SUMMARY

Potato production in Zimbabwe is severely constrained by soft rot pathogens, namely *Pectobacterium* and *Dickeya* species which can cause yield losses estimated to be between 20 and 60 % depending on environmental conditions. The bacteria produce cell wall degrading enzymes which break down pectin and release nutrients which are essential for microbial growth.

The objectives of this study were to identify the pathogens associated with blackleg and soft rot on potatoes in Zimbabwe; assess the impact and prevalence of blackleg and soft rot diseases in Zimbabwe; evaluate cultivars grown in Zimbabwe and South Africa for tolerance to *Pectobacterium* and *Dickeya* spp.; assess the role of calcium soil amendments in increasing resistance against blackleg and soft rot pathogens and to determine the genetic diversity of *Pectobacterium carotovorum* subsp. *brasiliensis* isolates from South Africa, Zimbabwe and from mini-tubers imported from China.

In chapter 3 bacterial isolates from infected plants were identified using biochemical and phenotypic characteristics, rep-PCR, Amplified Fragment Length Polymorphisms (AFLP) and sequences of *gyrB* and *recA* genes. The results revealed the identity of pectolytic enterobacteria infecting potatoes in Zimbabwe. *Dickeya dadantii* subsp. *dadantii* (*Dd*) was the most dominant pathogen followed by *Pectobacterium carotovorum* subsp. *brasiliensis* (*Pcb*). *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*) and *Pectobacterium atrosepticum* (*Pa)* were also isolated from infected plants. Phylogenetic analysis of the *recA* and *gyrB* gene sequences and rep-PCR fingerprinting of genomic DNA demonstrated high genetic homogeneity between the potato isolates from Zimbabwe and reference strains. This is the first report of *Dd* and *Pcb* on potato in Zimbabwe. Misdiagnosis of enterobacterial strains in Zimbabwe may have been due to limitations in diagnostic techniques to differentiate bacterial strains as earlier identification was solely based on biochemical and physiological assays.

AFLP analysis provides a basis for an evolutionary analysis of *Pcb* pathotypes. Genetic diversity of *Pcb* isolates from South Africa and Zimbabwe, and from mini-tubers imported from China was analysed using AFLP analysis. The analysis separated the strains into 12 clusters, reflecting subdivision in terms of geographic origin. A large degree of DNA polymorphism was evident between these 12 clusters, most strains collected from the same geographical area clustered together in the UPGMA tree, showing that *Pcb* populations from each area represent a distinct phenetic group. However in some cases, where numerous phenetic groups exist, it is possible that different founder effects occurred, probably because different potato cultivars are grown in diverse geographical areas in South Africa and Zimbabwe. Despite the genetic diversity evident in *Pcb*, a characteristic pattern of 2 to 3 bands was clearly visible in all isolates, providing potential molecular markers for identification and diagnosis.

The main feature distinguishing *Pectobacterium* and *Dickeya* species from othe enerobacteria is the production of large quantities of pectolytic enzymes. Pectinases, one of the enzymes produced, induces polyphenol oxidase (PPO) activity in the host. PPO subsequently oxidises phenols, forming a black margin around the infection foci and the margin restricts the pathogen from spreading. In chapter 4 the role of polyphenol oxidase, phenylalanine ammonia lyase, chlorogenic acid and total soluble phenols in imparting tolerance in potato to the soft rot pathogens was investigated. Potato varieties grown by farmers in South Africa and Zimbabwe were evaluated. Significantly higher enzyme activities of PPO and PAL as well as higher concentrations of chlorogenic acid and total soluble phenols were recorded in resistant cultivars. The resistance coincided with high PPO and PAL enzyme activity as well as higher concentrations of chlorogenic acid and total soluble phenols. The rotting zone diameter was significantly smaller and the viability of cell was relatively higher in varieties which recorded higher concentrations of these four compounds. The study showed that these compounds play a role in imparting resistance in potato to pectolytic pathogens.

Some of the soils in Zimbabwe have acidic low CEC and base saturation, possibly leading to calcium and / or magnesium deficiencies in the potato crop because most farmers do not fertilise with these nutrients. Calcium deficient potato plants are more susceptible to soft rot pathogens. Chapter 5 focussed on ways of increasing inherent resistance of potato plants and tubers to blackleg and soft rot pathogens by increasing the amount of calcium in the soil. The effect of calcium soil amendments on formation of phenolic compounds in the peel and ensuing tuber resistance to the pathogens was investigated. The results showed that calcium soil amendments increase concentrations of calcium in the plant, which result in increased chlorogenic acid concentrations and subsequently in improved resistance to soft rot pathogens. In addition, calcium significantly reduced the maceration effect of soft rot pathogens and soft rot losses of tubers in storage. Pre-plant application of calcium to the soil reduced blackleg and soft rot diseases in potato.

A major outbreak of the blackleg / soft rot disease complex occurred in the 2007/8 growing season in several potato growing seasons in Zimbabwe. A survey was carried out in the following year and samples of infected plants and tubers were also collected at the same time so that the pathogens responsible for the disease could be identified. The survey carried out in chapter 6 provided information which can be useful for researchers, growers and the potato industry. The information collected gave an insight of the epidemiology, population biology, economic status and control strategy for blackleg / soft rot disease complex in Zimbabwe.

The study has shown that the blackleg / soft rot disease complex in Zimbabwe is caused by several pathogens, namely *Dd*, *Pa*, *Pcb* and *cc*. The findings have indicated that PPO, POD, PAL, chlorogenic acid and total soluble phenols play a role in disease resistance against *P. atrosepticum*, *P. carotovorum*. subsp. *brasiliensis* and *D. dadantii*. Potato varieties with a high concentration of these compounds in tuber tissue can exhibit tolerance to pathogen attack.

Several researchers have reported the beneficial effects of calcium in increasing potato resistance against soft rot pathogens. This study has confirmed these findings under Zimbabwean conditions. It will be beneficial for growers to supplement with calcium in the field so as to reduce the intensity of the blackleg / soft rot complex since calcium improves tuber resistance against the pathogens. Useful information was gathered during the survey. Research in Zimbabwe should focus on development of effective control strategies and epidemiological studies in order to understand the disease development process in the country. The project enhanced the understanding of the disease complex and possible management strategies have been highlighted.

APPENDICES

Appendix A

A LIST OF BACTERIAL ISOLATES FROM POTATO (*SOLANUM TUBEROSUM*) PLANTS USED IN STUDY

Isolate no.	Scientific name	Isolation date	Host plant	Region isolated from
1	*P.c. subsp. brasilinsis*	15/12/2007	Potato	Gwebi
2	*P.c. subsp. brasilinsis*	15/12/2007	Potato	Gwebi
3	*Dickeya dadantii*	15/12/2007	Potato	Gwebi
4	*P. carotovorum subsp brasiliensis*	15/12/2007	Potato	Darwendale
5	*P.c. subsps brasilinsis*	15/12/2007	Potato	Harare
6	*P. carotovorum subsp brasiliensis*	10/02/2008	Potato	Harare
7	*P. carotovorum subsp carotovorum*	10/02/2008	Potato	Harare
8	*Not Identified*	10/02/2008	Potato	Harare

Isolate no.	Scientific name	Isolation date	Host plant	Region isolated from
9	*Not Identified*	10/02/2008	Potato	Harare
10	*Dickeya dadantii*	22/04/2008	Potato	Marondera
11	*Dickeya dadantii*	22/04/2008	Potato	Marondera
12	*Dickeya dadantii*	22/04/2008	Potato	Marondera
13	*Dickeya dadantii*	22/04/2008	Potato	Mazowe
14	*Dickeya dadantii*	22/04/2008	Potato	Mazowe
15	*Not Identified*	22/04/2008	Potato	Mazowe
16	*P.c. subsps brasilinsis*	6/5/2010	Potato	Mazowe
17	*Dickeya dadantii*	6/5/2008	Potato	Mazowe
18	*P.c. subsps brasilinsis*	6/05/200	Potato	Mazowe
19	*P.c. subsps brasilinsis*	6/5/2008	Potato	Mazowe

Isolate no.	Scientific name	Isolation date	Host plant	Region isolated from
20	*P. carotovorum subsp carotovorum*	6/5/2008	Potato	Mazowe
21	*P. carotovorum subsp carotovorum*	12/5/2008	Potato	Nyanga
22	*Not Identified*	12/5/2008	Potato	Nyanga
23	*P.c. subsps brasilinsis*	12/5/2008	Potato	Nyanga
24	*P.c. subsps brasilinsis*	12/5/2008	Potato	Nyanga
25	*P.c. subsps brasilinsis*	12/5/2008	Potato	Harare
26	*P.c. subsps brasilinsis*	12/5/2008	Potato	Harare
27	*Not Identified*	12/5/2008	Potato	Harare
28	*P. carotovorum subsp carotovorum*	12/5/2008	Potato	Harare
29	*Dickeya dadantii*	12/5/2008	Potato	Nyanga
30	*P. carotovorum subsp carotovorum*	12/5/2008	Potato	Nyanga
31	*P. carotovorum subsp carotovorum*	11/11/2008	Potato	Darwendale

Isolate no.	Scientific name	Isolation date	Host plant	Region isolated from
32	*P. carotovorum subsp carotovorum*	11/11/2008	Potato	Darwendale
33	*Not Identified*	11/11/2008	Potato	Gweru
34	*Not Identified*	11/11/2008	Potato	Gweru
35	*P. carotovorum subsp carotovorum*	9/12/2008	Potato	Gweru
36	*P.c. subsps brasilinsis*	9/12/2008	Potato	Mazowe
37	*P.c. subsps brasilinsis*	9/12/2008	Potato	Mazowe
38	*Dickeya dadantii*	9/12/2008	Potato	Mazowe
39	*P. carotovorum subsp carotovorum*	9/12/2008	Potato	Mazowe
40	*Dickeya dadantii*	28/01/2009	Potato	Gweru
41	*Not Identified*	28/01/2009	Potato	Gweru
42	*Not Identified*	28/01/2009	Potato	Gweru

Isolate no.	Scientific name	Isolation date	Host plant	Region isolated from
43	*Not Identified*	28/01/2009	Potato	Harare
44	*Dickeya dadantii*	28/01/2009	Potato	Harare
45	*Dickeya dadantii*	28/01/2009	Potato	Harare
46	*Dickeya dadantii*	28/01/2009	Potato	Harare
47	*Dickeya dadantii*	28/01/2009	Potato	Darwendale
48	*Dickeya dadantii*	18/02/2009	Potato	Gwebi
49	*Not Identified*	18/02/2009	Potato	Gwebi
50	*Dickeya dadantii*	18/02/2009	Potato	Gwebi
51	*P. carotovorum subsp brasiliensis*	18/02/2009	Potato	Shurugwi
52	*P. carotovorum subsp brasiliensis*	18/02/2009	Potato	Nyanga
53	*Pectobacterium atrosepticum*	18/02/2009	potato	Nyanga
54	*Pectobacterium atrosepticum*	18/02/2009	Potato	Nyanga

Isolate no.	Scientific name	Isolation date	Host plant	Region isolated from
55	*Pectobacterium atrosepticum*	4/03/2009	Potato	Nyanga
56	*P. carotovorum subsp carotovorum*	4/03/2009	potato	Nyanga
57	*P. carotovorum subsp brasiliensis*	4/03/2009	Potato	Shamva
58	*P. carotovorum subsp carotovorum*	4/03/2009	Potato	Shamva
59	*Dickeya dadantii*	4/03/2009	Potato	Nyanga
60	*Pectobacterium atrosepticum*	4/03/2009	potato	Nyanga
61	*P. carotovorum subsp carotovorum*	23/10/ 2009	Potato	Harare
63	*P. carotovorum subsp carotovorum*	23/10/2009	Potato	Harare
66	*P. carotovorum subsp brasiliensis*	28/10/2009	Potato	Marondera
67	*P. carotovorum subsp brasiliensis*	5/12/2009	Potato	Nyanga
68	*P. carotovorum subsp brasiliensis*	5/12/2009	Potato	Nyanga
69	*P. carotovorum subsp carotovorum*	5/12/2009	Potato	Nyanga

Isolate no.	Scientific name	Isolation date	Host plant	Region isolated from
70	*P. carotovorum subsp carotovorum*	18/12/2009	Potato	Darwendale
71	*P. carotovorum subsp carotovorum*	18/12/2009	Potato	Harare
72	*Dickeya dadantii*	18/12/2009	Potato	Harare
73	*Dickeya dadantii*	15/01/2010	Potato	Grweru
74	*Dickeya dadantii*	15/01/2010	Potato	Gweru
75	*P. carotovorum subsp carotovorum*	15/01/2010	Potato	Mazowe
76	*Dickeya dadantii*	17/03/2010	Potato	Harare
77	*Dickeya dadantii*	17/03/2010	Potato	Harare
78	*P. carotovorum subsp brasiliensis*	23/05/2010	Potato	Gweru
79	*P. carotovorum subsp brasiliensis*	23/05/2010	Potato	Gweru
80	*P. carotovorum subsp brasiliensis*	23/05/2010	Potato	Gweru
81	*P. carotovorum subsp brasiliensis*	23/05/2010	Potato	Shurugwi

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Appendix B

ERWINIA POTATO RESEARCH PROJECT QUESTIONNAIRE

Crop Science Department: University of Zimbabwe

Producer ……………………………………………………………………

Tel No. ……………………………………………………………………

Fax No. ……………………………………………………………………

Email address ……………………………………………………………………

Region ……………………………………………………………………

Name of Farm ……………………………………………………………………

Postal address ……………………………………………………………………

1. Average maximum temperature

Summer/Winter

	15 – 20^{0}C	21 – 25^{0}C	26 – 30^{0}C	> 30^{0}C
<10^{0}C	10 – 15^{0}C	16 – 20^{0}C	21 – 25^{0}C	> 25^{0}C

2. Average rainfall per year? (mm)

<250	250-500	501-750	751-1000	>1000

3. Are the potatoes irrigated?

Yes	No

How often?

Weekly	2x per week	Other

4. Do you use certified seed?

Yes	No

5. Are you familiar with: (Yes/No)

Soft rot – seed piece decay	Blackleg (Erwinia wilt)	Other

6. What measures are implemented on the farm to control *Erwinia*?

None	Crop rotation Which crops?	Removal of infected plants (rouging)	Other

7. Are potatoes treated with any chemicals to control *Erwinia*?

Yes	No

If yes: Chemicals used

………………………………………………………………………………………

Dosage

……………………………………………………………………………………..

Method of application

Dip	Spray	Dusting	Other

8. Please evaluate the cultivars that you are familiar with for their susceptibility to *Erwinia* diseases.

1 = resistant; 2 = moderately susceptible; 3 = susceptible

Cultivar	Soft rot (Post planting)			Blackleg			Soft rot (Post harvest)		
W									

9. hat are the estimated yield losses (in $ or %) of potatoes due to *Erwinia* diseases?

- Soft rot (seed piece decay, post planting)

  ………………………………………………………………………………………

- Blackleg (*Erwinia* wilt)

  ………………………………………………………………………………………

- Soft rot (post harvest)

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10. What aspects do you think are important for future *Erwinia* research?

………
………
………
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