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# Identification and control of potato soft rot and blackleg pathogens in Zimbabwe

by

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# Declaration

I, Elizabeth Ngadze declare that this thesis/dissertation, which I hereby submit for the degree PhD in Plant Pathology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE: ……

DATE: ……..

# Acknowledgements

I started this project in 2008 as someone set in my own ways of doing things but interactions with a number of people who have helped to see this project to completion, have changed my thinking. I now appreciate my limitations with humbleness and calmness and accept that when stuck always seek help from people who can provide answers and avoid wasting time looking for information or trying a new technique on my own.

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# Contents

## LIST OF FIGURES

## LIST OF TABLES PAGE

# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| AFLP | - | Amplified fragment length polymorphism |
| ATCC | - | American Type Culture Collection |
| BCC | - | Belgian Coordinated Collection of Microorganisms |
| Bp | - | base pair |
| ˚C | - | degrees Celsius |
| Cfu | - | colony forming units |
| *Dd* | - | *Dickeya dadantii* |
| DNA | - | deoxyribonucleic acid |
| dNTP's | - | deoxynucleotide triphosphate |
| Fig. | - | figure |
| *gyrB* | - | gene encoding DNA gyrase |
| LMG | - | Laboratory of Microbiology Ghent University |
| µl | - | microlitre |
| µm | - | micromolar |
| Ml | | militre |
| NCPPB | - | National collection of Plant Pathogenic Bacteria |
| NRF | - | National Research Foundation |
| *Pa* | | *Pectobacterium atrosepticum* |
| PAL | - | Phenylalanine ammonia lyase |
| *Pcb* | | *Pectobacterium carotovurum* subsp. *brasiliensis* |
| *Pcc* | | *Pectobacterium carotovurum* subsp. *carotovorum* |
| PCR | - | Polymerase chain reaction |
| POD | - | Peroxidases |
| PPO | - | poly phenol oxidase |
| *recA* | - | gene encoding recombinase A |
| rep-PCR | - | repetitive extragenic palindromic – PCR |
| subsp. | - | subspecies |
| sp | - | species |
| UPGMA | - | unweighted pair groups method using arithmetic average |
| V | - | volts |

# PREFACE

Potato (*Solanum tuberosum*) is one of the most popular food crops grown as a substitute staple in Zimbabwe. Its production is constrained by pest and diseases which reduce the yield drastically. The blackleg / soft rot disease complex caused by *Pectobacterium* and *Dickeya* species (formerly known as *Erwinia*) has been identified as a potential threat to potato production. The soft rot pathogens can cause systemic and vascular infections in potatoes, which result in the development of various symptoms on the stem and tubers. The symptoms that develop on the plant are not species specific but depend on climatic conditions prevailing at the infection stage. The pathogens affect the crop at all stages of production, which include in the field, storage and in transit.

The primary objectives of this study were to identify the pathogens which cause blackleg and soft rot on potatoes in Zimbabwe; document the grower's knowledge of blackleg and soft rot diseases in Zimbabwe; evaluate cultivars grown in Zimbabwe and South Africa for tolerance to *Pectobacterium* and *Dickeya* species; determine the role of calcium in blackleg and soft rot development; and determine the genetic diversity of *Pectobacterium carotovorum* subsp. *brasiliensis* isolates from South Africa, Zimbabwe and mini-tubers imported from China.

The first chapter introduces the research highlighting the importance of potatoes globally and in Zimbabwe. It discusses production constraints caused by soft rot pathogens, as well as the pathogenesis of *Pectobacterium* and *Dickeya* spp. It also summarises the research objectives and thesis outline. Chapter 2 provides an overview of published literature on morphology, pathogenicity and identification of blackleg and soft rot pathogens, epidemiology, host range, defense mechanisms in plants, host nutrition and disease management strategies.

Taxonomic revisions have led to the reclassification of pectolytic *Erwinia* into several genera. Strains formerly described as *Erwinia carotovora* have been incorporated into the genus *Pectobacterium*, and

strains classified as *Erwinia chrysanthemi* are now assigned to the genus *Dickeya.* Several species and subspecies of *Pectobacterium* and *Dickeya* have isolated from infected potatoes. In Zimbabwe only *Pectobacterium atrosepticum* and *Pectobacterium carotovorum* subsp. *carotovorum* have until now been identified as the causal agents of blackleg and soft rot diseases, respectively. Although other species and subspecies of *Pectobacterium* and *Dickeya* have been isolated from infected potato plants in Zimbabwe, their potential to cause diseases on potatoes should not be underestimated.

In chapter 3 bacterial isolates collected from infected plants showing typical blackleg / soft rot disease symptoms were identified using biochemical and physiological methods, as well as rep-PCR, Amplified Fragment Length Polymorphism (AFLP) and single gene sequencing using two genes, viz. *gyrB* and *recA.* Amplified Fragment Length Polymorphisms (AFLPs) were used to study the genetic diversity among potato isolates from Zimbabwe, South Africa and mini-tubers imported from China.

*Pectobacterium* and *Dickeya* species enter the plant through wounds and natural openings such as lenticels. When they invade the plant they colonise the vascular tissue and spaces between the thin walled parenchyma cells. They remain inside these cells as latent infections and disease symptoms develop when host resistance is impaired or when environmental conditions become conducive for disease development. Chapter 4 investigates the role of defense related enzymes, polyphenol oxidase, phenylalanine ammonia lyase, peroxidases, chlorogenic acid and total soluble phenols in host resistance to potato soft rot. The potato varieties were assayed for activity of polyphenol oxidase and phenylalanine ammonia lyase, peroxidases and concentration of chlorogenic acid and total soluble phenols in tuber tissue. The role of these four components in imparting resistance against the soft rot pathogens was also investigated.

Blackleg and soft rot diseases are seed-borne and difficult to control using chemical, physical and cultural methods. Several approaches aimed at controlling blackleg and tuber rot have been studied but the degree of success has been variable. Plant nutrition is an important component of natural

disease resistance. The effect of calcium soil amendments in enhancing resistance to potato soft rot pathogens was investigated in Chapter 5. A management strategy based on calcium-induced defense responses of potato to *Pectobacterium* and *Dickeya* spp. was investigated. Calcium promotes production of phenolics and strengthens the cell wall, making the plants resistant to maceration by pectolytic enzymes.

In Chapter 6 a survey was conducted in nine potato growing regions of Zimbabwe using an informal structured questionnaire. The findings highlighted the distribution and impact of blackleg and soft rot diseases on the Zimbabwean potato industry. A broad spectrum of information on blackleg / soft rot disease complex was gathered and this information can help growers to make informed decisions about control strategies to apply.

Each chapter in this thesis has been treated as an independent entity. Thus redundancy between chapters could not be avoided. It is my hope that the results of these studies on soft rot pathogens in Zimbabwe will contribute to a better understanding of the blackleg / soft rot disease complex that they cause. I also hope that these studies will form the basis of detailed and future investigations in epidemiology and disease control strategies.