morden and Winkler, MB microwave radiation was tested alone and in combination with copper hydroxide 53.8% and pyraclostrobin + fluxapyroxad + metalaxyl. Microwave radiation lowered seed emergence by up to 9%, but did not reduce disease infection or increase yield or return on investment when applied alone or in combination with a chemical treatment. Seed treatment with copper hydroxide slightly decreased the incidence and severity of halo blight, seed pick and increased hundred seed weight, yield, and return on investment, while pyraclostrobin + fluxapyroxad + metalaxyl had no effect on any of these parameters.

#### 3.2 Introduction

Halo blight (caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al.) (Psp), is a bacterial disease of dry bean (*Phaseolus vulgaris* L.) that is primarily spread via

infected seed. This bacterium can infect the leaves, stems, pods and seed of dry bean and reduce yields up to 43% in temperate areas under high humidity (Saettler and Potter, 1970; Saettler, 1989b). Initial infection by Psp usually appears on the lower leaf surface as a small water soaked lesion, which eventually becomes surrounded by a bright yellow halo (Schwartz et al., 2005). The initial infection foci becomes necrotic, but remains small while systemic chlorosis spreads from the infected leaflet to the trifoliolate leaves, and potentially the whole plant (Schwartz and Pastor-Corrales, 1989). This systemic chlorosis is caused by the production of phaseolotoxin, a toxin that is released into the extra-cellular space surrounding the infection foci, as well as into the plant phloem (Mitchell and Bielecki, 1977). With severe infections, the spread of the toxin can cause infected plants to become stunted and distorted (Mitchell and Bielecki, 1977). Lesions can also develop on the pods of infected plants and appear red-brown with a water soaked center (Schwartz et al., 2005). Infection of the pods can move to the developing seed and cause shrivelling and a yellow discolouration of the seed (Taylor et al., 1979a).

Once seed is infected, controlling halo blight is difficult, as there are few management strategies available that reduce or eliminate halo blight symptoms. The primary strategies for management are the use of disease-free seed and resistant cultivars (Bailey et al., 2003; Bozkurt and Soyly, 2011). However, obtaining disease-free seed can be costly, as seed has to be transported from seed production areas such as Idaho. The humid seed production areas in Canada are highly conducive for pathogen growth (Coyne and Schuster, 1974b; Gillard et al., 2009), which limits seed production. The use of resistant cultivars is an effective management strategy; however, no single cultivar can effectively control all of the races of Psp that have been identified (Bozkurt and Soyly, 2011). Breeding for halo blight resistance is also difficult, as multiple genes are reported to contribute to the resistance of a cultivar (Zaumeyer and Meiners,

1975). The use of other strategies, like cultural (Hall and Nasser, 1996), antibiotic (Taylor and Dudley, 1977), and chemical (Garrett and Schwartz, 1998) controls have also been used, but only have had limited effect in reducing inoculum spread and disease symptoms.

The use of bactericides is a standard practice for most conventional growers. Several products are readily available in Canada and are easy to apply. The current industry standard, copper hydroxide ( $\text{Cu}(\text{OH})_2$ ), is used as a foliar application and as a seed treatment. The effectiveness of  $\text{Cu}(\text{OH})_2$  as a foliar treatment is well understood, but multiple applications are required, which raises concerns for pathogen resistance (Garrett and Schwartz, 1998).

The use of alternative methods for controlling halo blight are needed as copper resistance has already been detected (Garrett and Schwartz, 1998). Thermotherapy treatments like dry heat, steam, and hot water soaks, control bacterial pathogens in other crops like tobacco (*Nicotiana tabacum*) ‘Consolidate L.’ (Hankin and Sands, 1977), cereals (Poaceae) and crucifers (Brassicaceae) (Grondeau et al., 1994). However, large seeded legume seeds like dry bean are sensitive to these treatments, as they can have a severe impact on seed germination and health (Spilde, 1989; Sinclair, 1993). One type of thermotherapy treatment that has been reported to have less of an effect on dry bean seed health and vigour is microwave radiation. The use of microwave radiation has effectively controlled bacterial and fungal pathogens in tobacco (*Nicotiana tabacum*), wheat (*Triticum* spp.), cassava (*Manihot esculenta* Crantz), and soybean (*Glycine max* L.) (Hankin and Sands, 1977; Cavalcante and Muchovej, 1993; Han, 2010; Knox et al., 2013). In dry bean, the effect of microwave radiation on bacterial pathogens has not been tested, but it did lower *Penicillium* spp. levels in a naturally infected seed lot (Tylkowska et al., 2010).

If effective, the use of microwave radiation as a seed treatment would be highly beneficial to growers as it would be an inexpensive addition or an alternative treatment for reducing or eliminating Psp. To test the efficacy of microwave treatment alone and in combination with chemical treatment, laboratory and field studies were conducted using navy and kidney bean seed naturally infected with Psp. Both market classes were chosen for their susceptibility to halo blight and their importance to dry bean production in Canada. Kidney bean is not as commonly grown as navy bean, but has a very high susceptibility to Psp and there are few resistant cultivars available. Studies were conducted at two field sites for a two-year period to determine the effect of microwave radiation on overall seed health and efficacy on halo blight.

### **3.3 Materials & Methods**

#### **3.3.1 Laboratory Study**

A two-year study was conducted using microwave radiation on seed naturally infected with Psp to determine the impact on seed germination and seed colonization. Two market classes, navy (cv. Envoy) and white kidney (cv. GTS 402) bean were used and randomized complete block designs (RCBD) experiments were replicated four times. Infected seed was obtained from field trials in Morden, MB in 2011 and 2012 for the 2012 and 2013 laboratory studies, respectively. To promote high disease pressure, seed was selected from plots where foliar ratings of halo blight were greater than 15%. Using a Fisher Scientific Isotemp Forced Air Oven (120 V, 60 Hz, 1800 w, 15.5 A) seed was dried down and moisture content was calculated once the seed had reached a stable weight. In navy bean and the 2012 kidney beans, moisture ranged between 7.4-8.6%, while in the 2013 kidney beans it was 9.2%. Each seed lot was exposed to ten radiation timings, divided into 10 s increments ranging between 0-90 s using an 1100W 2450 MHz microwave oven (General Electric Co., Fairfield, Connecticut, U.S.). When treating, 150



seeds were placed on a paper plate with a 450-500 ml beaker containing 200 ml of water in the center of the plate (Reddy et al., 2000). The temperature of the water was measured before and after each treatment to determine the change in temperature due to microwave exposure.

The effect of microwave radiation on seed germination was evaluated on 100 seeds from each experimental unit using the Canadian Food Inspection Agency (CFIA) methods and procedures for testing seed (CFIA, 2011). The seed was planted into 50 WG 60 Wellpak silica sand (B.P. Dust Control, Walton, ON) in 21.6 cm x 13.0 cm x 10.2 cm plastic clam lid containers (Par-Pak Ltd., Brampton, ON) and left to incubate for 6-7 d in a germination chamber. The incubation chamber was set at 25°C, had a 12L:12D photoperiod, and a relative humidity (RH) above 60%. After 6-7 d the seedlings were dried and the total dry weight of the germinated plant matter was recorded to assess vigour.

Disease plating was conducted in 2012 to determine the effect of microwave radiation on pathogen viability. Four replicates of plating were carried out with 50 seeds per treatment on King's B Medium, a specific media that causes *Pseudomonas spp.* to fluoresce (Chase, 1987). Prior to plating, seed was surface-sterilized in a 10% sodium hypochlorite solution for 2 min to prevent the growth of saprophytic species of fungi and bacteria. Once surface-sterilized, five seeds were plated per 100 x 15 mm petri plate (Fisher Scientific Company, Ottawa, ON) on 10 plates and allowed to germinate at room temperature for a minimum of 7 d. The seed was then evaluated for percentage of seed with visible bacterial colonization and germination.

### **3.3.2 Field Study**

Field trials using chemical and microwave treatments were conducted in Morden and Winkler, MB (1 navy and 1 kidney trial per site) over a two-year period (2012-2013). The trials consisted of ten treatments (Table 3.1) arranged in a randomized complete block design (RCBD)

with four replications. Seed for the field trials came from the same source as the laboratory study. The seed for the non-infected control, for both Envoy and GTS 402, were obtained from Gentec Seeds Inc. (Twin Falls, Idaho).

For the microwave radiation treatments, a maximum exposure rate (MER) of 50 s was set for navy bean (both years) and the 2012 kidney beans and 40 s for 2013 kidney beans, based on the seed germination results from the laboratory study (Figure 3.1). A half rate exposure time was set at 50% of the MER. Two chemical treatments were tested alone and in combination with microwave treatment. These treatments were pyraclostrobin + fluxapyroxad + metalaxyl (PFM) (BASF Canada, Mississauga, ON) and copper hydroxide 53.8% ( $\text{Cu}(\text{OH})_2$ ) (Kocide 2000, E.I. du Pont Canada Company, Mississauga, ON) applied at 14.0 and 30.4 g a.i. per 100 kg of seed, respectively. To minimize damping-off from soil pathogens such as *Pythium* spp., metalaxyl-M was included in all treatments at 4 g a.i. per 100 kg seed.

In Morden, plots were seeded 2 June 2012 and 8 June 2013 and were 1.2 m wide (four rows with a 30 cm spacing) and 5.0 m long and surrounded by a border of four rows of soybean (*Glycine max* (L.) Merr.). In Winkler, plots were seeded 30 May 2012 and 27 May 2013 and measured 1.2 m wide (four rows with a 30 cm spacing) and 4.0 m long with 0.6 m spacing between plots as no soybean borders due to limited space. For both sites, a four row cone-seeder was used. A seeding rate of 20 dry bean seeds  $\text{m}^{-1}$  of row and 25 soybean seeds  $\text{m}^{-1}$  were used.

To evaluate emergence, ratings were taken at 7, 10, 14, and 21 days after planting (DAP) in 2012 and at 7, 14, and 21 DAP in 2013. Ratings were not taken at 10 DAP in 2013, as no differences were noted in the previous year. Ratings on disease incidence and severity were taken 7 and 11 weeks after planting (WAP) on ten randomly selected plants per plot. Incidence was measured using a 1-9 scale where 1 equalled 0% infection and 9 equalled >25% infection

(Corrales and van Schoonhoven, 1987). Disease severity was measured using a 0-5 scale where 0 equalled no observable lesions and 5 equalled lesions that covered 50-100% of the leaf area (Mutlu et al., 2005). A pod infection rating was taken at 14 WAP and was based on percentage of pod area with lesions. In Winkler, plots received 12.7 mm of supplemental water weekly through overhead irrigation from the start of flowering until plant maturation. To prevent the development of white mold (*Sclerotinia sclerotiorum*) boscalid (BASF Canada, Mississauga, ON) was sprayed at a rate of 539.7 g a.i. ha<sup>-1</sup> on 13 August 2012 and 25 July 2013 at the Winkler trial sites.

A Wintersteiger NurseryMaster plot combine (Wintersteiger Ag, Ried im Innkreis, Austria) was used to harvest all the plots. Seed moisture was measured using a Motomco 919 Moisture Meter (Dickey-John Corp., Patterson, NJ). Hundred seed weight (HSW) was then measured and seed pick was evaluated. Pick was measured as the percentage of visibly discoloured seed in a random sample of 100 seeds to simulate the methods used for grading by the Ontario dry bean processors. All sample weights were adjusted to a standard storage moisture of 18% and converted to seed yield in kg ha<sup>-1</sup>. Using the modified equation below, return on investment (ROI) was calculated for each treatment (Gillard and Ranatunga, 2013):

$$\text{ROI} = ((\text{Seed Yield} - 2(\text{Pick})) * \text{Price kg}^{-1}) - \text{Cost of Seed Treatment}$$

Seed yield was adjusted for pick and market value price. Pick was doubled, once to account for poor seed quality and a second time for the cost of removal of infected seed. The market value prices, based on crop insurance values from 2012-2013, were \$0.84 kg<sup>-1</sup> and \$1.14 kg<sup>-1</sup> for navy and kidney beans, respectively (Agricorp, 2013). The cost of seed treatments were based on a manufacturer's suggested retail price (MSRP). A price of \$1.47 ha<sup>-1</sup> was used for copper hydroxide and \$77.33 ha<sup>-1</sup> for PFM. The elevated price of PFM was based on the MSRP for the

current industry standard seed treatment in dry bean, thiamethoxam + fludioxonil + metalaxyl-M + azoxystrobin (TFMA). This price was used to make PFM comparable in the market, as no price had been set for PFM as of yet. For the cost of the 1x Microwave treatments, \$2.90 ha<sup>-1</sup> was used for navy bean and \$4.22 and \$3.38 ha<sup>-1</sup> were used for 2012 and 2013 kidney beans, respectively. An increased cost was seen in the 2012 kidney beans compared to the 2013 kidney beans, as a higher MER was used. The 1x Microwave costs were reduced by 50% for the ½x treatments for all trials. The costs for microwave treatment accounted for the cost of electricity at a rate of 12.54¢ kWh<sup>-1</sup> based on Ontario Energy Board average daytime use.

### 3.3.3 Statistical Analysis

Data analysis was performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, U.S.A). To meet the assumptions of normality, transformations were performed when necessary and the highest Shapiro-Wilk's statistic was used to determine the best transformation and back-transformed estimates were presented. Lund's test for outliers was used to remove outliers (Rotondi and Koval, 2009). A Type I error rate of  $\alpha=0.05$  was set and models were assumed to be additive and linear where appropriate.

In the laboratory, analysis of variance (ANOVA) was performed using PROC MIXED where time was a fixed effect and replicate and year were random effects. Percentage germination and vigour were analyzed using a dosage response nonlinear regression in PROC NLIN:

$$Y = C + \frac{D - C}{1 + \exp[b(\log(X) - \log(I_{50}))]}$$

where a 50% dose response, microwave radiation and the upper and lower limits of percentage germination response ( $Y$ ) were represented by  $I_{50}$ ,  $X$  and  $C$  and  $D$ , respectively. An arcsine square root transformation was used for data on the navy bean percentage of germination.

In the field studies, ANOVA was performed using PROC MIXED where treatment was a fixed effect and environment, environment nested within replicate, environment x treatment and environment x year were random effects. Preplanned contrast comparisons were analyzed for various treatment combinations. To determine the effect on emergence and disease control all treatments were compared to the infected control (IC). The infected and disease-free controls were compared to determine the intensity of seed infection. The ½x and 1x rate of microwave radiation were compared to observe the impact of increased exposure time. To determine the efficacy of the two chemical products, PFM was compared to the industry standard, Cu(OH)<sub>2</sub>. Microwave and chemical treatments applied alone or in combination were compared to determine if there was any additional emergence or disease control when applied together. Percentage of emergence was arcsine square root transformed for both navy and kidney bean. The correlation between yield and ROI was determined through linear regressions using PROC GLM.s

### **3.4 Results & Discussion**

#### **3.4.1 Laboratory Study**

The effect of microwave radiation on seed germination is illustrated in Figure 3.1. A decrease in germination of between 2-15% was observed after 40 s of radiation in non-linear regressions for all the seed lots. The most significant decrease noted (15%) was in the 2013 kidney beans. This was attributed to the higher moisture content (MC) in that seed lot, which made the seed more susceptible to damage from lower levels of radiation, as microwave radiation produces energy through dielectric heating (Bouraoui et al., 1993). Dielectric heating uses high-frequency electromagnetic radiation to produce energy through the alignment of dipole water molecules (Bouraoui et al., 1993). The increased friction produced due to the higher number of water

molecules in the higher MC seed raised the seed temperature more than in the other seed lots, resulting in greater seed mortality at a lower exposure time (Appendix A) (Knox et al., 2013). Higher seed mortality (>10%) was also noted at exposure times over 50 s, where this prolonged exposure increased seed temperatures (Figure 3.1). The effect of microwave radiation on plant vigour, assessed as the total dry weight of germinated plant matter, also became more pronounced by 50 s of exposure (Figure 3.2). A decrease of 20-25% in vigour was observed at 50 s in both the navy and pinto bean experiments. Between 0-40 s, vigour decreased less than 14%. There was no correlation between microwave exposure and Psp colonization of the seed, as disease incidence was low at all exposure times (data not shown).

The use of microwave radiation as an alternate seed treatment for Psp showed potential as limited exposure had a minimal effect on seed germination and vigour. However, the lack of differences observed in pathogen colonization of the seed (data not shown) required further testing to determine the efficacy of microwave radiation. To determine a suitable level of microwave radiation for the field studies, non-linear regressions were performed to determine a MER, which was defined as the point where a <10% loss in germination occurred. This loss was considered acceptable if increased disease control was observed in the infected treatments in field studies, as there are few management strategies that are effective in controlling halo blight. In navy bean, a 50 s MER was set and for kidney bean a 50 s and 40 s MER were set for 2012 and 2013, respectively (Figure 3.1).

### **3.4.2 Field Study**

#### *Weather Conditions*

At both trial sites, seeding dates were early and temperatures ranged between 18-20°C, which was conducive for Psp growth. In 2012, moderate rainfall events occurred shortly after seeding

and helped to promote disease development throughout the season. In 2013, heavy rainfall shortly after seeding increased stress at both trial sites. This stress was greatest at the Morden trial site and resulted in the kidney bean trials having to be re-seeded. Conditions throughout the rest of the season were favourable for disease development and no other significant weather conditions occurred that negatively affected the trial sites. At the Winkler site, overhead irrigation was applied both years starting at early flower at a rate of 12.7 mm per week when natural rainfall events did not occur, to maintain a conducive environment for disease. The application of irrigation did increase disease incidence at this site compared to Morden, however this difference did not result in a significant environmental effect.

### *Emergence*

The NIC had the highest emergence at all ratings in kidney bean, except at the Winkler 2012 trial site at 7 DAP where no differences were observed between any of the contrast comparisons (Table 3.2). This trial was separated out due to lower emergence at 7 DAP, which was attributed to lower soil temperature and higher soil moisture. The higher emergence in the NIC observed in the other kidney bean trials was expected, as seed infection has been reported to lower seed health and emergence (Tu, 1996). However, no differences were observed in navy bean between the IC and NIC. The lack of differences between the controls was likely due to similarities in the percentage of germination in both seed lots and possibly a low pathogen colonization of the infected seed lot, based on observations in the laboratory study.

When comparing the IC to the seed treatments applied alone and in combination with microwave treatment, no differences in emergence were observed in either navy or kidney beans (Table 3.2). Differences between the two chemical treatments also were not detected. The absence of differences between these treatments suggests little phytotoxicity of the chemical

treatments on seedling emergence. The application of PFM has been reported to increase emergence and aid in controlling fungal pathogens (Barton, W. R., pers. comm., BASF Canada, Mississauga, ON); however, the presence of Psp in these seed lots may have masked the effect on emergence as its efficacy on Psp has not been determined. It is unknown if  $\text{Cu}(\text{OH})_2$  increases seed emergence, but an increase in emergence was expected as it is a bactericide known for controlling halo blight. In addition, the efficacy of  $\text{Cu}(\text{OH})_2$  as a seed treatment is not as well studied as its application as a foliar treatment (Garrett and Schwartz, 1998).

In comparing the microwave treatment to the chemical treatments, a decrease in emergence was observed in the majority of the trials (Table 3.2) and no additional control was detected in any of the combination treatments. In navy bean, a loss between 6-9% in emergence was detected at all rating dates when microwave treatment was compared to PFM +  $\text{Cu}(\text{OH})_2$  (ST) and ST + Microwave. A 9% delay in emergence was also observed at 7 and 10 DAP when comparing  $\text{Cu}(\text{OH})_2$  to  $\text{Cu}(\text{OH})_2$  + Microwave, but this was not evident at 20 DAP. This delay in emergence was likely due to increased stress on the seed from the 1x Microwave treatment (Table 3.2). Up to an 18% loss in emergence was measured when comparing the ½x Microwave and 1x Microwave treatments. This loss was above the 10% standard set for the MER, which suggests that a lower microwave exposure time was required. Despite these high losses in germination for the 1x Microwave treatment, no differences were observed between the IC and microwave treatment and emergence decreased less than the MER of 10% when microwave treatment was compared to the chemical treatments.

The application of microwave radiation had less impact on germination in kidney bean where emergence only decreased up to 6% when compared to ST at 10 and 20 DAP and PFM versus PFM + Microwave at 20 DAP (Table 3.2). The adverse effect of microwave treatment on the



emergence ratings for kidney bean occurred later than that observed in navy bean. The difference in the timing of these effects may be due to the differences in seed size. The kidney bean's larger seed size may have led to more uniform emergence early in the season as the larger seeded bean varieties have a greater food reserve to aid in seedling survival during germination (Conner et al., 2006b).

#### *Disease Incidence & Severity*

Few differences in leaf disease incidence were observed and no differences were detected for leaf disease severity or percent pod infection (Table 3.3). Disease incidence and severity were much higher in kidney bean than observed in navy bean. This was attributed to the higher susceptibility in the kidney market class to halo blight (Zaumeyer and Meiners, 1975; Singh et al., 2007). Treatment differences for the leaf disease incidence were only observed in kidney bean at 7 WAP. The NIC had lower disease incidence compared to the IC, but in later ratings no other differences were detected (Table 3.3). Even though the seed for the NIC was grown in an area that was suitable for the production of disease-free seed, moderate levels of halo blight infection were observed. The infection of these seed lots was likely due to latent infection of the seed.

In comparing the IC to the chemical treatments in kidney bean, increased disease control was observed when ST was used, but not when ST + Microwave was applied (Table 3.3). This suggests an negative effect resulted from the combination of the microwave and chemical treatment. However, when  $\text{Cu}(\text{OH})_2$  + Microwave treatment was compared to Microwave treatment alone, a benefit of the addition of  $\text{Cu}(\text{OH})_2$  was observed. The inconsistency between these two combination treatments may have been due to the lower efficacy of PFM compared to  $\text{Cu}(\text{OH})_2$ . The addition of PFM may have reduced the efficacy of ST and ST + Microwave

treatments compared to the IC. The application of either PFM or microwave treatment to  $\text{Cu}(\text{OH})_2$  did not increase disease control in any of the contrasts.

At 11 WAP, increased disease incidence was observed in navy bean, but no differences between contrasts were detected in navy or kidney bean (data not shown). The absence of differences at the later rating of the kidney bean suggests that the ST was unable to control halo blight under prolonged disease pressure. Disease severity at 11 WAP did not change from that observed at 7 WAP and no differences were observed (data not shown). Pod infection of both market classes was low and no differences were detected (Table 3.3).

#### *Hundred Seed Weight & Seed Pick*

In contrast comparisons for HSW and seed pick, all treatments were better than the IC in kidney bean, but no differences were detected in navy bean (Table 3.4). The absence of differences in navy bean was attributed to the uniform disease pressure observed among all treatments earlier in the growing season (Table 3.3). In kidney bean, the HSW and seed pick in the NIC, ST, Microwave, and ST + Microwave treatments were up to 19.5% better than the IC (Table 3.4). The lower seed weight in the IC was likely in response to halo blight infection (Taylor et al., 1979a), while the increased seed pick was attributed to higher disease pressure earlier in the growing season (Table 3.3). However, unlike in the ST and NIC treatments, lower disease pressure was not observed in the microwave and ST + Microwave treatments in the disease incidence ratings (Table 3.3). These results could be attributed to the  $\text{Cu}(\text{OH})_2$  component of ST, as increased disease control with  $\text{Cu}(\text{OH})_2$  was observed in other contrast comparisons. The increased HSW for microwave treatment compared to the IC may have been related to the lower seed pick, as the lower seed pick suggests some measure of disease control.

However, differences in disease control were not evident in the leaf disease ratings earlier in the season (Table 3.3).

No differences in contrast comparisons for HSW were observed between the microwave and chemical treatments applied alone or in combination, except for the Microwave versus  $\text{Cu}(\text{OH})_2$  + Microwave treatment (Table 3.4). An increased HSW was noted in the  $\text{Cu}(\text{OH})_2$  + Microwave treatment, which agrees with the better disease control observed earlier in the growing season (Table 3.3). The increase in control when  $\text{Cu}(\text{OH})_2$  and Microwave treatment were applied together, seen in early disease incidence ratings (Table 3.3), was not observed for seed pick (Table 3.4), indicating that season long control was not achieved.

#### *Yield & Return on Investment*

For navy bean, the Morden and Winkler trial sites were analyzed separately due to a significant ( $P < 0.05$ ) environment x treatment effect, which was likely due to differences in environmental conditions such as rainfall, soil conditions, and irrigation levels. For kidney bean, the Winkler 2012 trial site was presented separately due to decreased yield and ROI compared to the other site years.

In contrast comparisons for yield only a few differences were observed. The NIC had higher yields than the IC, but this only was observed in kidney bean trials (Table 3.5). Other treatment differences were only detected in the Winkler 2012 kidney trial, where ST and ST + Microwave treatment had yields 237-368  $\text{kg ha}^{-1}$  higher than the IC. This was attributed to the chemical components, as no increase in yield was seen when the microwave treatment was applied alone. The benefit of the chemical component was also observed in the Winkler navy bean trials, where both ST alone and PFM + Microwave treatment had yields 320-327  $\text{kg ha}^{-1}$  higher than microwave treatment alone. In kidney bean grown in Winkler 2012, yields were 235  $\text{kg ha}^{-1}$

higher for  $\text{Cu}(\text{OH})_2$  + Microwave compared to the microwave treatment. Microwave treatment applied alone did not increase yields at any of the trial sites and in the Winkler 2012 kidney bean trial it actually decreased yield when PFM was compared to PFM + Microwave. A similar difference was observed between the ½x and 1x Microwave treatments in the Winkler navy bean trials and the 2012 Winkler kidney bean trials. These differences were likely due to the differences in emergence, as described earlier.

For ROI, differences were only observed in the kidney bean trials (Table 3.6). The NIC had the highest ROI, which was up to 74% better than the IC. At the Winkler 2012 kidney trial site, the ST, Microwave, and ST + Microwave treatments all had higher ROI than the IC. This response was not observed in the other kidney bean trials. The larger ROI for microwave treatment when compared to the IC was not expected as no differences were detected for yield, but the lower percentage of seed pick for the microwave treatment (Table 3.4) may have been a contributing factor.

In the kidney bean trials excluding Winkler 2012, the  $\text{Cu}(\text{OH})_2$  treatment had a ROI  $\$352 \text{ ha}^{-1}$  greater than PFM, which was due to the large difference in cost for the two products, as no yield differences were observed (Table 3.5). Comparing Microwave to PFM + Microwave, a loss in ROI was also noted, which provides further evidence that the higher cost of PFM with no associated benefit on yield negatively impacted ROI (Table 3.6). In several contrast comparisons (PFM versus PFM + Microwave, ½x versus 1x Microwave, and Microwave versus  $\text{Cu}(\text{OH})_2$  + Microwave), a relationship between yield and ROI was observed, in that yield increases resulted in a higher ROI. This is due to the fact that yield is a large component in the ROI calculation. In Figure 3.3, a linear regression confirmed the high correlation of these factors ( $R^2 = 0.66-0.98$ ). In the Morden navy bean and kidney bean trials the correlation was greatest and for every  $1 \text{ kg ha}^{-1}$

increase in yield, the ROI increased between \$0.74 and \$0.82 ha<sup>-1</sup>, respectively. In the Winkler navy bean trials, this correlation was lower and return was not as high (Figure 3.3) due to lower seeding emergence early in the growing season (Table 3.2). No significant correlation could be made between yield and ROI in the Winkler 2012 kidney bean as variability between the treatments was too great (data not shown).

Microwave radiation had minimal impact on dry bean seed germination, vigour or pathogen viability following short (0-40 s) intervals of exposure. The application of Cu(OH)<sub>2</sub>, compared to the other treatments, slightly increased disease control early in the season in the kidney beans, which contributed to a lower seed pick and increased HSW, yield, and ROI at the end of the season. The use of PFM, however, did not provide equivalent control when compared to the industry standard and did not perform well enough to justify the increased cost. The addition of microwave radiation to chemical treatment was inexpensive, but did not provide a benefit for emergence, disease control, HSW, seed pick, yield or ROI. Further research into the benefits of Cu(OH)<sub>2</sub> as a seed treatment and alternative control methods are warranted, as there is little evidence that microwave radiation provided a measurable level of control for halo blight.

**Table 3.1. Treatments allocated for the dry bean halo blight seed treatment experiments in 2012 and 2013**

Treatments	Chemical Rate g a.i./100 kg seed	Microwave Time	
		Navy & Pinto 2012 seconds	Kidney 2013 seconds
1 Non-infected control	-	-	-
2 Infected control	-	-	-
3 PFM <sup>z</sup>	14.0	-	-
4 Cu(OH) <sub>2</sub> <sup>y</sup>	30.4	-	-
5 1/2x Microwave	-	25.0	20.0
6 1 x Microwave	-	50.0	40.0
7 1/2x Microwave + BAS720	14.0	25.0	20.0
8 1 x Microwave + PFM	14.0	50.0	40.0
9 1/2x Microwave + Cu(OH) <sub>2</sub>	30.4	25.0	20.0
10 1x Microwave + Cu(OH) <sub>2</sub>	30.4	50.0	40.0

<sup>z</sup>PFM= Pyraclostrobin + fluxapyroxad + metalaxyl

<sup>y</sup>Cu(OH)<sub>2</sub>= Copper Hyrdoxide 53.8%

**Table 3.2. Contrasts comparing percentage of emergence of navy and kidney beans for various seed treatments to control halo blight at Morden and Winkler, MB in 2012-2013**

Treatment comparison	Percentage of Emergence <sup>z</sup> (%)						
	7 DAP			10 DAP		20 DAP	
	Navy	Kidney	Kidney <sup>y</sup>	Navy	Kidney	Navy	Kidney
IC <sup>x</sup> vs NIC	71 vs 73	46 vs 72 **	8 vs 13	75 vs 77	62 vs 88 **	76 vs 77	70 vs 87 **
IC vs ST	71 vs 70	46 vs 42	8 vs 5	75 vs 76	62 vs 65	76 vs 76	70 vs 72
IC vs Microwave	71 vs 63	46 vs 39	8 vs 8	75 vs 67	62 vs 59	76 vs 68	70 vs 68
IC vs ST + Microwave	71 vs 64	46 vs 43	8 vs 6	75 vs 68	62 vs 62	76 vs 68	70 vs 69
PFM vs Cu(OH) <sub>2</sub>	71 vs 70	40 vs 43	4 vs 5	78 vs 74	65 vs 64	78 vs 73	72 vs 73
PFM vs PFM + Microwave	71 vs 66	40 vs 42	4 vs 5	78 vs 71	65 vs 61	78 vs 71	72 vs 66 *
Cu(OH) <sub>2</sub> vs Cu(OH) <sub>2</sub> + Microwave	70 vs 61 *	43 vs 43	5 vs 6	74 vs 65 *	64 vs 63	73 vs 66	73 vs 71
ST vs Microwave	70 vs 63 *	42 vs 39	5 vs 8	76 vs 67 *	65 vs 59 *	76 vs 68 *	72 vs 68 *
ST vs ST + Microwave	70 vs 64 *	42 vs 43	5 vs 6	76 vs 68 *	65 vs 62	76 vs 68 *	72 vs 69
<sup>1</sup> / <sub>2</sub> x Microwave vs 1x Microwave	69 vs 56 *	41 vs 38	9 vs 7	75 vs 58 **	61 vs 58	77 vs 59 **	70 vs 66
Microwave vs PFM + Microwave	63 vs 66	39 vs 42	8 vs 5	67 vs 71	59 vs 61	68 vs 71	68 vs 66
Microwave vs Cu(OH) <sub>2</sub> + Microwave	63 vs 61	39 vs 43	8 vs 6	67 vs 65	59 vs 63	68 vs 66	68 vs 71
Microwave vs ST + Microwave	63 vs 64	39 vs 43	8 vs 6	67 vs 68	59 vs 62	68 vs 68	68 vs 69

<sup>z</sup>Percentage of emergence was arcsine square root transformed for data analysis to satisfy the assumptions of normality and back-transformed estimates are presented.

<sup>y</sup>Winkler 2012 trial presented separately to meet the assumptions of normality.

<sup>x</sup>IC= infected control; NIC= non-infected control; Microwave= <sup>1</sup>/<sub>2</sub>x and 1x Microwave rates; ST= PFM and Cu(OH)<sub>2</sub>; PFM= Pyraclostrobin + fluxapyroxad + metalaxyl; Cu(OH)<sub>2</sub>= Copper Hydroxide 53.8%.

\*,\*\* Denotes significance at  $P < 0.05$  and  $P < 0.01$ , respectively, based on orthogonal contrasts.

**Table 3.3. Contrasts comparing percentage of leaf incidence and severity at 7 WAP and pod infection at 14 WAP on navy and kidney beans for various seed treatments to control halo blight at Morden and Winkler, MB in 2012-2013**

Treatment comparison	Leaf Infection (7WAP)				Pod Infection (14 WAP)	
	Incidence <sup>z</sup>		Severity <sup>y</sup>		%	
	Navy	Kidney	Navy	Kidney	Navy	Kidney
IC <sup>x</sup> vs NIC	2.7 vs 2.7	8.0 vs 5.0 **	2.1 vs 2.4	4.0 vs 3.0	1.8 vs 2.2	2.1 vs 1.8
IC vs ST	2.7 vs 2.9	7.6 vs 6.8 *	2.4 vs 2.2	3.9 vs 3.9	2.2 vs 1.6	1.8 vs 1.7
IC vs Microwave	2.7 vs 3.0	7.6 vs 7.4	2.4 vs 2.3	3.9 vs 4.0	2.2 vs 1.6	1.8 vs 1.9
IC vs ST + Microwave	2.7 vs 3.0	7.6 vs 7.2	2.4 vs 2.1	3.9 vs 3.9	2.2 vs 1.7	1.8 vs 2.0
PFM vs Cu(OH) <sub>2</sub>	3.0 vs 2.8	7.5 vs 6.2 **	2.3 vs 2.1	4.1 vs 3.6	1.7 vs 1.5	1.6 vs 1.8
PFM vs PFM + Microwave	3.0 vs 2.9	7.5 vs 7.6	2.3 vs 2.1	4.1 vs 4.0	1.7 vs 1.8	1.6 vs 1.9
Cu(OH) <sub>2</sub> vs Cu(OH) <sub>2</sub> + Microwave	2.8 vs 3.1	6.2 vs 6.8	2.1 vs 2.1	3.6 vs 3.8	1.5 vs 1.6	1.8 vs 2.0
ST vs Microwave	2.9 vs 3.0	6.8 vs 7.4	2.2 vs 2.3	3.9 vs 4.0	1.6 vs 1.6	1.7 vs 1.9
ST vs ST + Microwave	2.9 vs 3.0	6.8 vs 7.2	2.2 vs 2.1	3.9 vs 3.9	1.6 vs 1.7	1.7 vs 2.0
<sup>1</sup> / <sub>2</sub> x Microwave vs 1x Microwave	3.2 vs 2.8	7.6 vs 7.2	2.4 vs 2.3	3.9 vs 4.0	1.7 vs 1.5	2.0 vs 1.9
Microwave vs PFM + Microwave	3.0 vs 2.9	7.4 vs 7.6	2.3 vs 2.1	4.0 vs 4.0	1.6 vs 1.8	1.9 vs 1.9
Microwave vs Cu(OH) <sub>2</sub> + Microwave	3.0 vs 3.1	7.4 vs 6.8 *	2.3 vs 2.1	4.0 vs 3.8	1.6 vs 1.6	1.9 vs 2.0
Microwave vs ST + Microwave	3.0 vs 3.0	7.4 vs 7.2	2.3 vs 2.1	4.0 vs 3.9	1.6 vs 1.7	1.9 vs 2.0

<sup>z</sup>Data was collected using a 1-9 scale to evaluate incidence, percent of plant with symptoms, where 1= No visible lesions, 3= 2% of leaf area with round lesions and slight chlorosis, 5= 5% of leaf area with 5 mm round lesions and slight chlorosis, 7= 10% of leaf area with lesions and chlorosis with slight leaf distortion, and 9= 25% or more of leaf area with lesions and chlorosis with severe leaf distortion.

<sup>y</sup>Data was collected using a 0-5 scale to evaluate severity, the average area covered by a lesion, where 0= no lesion, 1= <5% of leaf, 2= 5-10% leaf, 3= 10-25% leaf, 4= 25-50% leaf, and 5= 50-100% of leaf.

<sup>x</sup>IC= infected control; NIC= non-infected control; Microwave= <sup>1</sup>/<sub>2</sub>x and 1x Microwave rates; ST= PFM and Cu(OH)<sub>2</sub>; PFM= Pyraclostrobin + fluxapyroxad + metalaxyl; Cu(OH)<sub>2</sub>= Copper Hydroxide 53.8%.

\*,\*\* Denotes significance at  $P < 0.05$  and  $P < 0.01$ , respectively, based on orthogonal contrasts.



**Table 3.4. Contrasts comparing hundred seed weight (HSW) and percentage of seed pick on navy and kidney beans for various seed treatments to control halo blight at Morden and Winkler, MB in 2012-2013**

Treatment comparison	HSW		Seed Pick (%)	
	Navy	Kidney	Navy	Kidney
IC <sup>z</sup> vs NIC	21.1 vs 21.0	41.0 vs 49.0 **	5.1 vs 3.4	23.1 vs 13.7 **
IC vs ST	21.1 vs 20.8	41.0 vs 43.9 **	5.1 vs 4.4	23.1 vs 19.4 *
IC vs Microwave	21.1 vs 21.1	41.0 vs 42.7 *	5.1 vs 5.0	23.1 vs 19.6 *
IC vs ST + Microwave	21.1 vs 21.4	41.0 vs 43.4 **	5.1 vs 4.5	23.1 vs 20.0 *
PFM vs Cu(OH) <sub>2</sub>	20.9 vs 20.7	43.4 vs 44.3	4.5 vs 4.3	20.2 vs 18.7
PFM vs PFM + Microwave	20.9 vs 21.3	43.4 vs 42.1	4.5 vs 4.8	20.2 vs 22.0
Cu(OH) <sub>2</sub> vs Cu(OH) <sub>2</sub> + Microwave	20.7 vs 21.4	44.3 vs 44.7	4.3 vs 4.2	18.7 vs 17.9
ST vs Microwave	20.8 vs 21.1	43.9 vs 42.7	4.4 vs 5.0	19.4 vs 19.6
ST vs ST + Microwave	20.8 vs 21.4	43.9 vs 43.4	4.4 vs 4.5	19.4 vs 20.0
<sup>1</sup> / <sub>2</sub> x Microwave vs 1x Microwave	21.3 vs 21.0	42.6 vs 42.7	5.2 vs 4.9	17.8 vs 21.3
Microwave vs PFM + Microwave	21.1 vs 21.3	42.7 vs 42.1	5.0 vs 4.8	19.6 vs 22.0
Microwave vs Cu(OH) <sub>2</sub> + Microwave	21.1 vs 21.4	42.7 vs 44.7 **	5.0 vs 4.2	19.6 vs 17.9
Microwave vs ST + Microwave	21.1 vs 21.4	42.7 vs 43.4	5.0 vs 4.5	19.6 vs 20.0

<sup>z</sup>IC= infected control; NIC= non-infected control; Microwave= <sup>1</sup>/<sub>2</sub>x and 1x Microwave rates; ST= PFM and Cu(OH)<sub>2</sub>; PFM= Pyraclostrobin + fluxapyroxad + metalaxyl; Cu(OH)<sub>2</sub>= Copper Hydroxide 53.8%.

\*,\*\* Denotes significance at  $P < 0.05$  and  $P < 0.01$ , respectively, based on orthogonal contrasts.

**Table 3.5. Contrasts comparing yield on navy and kidney beans for various seed treatments to control halo blight at Morden and Winkler, MB in 2012-2013**

Treatment comparison	Yield (kg ha <sup>-1</sup> )			
	Navy Morden	Navy Winkler	Kidney	Kidney <sup>z</sup>
IC <sup>x</sup> vs NIC	4119 vs 3981	4100 vs 3808	2144 vs 3363 **	558 vs 1371 **
IC vs ST	4119 vs 4050	4100 vs 4331	2144 vs 2372	558 vs 926 **
IC vs Microwave	4119 vs 3910	4100 vs 4004	2144 vs 2206	558 vs 741
IC vs ST + Microwave	4119 vs 4196	4100 vs 4159	2144 vs 2190	558 vs 795 *
PFM vs Cu(OH) <sub>2</sub>	3999 vs 4101	4366 vs 4295	2180 vs 2564	876 vs 975
PFM vs PFM + Microwave	3999 vs 4252	4366 vs 4324	2180 vs 2033	876 vs 641 *
Cu(OH) <sub>2</sub> vs Cu(OH) <sub>2</sub> + Microwave	4101 vs 4140	4295 vs 3995	2564 vs 2347	975 vs 950
ST vs Microwave	4050 vs 3910	4331 vs 4004 *	2372 vs 2206	926 vs 741
ST vs ST + Microwave	4050 vs 4196	4331 vs 4159	2372 vs 2190	926 vs 795
<sup>1</sup> / <sub>2</sub> x Microwave vs 1x Microwave	4131 vs 3688	4299 vs 3709 **	2271 vs 2141	892 vs 590 *
Microwave vs PFM + Microwave	3910 vs 4252	4004 vs 4324 *	2206 vs 2033	741 vs 641
Microwave vs Cu(OH) <sub>2</sub> + Microwave	3910 vs 4140	4004 vs 3995	2206 vs 2347	741 vs 950 *
Microwave vs ST + Microwave	3910 vs 4196	4004 vs 4159	2206 vs 2190	741 vs 795

<sup>z</sup>Winkler 2012 trial separated out to meet the assumptions of normality.

<sup>x</sup>IC= infected control; NIC= non-infected control; Microwave= <sup>1</sup>/<sub>2</sub>x and 1x Microwave rates; ST= PFM and Cu(OH)<sub>2</sub>; PFM= Pyraclostrobin + fluxapyroxad + metalaxyl; Cu(OH)<sub>2</sub>= Copper Hydroxide 53.8%.

\*, \*\* Denotes significance at  $P < 0.05$  and  $P < 0.01$ , respectively, based on orthogonal contrasts.

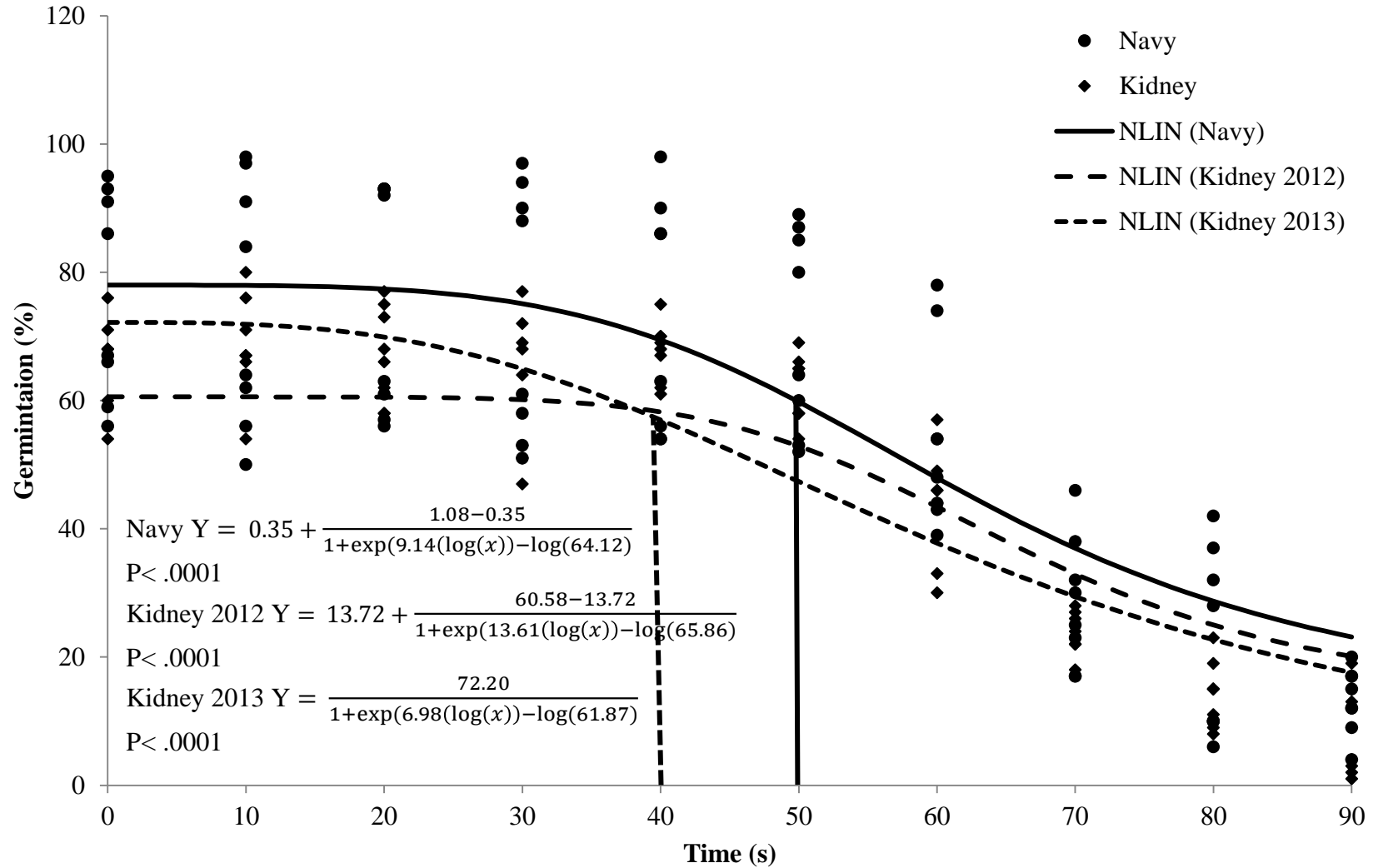
**Table 3.6. Contrasts comparing return on investment (ROI) on navy and kidney beans for various seed treatments to control halo blight at Morden and Winkler, MB in 2012-2013**

Treatment comparison	ROI (\$ ha <sup>-1</sup> )			
	Navy Morden	Navy Winkler	Kidney	Kidney <sup>z</sup>
IC <sup>x</sup> vs NIC	3223 vs 3173	2977 vs 2924	1388 vs 2696 **	286 vs 1108 **
IC vs ST	3223 vs 3183	2977 vs 3123	1388 vs 1538	286 vs 695 **
IC vs Microwave	3223 vs 3032	2977 vs 2930	1388 vs 1495	286 vs 522 *
IC vs ST + Microwave	3223 vs 3267	2977 vs 3020	1388 vs 1384	286 vs 553 *
PFM vs Cu(OH) <sub>2</sub>	3125 vs 3241	3102 vs 3145	1362 vs 1714 *	600 vs 791
PFM vs PFM + Microwave	3125 vs 3249	3102 vs 3098	1362 vs 1170	600 vs 357 *
Cu(OH) <sub>2</sub> vs Cu(OH) <sub>2</sub> + Microwave	3241 vs 3285	3145 vs 2943	1714 vs 1598	791 vs 750
ST vs Microwave	3183 vs 3032	3123 vs 2930	1538 vs 1495	695 vs 522
ST vs ST + Microwave	3183 vs 3267	3123 vs 3020	1538 vs 1384	695 vs 553
<sup>1</sup> / <sub>2</sub> x Microwave vs 1x Microwave	3193 vs 2872	3118 vs 2742	1562 vs 1427	681 vs 364 *
Microwave vs PFM + Microwave	3032 vs 3249	2930 vs 3098	1495 vs 1170 **	522 vs 357
Microwave vs Cu(OH) <sub>2</sub> + Microwave	3032 vs 3285	2930 vs 2943	1495 vs 1598	522 vs 750 *
Microwave vs ST + Microwave	3032 vs 3267	2930 vs 3020	1495 vs 1384	522 vs 553

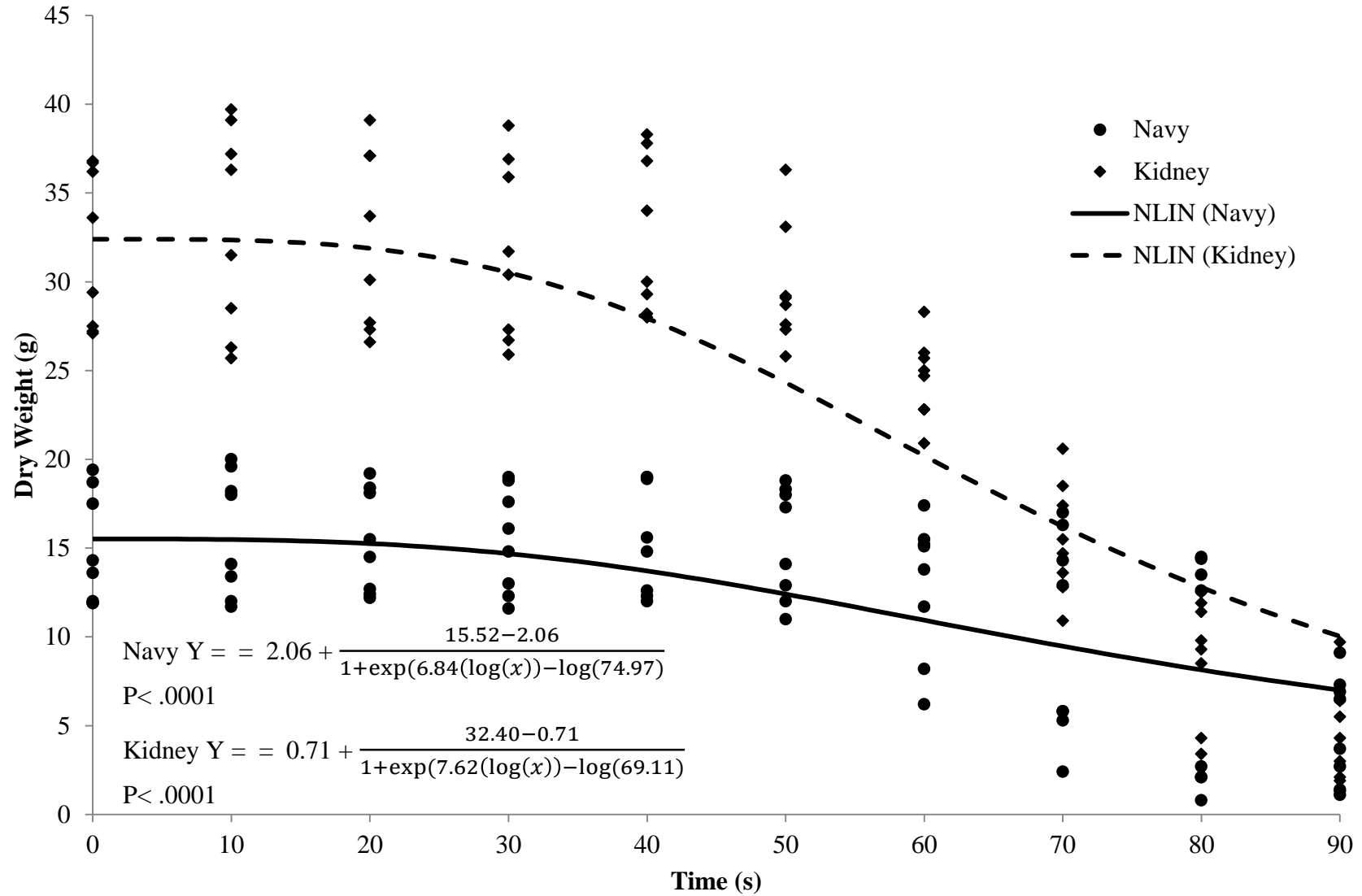
<sup>z</sup>Winkler 2012 trial separated out to meet the assumptions of normality.

<sup>x</sup>IC= infected control; NIC= non-infected control; Microwave= <sup>1</sup>/<sub>2</sub>x and 1x Microwave rates; ST= PFM and Cu(OH)<sub>2</sub>; PFM= Pyraclostrobin + fluxapyroxad + metalaxyl; Cu(OH)<sub>2</sub>= Copper Hydroxide 53.8%.

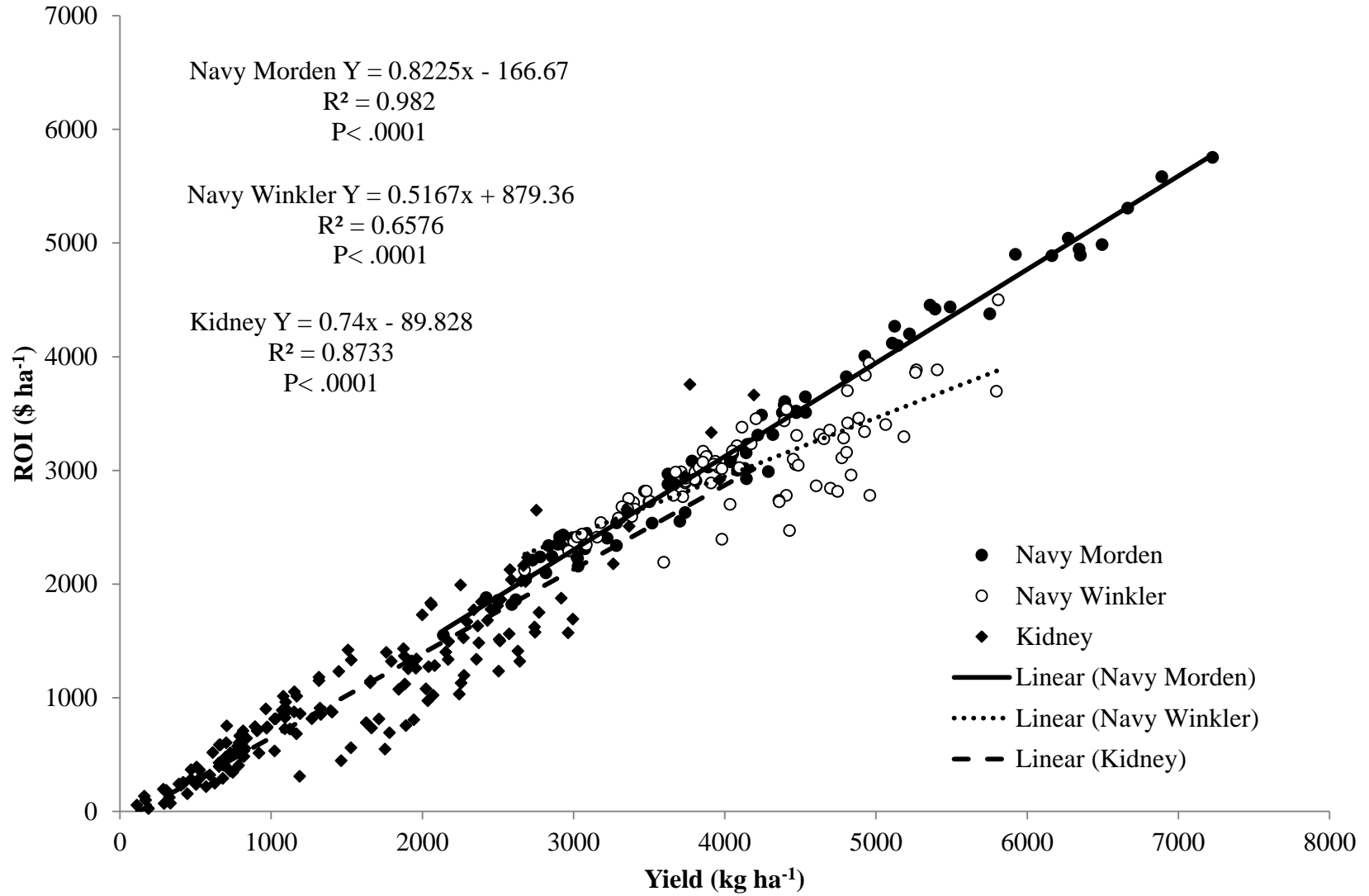
\*,\*\* Denotes significance at  $P < 0.05$  and  $P < 0.01$ , respectively, based on orthogonal contrasts.



**Figure 3.1.** Nonlinear regressions (NLIN) of percentage of germination of navy and kidney bean seed incubated in a germination chamber for 7 d at 25°C following various microwave radiation treatments in 2012 and 2013. Navy data was arcsine square root transformed for data analysis to meet the assumptions of normality and back-transformed estimates are presented.



**Figure 3.2.** Nonlinear regressions (NLIN) of plant vigour (dry weights of germinated material) from navy and kidney bean seed incubated at 25°C for 7 d in a germination chamber after various microwave radiation treatments in 2012 and 2013.



**Figure 3.3.** Linear regression of return on investment (ROI) and yield for navy and kidney bean seed treatment study for halo blight control in Morden, MB and Winkler, MB in 2012 and 2013.

## CHAPTER FOUR

### Effect of Microwave Radiation on Dry Bean Seed Infected with *Colletotrichum lindemuthianum* with and without the Use of Chemical Seed Treatment

#### 4.1 Abstract

Seed-borne anthracnose, caused by *Colletotrichum lindemuthianum*, is a serious disease that affects dry bean (*Phaseolus vulgaris* L.) seed quality and yield. A two-year study was conducted to examine the effect of microwave radiation on two market classes: navy (cv. Navigator) and pinto (cv. AC Ole) bean. In the laboratory, an exposure time between 40-50 s caused <10% decrease in seed germination and 0.14 and 0.10% s<sup>-1</sup> decrease in pathogen viability for navy and pinto bean, respectively. Field studies conducted at Ridgetown and Exeter, ON evaluated the effect of microwave radiation and two chemical seed treatments (thiamethoxam + fludioxonil + metalaxyl-M + azoxystrobin and pyraclostrobin + fluxapyroxad + metalaxyl), on emergence, percentage of infection, seed pick, yield and return on investment. Microwave treatment decreased emergence by <10% in both market classes and decreased disease symptoms by 17-30% for leaf and stem infection in the pinto bean when combined with chemical seed treatment. Chemical treatment alone decreased disease symptoms by 30% in navy bean. Microwave treatment did not affect pod infection, seed pick, yield, or the return on investment, yet chemical treatment increased yield between 6-25% in the navy bean.

#### 4.2 Introduction

*Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara is the causal agent of the seed-borne disease anthracnose in dry bean (*Phaseolus vulgaris* L.). When present, anthracnose can lower seed quality and reduce yields by up to 100% in environments with high humidity and

frequent rainfall (Schwartz et al., 2005). Disease symptoms can develop early in the growing season and occur primarily on petioles and veins of the lower leaf surface of seedlings. Later in the growing season, the lesions become black and sunken with a dark brown margin and can spread to the upper leaves, stems and pods (Schwartz et al., 2005). The development of lesions on the pods can lead to infection of the developing seed coats and cotyledons, resulting in discolouration and dark brown to black cankers on the seed (Tu, 1988).

Many management strategies have been suggested to reduce the spread of anthracnose via infected seed. The use of disease-free seed is the primary control measure recommended for anthracnose; however, sourcing this seed can be difficult and expensive. The use of other management strategies, such as cultural (Dillard and Cobb, 1993; Schwartz et al., 2005), chemical control (Trutmann et al., 1992) and genetic resistance (Ntahimpera et al., 1996) have also been utilized to reduce spread of anthracnose. Even with these control practices, anthracnose can still be difficult to manage as it can survive on infected crop debris (Dillard and Cobb, 1993). In addition, the anthracnose fungus possesses a high level of pathogenic variation, which limits the effectiveness of genetic control methods (Schwartz and Corrales, 1989).

Chemical seed treatment has been commonly used for controlling anthracnose as it has been shown to effectively reduce disease transmission from seed with small to medium sized anthracnose lesions (Tu, 1988). The use of thiamethoxam + fludioxonil + metalaxyl-M + azoxystrobin (TFMA) as a seed treatment has become the latest industry standard for controlling anthracnose as well as numerous soil-borne pathogens (Gillard and Ranatunga, 2013). The azoxystrobin component of TFMA, a strobilurin, has recently replaced the thiophanate-methyl component of DCT (diazinon + captan + thiophanate-methyl), the former industry standard in Ontario (Gillard et al., 2012a; Gillard and Ranatunga, 2013). However, even the application of



seed treatments like TFMA and DCT have been unable to fully eradicate anthracnose. Despite a reduction in incidence, disease symptoms are still frequently observed in treated fields (Gillard and Ranatunga, 2013).

The use of alternative seed treatment methods, such as thermotherapy, has been reported to effectively reduce or eliminate seed-borne pathogens from numerous crops (Grondeau et al., 1994). In dry bean, the use of alternative treatments like hot water or hot dry air decreased pathogen viability, but severely impacted bean germination (Sinclair, 1993). The use of microwave radiation as an alternate form of thermotherapy has successfully controlled pathogens in crops such as *Triticum* spp., tobacco (*Nicotiana tabacum* ‘Consolidate L.’), cassava true seed (*Manihot esculenta* Crantz) and soybean (*Glycine max* L.) (Hankin and Sands, 1977; Cavalcante and Muchovej, 1993; Han, 2010; Knox et al., 2013). The effect of microwave treatment on dry bean has been previously tested for its effect on seed germination and vigor and had little impact on seed germination at low levels of radiation (Spilde, 1989; Tylkowska et al., 2010). The use of microwave radiation was also tested for its effect on several different fungi (e.g. *Alternaria alternata* (Fr.) Keissler, *Fusarium* spp. and *Penicillium* spp.) and was effective in reducing the growth of *Penicillium* spp. on bean seed (Tylkowska et al., 2010). The authors hypothesized that microwave radiation would also control *C. lindemuthianum*, as microwave radiation appeared to have a greater effect on fungi with single-celled hyaline spores, but this theory was never tested (Cavalcante and Muchovej, 1993; Tylkowska et al., 2010).

The use of a treatment like microwave radiation could be highly beneficial to growers as a low cost treatment that could reduce or even eliminate the pathogen from infected seed while not severely impacting germination. It is unlikely that microwave radiation would be utilized alone for control of all pathogens, but it could be an inexpensive addition to a chemical seed treatment

to increase anthracnose control. Therefore, to further explore the hypotheses suggested by Tylkowska et al. (2010), the efficacy of microwave radiation on two dry bean market classes, navy and pinto bean, naturally infected with *C. lindemuthianum* were tested alone and in combination with chemical seed treatments. Navy and pinto bean market classes were selected due to their commercial importance in Canada, the obvious difference in seed size between dry beans (Swings and Civerolo, 1993) and the high susceptibility of many of the cultivars in these classes. The purpose of this study was to determine the effect of microwave radiation on seed germination and vigour and the effect on *C. lindemuthianum* viability and anthracnose development.

### **4.3 Materials & Methods**

#### **4.3.1 Laboratory Study**

A two-year thermotherapy study organized as a randomized complete block design (RCBD) was conducted on navy (cv. Navigator) and pinto (cv. AC Ole) bean seed naturally infected with *C. lindemuthianum* to determine the effect of microwave radiation on seed germination and *C. lindemuthianum* viability. Seed was obtained from field trials in 2011 in Morden, MB and from Exeter, ON in 2012 for the 2012 and 2013 laboratory studies, respectively. A Sortex electric eye (model 425BF, Gunson Sortex Ltd., London, UK) was used to sort blemished and unblemished seed in order to achieve a seed lot with a 10% discolouration rate. Blemished seed had lesions characteristic of anthracnose, which were identified as race 73 based on the pattern of disease severity on the 12 differential cultivars (Melotto et al., 2000). Seed was dried down using a Fisher Scientific Isotemp Forced Air Oven (120 V, 60 Hz, 1800 w, 15.5 A) and dry weights were taken until seed reached a stable weight to determine the seed moisture content for each seed lot. In the 2012 and 2013 season, the moisture content of navy bean and 2012 pinto bean ranged

between 6.6-7.6%, while in the 2013 pinto beans it was 10.6%. Four replicates of seed were exposed to ten microwave durations ranging from 0-90 s in 10 s increments using a consumer grade microwave oven (1100W 2450 MHz, General Electric Co. Fairfield, Connecticut, U.S.). Experimental units consisting of 150 seeds were placed on a paper plate. A 450-500 ml beaker containing 200 ml of water was placed at the center of the plate. The temperature of the water was measured before and after each microwave radiation to determine the change in temperature resulting from the microwave treatment (Reddy et al., 2000).

After treatment, 100 seeds per treatment were planted in 21.6 cm x 13.0 cm x 10.2 cm clam lid plastic containers (Par-Pak Ltd., Brampton, ON) using 50 WG 60 Wellpack silica sand (B.P. Dust Control, Walton, ON) as the growing medium. The seed was incubated in a germination chamber set at 25°C for 6-7 d using a 12L:12D photoperiod and relative humidity (RH) above 60%. Germination was evaluated using the Canadian Food Inspection Agency (CFIA) methods and procedures for testing seed (CFIA, 2011). Vigour was assessed as total dry weight of germinated plant matter in each container.

In 2012, four replicates of disease plating were conducted to evaluate seed infection for each seed lot, using potato dextrose agar (PDA) amended with 50% lactic acid. Microwave treated seed was surface-sterilized for 2 min in a 10% sodium hypochlorite solution and five seeds were placed per plate for a total of 50 seeds per experimental unit. Plated seed was left to germinate for a minimum of 7 d at room temperature (21-22°C) and evaluated for the percentage of germination and the percentage of seeds that produced visible colonies of *C. lindemuthianum*.

#### **4.3.2 Field Study**

Field trials were conducted in 2012 and 2013 to further evaluate the effects of microwave radiation on infected seed. Four studies were conducted at Exeter, ON (one navy and one pinto

per year) and three studies at Ridgetown, ON (one pinto and one navy in 2012 and one pinto in 2013). Ten treatments (Table 4.1) were arranged in a randomized complete block design with four replications, using infected seed from the same seed lots as the laboratory study. Non-infected control (NIC) treatments were planted using cv. AC Ole pinto bean seed obtained from disease-free plots in Manitoba and cv. Navigator navy bean seed produced in Idaho (Archer Daniels Midland Company, Decatur, IL).

The maximum microwave exposure rate (MER), determined from the microwave laboratory studies, resulted in <10% germination loss (Figure 4.1). This minor loss in germination was deemed acceptable if the microwave treatment could provide a sufficient level of anthracnose control. A MER of 50 s was established for the navy bean and 2012 pinto beans trials and 40 s for the 2013 pinto bean trials. The MER was used as the full microwave treatment (1x Microwave), while the half microwave ( $\frac{1}{2}$ x Microwave) treatment received half of the MER. Two chemical seed treatments were evaluated, namely pyraclostrobin + fluxapyroxad + metalaxyl (PFM) (BASF Canada, Mississauga, ON) and thiamethoxam + fludioxonil + metalaxyl –M + azoxystrobin (TFMA) (Syngenta Crop Protection Canada Inc., Guelph, ON) at rates of 14 g a.i. and 51 g a.i. per 100 kg of seed, respectively. All treatments included a treatment with metalaxyl-M (Bayer CropScience, Guelph, ON) at a rate of 4 g a.i. per 100 kg of seed to reduce any interference among treatments due to soil-borne pathogens, such as *Pythium* spp.

In Exeter, 1.5 m wide (four rows spaced 38 cm apart) by 6.0 m long plots were seeded 14 June 2012 and 21 June 2013. Plots were seeded using a six row cone-seeder in which the middle four rows were planted and the outer two rows left empty to provide space for evaluations. In Ridgetown, 1.3 m wide (three rows spaced 43 cm apart) and 6.0 m long plots were seeded 12

June 2012 and 17 June 2013. Plots were planted using a five row cone-seeder in which the three middle rows were planted and the outer two rows left unseeded. At Ridgeway and Exeter, plots were separated by 5 and 6 rows of soybean (*Glycine max* (L.) Merr.), respectively. Soybean was chosen as it is a non-host crop for *C. lindemuthianum* and minimized disease movement between plots (Gillard and Ranatunga, 2013). The seeding rate was 20 seeds m<sup>-1</sup> of row for the navy and pinto beans and 33 seeds m<sup>-1</sup> of row for soybeans. All plots were trimmed to 5.0 m in length after emergence.

Emergence and vigour were determined at 7, 10, 14 and 21 d after planting (DAP).

Emergence was measured as the total number of plants emerged in a 4 m length of the center row and then calculated as a percentage of the seeding rate of 20 seeds m<sup>-1</sup> of row. Vigour was evaluated using a 0-10 scale (0 = poor vigour and 10 = excellent vigour) and each treatment was compared to the infected control (IC). Non-destructive visual ratings of leaf vein, stem, and pod symptoms were based on the percentage of the plant tissue covered with anthracnose lesions. The first leaf vein and stem ratings were initiated when disease incidence had exceeded 3% plant infection in the IC. Two subsequent ratings were taken for leaf and stem infection at two and four weeks following the first rating. These ratings were used to calculate the area under the disease progress curve (AUDPC) using the modified equation:

$$\text{AUDPC} = \left( \frac{\mathbf{R}_1 + \mathbf{R}_2}{2} \right) (\mathbf{t}_2 - \mathbf{t}_1) + \left( \frac{\mathbf{R}_2 + \mathbf{R}_3}{2} \right) (\mathbf{t}_3 - \mathbf{t}_2)$$

where R<sub>1</sub> to R<sub>3</sub> are ratings matching to timing t<sub>1</sub>, t<sub>2</sub>, and t<sub>3</sub> (Wilcoxson et al., 1975). This equation was modified from the original where four ratings were used to calculate AUDPC. Pod infection was assessed at the same time as the last leaf and stem rating.

A blanket application of pyraclostrobin (BASF Canada, Mississauga, ON) at a rate of 100 g a.i. ha<sup>-1</sup> was made when 15% of the leaf veins were infected in the IC. The pyraclostrobin

application was used to maintain the treatment effects present in the experiments and to minimize additional disease spread. A pod destruction index (PDI) was carried out on the pods just prior to harvest using the equation (Gillard et al., 2012a):

$$\text{PDI} = (100 * \% \text{ destroyed pods}) + ((\% \text{ pod area with lesions}) * (1 - \% \text{ destroyed pods})) * 100$$

In Exeter, the plots were harvested using an Almaco SPC 40-2 (Almaco, Nevada, IA) small-plot combine. In Ridgetown, the plots were cut using a SRM-260 Echo power trimmer (Kioritz Crop., London, ON) and then seed was threshed with a stationary thresher (Almaco, Nevada, IA). For all plots, total seed weight was recorded and moisture content was determined using a Motomco 919 moisture meter (Dickey-John Corp., Patterson, NJ). Seed was cleaned using a 10/64 x 3/4 slotted screen to remove dockage (foreign material and split/undersized seed), which was then expressed as a percentage of the total seed weight (Canadian Grains Commission, 2010). After the dockage was removed, hundred-seed weight (HSW) and seed yield were determined and adjusted to 18% moisture. Seed pick was recorded as a percentage of seed with visible anthracnose lesions in a 100 seed sample. Evaluations on seed pick were made to mimic the methods used by Ontario dry bean processors in the grading of beans. The return on investment (ROI) for each treatment was calculated using the modified calculation (Gillard and Ranatunga, 2013):

$$\text{ROI} = ((\text{Seed Yield} - \text{Dockage} - 2(\text{Pick})) * \text{Seed Price kg}^{-1}) - \text{Cost of Seed Treatment}$$

Seed prices for navy and pinto beans were \$0.84 kg<sup>-1</sup> and \$0.73 kg<sup>-1</sup>, respectively (Agricorp, 2013). The percentage of pick was doubled, once to account for loss of poor quality seed and once for the cost of removing the infected seed. To determine the cost of the seed treatments, seeding density and manufacturer's suggested retail prices were used. Differences in row width resulted in a slight difference in seeding density, which led to differences in seed treatment costs

between the Ridgetown and Exeter sites. For TFMA, a cost of \$53.96 ha<sup>-1</sup> in Ridgetown and \$61.87 ha<sup>-1</sup> in Exeter were calculated. The same prices were used for PFM to make the treatment costs comparable, because no price has been set for this product. Microwave treatment costs were calculated using seeding density and electricity costs at a rate of 12.54¢ kWh<sup>-1</sup> according to the Ontario Energy Board average cost per kWh. In navy bean, 1x Microwave treatment costs were \$2.03 ha<sup>-1</sup> and \$2.32 ha<sup>-1</sup> for Ridgetown and Exeter, respectively. In pinto bean, 1x Microwave treatment costs were \$2.70 ha<sup>-1</sup> and \$3.10 ha<sup>-1</sup> in 2012 and \$2.32 ha<sup>-1</sup> and \$2.48 ha<sup>-1</sup> in 2013 for Ridgetown and Exeter respectively. Lower exposure times were used in 2013 than in 2012, resulting in differences in cost for pinto bean. The cost was divided by two in all trials for the ½x Microwave treatments.

#### 4.3.3 Statistical Analysis

Statistical analyses were performed with SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). When necessary, data was transformed to meet the assumptions of normality based on the highest Shapiro-Wilk's statistic and outliers were removed using Lund's test for outliers (Rotondi and Koval, 2009). Significance for all tests was determined by a Type I error rate of  $\alpha=0.05$  and it was assumed that the models were additive, and linear where appropriate.

In the laboratory studies, analysis of variance (ANOVA) was performed using PROC MIXED where length of microwave exposure was the fixed effect and replicate and year were random effects. A dosage response nonlinear regression was performed on percentage of germination and vigour using PROC NLIN to determine the exposure time at which germination and vigour start to decrease. The dosage response equation took the form:

$$Y = C + \frac{D - C}{1 + \exp[b (\log(X) - \log(I_{50}))]}$$

where  $C$  and  $D$  represented the upper and lower limit of the percentage of germination response ( $Y$ ), respectively,  $I_{50}$  was the time at which there was 50% response, and  $X$  represented microwave radiation time (Bowley, 2008). Linear regression was performed on disease incidence of seed colonization over time using PROC GLM to determine the decrease in percentage of colonization by *C. lindemuthianum* in the seed plating.

For the field studies, ANOVA was performed using PROC MIXED where treatment was the fixed effect and environment, environment nested in replicate, environment x treatment and environment x year were random effects. Preplanned contrasts were utilized to evaluate differences between treatments for all the variables. Comparisons between the NIC and IC were conducted to detect the absence and presence of disease, respectively. Microwave and chemical treatments were compared to the IC to determine the efficacy of each treatment on seed emergence and disease control. The 1x Microwave and ½x Microwave treatment were compared to observe the effect of increased microwave radiation. The two chemical treatments were compared to determine if PFM was equivalent to the current industry standard and the efficacy of both products on anthracnose. The combination of microwave and chemical treatments was also compared to the individual microwave and chemical treatments and the IC to determine if there were any additional effects on disease when they were applied in combination. Emergence and percentage of pod infection were arcsine square root transformed and the AUDPC for stem infection was square root transformed. Linear regressions were performed using PROC GLM to determine the correlation between ROI and yield and ROI and AUDPC.



## 4.4 Results & Discussion

### 4.4.1 Laboratory Study

Microwave radiation had little effect on seed germination when the seed was exposed to short time intervals of radiation. In non-linear regressions, a decrease of less than 10% in germination (Figure 4.1) and vigour (Figure 4.2) was observed when microwave radiation was below 40 s of exposure in the navy bean and 2012 pinto bean experiments; there was a decrease in germination after 30 s of exposure (Figure 4.1) in the 2013 pinto bean experiment. Using the regression formulas, a MER was determined for each of the seed lots based on <10% germination loss. A 50 s MER was used for the navy bean and the 2012 pinto bean experiments and 40 s MER was used for the 2013 pinto bean experiment (Figure 4.1). The lower MER in 2013 pinto beans was likely due to the difference in moisture content (MC) of the seed, compared to other seed lots. Microwave radiation produces energy through dielectric heating, which is a process that utilizes high-frequency electromagnetic radiation to heat dielectric materials through the aligning of dipole water molecules. When dielectric materials, such as seed, are exposed to microwave radiation the realignment of the water molecules creates friction, which produces heat and increases seed temperatures (Bouraoui et al., 1993). Therefore, when the seed MC increased, more heat was produced, which lowered the seeds tolerance to prolonged microwave radiation (Appendix) (Knox et al., 2013). Increased exposure time also increased seed temperature and it was observed that as microwave radiation exposure surpassed 30 s the percentage of germination (Figure 4.1) and plant dry weight for vigour (Figure 4.2) began to decrease slightly. Germination decreased up to 11% for navy bean and the 2012 pinto bean experiments and 8% for the 2013 pinto bean experiments in the 30-50 s exposure range. Vigour was not as severely affected at the 30-50 s for the navy bean experiments, but there was a similar trend in the percentage of

germination with a decrease in dry weight of 19% (i.e. 6.3 g) in the pinto bean trials at 50 s (Figure 4.2). After more than 50 s of exposure, percentage of seed germination (Figure 4.1) and vigour (Figure 4.2) decreased rapidly; which was likely due to increased seed temperature resulting in higher seedling mortality.

Microwave exposure also affected pathogen colonization of the seed in the disease plating experiments for both navy and pinto bean. A linear reduction in visible pathogen growth was observed and with every second of microwave exposure, pathogen colonization of the seed decreased 0.14% and 0.10% for navy and pinto bean, respectively (Figure 4.3). The reduction in colonization in combination with the slight decrease in seed germination between 30-50 s of exposure were indicative of the potential of microwave radiation as an alternative seed treatment option.

#### **4.4.2 Field Study**

##### *Weather Conditions*

In 2012, heavy rainfall occurred shortly after planting and was followed by hot dry weather in early July. The appearance of anthracnose symptoms was minimal until late July-early August after which frequent rainfalls promoted disease development. In Ridgetown, rainfall during pod set resulted in higher disease pressure than at the Exeter site. In 2013, recurrent rainfall and high humidity at 4 WAP resulted in heavy disease pressure on bean seedlings, which resulted in thinner stands, especially at the Exeter sites which received 113.9 mm of rainfall within 20 d of planting. However, after mid-July conditions were hot and dry and anthracnose symptoms were not as evident in the pod ratings.

## *Emergence*

Treatment comparisons showed that the application of microwave radiation and chemical treatment had a minimal effect on the percentage of emergence in the field trials. At 7 DAP no differences were observed in emergence or vigour for both the navy and pinto trials (data not shown). At subsequent ratings, treatment differences in emergence occurred and the results varied between the navy and pinto market classes (Table 4.2); however, no differences in vigour were observed (data not shown). The differences between the market classes may be a result of their difference in seed size and infection (Tu, 1996; Conner et al., 2006b). Pinto bean seed on average is two times the size of navy bean seed (Conner et al., 2006b). The increased seed size provides more food reserves, which can minimize differences in emergence between treatments compared to the smaller navy bean seed (Conner et al., 2006b; Conner et al., 2009). The more uniform emergence of the pinto bean and the minimal effect observed from microwave treatment may explain why fewer differences were seen in pinto bean.

In the navy bean trials, microwave radiation did not lower emergence compared to the IC. However, the microwave treatment decreased emergence by 5-11% in contrasts comparing ½x Microwave to 1x Microwave, PFM to PFM + Microwave and ST + Microwave to ST alone (Table 4.2). In comparing the efficacy of the ST and microwave treatments, a decrease in emergence was also noted at 10, 14 and 21 DAP. When the two chemical treatments were combined (i.e. ST) and compared to the microwave treatments, a delay in emergence was seen in the TFMA treatments. The lack of differences between the ST and microwave treatment at 10 DAP may have been due to TFMA, as the azoxystrobin component has been shown to delay early seed emergence (Gillard, C. unpublished). This delay was also observed when TFMA treatment was compared to PFM at 10 and 14 DAP. Overall, the lower percentage of emergence

observed due to microwave treatment in navy bean could be attributed to the MER that was set for the seed lots in the laboratory studies. This loss in emergence was close to the preplanned 10% standard reduction and did not appear to affect emergence any more than the IC, therefore it was considered an acceptable loss for the microwave treatments.

In pinto bean, the analysis on emergence was conducted on the combined results from both Exeter sites and the 2013 Ridgetown site. The 2012, the Ridgetown trial site was excluded from analysis as there was uneven and delayed emergence due to intense water stress caused by heavy rainfall during the first four weeks after planting. In the analysed trials, treatment differences were only observed at 10 DAP. The NIC had 9% lower emergence compared to the IC during the first 2 WAP; likely due to the fact that seed for the NIC control was produced and harvested in a different environment. Differences in seed size or seed coat thickness (Lush and Wien, 1980; Souza and Marcos-Filho, 2001), storage conditions (Spilde, 1989; TeKrony and Egli, 1991), mechanical damage (Moore, 1972) or other environmental factors could have contributed to the early delay in emergence. Additionally, the 1x Microwave treatment had 9% higher emergence than ½x Microwave treatment, opposite to that seen in navy bean. In previous studies, microwave exposure has been observed to speed up germination rates through the introduction of energy via radiation (Spilde, 1989; Aladjajian and Svetleva, 1997). The introduced energy has been suggested to stimulate molecular transformations that provide substances that speed up the germination rate (Aladjajian and Svetleva, 1997; Aladjajyan, 2007; Tylkowska et al., 2010). The increased energy introduced in the 1x Microwave treatment compared to the ½ x Microwave treatment may have attributed to the higher early emergence observed at the 1x Microwave treatment. However, these results were inconsistent between bean varieties in this study and with previous published work on dry bean emergence and vigour in both the laboratory and field

(Nelson et al., 1970; Nelson and Stetson, 1985; Spilde, 1989). Since no differences in emergence were observed for any of the other microwave treatments in this study, no firm conclusions can be made about increased germination in the field when microwave treatment was applied.

#### *Disease Incidence*

In all studies, disease symptoms on the leaves and stems were higher in the IC compared to the NIC (Table 4.3), which agrees with previous studies on anthracnose development from seed infected with *C. lindemuthianum* (Conner et al., 2009). Lower disease intensity over time was observed for ST and ST + Microwave treatment in comparison to the IC in both the leaf and stem evaluations (Table 4.3). The increase in disease control over time in this study was comparable to that observed in previous studies conducted using chemical seed treatments such as azoxystrobin and diazinon + captan + thiophanate-methyl (Gillard et al., 2012a; Gillard and Ranatunga, 2013). The PFM treatment provided similar disease control of leaf infection compared to the TFMA treatment, but lower control of stem infection in the navy bean.

The microwave treatments had less leaf infection than the IC in pinto bean. When ST and microwave treatments were compared, no differences in control were detected in pinto bean. However in navy bean, ST lowered the AUDPC value by more than 30% compared to the microwave treatments. The addition of microwave treatment to ST increased disease control when compared to ST in pinto bean and microwave treatment alone in both navy and pinto bean. Additional control was observed in pinto bean, when the combination of chemical treatment and microwave treatment (ST + Microwave, PFM + Microwave, and TFMA + Microwave) decreased disease severity between 17-30% compared to ST or microwave treatment alone (Table 4.3). In navy bean, chemical treatment and microwave treatment in combination had a lower AUDPC value than the microwave treatments alone. This suggests that additional control

occurred between these two treatments since no differences were seen when microwave treatment was applied alone compared to the IC and ST.

For pod infection, trends similar to the leaf and stem ratings were observed in navy bean, but no differences were observed in pinto bean (Table 4.4). The Ridgetown 2012 navy bean trial site was analyzed separately due to lower disease pressure until flowering and higher disease pressure during pod set. The only treatment differences observed for these studies were due to ST with no additional effect from the microwave treatment (Table 4.4). In comparing the two chemical treatments, the PFM treatment was less effective in reducing pod infection than the TFMA in navy bean.

#### *Pod Destruction Index & Seed Pick*

Contrast comparisons indicated that the NIC had the lowest PDI and seed pick values at the majority of the trial sites, compared with the IC (Table 4.5). The application of a ST + Microwave treatment lowered the PDI compared to the IC and the microwave treatment alone in both market classes, while the application of ST alone only lowered PDI in the pinto bean trials. No significant differences were detected for seed pick in the navy bean study. In pinto bean study, the TFMA treatment had higher percentage of seed pick compared to the PFM and TFMA + Microwave treatments. The increase in percentage of seed pick may be due to decreased pod shrivelling or fewer pods aborted by the plant before reaching maturity in the TFMA treatment since it had less leaf and pod disease.

#### *Yield & Return on Investment*

For yield, the 2012 and 2013 navy bean sites were separated due to a significant year x treatment reaction due to lower yields in the 2013 trial. The decrease in yield observed in 2013 was attributed to high disease pressure at 28 DAP, which resulted in stand thinning due to

seedling death in the infected treatments, which allowed for improved differentiation of the yield response among treatments in 2013. Yield was higher in the NIC than in the IC for the navy bean only (Table 4.6). In treatments where TFMA was applied (TFMA alone and ST) yields were increased by 6-25% when compared to the IC, PFM, and microwave treatments. Chemical treatments such as TFMA have been shown to effectively reduce disease symptoms early in the season, therefore increasing seedling survival and final yields (Gillard et al., 2012a).

In contrast comparisons of navy bean, the NIC increased ROI by \$1008 ha<sup>-1</sup> compared to the IC (Table 4.6). Although the microwave treatment was a low cost treatment, the only differences in ROI were seen when microwave and ST + Microwave were compared to ST alone in three of four pinto bean trials. The inconsistency between market classes and trials may have been due to the large differences in yield observed. Linear regressions were performed (Figure 4.4) and ROI was shown to be closely correlated with yield ( $R^2 = 0.84-0.94$ ), where an increase of \$0.63-0.86 occurred for every yield increase of 1 kg ha<sup>-1</sup>. In contrast comparisons, the application of TFMA + Microwave treatment increased ROI (Table 4.5) in navy bean, which was associated with the lower disease symptoms on leaf, stem (Table 4.3) and pods (Table 4.4). In linear regressions of ROI and AUDPC (Figure 4.5), increased leaf infection was shown to decrease ROI at a rate of \$6.66 ha<sup>-1</sup> and \$6.85 ha<sup>-1</sup> for navy and pinto bean, respectively, for every unit of increase in AUDPC ( $R^2 = 0.34-0.58$ ). The overall effect of microwave and chemical treatment on ROI was minimal and the lower cost microwave treatment did not increase ROI compared to the IC or chemical treatments.

In conclusion, microwave radiation is an inexpensive seed treatment method that has a minimal effect on seed germination and emergence at low levels of radiation. However, even though Tylkowska et al. (2010) predicted microwave radiation would control *C.*

*lindemuthianum*, the impact on pathogen viability was not consistent between the laboratory and field studies. Microwave radiation decreased leaf and stem infection in the pinto bean field trials when combined with seed treatment. However, in the navy bean field trials microwave treatment did not perform consistently compared to the chemical seed treatments. For both navy and pinto bean, microwave treatment provided little control of pod infection, PDI or seed pick, resulting in few differences in final yield and ROI. The difference between the two market classes may be attributed to differences in their seed size and moisture content. Clearly, further research is required to determine how microwave radiation affects different dry bean market classes and why disease control was more evident in the laboratory than in the field.



**Table 4.1. Treatments allocated for the dry bean anthracnose seed treatment experiments in 2012 and 2013**

Treatments	Chemical Rate	Microwave Time	
		Navy & Pinto 2012	Pinto 2013
	g a.i./100 kg seed	seconds	seconds
1 Non-infected control	-	-	-
2 Infected control	-	-	-
3 PFM <sup>z</sup>	14.0	-	-
4 TFMA <sup>y</sup>	51.0	-	-
5 1/2x Microwave	-	25.0	20.0
6 1x Microwave	-	50.0	40.0
7 1/2x Microwave + PFM	14.0	25.0	20.0
8 1x Microwave + PFM	14.0	50.0	40.0
9 1/2x Microwave + TFMA	51.0	25.0	20.0
10 1x Microwave + TFMA	51.0	50.0	40.0

<sup>z</sup>PFM= pyraclostrobin + fluxapyroxad + metalaxyl

<sup>y</sup>TFMA= thiamethoxam, fludioxinol, metalaxyl-M, azoxystrobin

**Table 4.2. Contrasts comparing percentage of emergence of navy and pinto beans for various seed treatments to control anthracnose at Ridgetown and Exeter, ON in 2012-2013**

Treatment comparison	Emergence <sup>z</sup> (%)					
	10 DAP		14 DAP		21 DAP	
	Navy	Pinto <sup>y</sup>	Navy	Pinto <sup>y</sup>	Navy	Pinto <sup>y</sup>
IC <sup>x</sup> vs NIC	85 vs 91	80 vs 71 *	91 vs 96 *	85 vs 81	92 vs 96	86 vs 82
IC vs ST	85 vs 88	80 vs 80	91 vs 95	85 vs 84	92 vs 95	86 vs 85
IC vs Microwave	85 vs 83	80 vs 75	91 vs 89	85 vs 81	92 vs 89	86 vs 82
IC vs ST + Microwave	85 vs 81	80 vs 76	91 vs 89	85 vs 84	92 vs 90	86 vs 85
PFM vs TFMA	93 vs 82 **	77 vs 82	97 vs 92 **	83 vs 85	97 vs 93	86 vs 84
PFM vs PFM + Microwave	93 vs 84 **	77 vs 75	97 vs 89 **	83 vs 82	97 vs 89 **	86 vs 84
TFMA vs TFMA + Microwave	82 vs 79	82 vs 78	92 vs 89	85 vs 85	93 vs 91	84 vs 86
ST vs Microwave	88 vs 83	80 vs 75	95 vs 89 **	84 vs 81	95 vs 89 **	85 vs 82
ST vs ST + Microwave	88 vs 81 **	80 vs 76	95 vs 89 **	84 vs 84	95 vs 90 **	85 vs 85
<sup>1</sup> / <sub>2</sub> x Microwave vs 1x Microwave	87 vs 79	70 vs 79 *	92 vs 86 *	76 vs 85	92 vs 86	78 vs 85
Microwave vs PFM + Microwave	83 vs 84	75 vs 75	89 vs 89	81 vs 82	89 vs 89	82 vs 84
Microwave vs TFMA + Microwave	83 vs 79	75 vs 78	89 vs 89	81 vs 85	89 vs 91	82 vs 86
Microwave vs ST + Microwave	83 vs 81	75 vs 76	89 vs 89	81 vs 84	89 vs 90	82 vs 85

<sup>z</sup>Emergence was arcsine square root transformed for data analysis to satisfy the assumptions of normality and back-transformed estimates are presented.

<sup>y</sup>Ridgetown 2012 trial not included as emergence was delayed due to water stress.

<sup>x</sup>IC= infected control; NIC= non-infected control; Microwave= <sup>1</sup>/<sub>2</sub>x and 1x Microwave rates; PFM= pyraclostrobin + fluxapyroxad + metalaxyl; TFMA= thiamethoxam, fludioxonil, metalaxyl-M, azoxystrobin; ST= PFM + TFMA.

\*,\*\* Denotes significance at  $P < 0.05$  and  $P < 0.01$ , respectively, based on orthogonal contrasts.

**Table 4.3. Contrasts comparing the area under the disease progress curve (AUDPC) for leaf and stem infection on navy and pinto beans for various seed treatments to control anthracnose in Ridgetown and Exeter, ON in 2012-2013**

Treatment comparison	AUDPC			
	Leaf Infection		Stem Infection	
	Navy	Pinto	Navy	Pinto <sup>z</sup>
IC <sup>y</sup> vs NIC	145 vs 42 **	101 vs 25 **	80 vs 25 **	33 vs 8 **
IC vs ST	145 vs 103	101 vs 80 *	80 vs 56 *	33 vs 24 **
IC vs Microwave	145 vs 161	101 vs 83 *	80 vs 82	33 vs 27
IC vs ST + Microwave	145 vs 92 *	101 vs 64 **	80 vs 52 **	33 vs 20 **
PFM vs TFMA	124 vs 82	71 vs 88	68 vs 44 *	21 vs 27
PFM vs PFM + Microwave	124 vs 112	71 vs 61	68 vs 60	21 vs 19
TFMA vs TFMA + Microwave	82 vs 73	88 vs 68 *	44 vs 45	27 vs 22
ST vs Microwave	103 vs 161 *	80 vs 83	56 vs 82 **	24 vs 27
ST vs ST + Microwave	103 vs 92	80 vs 64 *	56 vs 52	24 vs 20 *
<sup>1</sup> / <sub>2</sub> x Microwave vs 1x Microwave	156 vs 165	81 vs 84	86 vs 78	27 vs 27
Microwave vs PFM + Microwave	161 vs 112 *	83 vs 61 **	82 vs 60 *	27 vs 19 **
Microwave vs TFMA + Microwave	161 vs 73 **	83 vs 68 *	82 vs 45 **	27 vs 22 *
Microwave vs ST + Microwave	161 vs 92 **	83 vs 64 **	82 vs 52 **	27 vs 20 **

<sup>z</sup> AUDPC data for stem infection in pinto bean were square root +0.5 transformed to satisfy the assumptions of

<sup>y</sup> IC= infected control; NIC= non-infected control; Microwave= <sup>1</sup>/<sub>2</sub>x and 1x Microwave rates; PFM= pyraclostrobin + fluxapyroxad + metalaxyl; TFMA= thiamethoxam, fludioxonil, metalaxyl-M, azoxystrobin; ST= PFM + TFMA.

\*,\*\* Denotes significance at  $P < 0.05$  and  $P < 0.01$ , respectively, based on orthogonal contrasts.

**Table 4.4. Contrasts comparing the percentage of pod infection on navy and pinto beans for various seed treatments to control anthracnose in Ridgetown and Exeter, ON in 2012-2013**

Treatment comparison	Pod Infection <sup>z</sup> (%)		
	10 WAP		
	Navy	Navy <sup>y</sup>	Pinto
IC <sup>x</sup> vs NIC	2.0 vs 0.0 **	5.0 vs 0.0 *	2.0 vs 0.0
IC vs ST	1.8 vs 0.9 *	4.9 vs 2.5	2.4 vs 4.0
IC vs Microwave	1.8 vs 2.6	4.9 vs 6.8	2.4 vs 2.6
IC vs ST + Microwave	1.8 vs 1.1 *	4.9 vs 2.3	2.4 vs 2.7
PFM vs TFMA	1.3 vs 0.6 *	5.6 vs 0.6 *	2.9 vs 5.3
PFM vs PFM + Microwave	1.3 vs 1.2	5.6 vs 5.3	2.9 vs 2.9
TFMA vs TFMA + Microwave	0.6 vs 0.9	0.6 vs 0.6	5.3 vs 2.6
ST vs Microwave	0.9 vs 2.6 **	2.5 vs 6.8 *	4.0 vs 2.6
ST vs ST + Microwave	0.9 vs 1.1	2.5 vs 2.3	4.0 vs 2.7
<sup>1</sup> / <sub>2</sub> x Microwave vs 1x Microwave	2.9 vs 2.3	5.5 vs 8.1	3.0 vs 2.3
Microwave vs PFM + Microwave	2.6 vs 1.2 **	6.8 vs 5.3	2.6 vs 2.9
Microwave vs TFMA + Microwave	2.6 vs 0.9 **	6.8 vs 0.6 **	2.6 vs 2.6
Microwave vs ST + Microwave	2.6 vs 1.1 **	6.8 vs 2.3 *	2.6 vs 2.7

<sup>z</sup>Pod infection data were arcsine square root transformed to satisfy the assumptions of normality and back-transformed estimates are presented

<sup>y</sup>Ridgetown 2012 trial presented separately to meet the assumptions of normality.

<sup>x</sup>IC= infected control; NIC= non-infected control; Microwave= <sup>1</sup>/<sub>2</sub>x and 1x Microwave rates; PFM= pyraclostrobin + fluxapyroxad + metalaxyl; TFMA= thiamethoxam, fludioxonil, metalaxyl-M, azoxystrobin; ST= PFM + TFMA.

\*,\*\* Denotes significance at  $P < 0.05$  and  $P < 0.01$ , respectively, based on orthogonal contrasts.

**Table 4.5. Contrasts comparing pod destruction index and percentage of seed pick for navy and pinto beans for various seed treatments to control anthracnose in Ridgetown and Exeter, ON in 2012-2013**

Treatment comparison	Pod Destruction Index		Seed Pick (%)	
	Navy	Pinto	Navy	Pinto
IC <sup>z</sup> vs NIC	5.0 vs 1.8 **	6.3 vs 1.6 **	7.0 vs 2.0	7.0 vs 4.0 **
IC vs ST	5.5 vs 4.5	6.3 vs 4.7 *	6.9 vs 5.5	6.7 vs 6.4
IC vs Microwave	5.5 vs 5.1	6.3 vs 5.3	6.9 vs 7.2	6.7 vs 6.5
IC vs ST + Microwave	5.5 vs 3.8 *	6.3 vs 4.2 **	6.9 vs 5.2	6.7 vs 5.6
PFM vs TFMA	4.6 vs 4.3	4.2 vs 5.3	6.0 vs 4.9	5.2 vs 7.6 *
PFM vs PFM + Microwave	4.6 vs 4.3	4.2 vs 4.0	6.0 vs 5.9	5.2 vs 5.4
TFMA vs TFMA + Microwave	4.3 vs 3.3	5.3 vs 4.3	4.9 vs 4.4	7.6 vs 5.7 *
ST vs Microwave	4.5 vs 5.1	4.7 vs 5.3	5.5 vs 7.2	6.4 vs 6.5
ST vs ST + Microwave	4.5 vs 3.8	4.7 vs 4.2	5.5 vs 5.2	6.4 vs 5.6
<sup>1</sup> / <sub>2</sub> x Microwave vs 1x Microwave	5.2 vs 5.0	5.2 vs 5.3	7.3 vs 7.1	6.2 vs 6.8
Microwave vs PFM + Microwave	5.1 vs 4.3	5.3 vs 4.0 *	7.2 vs 5.9	6.5 vs 5.4
Microwave vs TFMA + Microwave	5.1 vs 3.3 **	5.3 vs 4.3 *	7.2 vs 4.4	6.5 vs 5.7
Microwave vs ST + Microwave	5.1 vs 3.8 *	5.3 vs 4.2 *	7.2 vs 5.2	6.5 vs 5.6

<sup>z</sup>IC= infected control; NIC= non-infected control; Microwave= <sup>1</sup>/<sub>2</sub>x and 1x Microwave rates; PFM= pyraclostrobin + fluxapyroxad + metalaxyl; TFMA= thiamethoxam, fludioxonil, metalaxyl-M, azoxystrobin; ST= PFM + TFMA.

\*,\*\* Denotes significance at  $P < 0.05$  and  $P < 0.01$ , respectively, based on orthogonal contrasts.

**Table 4.6. Contrasts comparing yield and return on investment for navy and pinto beans for various seed treatments to control anthracnose in Ridgetown and Exeter, ON in 2012-2013**

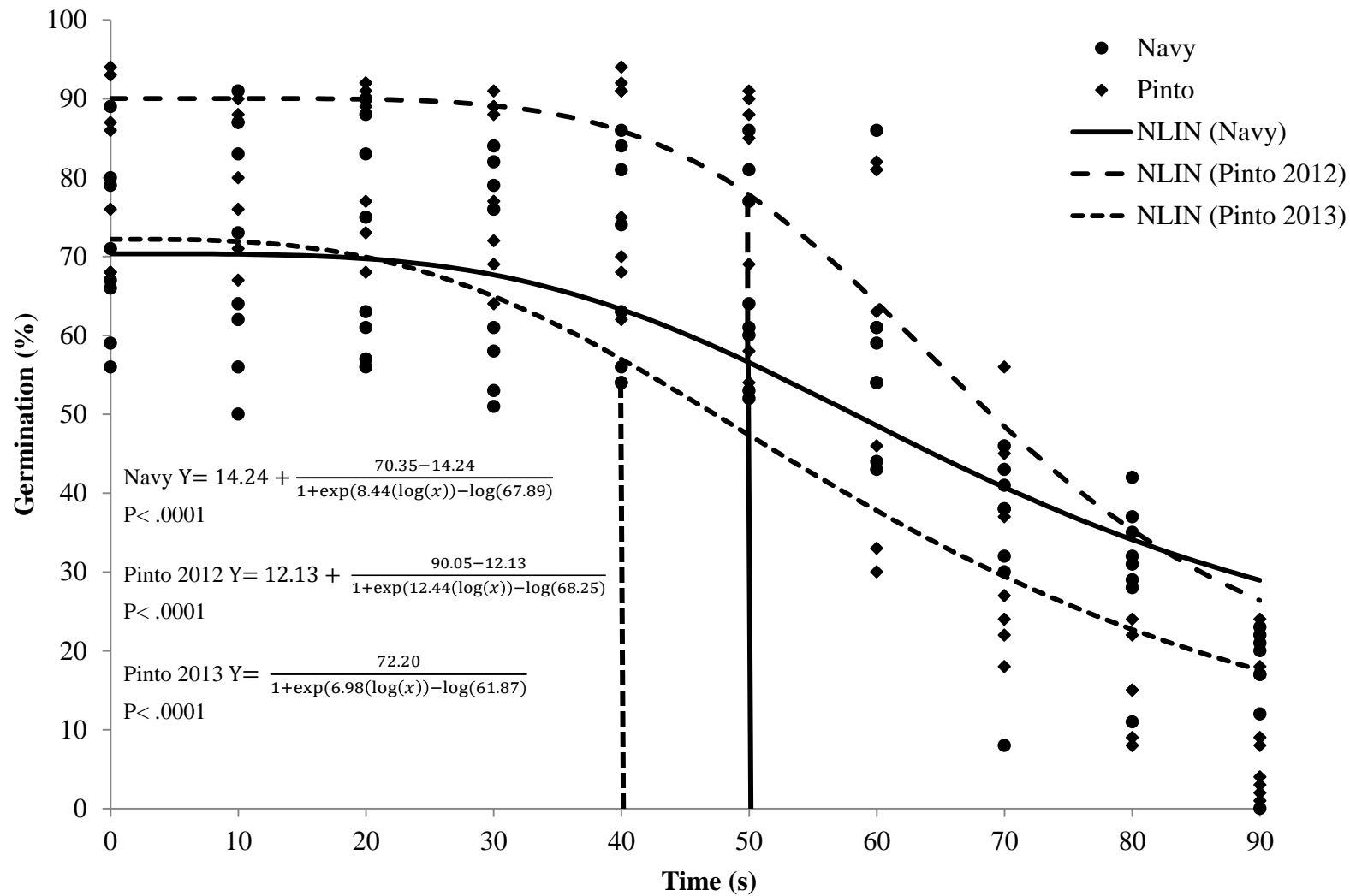
Treatment comparison	Yield (kg ha <sup>-1</sup> )			Return on Investment (\$ ha <sup>-1</sup> )		
	Navy 2012 <sup>z</sup>	Navy 2013 <sup>z</sup>	Pinto	Navy	Pinto	Pinto <sup>y</sup>
IC <sup>x</sup> vs NIC	2494 vs 3712 **	2063 vs 2313 **	3102 vs 3205	1416 vs 2424 **	1635 vs 1891	2571 vs 3237
IC vs ST	2494 vs 2964	2063 vs 2232 *	3102 vs 3124	1416 vs 1710	1635 vs 1423	2571 vs 3200
IC vs Microwave	2494 vs 2629	2063 vs 2046	3102 vs 3095	1416 vs 1467	1635 vs 1632	2571 vs 2842
IC vs ST + Microwave	2494 vs 3098 *	2063 vs 2163	3102 vs 3267	1416 vs 1739	1635 vs 1599	2571 vs 3127
PFM vs TFMA	2724 vs 3204	2095 vs 2368 **	3199 vs 3048	1502 vs 1917	1528 vs 1318	3247 vs 3154
PFM vs PFM + Microwave	2724 vs 2918	2095 vs 2154	3199 vs 3308	1502 vs 1627	1528 vs 1611	3247 vs 3251
TFMA vs TFMA + Microwave	3204 vs 3278	2368 vs 2172 *	3048 vs 3227	1917 vs 1851	1318 vs 1588 *	3154 vs 3003
ST vs Microwave	2964 vs 2629	2232 vs 2046 **	3124 vs 3095	1710 vs 1467	1423 vs 1632 *	3200 vs 2842
ST vs ST + Microwave	2964 vs 3098	2232 vs 2163	3124 vs 3267	1710 vs 1739	1423 vs 1599 *	3200 vs 3127
<sup>1</sup> / <sub>2</sub> x Microwave vs 1x Microwave	2666 vs 2592	1994 vs 2098	3161 vs 3029	1498 vs 1437	1733 vs 1530	2891 vs 2793
Microwave vs PFM + Microwave	2629 vs 2918	2046 vs 2154	3095 vs 3308	1467 vs 1627	1632 vs 1611	2842 vs 3251
Microwave vs TFMA + Microwave	2629 vs 3278 *	2046 vs 2172 *	3095 vs 3227	1467 vs 1851 *	1632 vs 1588	2842 vs 3003
Microwave vs ST + Microwave	2629 vs 3098 *	2046 vs 2163 *	3095 vs 3267	1467 vs 1739	1632 vs 1599	2842 vs 3127

<sup>z</sup>Yield data for 2012 and 2013 were separated out to satisfy the assumptions of normality.

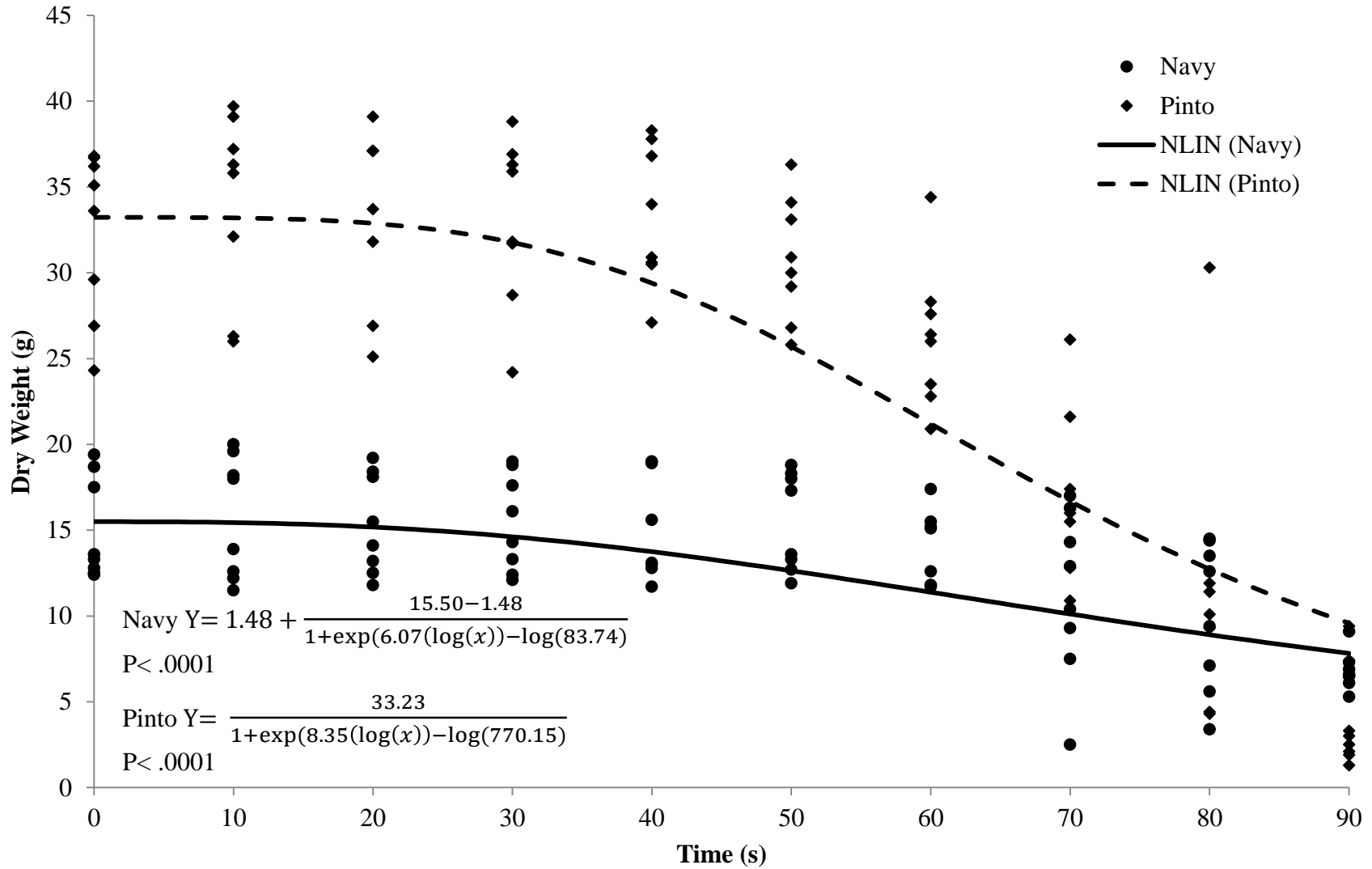
<sup>y</sup>Return on investment data for the Ridgetown 2013 trial were separated out to meet the assumptions of normality.

<sup>x</sup>IC= infected control; NIC= non-infected control; Microwave= <sup>1</sup>/<sub>2</sub>x and 1x Microwave rates; PFM= pyraclostrobin + fluxapyroxad + metalaxyl; TFMA= thiamethoxam, fludioxonil, metalaxyl-M, azoxystrobin; ST= PFM + TFMA.

\*,\*\* Denotes significance at  $P < 0.05$  and  $P < 0.01$ , respectively, based on orthogonal contrasts.

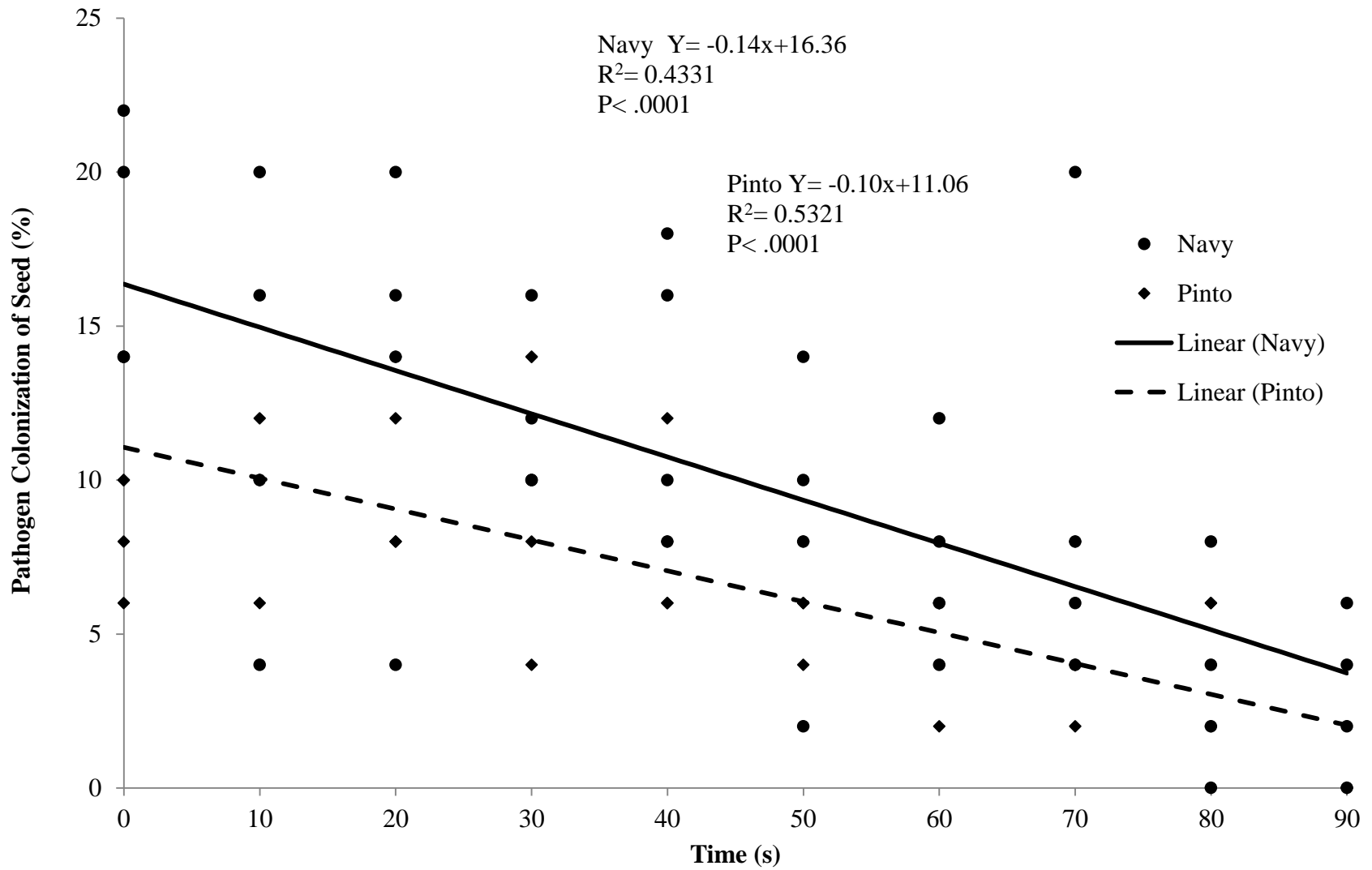


**Figure 4.1.** Nonlinear regressions (NLIN) of percentage of germination of navy and pinto bean seed incubated in a germination chamber for 7 d at 25°C following various microwave radiation treatments in 2012 and 2013.

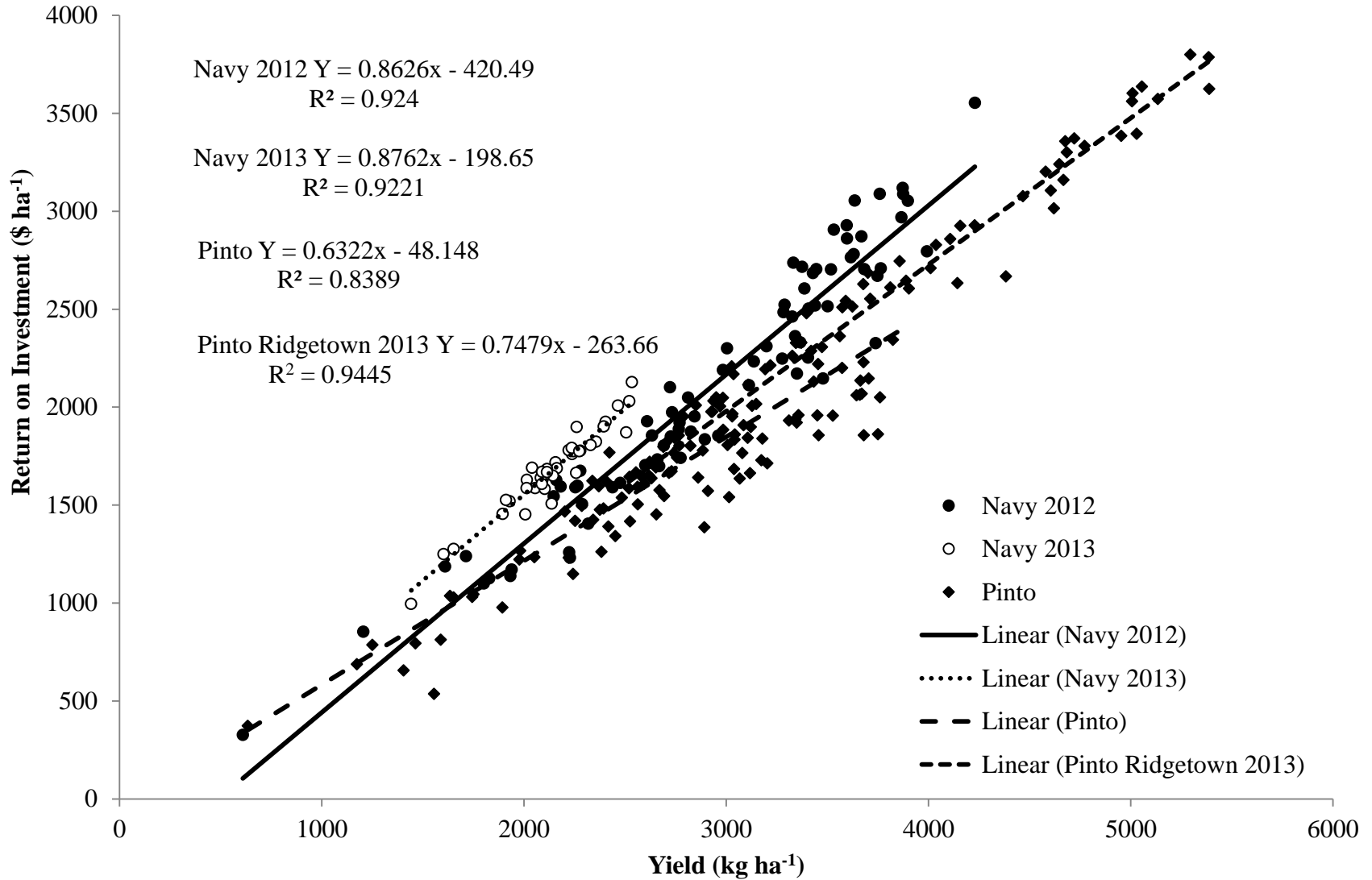


**Figure 4.2.** Nonlinear regressions (NLIN) of plant vigour (dry weights of germinated material) from navy and pinto bean seed incubated in a germination chamber for 7 day at 25°C following various microwave radiation treatments in 2012 and 2013.

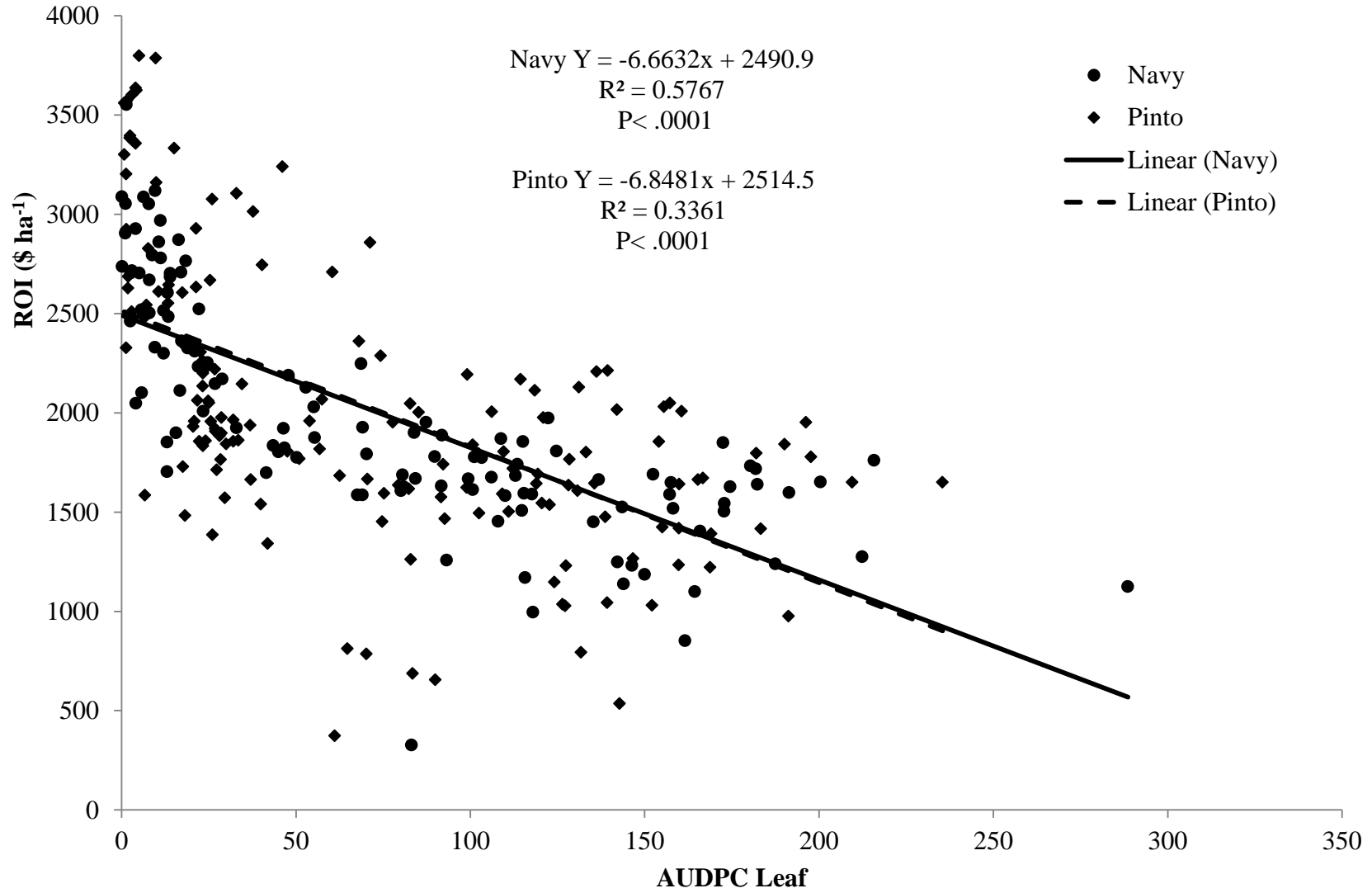




**Figure 4.3.** Linear regressions of percentage of pathogen colonization of seed by *Colletotrichum lindemuthianum* on potato dextrose agar following various microwave radiation treatments in 2012.



**Figure 4.4.** Linear regression of return on investment (ROI) and yield for navy and pinto bean seed treatment study for anthracnose control in 2012 and 2013.



**Figure 4.5.** Linear regression of area under the disease progress curve (AUDPC) for leaf infection and return on investment (ROI) for navy and pinto bean seed treatment study for anthracnose control in 2012 and 2013.

## CHAPTER FIVE

### General Discussion

#### 5.1 Research Contributions

This study was the first to examine the effect of microwave radiation on dry bean seed germination and vigour as well as the control of the seed-borne pathogens *Xap*, *Psp*, and *C. lindemuthianum* in both the laboratory and the field. Various dry bean market classes were used to better understand how seed size, moisture content, and pathogen colonization impacted the effect of microwave treatment. These studies also studied the potential of microwave radiation as an option for organic growers, or as a complimentary treatment for the conventional control methods currently being practiced.

The effect of microwave radiation on seed germination and emergence was consistent between the laboratory and field studies and indicative of how microwave radiation has little adverse effect on seed health when applied at an optimal exposure time for the seed lot. Determining the optimal microwave radiation exposure time for use under field conditions depended on the moisture content of the seed. Previous research on this relationship had been conducted in cereal crops (Knox et al., 2013), but this was the first study to examine the use of microwave radiation on various dry bean market classes. The effect of microwave radiation on the various market classes was also informative, as the larger seeded class tended to be more sensitive to prolonged microwave radiation than the smaller navy bean class.

The effect of microwave radiation on pathogen colonization and disease control in the laboratory and the field was less consistent. The lack of disease control observed with microwave radiation throughout the entire season suggests it has little effect on the seed-borne

pathogens tested and would not be highly beneficial to organic growers. In majority of the field trials, no differences in disease control were observed when the microwave treatment was applied in combination with chemical treatment. The lack of improvement in control would not justify the addition of an inexpensive microwave treatment to growers, as yield and ROI did not improve. Chemical treatments increased disease control in the *C. lindemuthianum* experiments, but were largely ineffective for the bacterial diseases. Despite the efficacy of the chemical treatments, there is concern regarding the overuse of products in the same chemical family if they are applied both as a seed and foliar treatment (Garrett and Schwartz, 1998; Gillard et al., 2012a).

## **5.2 Research Limitations**

The use of laboratory and field studies in this project allowed for the observation of the effect of microwave radiation in both a controlled and natural environment. However, the methodology used may have limited some of the evaluations. In the laboratory, the use of plastic tray containers limited the growth of the seedling roots and shoots. The use of deeper, open pots may have allowed for greater growth and the option to assess vigour based on root and shoot growth, rather than only as the dry weight of germinated plant matter, which was closely associated with seed mortality. In the field, environmental conditions played a large role in the variability in disease pressure between sites. Increasing the buffer area between the plots may have helped to decrease disease spread from rain splash and plant to plant contact between plots. However, a larger trial area would increase variability due to soil type and topography.

The use of two market classes for each disease studied allowed the examination of the effect of microwave radiation on a small- and large-seeded dry bean class. However, the reactions between the market classes were not always consistent and suggest that further testing of more

market classes is warranted. The moisture content of the seed lots also varied between market classes, which affected the maximum microwave exposure rate. A consistent seed moisture content between the seed lots would have provided a more uniform exposure time, which may have provided a more consistent measure of disease control from the microwave treatment. The infected seed used was obtained from areas where disease symptoms were visibly apparent and infection rates were high. The disease incidence in infected seed and the NICs were only determined based on the presence and absence of visible infection on the seed in 2013. This limited the understanding of actual disease incidence in the infected lots for the second season. The disease-free nature of the NICs was assumed based on the area where they were produced, but seed infection levels were not tested in the laboratory prior to seeding. The lack of seed plating for the disease-free seed lots may have limited the differences observed between the IC and NIC, as seen with the bacterial blights. Obtaining the NIC treatments from a different environment than that of the IC also affected emergence results in the field trials. In these trials, the cost for the NIC was also not considered in the ROI calculation and limits the actual gain in ROI observed when disease-free is obtained from less conducive growing areas.

Microwave radiation was only tested at one power setting in this study. Simplifying the microwave settings was done to fully examine the radiation effect on six seed lots and three seed-borne diseases, but this limited the exposure time that could be applied to the seed. The use of time intervals as a measure of exposure may have also limited the effectiveness of microwave treatment. Currently, the mode of action for a microwave radiation treatment is assumed to be heat production; however, this has not been scientifically proven. If heat production is the main mode of action, maximizing the temperature increase of exposed seed would have been more effective than maximizing the exposure time.

The mode of action for the chemical treatments was also limited in the *C. lindemuthianum* trials where both compounds were based on the strobilurin family. The diversification for anthracnose control is required as strobilurin fungicides were the primary foliar treatment as well, to prevent resistance in the future. The use of a chemical like DCT may have diversified this study more than the use of a new treatment with a strobilurin MOA as well.

### **5.3 Future Research**

Further research into the effect of microwave radiation and other thermotherapy methods as a treatment for seed-borne diseases in dry bean would be beneficial in the seed production industry as microwave radiation proved to have minimal effect on seed health and potential control of some fungal pathogens based on the laboratory results. The use of microwave radiation or other radiation treatments as alternative control methods would be valuable to growers as they tend to be inexpensive and have a minimal adverse impact on the seed and the environment. Microwave radiation offers two advantages; it would be a simple addition to the current seed treatment methods, and based on these studies no negative effects on the efficacy of the chemical treatment was observed.

The effect of microwave radiation could be further explored to confirm the mode of action of this treatment as well as experimentation with different microwave power levels where exposure time could be extended without harming seed germination. Research into other forms of radiation, like infrared and ultraviolet radiation, could also provide more options for alternative control methods. These treatments were tested previously as a post-harvest treatment for dry bean storage (Cunha et al., 1993) and for the sterilization of processing plants (Bintsis et al., 2000), but could aid in the control of disease as a pre-plant treatment as well. However, the effect of these types of treatment would also need to be tested to determine the effect they would

have on dry bean seed health. Studies using electron beam and plasma technology are also a promising front for a thermotherapy control in dry bean (Rogner, 2011). This technique has been tested on cereal crops and reported to eliminate pathogenic bacteria and fungi and should be further explored for leguminous crops.

Future studies should also concentrate on the modes of action of the chemical seed treatments tested in this study. For anthracnose control, the current industry standard (TFMA) and PFM were from the strobilurin family and provided some efficacy in field. For the bacterial blights, the  $\text{Cu}(\text{OH})_2$  treatments had fairly low efficacy and PFM was ineffective and therefore should not be considered as an alternative control. Research into the benefits of these seed treatments and the stacking of the same mode of action in the seed treatment and foliar application is required to prevent fungicide resistance in the future.



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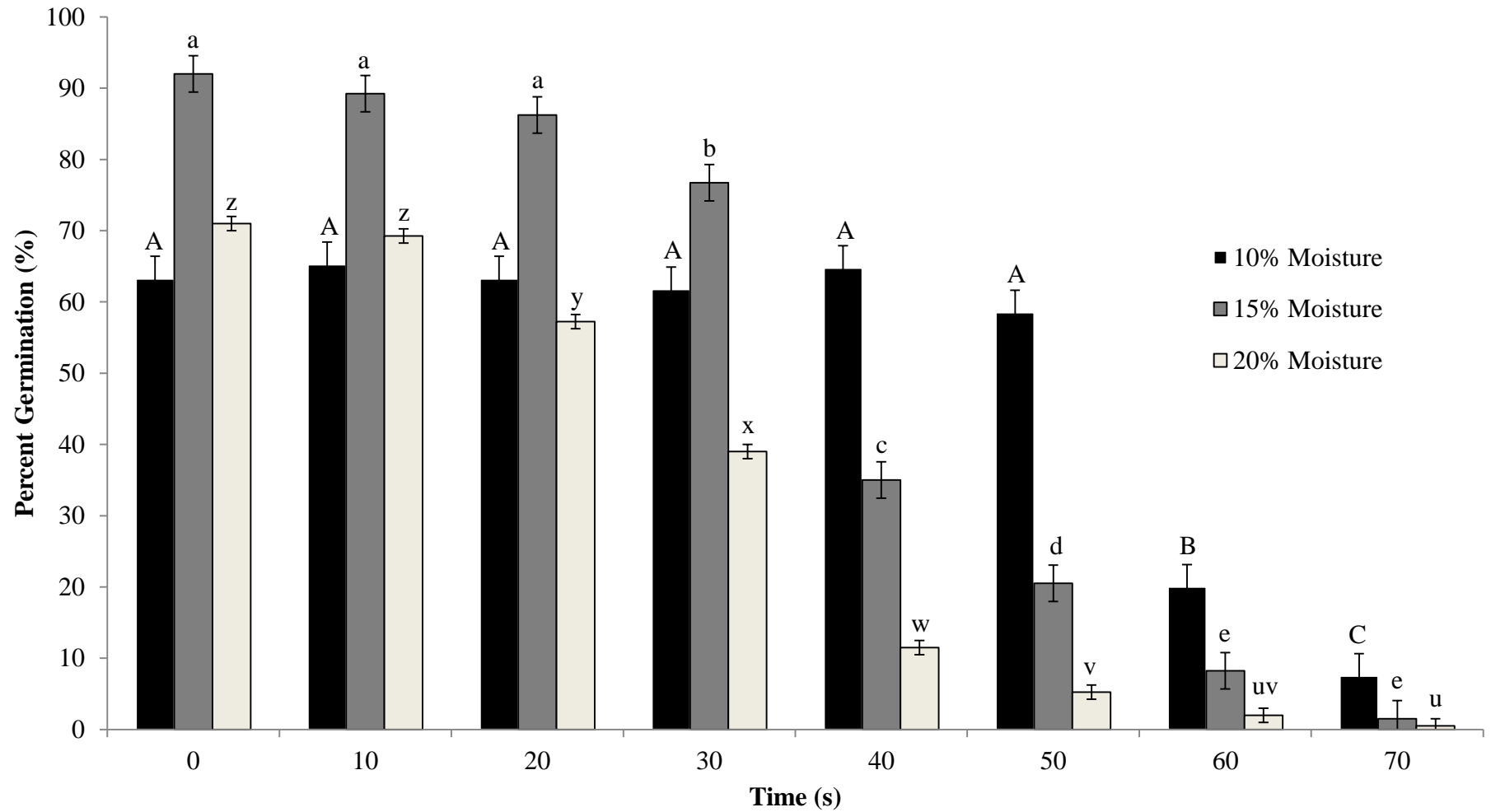


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## LIST OF APPENDICES

### APPENDIX A: Effect of Microwave Radiation on Seed with Increasing Moisture Content



**Figure A.1.** Influence of microwave radiation at varying exposure lengths on dry bean seed with increasing seed moisture content. Columns within the same moisture content with the same letter are not significantly different ( $P \geq 0.05$ ); A-C 10%; a-e 15%; z-u 20%.