AN ASSESSMENT OF POTATO (*Solanum tuberosum* L.*)* VARIETAL TOLERANCE TO BACTERIAL SOFT ROT (*Pectobacterium carotovorum* subsp. *carotovorum*) DISEASE

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP PRODUCTION

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ABSTRACT

Potato production in Zimbabwe is an important element of the Agricultural Production sector. The local industry is however affected by low yields due to soft rot disease (*Pectobacterium carotororum* subsp *carotovorum)* and poor quality produce. The main thrust of this study was to come up with recommendations that would increase potato production through improved knowledge of cultivar choices according to ranked performance in terms of soft rot infestations. Four experiments were conducted, in the field; in the greenhouse, in storage at 10^0C and at room temperature on soft rot inoculated and uninoculated potato tubers. The experiments were carried out on five locally available varieties in Zimbabwe. Seeds were screened for soft rot infection using counts and weights of infected tubers and BP1 showed significant difference ($p<0.05$) interms of soft rot infestation on counted tubers. The field experiment treatments of Amethyst, Mnandi, BP1, Montclare and Jasper were laid in Randomised Complete Block Design (RCBD). Germination percentages for planted varieties were recorded and Jasper had 70.3%, Amethyst had 56.7%, Mnandi had 57.7%, BP1 had the lowest percentage of 50.7% and Montclare had 55.5%. On the Area under Disease Progress Curve (AUDPC) for potato blight BP1 was significantly different ($p<0.05$) from other varieties as it was most infected by early blight. For disease scores, incidence of soft rot on harvested tubers BP1 and Amethyst displayed significant difference ($P<0.05$) as they were more susceptible to the disease. The greenhouse pot experiment had five soft rot inoculated and uninoculated potato tubers and treatments were laid in Complete Random Design (CRD). Percentage emergence for inoculated pots was below 50% for all varieties except for Diamond which was less suceptible to soft rot and had 83.3% germination. Emergence was above 83% in uninoculated pots across all varieties. For Amethyst a significant difference ($p<0.05$) was observed as there was zero emergence for soft rot inoculated pots. In the storage experiment, three tubers per variety of the five cultivars were placed in a polythene bag and replicated 3 times. The soft rot inoculated tubers were stored at 10^0C and at room temperature an uninoculated treatment was included as the control. Weekly weight (g) reading were collected and the weights showed significant differences ($p<0.05$) as soft rot infestation was more at room temperature in the first week than at 10^0C for BP1 and Mnandi varieties. Soft rot was expressed in week four and five at 10^0C and a significant difference ($p>0.05$) was observed. Specific gravity of soft rot inoculated and uninoculated tubers at room temperature indicated more weight reduction and a significant difference ($p<0.05$) was indicated on Amethyst and Montclare varieties than specific gravity weight at 10^0C which showed no weight reduction hence there were significant difference ($p>0.05$) amongst varieties. Tubers were graded after harvesting and BP1 had the largest tuber size 31.1% and Amethyst at 8.82% .The least susceptible varieties to soft rot disease were Diamond and Montclare. BP1 variety showed the most susceptibility in storage. This study shows that BP1 variety rank in the potato seed certification industry needs to be revised according to soft rot tolerance to update previous ranks of the cultivar; farmers are advised to adopt new cultivars and have reliable seed sources.

DECLARATION

I.....do hereby declare that this dissertation is my original work accepted by Africa University, Mutare, Zimbabwe in partial fulfilment of the requirements for the degree of Master of Science in Crop Production and has not been submitted to any university for the award of any other degree. All the work was written by other authors and used in the present thesis is fully acknowledged.

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Student's signature

Approved for submission by

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Name of supervisor Date

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DEDICATION

I dedicate this dissertation to my family and friends who were there for me through my journey on this project. I want to thank the Nzula family, Patience and Wilson for encouraging me and contributing to the success of my career. Finally I thank the Lord, God almighty for bringing me this far.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progress Curve
CIP	International Potato Center
FAO	Food and Agriculture Organisation
FANR	Faculty of Agriculture and Natural Resourses
g	grams
ha	Hectare
kg	Kilogram
MOAMID	Ministry of Agriculture Mechanisation and Irrigation Dvelopment
NGOs	Non Governmental Organizations
OECD	Organisation for Economic Co-Operation and Development
Pa	Pectobacterium atrosepticum
Pcc	Pectobacterium carotovora ssp. carotovora
RCBD	Randomized Complete Block Design
Zim-STAMP	Zimbabwe Smallholder Technology and Access to Markets Program

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CHAPTER 1

INTRODUCTION

1.1 Background

Potato (S*olanum tuberosum* L.) is the third most important food crop after rice and wheat in terms of consumption (CIP, 2015) and fourth after maize in terms of production (Mantsebo *et al*, 2014). The potato crop is a nutritionally superior vegetable because of the dry matter, edible energy and edible protein content and minerals like calcium, phosphorus and iron, and vitamins B1, B2, B6 and C (CIP, 2015). Potato produces more quantity of dry matter, edible energy and edible protein in lesser duration of time than cereal crops like wheat and maize. This crop offers a sure avenue of achieving global nutritional food security. Historically, potato is temperate crop but now widely cultivated in warm regions of the world (Czajkowski *et al*., 2011a). In Zimbabwe, potato is increasingly becoming a major food and cash crop but production continues to be hampered by diseases (Ngadze *et al*., 2012). Potato tubers being nearly 80% water are especially susceptible to diseases caused by bacteria. Bacterial pathogens that cause soft rot of potato have been reported to cause losses of up to 90 % in the field, and in storage (Czajkowski *et al*., 2011b).

The enterobacterial plant pathogen *Pectobacterium* (formerly *Erwinia carotovora*) causes soft rot diseases in monocot and dicot host plants in at least 35% of angiosperms (Faquihi *et al*., 2014). In potato, *Pectobacterium* and *Dickeya* genera cause wilt, soft rot, and blackleg and affects plant health during field production and storage (Pe´rombelon, 2002). Tuber soft rot and aerial stem rot often occur after plants are wounded by tools, poor handling, insects and severe weather such as hail. Tuber soft rot is promoted by low oxygen conditions (Bassoriello, 2010). In contrast, blackleg is considered a tuber-borne disease, with the

bacterial pathogen causing an inky black decay on the lower part of the potato stem (Czajkowski *et al*., 2011a).

Although infested crop residues and rotting tubers are among the important sources of inoculum, latent infections in seed tuber provide the major source of infection in potato production (van deh Wolf and Bergsma Vlami, 2013). Copper sprays may be used to prevent infection of wounded plant stems and leaves, but once the plant is colonized, there is no chemical control available for this pathogen (Elphinestone, 1987). Resistance genes active against *Pectobacterium* have been found in multiple host species, but their sequences and mechanisms remain unknown (Ngadze, 2010; Salas *et al*., 2003; Wright and Anderson, 2004). No currently grown commercial potato variety has an effective level of resistance to soft rot, stem rot, or blackleg. *Pectobacterium* pathogenesis has been studied for over a century, however tolerance can still be determined (Pasco *et al*., 2006).

To promote rot, soft rot pathogens employ a wide range of plant cell wall degrading enzymes to disrupt and metabolize plant cells (He´lias *et al*., 2000). Additional virulence determinants also have been described as contributing to bacterial invasion, establishment, multiplication, and host resistance evasion. These include the flagella system, putative phytotoxins, quorum-sensing system (Mantsebo *et al*., 2014), efflux pumps (Ali *et al*., 2010), the type III secretion system, and plant antimicrobial resistance systems (Bassoriello, 2010). Conducive environmental factors are also critical for the infection process, such as water availability, low oxygen levels, and optimal temperatures for bacterial growth (Pe´rombelon, 2002).

A survey was carried out in the potato (*Solanum tuberosum* L.) growing regions of Zimbabwe in April 2009 to assess the prevalence of bacterial soft rot (Ngadze, 2010). Isolations from diseased tubers were tested and identified. One-microliter suspensions (108 CFU per ml) of 20 samples were injected into the stolon end of potato tubers (*S. tuberosum*

L.). Soft rot symptoms identical to those observed in the field and in storage appeared on all inoculated tubers 1 to 2 days after inoculation but not on the control tubers. This was the first published report of soft rot on potato in Zimbabwe caused by *D. dadantii*, formerly referred to as *E. chrysanthemi* (Ngadze, 2010). The centre of potato production in Zimbabwe located in, Manicaland province, Nyanga district. The seed potato certification scheme originates in the Nyanga quarantine area comprising large scale commercial enterprises. Ware potato producers are mainly characterised by A2 and smallholder communal farmers. Potato is a cool climate crop (Wright *et al*., 1991) which makes Nyanga the ideal production area, coupled with good soils, high rainfall and abundant irrigation water. The cool temperatures ensure an aphid free growing environment.

In Zimbabwe, eight commercial varieties of potato are predominantly grown. These are Amethyst, BP1, Diamond, Garnet, Jasper, Montclair, Mondial and Pimpernel. Seed potato production is governed by laws under the Plant and disease act (Chapter 19:08) which stipulates that all seed potato is produced in Nyanga Quarantine Area. The Seed Potato Regulations contained in the Seed Act and Seeds Certification Scheme Notice, administered by Seed Services Institute and Plant Protection Research Institute of the department of Research and Specialist Services ensure growers obtain clean disease free planting seed. The main objective of the scheme is disease control through monitoring of the seed tuber contamination in certification programmes. However, there also exists an informal seed system whereby farmers sell or exchange seed amongst themselves.

The presence and or absence of varietal tolerance or partial resistance to soft rot causing bacteria among the cultivated commercial varieties are not known. Ranking of cultivars for resistance and tolerance to tuber soft rot is a key to improving growers' decision making process with regards to disease management strategies. Growers are encountering varying magnitude of losses due to these bacterial pathogens which are further amplified by poor

storage, handling and agronomic practices (Elphinestone, 1987; Pe´rombelon, 2002; Ali *et al*., 2010; Czajkowski *et al*., 2011a, 2015; Ngadze *et al*., 2012; Mantsebo et *al*., 2014; Onkendi and Moleleki, 2014). These findings have implications for import and export of potato. Zimbabwe imports seed from various countries because of the current seed shortage and it also exports table potatoes to other African states (Ngadze, 2010). The levels of tolerance to soft rot differ amongst commercial varieties under cultivation. An inadequate level of tolerance to tuber soft rot caused by bacteria *Pectobacterium* spp. in potato cultivars has raised the interest in breeding for more tolerant and resistant cultivars (Pasco *et al*., 2006). Future plant breeding efforts can thus be formulated to produce commercial potato varieties that can tolerate potato soft rot and still give an economic yield (Czajkowski *et al*., 2011b).

Commercial potato cultivars which are naturally immune to blackleg and soft rot caused by *Dickeya* and *Pectobacterium* species do not exist, but some cultivars show a partial resistance. This study will evaluate the incidence and tolerance of bacterial soft rot among the different potato varieties currently under cultivation in Zimbabwe. Assessment of the effect of bacterial soft rot on growth and development of potato, yield and storage management will contribute to the knowledge gap on the economic importance of the disease complex. The disease continues to cause severe economic losses in seed and commercial potato production enterprises, prompting research on its epidemiology and management. Several approaches have been studied to control blackleg and tuber soft rot (Czajkowski *et al*., 2011a), but the degree of success has been variable. Methods based on avoiding contamination and reliance on seed certification schemes are widely used and have been partially successful. Improved store management can reduce bacterial load on tubers and tuber rotting (Mantsebo *et al*., 2014). The significance of storage temperature on incidence of the disease will also be studied. Competition within rotting mother tubers due to environmental conditions,

temperature in particular, determines which pathogen will dominates if more than one is present (Ngadze *et al*., 2012). The soft rot bacteria can also interact with other pathogens, especially vascular infecting ones, such as *Ralstonia solanaecearum*, *Fusarium* spp., *Verticillium* spp. and *Rhizoctonia solani.* Weakening of the host resistance by one pathogen may render the plant susceptible to another.

1.2 Statement of the problem

Potato production in Zimbabwe is being increasingly threatened by bacteria pathogens of *Pectobacterium* and *Dickeya* genera (Mantsebo *et al*., 2014) which causes wilt, soft rot, and blackleg.The pathogen affects plant health during field production and storage. High yield losses of stored ware and seed potatoes are attributed mostly to *Pectobacterium* and *Dickeya* genera (Mantsebo *et al*., 2014; Ngadze, 2010). In Zimbabwe, potato growers face the challenge of significant post-harvest losses of tubers ranging from 20 to 80% (Ngadze, 2010) leading to significant financial losses. Potato being mainly propagated vegetatively is affected by diseases as they are transferred from one generation to the next or may even accumulate in the seed. Yield losses of up to 90 % as a result of planting diseased seed have been recorded (Ngadze *et al*., 2012). There is abundance of evidence on the role of the store environment on disease incidence. It is clearly established that tuber decay is high in humid conditios , when a film of moisture is on the tuber surface, temperatures are high and anaerobic conditions (Mantsebo *et al*., 2014).

Sustainable seed and ware potato production in Zimbabwe hinges on effective integrated disease management approaches to reduce the effects of these bacterial pathogens (Ngadze, 2010). Information on varietal differences with regards to bacterial soft rot tolerance is scant. In view of the knowledge gap on varietal differences in tolerance to the disease for which no effective chemical control exists, this study provides a platform for initiation of further

research on future breeding strategies and integrated disease management programs for bacterial soft rots.

1.3 Justification for the study

In Zimbabwe more than eight commercial varieties of potato are predominantly grown and these include Amethyst, BP1, Diamond, Garnet, Mnandi, Jasper, Montclare, Mondial and Pimpernel. The varieties vary in yield, taste, food value, cooking quality, tuber size and shape as well as tolerance to bacterial soft rot. Potato is increasingly becoming a significant cash and food security crop (CIP, 2015). Yield loss is reaching up to 90% due to soft rot disease. The evaluation and identification of bacterial soft rot tolerance is important for purposes of germplasm conservation and genetic diversity assessment for future breeding. Lack of resistant commercial varieties has raised the interest in breeding for more tolerant and resistant cultivars. Soft rot is economically important as it reduces potato yield and cause downgrading or rejection of seed potato in certification schemes (Elphinestone, 1987; Pe´rombelon, 2002; Ngadze, 2010; Mantsebo *et al*., 2014). Several control strategies thus far have been studied, but with limited success. The purpose of this study is to evaluate soft rot tolerance across different potato varieties and the relationship between tolerances to soft rot. Basic agronomic traits will be evaluated in these varieties in the field and in storage.

1.4. Main objective

To evaluate the incidence and tolerance of bacterial soft rot in different potato varieties grown in Zimbabwe.

1.4.1 Specific objectives

1. To assess the effect of bacterial soft rot (*Pectobacterium carotovorum* subsp *carotovorum*) on growth of potato (*Solanum tuberosum*) varieties grown in Zimbabwe.

2. To evaluate the incidence of bacterial soft rot on different varieties of seed potato at the farmers level.

3. To evaluate the incidence of bacterial soft rot on different potato varieties when stored at different temperatures.

4. To evaluate the severity of bacterial soft rot on different varieties of potato in the field.

1.4.2 Research questions

1. Is there significant difference among potato varieties grown in Zimbabwe to soft rot tolerance?

2. Is there significant difference in potato soft rot incidence at the farmer's level?

3. Is there significant difference in the effect of storage temperature on potato soft rot incidence?

4. Is there a significant difference in bacterial soft rot disease on potato varieties in the field ?

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy of potato (*Solanum tuberosum* L.)

Potato belongs to the family Solanaceae, genus *Solanum* and species *tuberosum.* Modern potatoes are derived from the domestication of wild species and hybridization (Mantsebo *et* al., 2014). The potato (*Solanum tuberosum* L.) is a herbaceous, dicotyledonous, starchy, tuberous perennial grown as an annual vegetable crop that reproduces sexually and asexually. Potato is grown by means of tubers. The tuber is a short, swollen, starchy underground stem with minute scale leaves and buds. Tubers produce shoots and stolons. The plant produces small green or purplish green fruits (berry) 1.3-2cm in diameter, which are poisonous. Seeds are used in breeding and production of true potato seed (TPS). The edible part of the plant is the tuber comprising a fleshy stem with buds or eyes. The tuber is commonly known as potato. Tubers are round to long oval. The flesh is generally white or cream to yellow and the skin colour light brownish to red. Tubers can contain high levels of solanine, a toxic alkaloid (OECD, 1997).

Solanum tuberosum is divided into two subspecies; subsp. *tuberosum* and subsp. *andigena*. *S. tuberosum* subsp. *andigena* is specifically suited to cultivation at high altitudes and short daylight hours whereas subsp. *tuberosum* prefers cultivation at lower altitudes and a longer day length (OECD, 1997).

2.2 Overview of potato production in the world

The Irish potato is one of the most widely grown tuber crops in the world and contributes immensely to human nutrition and food security. More than a billion people worldwide eat potato, and global total potato production exceeds 300 million metric tons. Among the major

potato growing countries of the world, China ranks first in area, followed by the Russian Federation, Ukarine and Poland (He´ lias *et al*., 2000). Potatoes can grow from sea level up to 4,700 meters above sea level; from southern Chile to Greenland (CIP, 2015)

One hectare of potato can yield two to four times the food quantity of grain crops. Potatoes produce more food per unit of water than any other major crop. It is up to seven times more efficient in using water than cereals (Elphinestone, 1987). Potatoes are produced in over 100 countries worldwide. Since the early 1960s the potato production area has rapidly overtaken all other food crops in developing countries (Elphinestone, 1987). It is a fundamental element in the food security for millions of people across South America, Africa, and Asia, including Central Asia (CIP, 2015). Presently, more than half of global potato production now comes from developing countries comparison with other roots and tubers; the protein content of potato is very high but almost similar to that of cereals. Starch makes up about 85 percent of this solid mass and the rest is protein. After wheat, maize and rice, the potato is ranked world's fourth most important food because of its nutritive richness. It provides a balanced source of starch, vitamins and minerals to many communities in the global village (Zim-STAMP, 2011).

Plant pathogenic diseases remain the major constraint to world potato production. About a million people died of starvation while another emigrated mostly to the United States of America during the European potato famine of 1844 -1845 and the Irish potato famine of 1845 –1848 caused by late blight (*Phytopthora infestans*). Other pathogens of economic importance are early blight, Alternaria *solani*; brown rot or bacterial wilt *Ralstonia solanacearum;* black leg and soft rot, *Pectobacterium carotovora* sub sp *atroseptica;* ring rot, *Clavibacter michiganense* sub sp. *sepedonicum* and common scab *Streptomyces scabies.* An estimated 22% of potatoes are lost per year to viral, bacterial and fungal diseases and pests, which is equivalent to an annual loss of over 65 million tonnes (Perombelon, 2002).

2.3 Status of potato production in Zimbabwe

2.3.1 Production

Potato was introduced in Zimbabwe in the early 20th century (Ngadze *et al*., 2012). Importation of fresh seed potato from Scotland was the regular practice. The seed was bulked up for one or more generations for the commercial crop. The first foundation seed was produced in the summer of 1966/67 at Nyanga Experiment station (Ngadze *et al*., 2012). The main varieties were Up-to-Date and King Edward. Over the years due to continued efforts in research locally bred higher yielding varieties have been developed. Zimbabwe is divided into two major potato producing areas; the Eastern Highlands (above 1800 m) and the Highveld areas (above 1200m) (Ngadze *et al*., 2012).

Traditionally, potatoes have been grown by large commercial farmers in Zimbabwe, but more and more smallholders are growing the crop. Currently, small holder farmers and communal areas around Nyanga, Mutasa, Domboshawa, Chiweshe, Wedza, Goromonzi and Mhondoro are producing significant quantities of table potatoes (Ngadze *et al*., 2012). However, there is a lack of statistical data, and it is very difficult to estimate national potato production or what percentage of were approximately 900-1000 hectares under production for potatoes in Zimbabwe per year (Ngadze *et al*., 2012). This figure may be much higher since many growers also obtain their seed through informal arrangements. Nyanga quarantine area is the main area where seed potatoes are grown as it is too cold for insect vectors to survive, so the crops remain virus free (Ngadze *et al*., 2012).

2.3.2 Varieties

i. Montclare

Montclare is a Zimbabwean bred (1972) very high yielding variety, producing medium quality tubers which tend to be large and of poor shape with deep eyes (Ngadze *et al*., 2012). It is a late maturing variety and it has uneven sprouting habit. White skin and flesh, round to pear shape. Abundant purple flowers but rarely gives berries (Spooner, 2013). Montclare has high tolerance to late blight but susceptible to viral diseases.It is ideal for irrigated and rain fed plantings. The variety is popular in the Midlands and Matebeleland (Zim-STAMP, 2011).

ii. BP1 (Blight proof 1)

A South African bred medium-early variety taking 14-15 weeks in the ground. It is deal for winter and spring irrigated plantings (Spooner, 2013). The variety has white flesh, hard skin, and good oval shape and is very high yielding. BP1 has similar quality to Up-to-date variety. They are both moderately tolerant to late blight but susceptible to early blight in summer. They are also even sprouters with a moderate sprouting habit (Bassoriello, 2010). They have upright haulms with dense foliage. They both produce abundant blue heliotrope flowers and unfavourable conditions give a few berries. Under very fertile conditions BP1 sometimes gives large tubers which may suffer from moon shaped cracks (Zim-STAMP, 2011)

iii. Pimpernel

Pimpernel is a Dutch bred late variety, medium yielding, and red skinned. It is yellow fleshed and is mainly for ‘chip’ trade. Pimpernel is used primarily for processing. It has been rated highly for its culinary quality. It keeps well and has good field resistance to Late blight. Pimpernel is fairly tolerant of virus diseases (Zim-STAMP, 2011).

iv. Inyanga Amethyst

Amethyst is a Zimbabwean variety and was bred in 1986. It is late maturating (18-20 weeks) and a consistent high yielder under summer and irrigated plantings. (Ngadze *et al*., 2012). It performs well in all Zimbabwean potato production regions. Its tubers have white skin and flesh, flat, are oval and have shallow eyes and a russetted skin. Sprouting is quick (about 65 days) and foliage is upright haulms with dense canopy. Leaves are numerous, small and dark green in colour. It has a high level of tolerance to late blight (Zim-STAMP, 2011).

v. Jasper

Jasper is Zimbabwean bred and was in 1993.It is high yielding and late maturating (18-20 weeks). Jasper is ideal for winter and spring irrigated plantings (Ngadze *et al*., 2012). Tubers have white skin and flesh are, round to oval, and have slight surface roughness with shallow eyes and moderate sprouting habit. It is vigorous growing with up right haulms of up to 1.5 metres (Zim-STAMP, 2011).

vi. Garnet

Garnet is a Zimbabwean bred, late maturing (17-19 weeks) variety.It is moderately yielding, ideal for irrigated and rain fed plantings. Garnet has good crisping qualities (Ngadze *et al*., 2011). The tubers have white skin and yellow flesh, are round and medium sized with shallow eyes. It has a moderate sprouting habit. The Foliage has thin tall haulms with narrow leaves and white flowers with high tolerance to late blight (Zim-STAMP, 2011)

vii. Diamond

Diamond is a Zimbabwean bred (2005) variety of early to medium maturing (14– 15 weeks). Diamond is deal for irrigated and rain fed plantings. It is high yielding with good crisping qualities. Tubers are white, rough skinned and yellow fleshed, oval, smooth with shallow eyes. It has an early sprouting habit. Foliage has upright haulms with blue flowers. Diamond is moderately tolerant to early and late blight (Zim-STAMP, 2011).

2.3.3 Climatic requirements

The potato originated in the cool mountain climate of the Andes in South America (OECD, 1997). While it can thrive where day temperatures are warm, cool nights are needed for adequate tuber formation. Mean optimum temperatures for tuber production are between 15^{o} C and 20^{o} C. Above 32^{o} C, both tuber formation and yield are poor (Spooner, 2013). There are few production regions in Zimbabwe that meet these requirements with the result that most potatoes are cultivated under temperature stress (Ngadze *et al*., 2012). This is one of the main reasons that has contributed to poor yields, quality and keeping quality (del Pilar Marquez-Villavicencio, *et al*., 2011). Competition within rotting mother tubers, catalysed by environmental conditions, temperature especially, determines which pathogen will predominate if more than one is present (Czajkowski *et al*., 2015).

Environmental conditions under which *S. tuberosum* can be successfully grown are very diverse, as can be concluded because potatoes are cultivated in many parts of the world (OECD, 1997).

The *S. tuberosum* subsp. *tuberosum* tuber cannot survive a temperature of -3°C and lower. The foliage dies at temperatures of -4°C (Spooner, 2013). Potato tubers are destroyed by a

frost period of 25 hours at -2°C or a frost period of five hours at -10°C (OECD, 1997). Latin American *Solanum* species can be much more frost-resistant. *S. tuberosum* subsp. *tuberosum* is a daylight neutral crop, which means that tubers are made independent of the day length. But variation for daylight sensitivity can be found among *S. tuberosum* subsp. *tuberosum* cultivars. Extreme low or high temperatures, in particular the night temperature, can obstruct tuber formation (Spooner, 2013). Short days less than (14 hours) and moderate ground temperatures (15-18°C) enhance tuber formation. Longer days (14-16 hours) and higher day temperatures (20-25°C) enhance flowering and seed formation (Spooner, 2013).

2.4 Plant disease caused by bacteria

2.4.1 Background

Bacterial diseases of plants occur in every place that is reasonably moist or warm, and they affect all kinds of plants. Bacteria are ever present whenever fleshy plant tissues are rotting in the field or in storage, and the foul smell given off by such rotting tissues is due, usually, to volatile substances released during the disintegration of plant tissues by such bacteria. Rotting tissues become soft and watery, and slimy masses of bacteria and cellular debris frequently ooze out from cracks in the tissues, hence the name bacterial sot rots. In many soft rots, the bacteria involved are not plant pathogenic, rather they are saprophytic or secondary parasites. Some bacteria, however, attack living plant tissues and cause soft rots in the field or in storage (Agrios, 2005).

Pectobacterium, the "carotovora" or "soft rot" group, causing soft rots of numerous fleshy fruits, vegetables, and ornamentals (*P. carotovora* pv. *carotovora*), and blackleg of potato (*P. carotovora* pv. *atroseptica*) also causing soft rots of fleshy fruits and fleshy vegetables (*P. fluorescens*). *Pseudomonas* cause pink eye disease of potato, slippery skin disease of onion, and the sour skin of onion. *Bacillus,* causes rotting of potato and tobacco leaves in storage.

Clostridium of tomato seedlings and soybeans, also causes rotting of potato and tobacco leaves in storage and the wet wood syndrome of poplar and elm (Agrios, 2005).

2.5 Bacterial soft rots of potato

2.5.1 Bacteria

The main bacteria causing blackleg, which affects the growing plant, and tuber soft rot of potato are the soft rot bacteria *Pectobacterium atrosepticum* (Pa), *P. carotovorum* subsp. *carotovorum* (Pcc) and *Dickeya* species (Czajkowski *et al*., 2015), formerly belonging to the genus *Erwinia* (*E. carotovora* subsp. *carotovora*, *E.carotovora* subsp. *atroseptica* and *E. chrysanthemi*) (Czajkowski *et al*., 2015b).

2.5.2 Identification of bacteria

Pectobacterium spp. are pectinolytic Gram-negative, facultative anaerobic, non-sporing, motile, straight rods with peritrichous flagellae (Bassoriello, 2010). *Pectobacterium carotovora* ssp. *carotovora* (*Pcc*) is a gram-negative plant pathogenic bacterium measuring 0.5 to 0.8 μm by 1.0 to 3.0 μm (Talib Sahi *et al*., 2007). *Pcc* has a rod-shape consistent with other gram-negative bacteria, along with peritrichous flagella and fimbrae that allow for motility and adherence to host tissues.

Pectobacterium spp. belong to the *Proteobacteria* subdivision and are clustered in the *Enterobacteriaceae* family (Degefu *et al*., 2006). They characteristically produce a variety of cell wall degrading enzymes that allow infiltration and maceration of plant tissues on which they feed (Mantsebo *et al*., 2014).

2.5.3 Host range and distribution

While Pcc has a wide host range worldwide, Pa is restricted only to potato predominantly in temperate regions. In contrast, *Dickeya* spp. affect a restricted number of host species in temperate, sub-tropical and tropical regions (Pe´rombelon, 2002). It is now known that the three bacterial species can cause tuber soft rot but previously only Pa was believed to cause blackleg in temperate and *Dickeya* spp. in warmer regions (Elphinestone, 1987). However, recently Pcc has been shown to infect potato plants causing typical blackleg symptoms (He´lias *et al*., 2000) as observed in Colorado and Arizona in the USA with hot summers.

Although *Dickeya* spp. have long been associated with blackleg in tropical and subtropical regions, only strains of *Dickeya dianthicola* were isolated from blackleg-diseased plants in Western Europe in the past twenty to thirty years (Perombelon, 2002). Since 2005, a new genetic clade, has been isolated and detected in France, Finland, Poland, The Netherlands and Israel Europe of a highly virulent *Dickeya* species belonging to biovar 3 (van deh Wolf and Bergsma Vlami, 2013) . In many of these countries, the pathogen was introduced via the international movement of seed potatoes.

All isolates were clonal, which suggests a common origin and possibly a single introduction event. The same genetic clade has been found in hyacinth (Slawiak *et al*., 2009). One might speculate that in the recent past, this genetic clade was introduced from hyacinth into potato, possibly via the use of contaminated irrigation water (Mantsebo *et al*., 2014). However, the role of flower bulbs in the dissemination of the pathogen is still unknown.

Two new subspecies of Pcc were described as potato blackleg causing organisms. *Pectobacterium. carotovorum.* subsp. *brasiliensis*, a highly aggressive bacterium, is responsible for the majority of blackleg incidences in Brazil and South Africa (Pe´rombelon, 2002). In New Zealand *P.c*. subsp. *wasabiae* has been described as a new potato pathogen

responsible for high blackleg levels. *P.c. wasabiae* was found only in association with soft rot on Japanese horseradish (He´ lias *et al*., 2000).

A survey carried out in the potato (*Solanum tuberosum* L.) growing regions of Zimbabwe in April 2009 to assess the prevalence of bacterial soft rot (Ngadze, 2010) led to the first report of soft rot on potato in Zimbabwe caused by *D. dadantii*, formerly referred to as *E. chrysanthemi* (Mantsebo *et al*., 2014). This finding has implications for import and export of potato material into and out of Zimbabwe.

Zimbabwe imports seed from various countries because of the current seed shortage and exports table potatoes to other African states. Ngadze and Icishahayo (2014) report that *Pectobacterium atrosepticum* and *Pectobacterium carotovorum* subspecies *carotovorum* have been listed as the major pathogens causing blackleg and tuber soft rot diseases respectively and recently *P. carotovorum* subspecies *brasiliensies* and *D. dadanti* (Ngadze and Icishahayo, 2014).

2.5.4 Epidemiology and aetiology of soft rot in potato

Soft rot bacteria do not overwinter in soil. The survival in soil is restricted to between 1 week to 6 months (Elphinestone, 1987) depending on environmental conditions in the absence of any potato plant material. Survival is affected by soil temperature, moisture and pH (Pe´rombelon, 2002). Survival can be longer in association with plant material including volunteers (Czajkowski *et al.*, 2011a). A crop rotation system of 3–8 years kills bacteria (Mantsebo *et al*., 2014). Current knowledge has shown the major source for blackleg infection to be latently infected seed (mother) tubers (Czajkowski *et al*., 2015).

A rotting mother tuber, releases the bacteria into the soil and inoculum are transmitted by soil water to contaminate neighbouring progeny tubers. The bacteria has been shown to also

invade potato roots and then move through the plant system into progeny tubers (Ali *et al*., 2010). The bacteria can survive in latent form in plant stem without causing black leg. Transmission of bacteria from diseased plants by winged insects over long distances can result in contamination of other potato crops. Fruit flies and ants have also been associated with Pcc (Czajkowski *et al., 2011b*). After insects have laid eggs over seed tubers already infected by Pcc, contaminated larvae carry the bacteria into the tuber (Pe´rombelon, 2002).

Aerosols produced by rain drop impact on blackleg plants and by haulm pulverization prior to harvest are also sources of inoculum (Fraaije *et al*., 1997). Irrigation water from surface sources can be a source of disease inoculum for the pathogen, and may also be a source of new strains of the pathogen (Prajapat *et al*., 2013).

Contamination of healthy tubers can occur during harvesting and handling (grading) in store as a result of the disintegration of infected tubers and the spread of infected tissue on handlers, tools and machinery into wounds inflicted during handling (Pe´rombelon, 2002). Due to vegetative propagation of tubers, there is a high risk of pathogen survival from one generation to the next (Ngadze *et al*., 2012). Anaerobic conditions that favour bacterial multiplication and initiation of rotting in the mother tubers are caused by presence of a water film on the tuber surface (Mantsebo *et al*., 2014). Anaerobiosis affects oxygen-dependent host resistance, allowing unhindered bacterial multiplication and production of cell-wall-degrading enzymes, resulting in a rotting lesion (Bassoriello, 2010).

The disease blackleg develops after rotting of seed tubers including conditions favoring decay. Another critical condition in soft rot disease development is the level of seed contamination as shown in the case of Pa (Talib Sahi *et al*., 2007). The higher the bacterial density is, the more likely virulent the pathogen and the faster the incipient lesion and the earlier the rotting. Progeny tuber contamination is related to seed tuber contamination as well

as blackleg disease. Data available indicate *Dickeya* spp. are dependent on the level of seed contamination but is less important for blackleg development possibly because of their higher virulence (Fraaije *et al*., 1997).

The soft rot bacteria can also interact with other pathogens, especially vascular ones, such as *Ralstonia solanaecearum, Fusarium* spp., *Verticillium* spp. and *Rhizoctonia solani* (Prajapat *et al*., 2013). Weakening of the host resistance by one pathogen may favour the development of another.

2.6 Impact of agronomic practices on soft rot incidence

2.6.1 Soil and land preparation

Potatoes can be grown on most soil types, but ideal soils are medium textured loamy soils with good organic matter content and a pH of between 5.0 and 5.5 ($CaCl_2$). It is not advisable to lime just before planting as the high pH will predispose the crop to potato scab. Pe´rombelon (2002) reported soil pH to affect survival time of the pathogen in the soil. Thus, a crop rotation system of 3 to 6 years currently practiced can deter the carryover of the bacteria (Czajkowski *et al*., 2011a). Lime should thus be applied in rotation with other crops. Use of well drained fields and conservation reduces the risk of tubers being surrounded by a water film that can result in anaerobiosis and consequently tuber decay in the field (Talib Sahi *et al*., 2007).

The land should be ploughed to a fine tilth which is necessary for good tuber development. Potatoes are shallow rooted, but however, a soil depth of at least 600 mm is preferable. In cases where the crop is being planted on virgin soil or green-manured lands, the land should be prepared at least several months in advance, preferably while moisture is still available to allow for the decomposition of organic matter. An application of 100 kg/ha Ammonium

nitrate will assist the breakdown. In areas where eelworm is suspected, the soil should be fumigated with EDB or any other nematicide available (Masarirambi *et* al., 2012)

2.6.2 Plant population and spacing

During planting, potato tubers are mechanically or manually placed in the rows, 20 cm to 30 cm apart, with a row to row spacing maintained at between 60 to 120 cm. Spacing is influenced by seed size and soil fertility. High plant population will result in a dense crop canopy (Masarirambi *et al*., 2012), which in turn gives rise to a continually humid canopy environment that favours soft rot spread (He´lias *et* al., 2000). Latently infected seed (mother) tubers results in poor germination percentages (Czajkowski *et al*., 2015) and closer spacing will increase rate of spread by soil water to contaminate tubers (Johnson, 1999). Bruising, potato tubers during mechanical or manual planting was shown to predispose seed tubers to soft rot attack and increase susceptibility (Wright and Anderson, 2004).

2.6.3 Harvesting processes

Harvesting equipment can lead to contamination of crops (Pe´rombelon, 2002). Inoculum released by rotting tubers due to impact with potato diggers, harvesters and handling (grading) in store are most important sources of contamination of tubers (Carputo *et al*., 1997). This presents a greater risk that one or several soft rot bacteria contaminate the commercially produced tubers, on which bacteria can survive from one generation to the next (Pasco *et al*., 2006). Late harvesting allows bacterial multiplication on leaves and in debris left on the ground following haulm falling. This may result in contamination of progeny tubers underground during wet weather conditions. Spreading and smearing of the bacteria in a seed lot can be reduced by removal of rotten tubers during harvesting and grading (Slawiak *et al*., 2009). Avoidance of wounding by correct machinery calibration during harvesting and

grading is important to reduce the risks of wounding in which bacteria can survive after wound healing (Pe´rombelon, 2002). Harvesting of mature tubers with a well-developed periderm will also reduce risks on wounding (Wright *et al*., 1991).

2.6.4 Storage conditions

Farmers store seed potato in storage shed of brick or timber under asbestos. No artificial ventilation is employed. The tubers are packed in chitting trays. Where sheds are inadequately ventilated or chitting trays are overloaded, rotting can spread to adjoining tubers as liquid from the rotting tubers percolate onto others, leading sometimes to massive rotting pockets in the stored tuber lot (Pe´rombelon, 2002). Seed storage sheds should be well-ventilated at low temperatures to avoid condensation on tuber surface which in turn will prevent multiplication of the blackleg pathogen (Mantsebo *et al*., 2014). If the tubers remain wet long enough, tuber decay can ensure that result in further spread of the bacteria when tubers are graded and sometimes massive tuber decay (Prajapat *et al*., 2013). It is critical to dry rapidly the tubers in warm air as this favour wound healing followed by cooler air for controlling sprouting and long term storage (Talib Sahi *et al*., 2007). Good storage management is of importance not only to prevent tuber decay but also avoid increasing the tuber inoculum load which would result subsequently in greater disease risks (Czajkowski *et al*., 2011a)

CHAPTER 3

MATERIALS AND METHODS

3.1 Survey of farms in the Nyanga plant quarantine area

A survey was conducted in Manicaland Province, Nyanga district, Quarantine farming area. The site is in Natural Region Ia with an altitude of 2 100m above sea level. The mean annual rainfall ranges from 800-1000 mm. The mean annual temperature ranges from 15-27^{o}C. The soil type is predominantly red clay.

3.1.1 Survey.

A total population of 9 farmers was chosen at random in the area under investigation. A questionnaire was administered to the individual farmers and a face to face interview was done. Data was collecteda relating to their general experience with bacterial soft rots and its economic impact on potato production management strategies. The questionnaire is presented in Appendix 1. Samples of 5 different potato varieties; Amethyst, Jasper, Mnandi, Bp1 and Montclare were collected based on availability. The tubers were brought to the Africa University laboratory where biochemical analysis of soft rot was done.

3.2 Ethical consent of the responsences in the survey

Before administering the questionnire in the survey, all participants in the survey received adequate explanations on their ethical rights in the survey. It was also disclosed that the information gathered was confidential and they could choose not to answer questions as they wished. The data was collected after the explicit consent of the individual interviewed.

3.3 Field experiment to assess farmers' seed

A trial was set up to assess the tolerance to Bacterial soft rot of potato varieties at Africa University farm, Mutare Zimbabwe during the 2014-2015 farming season. The area is under

agroecological region 2 ($18^0$53'70, 3" S: 32^0 36'27'9"E) at an elevation of 1131 m above sea level. Average day length is 14 hours in summer to 11 hours in winter and annual rainfall ranges from 750 mm to 1200 mm. Rain falls mostly in the months of December to February although heavy showers are possible before and after this period. The averages temperature ranges from 18^0C (July) to 32^0C (October). The hot summer is between September and December. The soils are classified as sandy clay loam of the red Fersiallitic 5E series under Zimbabwe soil classification (Nyamapfene, 1991).

3.3.1 Field experimental design

Five varieties of seed potato, Amethyst, Montclare, Mnandi, Jasper and BP1 were evaluated for soft rot varietal tolerance in a field experiment laid out in a Randomised Complete Block Design (RCBD) with three replicates. The distance between blocks was 1metre and the distance between plots in a block was 1 meter. Each plot was 4 m x 3 m with 4 rows. Each row had 10 plant stations spaced at an inter-row spacing of 0.90 m and an in-row spacing of 0.30 m. The blocking factor was the slope of the land. A potato border crop was planted right round the experimental field. Compound D ($7N:14P_2O_5:7K_2O$) was applied as a basal dressing at a rate of 1500 kg ha^{-1} and one tuber was planted at each planting station. Weeding was done by hand hoeing. Top dressing using ammonium nitrate at a rate of 300 kg ha^{-1} was split applied twice. The experimental site was rain-fed and irrigation water applied to supplement the rains. The potatoes were harvested after (3-4 months) and graded according to tuber sizes.

3.4 Greenhouse experiment

The experiment was carried out at the Africa University farm at the Horticulture section. An area of 2 m x 2 m was allocated in a 4 m x 5 m greenhouse. The pots were placed on the concrete floor of the green house and irrigated manually using a 20 litre watering can. The

five potato varieties were inoculated with a solution of a soft rot bacterium.Two tuber were planted per variety in three pots each and replicated three times. A set of uninoculted tubers was also used in the trial. The five varieties evaluated were, Amethst, Bp1, Mnandi, Montclare and Diamond. The germination assessment was conducted 2 weeks after planting.

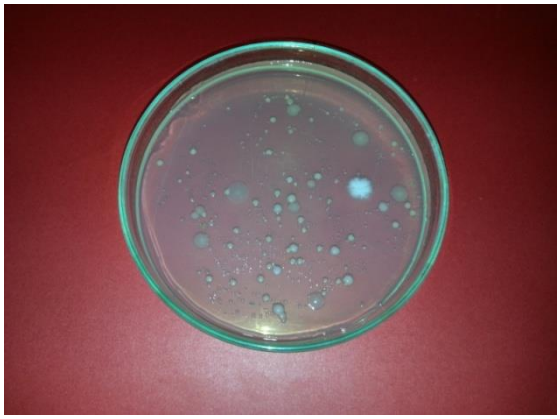
3.4.1 Greenhouse experimental design

There were three replicates for each of the 5 potato varieties. An autoclave was used to sterilize 150 kg of soil against any soil pathogens. Seed potato cultivars Amethyst, Mnandi, BP1, Montclare and Diamond were grown in 5 kg black plastic pots on sandy loam soil. A total of 15 uninoculated pots were planted with two tubers each, and 15 pots with two inoculated tubers per pot were also planted.

3.5 Evaluation of the effects of storage temperature on soft rot disease progress on stored tubers

Potato tuber portions infested with soft rot bacteria were cut and washed with distilled water. The infested portions were ground using a kitchen blender and the inoculum was plated with the supposed soft bacteria in nutrient agar. The samples were left in the laboratory under sterile conditions for 48 hours at 25^0C. The bacterial colonies from the nutrient agar were isolated and multiplied and incubated for 24 hours (Figure 3.1 a and b). Potato tuber slices were cut at 7-8 mm asceptically. The slices were placed on moistened, filter paper in a petri dish. A nick was made in the center and bacterial growth for *Pectobacterium* inoculum was placed on the slice nick point. The soft rot test was graded positive as symptoms and characteristics of soft rot bacteria were observed on inoculated tuber slice as shown in (Figure 3.1 c). After observing the presence of soft rot in the test, five potato tubers of different varieties were placed in Randomised Complete Design (RCD) with 3 replicates. Each replicate had 3 tubers placed in 15 Khakhi polythene bags. The 15 tubers were inoculated by

dipping in the suspensions of soft rot bacterium concentration (200 ml inoculum: 2l water), for 30 minutes and air-dried separately. For five potato varieties, three uninoculated tubers per bag were dipped in distilled water were a control. The replicates were placed under room temperature and weights were taken every week per replicate.



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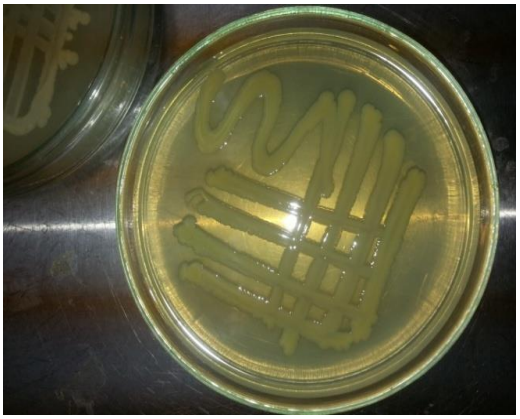

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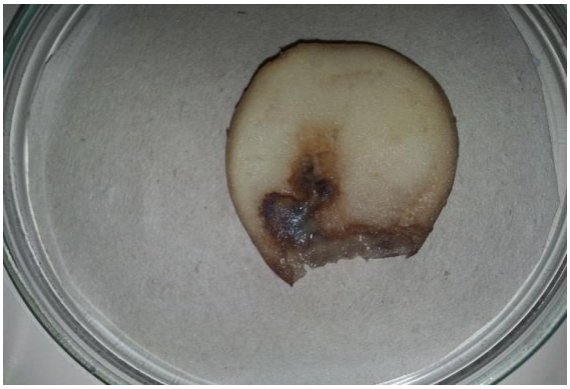

c

Figure 3.1. (a) Isolated bacterial colonies, (b) Streaked bacterial colonies for inoculums multiplication, (c) Positive soft rot test on slice of BP1.

3.5.1 Room temperature

A laboratory storage room facility was used to store 5 potato tuber varieties Amethyst, Mnandi, BP1, Montclare and Diamond under ambient temperatures.The room was enclosed. Each potato variety had 3 packs containing three tubers of the same physiological age.

3.5.2 10^0C temperature

The cold storage facility at Manica Produce (Pvt) Ltd in Mutare regulated at 10^0C was used to store five potato varieties Amethyst, Mnandi, BP1, Montclare and Diamond. A set of inoculated and uninoculated varieties had three packs containing three tubers of the same physiological age and size.

3.6 Potato tuber screening for soft rot infection

A physical count of the tubers was done in the laboratory. Damaged tubers were separated from healthy ones. For each variety, damaged potato tubers showing signs of soft rot were separated, counted and recorded against the healthy potato tubers. A percentage of infection was noted against healthy ones. Potato variety tubers were weighed into 3 kg pockets and replicated three times. The potato tubers were left in storage under room temperature for 5 weeks. The same inoculation procedure was repeated in another experiment where storage temperature was set at 10^0C.

3.7 Data collection

3.7.1 Description of survey measurements

Data was collected by administering a questionnare (Appendix 1) to respondents based on the educational qualifications; soft rot control methods, soft rot knowledge, potato cultivar preferences and cultivar ranks, water sources and irrigation method used by farmers were recorded.

3.7.2 Description tuber screening measurements

The number of infected potato tubers were counted from each replicate and weighed then they were converted to percentages after 2 weeks.

3.7.3 Description of field experiment measurements

Date of emergence was recorded emergence percentages were calculated two weeks after planting. Soft rot, early blight incidence and severity scores were collected weekly. For each cultivar a score (percentage of leaf area infected) based on the soft rot and blight severity was used. The net plots were used for data collection and five plants per plot were used at all growth and development stages.

3.7.4 Description of greenhouse experiment measurements

Data on soft rot emergence percentages was collected 2 weeks after planting in the pots. Incidences of soft rot were recorded after emergence after every seven days for four weeks.

3.7.5 Description of storage temperature experiment measurements

The weights (g) of inoculated and uninoculated tubers for 3 replicates per variety were recorded after every seven days from week 1 to week 5 for weights (g) of inoculated and uninoculated potato tubers at room temperature and at 10^0C. Specific gravity weights (g) taken were from the same inoculated and uninoculated tuber weight at 10^0C and room temperature.

3.8 Data analysis

Data from the survey were analysed using SPSS version 16.0. Data from the field experiment and screening were analysed using Genstat version 5 statistical packages. The data were subjected to analysis of variance (ANOVA) and the means of the parameters were separated using the least significant difference (LSD) at P=0.05. Data from the green house experiment

and storage temperature experiment were analysed using Genstat version 5 (t-test statistic) to compare inoculated and uninoculated tuber parameters.Yield was graded according to tuber sizes per variety.

CHAPTER 4

RESULTS

4.1 Survey results in nyanga for gender distribution

Of the 9 farmers interviewed, 89% of questionnaire respondents were male and 11% were female.

4.2 Farmers' level of education

In terms of educational level, 44.4% of the farmers obtained secondary education and 22.2% reached diploma level of education. Some 33.3% had University qualification. However, all farmers were interested in participating in knowledge training of soft rot disease.

4.3 Farmers' knowledge of soft rot management

In Nyanga area, all the farmers used natural seed store temperatures to manage the soft rot disease. No specific disease management has been adopted by farmers. The survey showed that 55.6% of farmers use cultural control methods like use of disinfectants or sanitation. 44.4% of farmers do not practice any softrot control method.

4.4 Cultivars grown by farmers

Three groups of farmers were questioned about their cultivar preferences. All groups had thirty three percent preferences of the varieties they grow from the total population of farmers interviewed. The three groups of farmers ranked there preferences of potato cultivars in relation to tolerance to soft rot disease. The farmers that prefer to grow Amethyst, Garnet, Jasper, Diamond, BP1, and montclare were seventy seven percent and twenty-three percent

prefered to grow Mondial and Mnandi. Farmers experienced problems with potato tuber moth, root knot nematode and aphids' .The percentage of effect of the pest to the farmer was the same at thirty- three point three percent for all pests.

Table 4.1 Percentage of cultivars grown by respondents, ranks according to preference and pest problems experienced.

Cultivars grown by respondents	Responses (%)
Group 1.Amethyst, Garnet, Jasper, Pimpernel, BP1, Montclare	33.3
Group 2.Amethyst,Garnet,Diamond, Pimpernel, BP1, Montclare	33.3
Group 3.Amethyst, Garnet, Jasper, Diamond, Pimpernel, BP1, Montclare, Mnandi	33.3

4.2 Water sources for irrigation

Table 4.2 Percentage of water source used by respondents and irrigation methods adopted

Water source	Responses (%)
Dam/Weir	44.4
Borehole	11.1
Other	44.4
Irrigation method	
Sprinkler	72.8
Perforated pipes	22.2

4.3 Quality of seed tubers collected from farmers

There was no significant difference ($p>0.05$) between Amethyst, Mnandi and Diamond potato varieties interms of softrot infection on seed tubers. There was significant difference ($p<0.05$) between BP1 and other varieties as BP1 showed a high infestation of soft rot infection on seed tubers (Table 4.3). Based on weights (g) of screened tubers, there was no

significant difference (p>0.05) for Amethyst, Mnandi, Montclair and Diamond as seed had less infected tubers when they were counted from 3 kg pockets.

Table 4.3 Average number and weights of infected tubers obtained from seed potato farmers in Nyanga

Variety	Average number of infected tubers of 3kg potato seed	Weights (g) of infected tubers of 3 kg potato seed packs
Amethyst	0.67[a]	0.0150[a]
BP1	3.33[b]	0.1133[b]
Mnandi	1.67[a]	0.0150[a]
Montclare	1.33[a]	0.0100[a]
Diamond	0.97[a]	0.0135[a]
Significance of F	0.045	0.039
LSD	1.087	0.02015
CV %	33.0	27.9

Means followed by the same letter in the column are not significantly different from each other at P=0.05.

4.7 Germination percentage, AUDPC of early blight, yield t/ha and soft rot incidence for field experiment

There was no significant difference (p>0.05) across the varieties for germination percentage. Amethyst, Mnandi and Montclare were not significantly different (p>0.05) from each other. On the AUDPC, BP1 was significantly different (p<0.05) from other varieties as it was more susceptible to early blight. Jasper was significantly different from other varieties on the AUDPC as it was less susceptible to blight. No significant difference (p>0.05) was noted between Amethyst and Montclare and also between BP 1 and Mnandi on yield (t/ha). Jasper was significantly different (p<0.05) from the other varieties as it had the highest yield. There was no significant difference (p>0.05) for incidence of soft rot on Amethyst and BP1 varieties although Montclare, Jasper and Mnandi were siginificantly different (p<0.05) to Amethyst and BP1 interms of infected tuber counts. Mnandi was not significantly different

($p>0.05$) from Montclare but a significant difference ($p<0.05$) from Amethyst, Jasper and Mnandi in terms of weights of infected tubers.

Table 4.4 Germination percentages, AUDPC and yield (t/ha) and softrot yield incidence % for 5 potato varieties

Variety	Germination (%)	AUDPC of early blight	Yield t/ha	Incidence of soft rot on yield	
				Weights (g)	Per tuber Counts
Amethyst	56.7	8.12[b]	18.58[a]	0.867b	5.00b
BP1	50.7	10.04[c]	27.47[b]	0.950b	5.00b
Jasper	70.3	6.81 [a]	34.01[c]	1.333b	1.67a
Mnandi	57.7	8.36[b]	26.79[b]	0.683a	2.33a
Montclare	55	8.10[b]	20.62[a]	0.300a	1.33a
Significance of F	0.103	0.003	0.055	0.024	0.002
LSD	14.65	1.26	4.23	0.542	1.770
CV%	13.9	8.4	32.5	12.7	10.0

Means followed by the same letter in the column are not significantly different from each other at (p=0.05)

4.8 Greenhouse pot experiment

The percentage number of emerged tubers per pot in control plots was higher in all treatments than in inoculated pots (Table 4.5). A significant difference ($p<0.05$) was observed for Amethyst variety for the average emergence percentage of inoculated and uninoculated tubers. The other varieties were not significantly different ($p>0.05$).

Table 4.5 Mean percentage of emerged tubers of inoculated and uninoculated pots

Variety	Percentage		Difference	Test statistic (t)	Probability (p)
	Uninoculated	Inoculated			
Diamond	33.30	27.73	1.00	5.567	0.423
BP1	27.73	5.533	2.00	22.20	0.184
Montclare	22.31	5.53	1.02	16.78	0.417
Amethyst	27.73	0	4.98	27.73	0.038*
Mnandi	27.73	5.53	2.00	22.20	0.184

* Denotes significant difference at (p=0.05).

4.9 Laboratory storage experiment of 3 tubers per variety inoculated and uninoculated

Table 4.6 indicates that Bp1 and Mnandi varieties were significantly different ($p<0.05$) at room temperatures in week 1, and week 5 as weight reduction and tubers showed deteriorarion in storage as weeks progressed. Bp1 tuber weights showed significant difference ($p<0.05$) as weights continued to reduce at a decreasing rate at weeks three. During week four and week five both Mnandi and BP1 were significantly different ($p<0.05$) at storage room temperature as tubers were completely disintergrated by soft rot bacteria.

Table 4.6 Storage at room temperature for 3 tubers (g) of control and inoculated tubers of 5 potato varieties at week 1

Variety	Weights (g)		Difference	Test statistic	Probability
Week 2	Uninoculated	Inoculated		(t)	(p)
Diamond	0.23	0.29	0.06817	0.46	0.664
BP1	0.14	0.41	0.2682	2.77	0.039*
Montclare	0.64	0.72	0.0773	1.20	0.283
Amethyst	0.57	0.38	-0.1870	0.84	0.438
Mnandi	0.62	0.47	-0.1472	2.80	0.038*

*Denotes significant differences at (p=0.05)

Table 4.7 Storage at room temperature for 3 tubers (g) of control and inoculated tubers of 5 potato varieties at week 5

Variety	Weights (g)		Difference	Test statistic	Probability
Week 5	Uninoculated	Inoculated		(t)	(p)
Diamond	0.18	0.28	0.107	0.82	0.053
BP1	0.10	0.61	0.5095	4.56	0.006*
Montclare	0.67	0.55	0.1133	2.53	0.449
Amethyst	0.63	0.69	0.0633	0.42	0.691
Mnandi	0.88	0.69	-0.1825	4.26	0.008*

*Denotes significant differences at (p=0.05)

4.10 Storage weights (g) for 3 tubers at temperature 10º c of inoculated and uninoculated tubers of 5 potato varieties

At week 1, 2 and 3 there were no significant differences (p>0.05) across all the potato varieties at 10^0C. Tuber weights of inoculated and uninoculated at temperature 10^0C indicated a significant difference in week 4 and 5 for Mnandi variety at (p<0.05). Storage at temperature (10^oC) for 3 tubers (g) of control and inoculated tubers of 5 potato varieties at week 1

Table 4.8 Storage at temperature (10^oC) for 3 tubers (g) of control and inoculated tubers of 5 potato varieties at week 1

Variety	Weights (g)		Difference	Test statistic	Probability
Week 1	Uninoculated	Inoculated		(t)	(p)
Diamond	0.22	0.32	0.1040	0.85	0.432
BP1	0.37	0.65	0.2808	2.22	0.077*
Montclare	0.61	0.52	0.00897	0.37	0.726
Amethyst	0.49	0.50	0.0500	0.03	0.980
Mnandi	0.60	0.61	0.0100	0.06	0.956

*Denotes significant differences at (p=0.05)

Table 4.9 Storage at temperature (10^oC) for 3 tubers (g) of control and inoculated tubers of 5 potato varieties at week 5

Variety	Weights (g)		Difference	Test statistic	Probability
Week 5	Uninoculated	Inoculated		(t)	(p)
Diamond	0.39	0.47	0.0750	0.59	0.583
BP1	0.36	0.45	0.0891	0.58	0.585
Montclare	0.56	0.52	-0.0383	0.25	0.812
Amethyst	0.38	0.56	0.1867	1.25	0.267
Mnandi	0.61	0.61	-0.0060	0.03	0.008*

*Denotes significance at (p=0.05)

4.11 Comparison of tuber weights (g) for inoculated and uninoculated potato varieties at week 1 and week 5 at room temperature

At week 1 and week, there was no significant difference (p>0.05) across all inoculated and uninoculted potato varieties except for BP1 which was significantly different (p<0.05) from other inoculated and uninoculated potato varieties at storage room temperature (Table 4.10).

Table 4.10 Weights (g) at room temperature for 3 tubers, per five varieties of inoculated and uninoculated potato tubers for week 1 and week 5

Variety	Treatment	Week 1	Week 5	P
		Mean	Mean	
Mnandi	Inoculated	0.51	0.69	0.232
	Uninoculated	0.25	0.73	0.363
Diamond	Inoculated	0.23	0.14	0.887
	Uninoculated	0.23	0.19	0.099
BP1	Inoculated	0.40	0.61	0.006*
	Uninoculated	0.13	0.10	0.039*
Montclair	Inoculated	0.61	0.40	0.456
	Uninoculated	0.56	0.44	0.102
Amethyst	Inoculated	0.38	0.53	0.263
	Uninoculated	0.56	0.33	0.246

*Denotes significancant differences at (P=0.05)

4.12 Comparison of tuber weights (g) for inoculated and uninoculated potato varieties at week 1 and week 5 at temperature10^0c

At week 1 and week 5, no significant differences ($p>0.05$) were observed across all inoculated and uninoculted potato varieties. There was significant difference ($p<0.05$) for inoculated BP1 potato variety at 10^0C (Table 4.11).

Table 4.11 Weights (g) at10^0c temperature for 3 tubers, per five varieties of inoculated and uninoculated potato tubers for week 1 and week 5

Variety	Treatment	Week 1	Week 5	P
		Mean	Mean	
Mnandi	Inoculated	0.60	0.61	0.984
	Uninoculated	0.59	0.61	0.264
Diamond	Inoculated	0.32	0.46	0.354
	Uninoculated	0.29	0.39	0.268
BP1	Inoculated	0.64	0.44	0.025*
	Uninoculated	0.36	0.57	0.226
Montclair	Inoculated	0.51	0.52	0.977
	Uninoculated	0.60	0.55	0.793
Amethyst	Inoculated	0.49	0.56	0.642
	Uninoculated	0.49	0.37	0.384

*Denotes significance at (P=0.05)

4.13 Specific gravity weight of 5 inoculated and uninoculated potato varieties at room temperature

There was no significant difference ($p>0.05$) across Diamond, BP1 and Mnandi inoculated and uninoculated potato varieties interms of weight reduction due to soft rot infection. The weight reduction showed a significant difference ($p<0.05$) for soft rot inoculated and uninoculated tubers for Montclare and Amethyst at room temperature (Table 4.12).

Table 4.11 Specific gravity of inoculated and uninoculated 3 tubers per variety at room temperature

Variety	Weights		Difference	Test statistic (t)	Probability (p)
	uninoculated	inoculated			
Diamond	0.1948	0.2667	-0.07190	0.15	0.896
BP1	0.1631	0.3500	-0.1869	0.28	0.807
Montclare	-0.4667	0.5430	1.010	7.88	0.016*
Amethyst	-0.1667	0.3345	0.5011	9.73	0.010*
Mnandi	-0.500	0.2809	0.7809	3.04	0.093

*Denotes significant differences at (P=0.05)

4.14 Specific gravity of 5 inoculated and uninoculated potato varieties at 10^0C

Table 4.13 Shows that there ware no significant differences ($p>0.05$) on the weight reduction across all soft rot inoculated and uninoculated potato varieties at 10^0C temperature.

Table 4.13 Specific gravity of inoculated and uninoculated 3 tubers per variety at 10^0C

Variety	Weights		Difference	Test statistic (t)	Probability (p)
	uninoculated	inoculated			
Diamond	0.0500	0.1567	0.1067	0.87	0.474
BP1	0.1333	0.1433	0.0100	1.00	0.423
Montclare	0.0500	0.0900	0.0400	1.31	0.321
Amethyst	0.06667	0.1267	0.0600	0.81	0.501
Mnandi	0.0500	0.1067	0.05667	1.63	0.245

***Denotes significance at (p=0.05)**

4.15 Graded percentage weights (kg) of harvested tubers for 5 potato varieties

BP1 recorded the highest percentage of large tuber sizes and Amethysts had the lowest (8.82%) for larger tuber size. As for large medium size, Jasper recorded the highest (30.12 %) and Mnandi had the lowest 19.81%. For medium sized tubers, Mnandi had the highest percentage and BP1 had the lowest percentage. The grade of small and baby tuber sizes, Mnandi had the highest percentage and BP1 had the least tuber sizes for small and baby tuber sizes (Table 4.14).

Table 4.14 Percentage weights (kg) of graded tubers after harvesting

Weights in (gms)	Tuber sizes	Bp1 %	Amethyst %	Montclare %	Jasper %	Mnandi %
>250	Large	31.1	8.82	22.1	17.06	11.52
150-250	Large medium	26.66	29.4	20.35	30.12	19.81
90-170	Medium	22.2	23.5	25.7	26.37	27.65
50- 100	Small	14.66	21.17	17.96	25.05	26.27
5-50	Baby	5.33	13.52	13.77	14.07	15.20

CHAPTER 5

DISCUSSION

5.1 Survey results

This research's philosophical perspective leaned towards the phenomenology paradigm which was centered on considering the respondent's feelings, attitudes, perceptions, and experiences towards the impacts of soft rot bacteria and the constraints they suffer due to bacterial effect in the potato. According to Fraaije *et al* (1997) response rate of above fifty percent for phenomenological research is sufficient enough to allow the researcher to gather valid and reliable data that is representative of the population.

The results of the baseline survey to assess prevalence of soft rot disease complex among small scale seed potato farmers in the Nyanga area confirmed that the disease is present as infected tubers were isolated from the batches of seed obtained from the farmers. From the recorded responses, the source of the initial inoculum is difficult to acertain as the seed potato certification scheme relies solely on visual inspection of the crop in the field and the harvested tubers. As a result, latently infected tubers cannot be detected visually and thus require sampling and testing of seed stocks (Czajkowski *et al*., 2011a). Washing and disinfection of farm equipment used when planting, ridging, spraying, haulm destruction, harvesting and grading in store will aid to reduce risks of introducing soft rot bacteria in a pathogen-free crop (Pe´rombelon, 2002).

Susceptibility of presently cultivated genotypes lends farmers to solely depend on avoidance mechanisms to prevent disease spread. Jasper, Amethyst and Montclare were ranked by all respondents as moderately susceptible, BP1 and Mnandi ranking as most susceptible. Moderate susceptibility of the genotypes was also observed in their ability to carry profitable

yields under disease pressure. Inability of some famers to positively diagnose soft rot in the field and in storage has impeded effective control through cultural practices. Farmers' knowledge of the aetiology and epidemiology is key to management of the soft rot disease complex. Such knowledge has greatly reduced occurrence of the disease in developed countries (Czajkowski *et al.,* 2011b). Studies in Scotland showed that an initially bacteria-free potato stock became progressively more contaminated after the third year in the field (Czajkowski *et al*., 2011a).

Contamination occurred at the time that mechanical crop handling at harvest and grading in store became necessary (Mantsebo *et al*., 2014) . It is likely therefore that initial inoculum came from farm equipment already contaminated, although contamination may result by airborne bacteria or irrigation water. Late and early blight as well as bacterial wilt do occur in the crop at the same time with soft rot which then necessitates training of famers in order to enable them to differentiate one disease pathogen from the other (del Pilar Marquez-Villavicencio, *et al*., 2011). The survey results indicated that there is indeed a knowledge gap which when filled, yield losses caused by blackleg and soft rot will be reduced. This knowledge will allow a more focused approach to reducing risks of introducing the bacteria at different stages of seed production

Source of irrigation water and method of irrigation also tend to compound the soft rot disease complex (Elphinestone, 1987). The rainfall received in the Nyanga region is adequate for potato production in a good season. Farmers tend to supplement their crop during mid season droughts as seed crops are only grown in summer. Seed crops are only grown in summer to ensure that they are rainfed which eliminates irrigation water or sprinkler splash as a source and mode of spread of diseases in the crop. Surface water in the USA and Scotland was found to be contaminated with *Pcc* and to a lesser extent with *Pa* (Czajkowski *et al.,* 2011a).

In Europe, *Dickeya* spp. was found in river water (Czajkowski *et al*., 2011b).Surface water used for irrigation purposes is likely to be a source for the pathogen.

Soils in the Nyanga area are characteristically deep and well drained and thus reduces the risk of tubers being surrounded by a water film that can result in anaerobiosis and consequent tuber decay in the field (Fraaije *et al*., 1997). Ngadze and Icishahayo (2014) reported that Nyanga has the lowest disease incidence of blackleg and soft rot. Disease incidence and severity of blackleg and soft rot diseases depend on temperature and free water (Pe´rombelon, 2002).

Seed store temperature and moisture management practices were the same for all respondents, seed was placed in stacked chitting boxes under *Pinus* spp. and in sheds with natural temperature control. Well ventilated seed stores at low temperatures have been shown to avoid condensation on tuber surfaces, which in turn will prevent multiplication of the blackleg pathogen (Elphinestone, 1987). Conditions optimal for blackleg and soft rot development are between 15 and 25 ˚C with prevailing wet conditions (Agrios, 2005). When tubers remain wet long enough, tuber decay can follow, resulting in further spread of the bacteria when tubers are graded, and extensive tuber decay (Pe´rombelon, 2002) .

In developed countries, tubers are dried rapidly by forced ventilation with warm air for wound healing, followed by cooler air to control sprouting and for long-term storage (Talib Sahi *et al*., 2007). Good seed storage management is of importance, not only to prevent tuber decay, but also to avoid increasing the tuber inoculum load, which would result in greater subsequent disease risks.

BP1 variety was the most susceptible while Mnandi and Amethyst were moderately susceptible to soft rot under room temperature regime. Similarly, Montclaire, which is more

resistant to late blight, was more susceptible to soft rot than Diamond. Variation was not carefully controlled as the tubers used were not of the same grade size despite being of the same physiological maturity (Talib Sahi *et al*., 2007). It is possible that tuber size differences account for our results, but it is also possible that late blight resistance also affects soft rot resistance in these two genotypes.

Although variation was controlled for in the tuber storage, tuber to tuber variation was still found. This variation observed cannot be credited to genetic differences among cultivated genotypes, since potatoes are vegetatively propagated, and is therefore likely to be due to physiological differences among tubers. If physiological differences that affect tuber susceptibility can be identified and controlled, farmers may be able to reduce the incidence and severity of soft rot. Research has shown more mature tubers tend to have better developed periderm and thus resist injuries that can inoculate bacteria into the tuber flesh (He´ lias *et al.*, 2000). However, neither initial tuber weight nor harvest date can account for the variation we observed within varieties in our tuber experiments since these variables were controlled.

Tuber maturity may still be a factor, since tubers develop at different times under potato plants and some of the tubers examined may be older than others, even though they are the same size. Soil adhered to the collected tubers was not examined, with the hypothesis that variation in soil characteristics that affect plant nutrition, calcium deficiency in particular might account for differences in susceptibility (Mantsebo *et al*., 2014).

Due to low education levels, farmers were not able to determine just by observation, varieties that are more susceptible to the disease. Hesitance to adopt new cultivars was observed and due to this a decline in potato yield is enevitable.

5.2 Tuber screening

Among varieties collected from farmers, BP1 was more affected during tuber screening as compared to other varieties and amethyst was the least infected (Table 4.5). It could be that the condition that these farmers store their seed was good for the prevalence of soft rot infestation. According to Agrios (1997), control of bacterial soft rots depends on keeping storage tissues dry and cool. Proper sanitation and avoiding any form of tuber injuries is a major contribution to managing soft rot. Due to the limited level of education of farmers, there is lack of knowledge on the soft rot disease and some of the farmers struggle to identify and what really it is. It was noticed that some farmer use chemicals to control soft rot towards the disease. However, according to Agrios (1997) chemical sprays are not recommended for the control of soft rots.

It was noticed that the traits of soft rot are not easily noticed in the field but emanate and are more expressed in storage. However, it is possible that farmers obtain the seed with inoculum of the soft rot with latent infection and after planting and harvesting thus when they observe the traits of the bacteria. In the field it is difficult to determine and differentiate between a crop affected by soft rot, bacterial wilt and blights.

5.3 Field experiment

The crop emergence percentages were generally low across all varieties. Bp1 and Montclare had the lowest (Table 4.6). It could be that the seed was affected latently by soft rot bacteria. According to Ngadze (2014), crop emergence and yield are affected by soft rot bacteria negatively. However, farmers in Nyanga are experiencing a decline in yield. It is assumed that the poor crop emergence in the field and in the greenhouse may have been due to soft rot bacteria. The situation in the field was similar to that of the greenhouse. The only difference was knowledge on the source of the bacteria. For the greenhouse, the soils were sterilised and infection was administered deliberately. It was observed that soft rot on tubers cannot migrate

but takes advantage of water sources, insect pests, and any form of injury to the tubers for the infection. The Area under diseases was calculated for potato blight and it was noticed that the variety which was highly infected by blight also had the least emergence percentages. Disturbed plant physiology contributes to the tolerance levels of the crop soft rot bacteria. Soft rot bacteria on its own can not penatrate plant haulms (Perombelon 2002) but it migrates on to insect damaged plant parts or takes advantage of lenticel openings and cracks. Soft rot develops from injured plant parts until it has completely destroyed the plant inner periderm. At harvesting, observation of soft rot infected tubers were noticed the latent infection started expressing the disease when tubers where in storage after harvesting.

In a plot, tuber sizes were not uniform and this is a sign that soft rot was present in the field. The tendancy of soft rot to affecting emergence and growth of the crop was observed in the greenhouse as emergence percentages were low and close to zero on some of the inoculated tuber varieties. When the mother tuber rots, the bacteria are released into the soil and transmitted by the soil to contaminate neighbouring Progeny (Czajkowski *et al*. 2011a). However, excess water from irrigation can easily transport softrot infection throughout the whole field. The bacteria can colonise potato roots and subsequently move via the vascular system into progeny tubers. Once in the stem the bacteria does not necessarily cause stem rots but will survive as latent infection in the tubers.

Over irrigating the field is discouraged as soft rot thrives in wet conditions. According to Perombelon (2002), soft rot bacteria do not over winter in soil and survival in the soil is restricted to 1 week to 6 months, depending on environmental conditions. Survival can only be when the bacteria is in association with plant material including volunteers. It is possible that cultivars highly suscuptible to softrot may be prone to bacterial soft rot as these diseases affect potato yields.

In the field experiment BP1 was more susceptible to early blight and in the greenhouse, the variety's physiology deteriorated, making it more susceptible to soft rot infection. Perombelon (2002) noticed that tubers can be infected initially. A shallow, light reddish - brown dry rot lesion caused by early blight develops to soft rot which develops and destroys the tuber rapidly.

5.3.1 Grading of tubers

Tuber size and maturity affect the susceptibility of potato tubers to soft rot (Marquez *et al.* 2011). The varieties that had small and medium tubers like Montclare, Amethyst and Mnandi showed less susceptiblity to soft rot and it can be assumed that smaller tubers are less prone to handling damages than large tubers generally when harvesting. When tubers are smaller, they tend to be easier to handle and package making handling easy. The tubers of Mnandi, Amethyst and Montclare which were ranging from large to large medium size were heavily infested with soft rot indicating it maybe tuber size is a factor of concern when determining tolerance to soft rot and cultivar choice should be revised by farmers. BP1 cultivar has large tuber sizes, it was noted as the cultivar most susceptible to soft rot bacteria.

5.4 Greenhouse experiment

Pectobacterium colonies on nutrient agar medium were transparent, circular, shining, raised and creamy white. These colony characteristics helped in the identification of the isolates to be belonging to *Pectobacterium* spp. and in particular *Pectobacterium carotovorum* subsp *carotovorum.* The soft rot test was positive, surerity of soft rot presence was certain in the greenhouse. Infection of seed tubers by pectinolytic *Pectobacterium* species lead to the development of various symptoms during vegetative growth of potato crops in the greenhouse.

Poor emergence of plants, chlorosis, wilting, and haulm desiccation are observations that were noted. The germination percentages were very low generally. Diamond had the highest crop emergence for both inoculated and uninoculated tubers. This could be because of its early emergence characteristic which does not give the bacteria a chance to set before completely destroying the tubers underground. For Diamond variety, the plant vigor for the inoculated pots was slower than that of uninoculated pots but lesions were noticed on the leaves of the inoculated pots. For Mnandi, Amethyst, Montclare and BP1 the crop emergence percentages was low. The inoculated pots did not have any plants emerging. Mnandi and Montclare had one plant that emerged out of six planted tubers. The haulms of tubers inoculated were present in the soil.

5.5 Storage experiment at room temperature and at 10^0C

BP1, Mnandi, and Amethyst were more susceptible to soft rot under the room temperature regime. Similarly, Montclaire, which is more resistant to late blight, was more susceptible to soft rot than Diamond. If physiological differences that affect tuber susceptibility can be identified and controlled, Nyanga farmers may be able to reduce the incidence and severity of soft rot. In storage, it was noticed that more mature tubers with a better developed periderm resist injuries and could have a higher level of resistance to bacteria inoculated into the tuber flesh. Different temperature regulations for the inoculated and uninoculated tubers could account for soft rot influence. The fact that tubers of the same physiological age were used, development and management could have differed from farmer to farmer. Hence, the differences in responce of inoculated and uninoculated tubers in storage. Also the differences in responce can be due to genetic differences of the potato varieties. Variation was not carefully controlled as the tubers used were not of the same grade size despite being of the same physiological maturity (Talib Sahi *et al*., 2007). It is possible that tuber size differences account for our results, but also possible that late blight resistance also affects soft rot

resistance in these two genotypes. When the bacteria has migrated and set on tubers, aggressive effect of the soft rot bacteria was the same across all varieties inoculated. The issue of tuber sizes could be a factor influencing tuber susceptbility. Development of partial anaerobic conditions in stored potatoes could predispose them to bacterial soft rot. Low levels of oxygen could increase the amount of tuber decay. However the rate of decay observed by the researcher indicates that the level differs across and within the varieties. The consistency of tuber decay for tubers stored under room temperature was generally more aggresive than for tubers stored at 10^0C temperature. It could be that the latent infection in the tubers is highly activated at high temperatures. The weights of infected tubers reduced constantly at room temperatures weekly.

5.5.3 Specific gravity at room temperature and at 10^0C

Specific gravity is a dimensionless measurement. Room temperature affected the specific gravity of inoculated tubers as there was weight reduction of tubers by soft rot infecton. For the potato tubers specific gravity, displacement accounts for the whole tuber affected areas the weight is emphasized unlike the weight straight from the scale. The inoculated potato tubers administered to a temperature of 10^0C in storage were observed to have deteriorated but at slower rate. Low humidity and temperatures minimise the rate of deterioration by soft rot disease. Specific gravity at 10^0C weight and signs of soft rot disintergrating of inoculated tubers was noticed in the last weeks as the infection was taking in and established.This then explains that soft rot is not very completely active at lower temperature and it is crititcal for farmers to have a cold storage facility.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

BP 1 is a variety which is more susceptible to soft rot bacteria among all varieties studied. The deterioration of tubers by soft rot bacteria for BP1 was more at room temperature. Maximum hygiene is required when handling seed or throughout field operations so that softrot incidence can be reduced to allow farmers to achieve high yields. It was noted that continuous use of potato seed results in the bacteria developing and getting accustomed to the variety resulting in high susceptibility levels of soft rot. The issue of crop rotation should not be ignored as small scale farmers have land as a limitation and the bacteria develop over time in the soil. Temperature and soft rot bacteria highly contribute to the effect on the response of potato tubers shelf life and susceptibility. The lower the temperatures the less deterioration tubers by soft rot infection. Diamond variety was more tolerant amongst all varieties studied and thus good yield can be realized. Other varieties that were unoculated with the bacteria were still affected by soft rot showing that tubers with latent infection cannot be observed by the physical eye. However, crop emergence can be affected nagatively if infected tubers are planted. Control measures which reduce bacterial contamination on seed tubers also reduce the risk of soft rot. Disease development is very dependant on the temperature and moisture levels in both the field and store. BP1 may attain high yields in the field but in storage its

response to soft rot infection is poor. A consideration of revising BP1 performance is necessary, slowly encouraging farmers to adopt new cultivars and eliminate BP1 is adviced, as it is more susceptible to soft rot infection in storage. New updated releases by breeders of improved cultivars would assist in soft rot management.

6.2 Recommendations

6.2.1 to the farmers

1. Farmers should seek advice on the choice of cultivars with regards to soft rot from experienced people

2. The potato seed source and quality should be approved as physiological health of the seed determines performance of the cultivar.

3. Storing seed carefully before planting is very important and low temperatures of 10-20^{0}C are recommended.

4. Avoid condensation during storage is adviced as it influences a conducive environment for soft rot bacteria to survive

5. Farmer should be flexible to change and adopt new cultivars as varieties such and BP1 and Amethyst are not performing to expected standards but are still popular varieties grown.

6.2.2 Recommendations to Africa University

1. Experiments on softrot bacteria behaviour are needed and new findings would give the farmers new information.

2. Sanitisation of equipment should be practised at the University farm to avoid soft rot soil contamination.

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APPENDICES

Appendix 1: Survey questionnaire to assess Potato (*Solanum tuberosum* L*)* varietal tolerance to diseases with emphasis on bacterial soft rot

STRICTLY PRIVATE AND CONFIDENTIAL

Please answer the following questions on your own with no consultation with anyone else doing the questionnaire. These data will be treated as private and confidential. Please answer the questions truthfully and to the best of your ability. Please ask if you are unsure about how to answer any of the questions.

1. Year of Birth: _____ Sex: Male: ___ or Female: ___

2. Marital Status (Single=1, Married=2, Divorced=3, Widowed=4): ____

3. Education Level (Highest academic qualification): ____

4.Permanent Residence (name the city/town or district): _____

5.Plant diseases affecting potato :

(a) _____

(b) _____

(c) _____

6. Have you received any education/information on soft rot? Yes: ____ No: ____

If yes, please specify:

7. Would you like to receive education/information on softrot? Yes: ____ No: ____

8. How do you manage /control soft rot diseases

9. Which varieties of potato do you grow?

10. How do you rank them in terms of tolerance to soft rot?

11. How do manage diseases in general which other diseases are most prevelant?

12. What insect pests are problematic and how do you manage them?

Appendix 2: Field plan map

BOADER CROP

PATHWAY

BOADER CROP		CV 1 P1		CV5 P2		CV 2 P3		CV 4 P4		CV 3 P5		BOADER CROP

PATHWAY 1m

		CV 3 P6		CV 4 P7		CV 1 P8		CV 2 P9		CV 5 P10		

PATHWAY

		CV 1 P11		CV 3 P12		CV 5 P13		CV4 P14		CV2 P 15		

PATHWAY

BOADER CROP POTATO

Key: CV 1 Amethyst

CV 2 BP1

CV 3 Mnandi

CV 4 Jasper

CV Montclair

Appendix 3: Data analysis output ANOVA, T-test

Number of infected tubers

**** Analysis of variance *****

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
variety	3	11.5833	3.8611	11.58	0.003
Residual	8	2.6667	0.3333		
Total	11	14.2500			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	3	0.0225500	0.0075167	65.60	<.001
Residual	8	0.0009167	0.0001146		
Total	11	0.0234667			

Yield of harvested potato plants

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
VARIETY stratum	4	14036.58	3509.15	41.56	
VARIETY. stratum					
REP	2	498.50	249.25	2.95	0.055
Residual	143	12075.17	84.44		
Total	149	26610.25			

Number of infected yield tubers per replicate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicat stratum	2	0.9333	0.4667	0.53	
replicat.*Units* stratum					
Variety	4	38.9333	9.7333	11.02	0.002
Residual	8	7.0667	0.8833		
Total	14	46.9333			

Weights of infected yield tubers

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicat stratum	2	0.11033	0.05517	0.66	
replicat.*Units* stratum					
Variety	4	1.71433	0.42858	5.16	0.024
Residual	8	0.66467	0.08308		
Total	14	2.48933			

Inoculated and uninoculated weights at room temperature

Identifier	Minimum	Mean	Maximum	Values	Missing
UNINOCUL	0.1050	0.4725	0.7300	6	0

Identifier	Minimum	Mean	Maximum	Values	Missing
INOCULTE	0.1180	0.6197	0.9000	6	0

***** One-sample T-test *****MNANDI AT ROOM TEMPERATURE

Sample	Size	Mean	Variance
Q[1]	6	0.1472	0.01654

*** Test for evidence that mean (Q [1]) is different from 0 ***

Test statistic t = 2.80 on 5 df.

Probability level (under null hypothesis) p = 0.038

Identifier	Minimum	Mean	Maximum	Values	Missing
UNINOCUL	0.7300	0.7300	0.7300	1	0

Identifier	Minimum	Mean	Maximum	Values	Missing
UNINOCUL	0.1400	0.3277	0.9000	6	0

Identifier	Minimum	Mean	Maximum	Values	Missing
INOCULTE	0.1500	0.2237	0.2900	6	0

***** One-sample T-test *****DIAMOND AT ROOM TEMPERATURE

Sample	Size	Mean	Variance
Q[1]	6	-0.1040	0.08898

*** Test for evidence that mean(Q[1]) is different from 0 ***

Test statistic t = 2.80 on 5 df.

Probability level (under null hypothesis) p = 0.664

Identifier	Minimum	Mean	Maximum	Values	Missing
0 UNINOCUL	0.2200	0.2200	0.2200	1	

Identifier	Minimum	Mean	Maximum	Values	Missing
0 UNINOCUL	0.1500	0.6467	0.9800	6	

Identifier	Minimum	Mean	Maximum	Values	Missing
0 INOCULTE	0.1000	0.3658	0.9300	6	

***** One-sample T-test *****BP1 AT ROOM TEMPERATURE

Sample	Size	Mean	Variance
Q[1]	6	-0.2808	0.09628

*** Test for evidence that mean(Q[1]) is different from 0 ***

Test statistic t = 2.77 on 5 df.

Probability level (under null hypothesis) p = 0.039

Identifier	Minimum	Mean	Maximum	Values	Missing
0 UNINOCUL	0.9500	0.9500	0.9500	1	

Identifier	Minimum	Mean	Maximum	Values	Missing
0 UNINOCUL	0.2300	0.5167	0.8700	6	

Identifier	Minimum	Mean	Maximum	Values	Missing
0 INOCULTE	0.1150	0.6058	0.9000	6	

***** One-sample T-test *****MONTCLARE AT ROOM TEMPERATURE

```
      Sample      Size      Mean      Variance
      Q[1]        6         0.08917   0.3457

*** Test for evidence that mean(Q[1]) is different from 0 ***

      Test statistic t = 1.20   on 5 df.

      Probability level (under null hypothesis) p = 0.283


    Identifier     Minimum          Mean     Maximum       Values
Missing
      UNINOCUL      0.6500      0.6500      0.6500             1
0

    Identifier     Minimum          Mean     Maximum       Values
Missing
   0   UNINOCUL    0.1000    0.4958    0.9500         6

    Identifier     Minimum          Mean     Maximum       Values
Missing
  0    INOCULTE    0.1100    0.4908    0.9000         6

***** One-sample T-test ***AMETHYST AT ROOM TEMPERATURE

      Sample      Size      Mean      Variance
      Q[1]        6         -0.005000 0.2204

*** Test for evidence that mean (Q [1]) is different from 0

      Test statistic t = 0.03   on 5 df.

      Probability level (under null hypothesis) p = 0.438
```

Inoculated and uninoculated weights at 10^0C

```
    Identifier     Minimum          Mean     Maximum       Values
Missing
    0 Inoculat   0.06300   0.09100   0.11000         3

    Identifier     Minimum          Mean     Maximum       Values
Missing
   0   Uninocul    0.0850    0.1067    0.1200         3


***** Two-sample T-test *****MNANDI WK 1 AT 10 DEGREES

      Sample      Size      Mean      Variance
```

```
      Inoculat    3         0.09100   0.0006130
      Uninocul    3         0.1067    0.0003583

***  Test  for  evidence  that  the  distribution  means  are
different ***

      Test statistic t = 0.87   on 4 df.

      Probability level (under null hypothesis) p = 0.433



 Identifier   Minimum      Mean   Maximum    Values   Missing
      Inoculat    0.06300    0.06300    0.06300             1
0
    Identifier     Minimum          Mean     Maximum       Values
Missing
      Inoculat      0.1800      0.2100      0.2600             3
0
    Identifier     Minimum          Mean     Maximum       Values
Missing
  0    Uninocul    0.2300    0.2433    0.2550              3


***** Two-sample T-test *****DIAMOND WEEK1 AT 10 DEGREES

      Sample      Size      Mean      Variance
      Inoculat    3         0.2100    0.001900
      Uninocul    3         0.2433    0.0001583

***  Test  for  evidence  that  the  distribution  means  are
different ***


  Test statistic t = 1.27   on 4 df.

  Probability level (under null hypothesis) p = 0.432


Identifier   Minimum      Mean   Maximum    Values   Missing
   0   Inoculat    0.1800    0.1800    0.1800         1

 Identifier   Minimum      Mean   Maximum    Values   Missing
   0   Inoculat   0.07500   0.09167   0.12000              3

    Identifier     Minimum          Mean     Maximum       Values
Missing
   0   Uninocul   0.08000   0.09000   0.10000              3
```

***** Two-sample T-test ***** BP WEEK1 AT 10 DEGREES

Sample	Size	Mean	Variance
Inoculat	3	0.09167	0.0006083
Uninocul	3	0.09000	0.00010000

*** Test for evidence that the distribution means are different ***

Test statistic t = 0.11 on 4 df.

Probability level (under null hypothesis) p = 0.077

Identifier	Minimum	Mean	Maximum	Values	Missing
0 Inoculat	0.1200	0.1200	0.1200	1	

Identifier	Minimum	Mean	Maximum	Values	Missing
0 Inoculat	0.06000	0.07400	0.08200	3	

Identifier	Minimum	Mean	Maximum	Values	Missing
0 Uninocul	0.0800	0.1000	0.1300	3	

***** Two-sample T-test *****MONTCLARE WEEK 1 AT 10 DEGREES

Sample	Size	Mean	Variance
Inoculat	3	0.07400	0.0001480
Uninocul	3	0.1000	0.0007000

*** Test for evidence that the distribution means are different ***

Test statistic t = 1.55 on 4 df.

Probability level (under null hypothesis) p = 0.726

					Missing
0 Inoculat	0.08000	0.08000	0.08000	1	

Identifier	Minimum	Mean	Maximum	Values	Missing
0 Inoculat	0.0950	0.1017	0.1100	3	

Identifier	Minimum	Mean	Maximum	Values	Missing
0 Uninocul	0.1050	0.1083	0.1150	3	

***** Two-sample T-test *****AMETHYST WEEK1 AT 10 DEGREES

Sample	Size	Mean	Variance
Inoculat	3	0.1017	0.00005833
Uninocul	3	0.1083	0.00003333

*** Test for evidence that the distribution means are different ***

Test statistic t = 1.21 on 4 df.

Probability level (under null hypothesis) p = 0.980

Weights of specific gravity inoculated and uninoculated room temperature

	Identifier	Minimum	Mean	Maximum	Values	Missing
0	SP_GRV_U	-0.1127	0.1631	0.6571	3	

	Identifier	Minimum	Mean	Maximum	Values	Missing
0	SP_GR_IN	-0.2500	0.3500	1.3500	3	

***** One-sample T-test *****BP 1 specific gravity

Sample	Size	Mean	Variance
Q[1]	3	-0.1869	1.348

*** Test for evidence that mean(Q[1]) is different from 0 ***

Test statistic t = 0.28 on 2 df.

Probability level (under null hypothesis) p = 0.807

Identifier	Minimum	Mean	Maximum	Values	Missing
SP_GRV_U	0.6571	0.6571	0.6571	1	0

Identifier	Minimum	Mean	Maximum	Values	Missing

```
 0     SP_GRV_U   -0.5591    0.1948    0.8169         3

    Identifier     Minimum          Mean     Maximum       Values
Missing
 0     SP_GR_IN   -0.2500    0.2667    1.1000         3



***** One-sample T-test *****DIAMOND specific gravity

      Sample      Size      Mean      Variance
      Q[1]        3         -0.07190  0.7150

*** Test for evidence that mean(Q[1]) is different from 0 ***


      Test statistic t = 0.15   on 2 df.

      Probability level (under null hypothesis) p = 0.896


    Identifier     Minimum          Mean     Maximum       Values
Missing
      SPG_INOC      -69.99      -23.17        0.26             3
0
    Identifier     Minimum          Mean     Maximum       Values
Missing
      SPG_UNIN     -1.0000     -0.5000      0.0500             3
0

***** One-sample T-test *****MNANDI SPECIFIC GRAVITY

      Sample      Size      Mean      Variance
      Q[1]        3         -22.67    1683

*** Test for evidence that mean(Q[1]) is different from 0 ***

      Test statistic t = 0.96   on 2 df.

      Probability level (under null hypothesis) p = 0.093



    Identifier     Minimum          Mean     Maximum       Values
Missing
      SPG_INOC      0.4269      0.5430      0.6611             3
0
```

```
    Identifier     Minimum          Mean     Maximum       Values
Missing
      SPG_UNIN     -0.6000     -0.4667     -0.3000              3
0


***** One-sample T-test *****MONTCLARE SPECIFIC GRAVITY

      Sample       Size       Mean       Variance
      Q[1]         3          1.010      0.04927

*** Test for evidence that mean(Q[1]) is different from 0 ***


      Test statistic t = 7.88    on 2 df.

      Probability level (under null hypothesis) p = 0.016


Identifier   Minimum      Mean   Maximum    Values   Missing
      SPG_INOC      0.2000      0.3345      0.5409             3
0


    Identifier     Minimum          Mean     Maximum       Values
Missing
      SPG_UNIN     -0.3000     -0.1667     -0.0500              3
0

***** One-sample T-test *****AMETHYST SPECIFIC GRAVITY

      Sample       Size       Mean       Variance
      Q[1]         3          0.5011     0.007958

*** Test for evidence that mean(Q[1]) is different from 0 ***


      Test statistic t = 9.73    on 2 df.

      Probability level (under null hypothesis) p = 0.010
```

Specific gravity at 10^0C temperature

```
    Identifier     Minimum          Mean     Maximum       Values
Missing
   0   SPG_UNIN    0.0500    0.1333    0.2500          3

    Identifier     Minimum          Mean     Maximum       Values
Missing
    0   SPG_INO    0.0500    0.1433    0.2800          3


 ***** One-sample T-test *****SPG BP 10 DEG

      Sample      Size      Mean      Variance
      Q[1]        3         0.01000   0.0003000

*** Test for evidence that mean(Q[1]) is different from 0 ***

      Test statistic t = 1.00   on 2 df.

      Probability level (under null hypothesis) p = 0.423


    Identifier     Minimum          Mean     Maximum       Values
Missing
   0   SPG_UNIN   0.05000   0.05000   0.05000          3


    Identifier     Minimum          Mean     Maximum       Values
Missing
    0   SPG_INO    0.0200    0.1567    0.4000          3

***** One-sample T-test *****SPG DIAMOND 10 DEG

      Sample      Size      Mean      Variance
      Q[1]        3         0.1067    0.04463

*** Test for evidence that mean(Q[1]) is different from 0 ***


     Test statistic t = 0.87   on 2 df.

      Probability level (under null hypothesis) p = 0.474


    Identifier     Minimum          Mean     Maximum       Values
Missing
```

```
   0   SPG_UNIN   0.05000   0.05000   0.05000         1


    Identifier     Minimum          Mean     Maximum       Values
Missing
  0    SPG_UNIN   0.05000   0.05000   0.05000         3

    Identifier     Minimum          Mean     Maximum       Values
Missing
       SPG_INO      0.0500      0.1067      0.1700             3
0

***** One-sample T-test *****SPG MNANDI 10 DEG

      Sample      Size      Mean      Variance
      Q[1]        3         0.05667   0.003633

*** Test for evidence that mean(Q[1]) is different from 0 ***

      Test statistic t = 1.63   on 2 df.

      Probability level (under null hypothesis) p = 0.245


    Identifier     Minimum          Mean     Maximum       Values
Missing
      SPG_UNIN     0.05000     0.05000     0.05000             1
0

    Identifier     Minimum          Mean     Maximum       Values
Missing
      SPG_UNIN     0.05000     0.05000     0.05000             3
0

    Identifier     Minimum          Mean     Maximum       Values
Missing
       SPG_INO     0.05000     0.09000     0.15000             3
0

***** One-sample T-test *****SGP MONTCLARE  10 DEG

      Sample      Size      Mean      Variance
      Q[1]        3         0.04000   0.002800

*** Test for evidence that mean(Q[1]) is different from 0 ***

      Test statistic t = 1.31   on 2 df.

      Probability level (under null hypothesis) p = 0.321
```

```
    Identifier     Minimum          Mean     Maximum       Values
Missing
   0   SPG_UNIN   0.05000   0.06667   0.10000         3

    Identifier     Minimum          Mean     Maximum       Values
Missing
   0    SPG_INO    0.0500    0.1267    0.2500         3


***** One-sample T-test *****SPG AMETHYST 10 DEG

      Sample      Size      Mean      Variance
      Q[1]        3         0.06000   0.01630

*** Test for evidence that mean(Q[1]) is different from 0


      Test statistic t = 0.81   on 2 df.

      Probability level (under null hypothesis) p = 0.501
```

Uninoculated and inoculated weights at 10 degrees week 1 and week t –test

```
    Identifier     Minimum          Mean     Maximum       Values
Missing
  0    Week_1_c                                          6
Identifier   Minimum      Mean   Maximum    Values   Missing
    0  Week_5_c                                          6

    Identifier     Minimum          Mean     Maximum       Values
Missing
    0  Week_5_c    0.1000    0.6133    0.9800         6


    Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_1_c      0.1500      0.6083      0.9500             6
0

***** One-sample T-test *****mnandi uninoculated

      Sample      Size      Mean      Variance
      Q[1]        6         -0.005000 0.3576

*** Test for evidence that mean(Q[1]) is different from 0 ***

      Test statistic t = 0.02   on 5 df.
```

```
      Probability level (under null hypothesis) p = 0.984


    Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_1_c      0.6000      0.6000      0.6000             1
0
    Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_1_c      0.1100      0.5983      0.9000             6
0

    Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_5_c      0.1050      0.6193      0.9300             6
0

***** One-sample T-test ***** mnandi inoculate

      Sample      Size      Mean      Variance
      Q[1]        6         -0.02100  0.001676

*** Test for evidence that mean(Q[1]) is different from 0 ***

      Test statistic t = 1.26   on 5 df.

      Probability level (under null hypothesis) p = 0.264


    Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_1_c      0.7500      0.7500      0.7500             1
0
    Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_1_c      0.1500      0.6467      0.9800             6
0
    Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_5_c      0.1100      0.4492      0.9500             6
0

***** One-sample T-test *****BP 1 UNOCULATED

      Sample      Size      Mean      Variance
      Q[1]        6         0.1975    0.02342

*** Test for evidence that mean(Q[1]) is different from 0 ***

      Test statistic t = 3.16   on 5 df.
```

```
      Probability level (under null hypothesis) p = 0.025


    Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_1_c      0.1500      0.1500      0.1500             1
0

    Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_1_c      0.1000      0.3658      0.9300             6
0

    Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_5_c      0.1000      0.5750      0.9000             6
0

***** One-sample T-test *****BP 1 INOCULATED

      Sample      Size      Mean      Variance
      Q[1]        6         -0.2092   0.1379

*** Test for evidence that mean(Q[1]) is different from 0 ***

      Test statistic t = 1.38   on 5 df.

      Probability level (under null hypothesis) p = 0.226


    Identifier     Minimum          Mean     Maximum       Values
Missing
   0   Week_1_c    0.1400    0.3277    0.9000         6

    Identifier     Minimum          Mean     Maximum       Values
Missing
   0   Week_5_c    0.1200    0.4667    0.8500         6

***** One-sample T-test *****DIAMOND UNINOCULATED

      Sample      Size      Mean      Variance
      Q[1]        6         -0.1390   0.1114

*** Test for evidence that mean(Q[1]) is different from 0 ***

      Test statistic t = 1.02   on 5 df.

      Probability level (under null hypothesis) p = 0.354


***** One-sample T-test *****DIAMOND INOCULATED
```

```
      Sample      Size      Mean      Variance
      Q[1]        6         -0.1680   0.1441

*** Test for evidence that mean(Q[1]) is different from 0 ***

      Test statistic t = 1.08   on 5 df.

      Probability level (under null hypothesis) p = 0.328


    Identifier     Minimum          Mean     Maximum      Values
Missing
      Week_5_c      0.1300      0.1300      0.1300             1
0

    Identifier     Minimum          Mean     Maximum      Values
Missing
      Week_1_c      0.1000      0.4958      0.9500             6
0
    Identifier     Minimum          Mean     Maximum      Values
Missing
      Week_5_c      0.1200      0.5617      0.9000             6
0

***** One-sample T-test *****AMETHYST UNINOCULATED

      Sample      Size      Mean      Variance
      Q[1]        6         -0.06583  0.1066

*** Test for evidence that mean(Q[1]) is different from 0 ***

      Test statistic t = 0.49   on 5 df.

      Probability level (under null hypothesis) p = 0.642

    Identifier     Minimum          Mean     Maximum      Values
Missing
      Week_5_c       9.000       9.000       9.000             1
0
    Identifier     Minimum          Mean     Maximum      Values
Missing
      Week_5_c      0.1500      0.3750      0.7500             6
0
    Identifier     Minimum          Mean     Maximum      Values
Missing
      Week_1_c      0.1100      0.4908      0.9000             6
0

***** One-sample T-test *****AMETHYST INNOCULATED
```

```
      Sample      Size      Mean      Variance
      Q[1]        6         0.1158    0.08844

*** Test for evidence that mean(Q[1]) is different from 0 ***

      Test statistic t = 0.95   on 5 df.

      Probability level (under null hypothesis) p = 0.384


    Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_5_c      0.1500      0.1500      0.1500             1
0

     Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_1_c      0.2300      0.5167      0.8700             6
0

    Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_5_c      0.1200      0.5200      0.8000             6
0

 ***** One-sample T-test *****MONTCLARE UNINOCULATED

      Sample      Size      Mean      Variance
      Q[1]        6         -0.003333 0.07208

*** Test for evidence that mean(Q[1]) is different from 0 ***

      Test statistic t = 0.03   on 5 df.

      Probability level (under null hypothesis) p = 0.977

    Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_1_c      0.6500      0.6500      0.6500             1
0

    Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_5_c      0.1000      0.5583      0.8500             6
0

    Identifier     Minimum          Mean     Maximum       Values
Missing
      Week_1_c      0.1150      0.6058      0.9000             6
0
```

***** One-sample T-test *****MONTCLARE INOCULATED

Sample	Size	Mean	Variance
Q[1]	6	0.04750	0.1769

*** Test for evidence that mean(Q[1]) is different from 0 ***

Test statistic t = 0.28 on 5 df.

Probability level (under null hypothesis) p = 0.793

Greenhouse germination percentage

Identifier	Minimum	Mean	Maximum	Values	Missing
UNINOCUL	2.000	2.000	2.000	3	0

Identifier	Minimum	Mean	Maximum	Values	Missing
INOCULAT	0.0000	0.3333	1.0000	3	0

***** Two-sample T-test *****MONTCLARE GREEN HOUSE

Sample	Size	Mean	Variance
UNINOCUL	3	2.000	0
INOCULAT	3	0.3333	0.3333

*** Test for evidence that the distribution means are different ***

Test statistic t = 5.00 on 4 df.

Probability level (under null hypothesis) p = 0.007

Identifier	Minimum	Mean	Maximum	Values	Missing
UNINOCUL	1.000	1.667	2.000	3	0

Identifier	Minimum	Mean	Maximum	Values	Missing
INOCULAT	0.0000	0.3333	1.0000	3	0

***** Two-sample T-test *****MNANDI GREEN HSE

Sample	Size	Mean	Variance
UNINOCUL	3	1.667	0.3333
INOCULAT	3	0.3333	0.3333

*** Test for evidence that the distribution means are different ***

Test statistic t = 2.83 on 4 df.

Probability level (under null hypothesis) p = 0.047

Identifier	Minimum	Mean	Maximum	Values	Missing
UNINOCUL	2.000	2.000	2.000	3	0

Identifier	Minimum	Mean	Maximum	Values	Missing
INOCULAT	1.000	1.667	2.000	3	0

***** Two-sample T-test *****DIAMOND

Sample	Size	Mean	Variance
UNINOCUL	3	2.000	0
INOCULAT	3	1.667	0.3333

*** Test for evidence that the distribution means are different ***

Test statistic t = 1.00 on 4 df.

Probability level (under null hypothesis) p = 0.374

Identifier	Minimum	Mean	Maximum	Values	Missing
UNINOCUL	1.000	1.667	2.000	3	0

Identifier	Minimum	Mean	Maximum	Values	Missing
INOCULAT	0	0	0	3	0

***** Two-sample T-test *****Amethyst green house

Sample	Size	Mean	Variance
INOCULAT	3	0	0
UNINOCUL	3	1.667	0.3333

*** Test for evidence that the distribution means are different ***

Test statistic t = 5.00 on 4 df.

Probability level (under null hypothesis) p = 0.007

	Identifier	Minimum	Mean	Maximum	Values	Missing
0	UNINOCUL	1.000	1.667	2.000	3	

	Identifier	Minimum	Mean	Maximum	Values	Missing
0	INOCULAT	0.0000	0.3333	1.0000	3	

***** Two-sample T-test *****BP1 GREEN HSE ACTUAL

Sample	Size	Mean	Variance
UNINOCUL	3	1.667	0.3333
INOCULAT	3	0.3333	0.3333

*** Test for evidence that the distribution means are different ***

Test statistic t = 2.83 on 4 df.

Probability level (under null hypothesis) p = 0.047