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Emerging diseases of maize and onion caused by bacteria belonging to the Genus *Pantoea*

by

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Co-supervisor: Prof. S.N. Venter

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SUMMARY

Center rot of onion, caused by *Pantoea ananatis*, was first described in the USA, in 1997. *P. ananatis* is seed-borne in onions and it was suggested that it was introduced into the USA on infected seed lots from South Africa. Center rot has not been observed in South Africa and it was essential to determine if *P. ananatis* is present in local onion seed. Colonies resembling those of *P. ananatis* were isolated from four South African seed lots on PA 20, a new semi-selective medium. Pathogenicity tests demonstrated that the South African and America strains induced the same symptoms on onion. Phenotypic and genotypic analyses identified the strains from seed as *P. ananatis*. In 2004/2005, an unreported disease of maize, brown stalk rot, was observed on commercial fields in South Africa. The representative strains induced disease symptoms similar to those observed in the field. The phenotypic and genotyping tests showed that the strains belonged to the genus *Pantoea* and separated them into two groups. The first group was identified as *P. ananatis*. The F-AFLP genomic fingerprints generated by the second group of strains, were distinctly different from those generated by known *Pantoea* species. To resolve the taxonomic position of *Pantoea* isolated

from onion and maize, sixty-seven strains were subjected to a polyphasic study. The methods used included phenotypic characterisation, genomic fingerprinting, 16S rRNA gene sequence analysis and DNA-DNA hybridisation. The results revealed that the strains belong to three different species within the genus *Pantoea*: *P. ananatis*, *P. vagens* and a novel species, *Pantoea allii* sp. nov.

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CONTENTS

PREFACE

Maize and onion are important agricultural crops in South Africa. The country is one of the biggest producers of onion seed in the world. In the 2004/2005 growing season 358 tons of onion seed were produced, of which 282 tons were for the export market. Maize is the most important grain crop in South Africa, being both the major animal feed grain and the staple food of the majority of the population. For the 2003/2004 marketing year maize was responsible for the second largest contribution to the gross value of agricultural production in the country. The South African maize industry is also the largest maize industry in Africa. Commercial farmers are cultivating nearly three million hectares of maize per year. In the past five years, South Africa produced between 7.2 and 10.1 million tons of maize per annum, with an average of 9.2 million tons. The main maize production areas in South Africa are the Free State, Northwest and Mpumalanga Provinces. These three provinces are responsible for 85% of the total maize produced in the country.

Numerous fungal diseases cause excessive damage to maize and onion in South Africa and efforts to control the quality of these crops concentrated on the detection and control of fungal pathogens. Consequently, little is known about plant pathogenic bacteria that may be present.

In 1981, leaf and seed stalk necrosis of onion, caused by *Erwinia herbicola* (syn. *Pantoea agglomerans*) was reported in the onion seed production areas. In 2000, leaf blight of onion, caused by *Xanthomonas axonopodis* pv. *allii* was observed in a few commercial fields of the Limpopo Province. These two diseases have not been observed again and no attempts have been made to screen the locally produced onion seed for the presence of *P. agglomerans* and *X. axonopodis* pv. *allii*.

In 1997, a disease similar to leaf and seed stalk necrosis was observed in onion fields in Georgia, USA. The disease, named center rot, has occurred in commercial fields in Georgia every year since 1997 and accounted for 100% loss in some fields. The causal agent of center rot is a gram-negative, facultatively anaerobic, seed-borne bacterium, identified as *Pantoea ananatis*. The seed associated with the first outbreak of center rot of onion in Georgia, USA, was produced in South Africa and it was suggested that the center rot

pathogen was possibly introduced on infested seed lots. *P. ananatis* is the causal agent of bacterial blight and dieback of *Eucalyptus* in South Africa, but center rot of onion has not been reported from this country.

The first goal of this study was to determine if pathogenic *P. ananatis* was present in South African onion seed and to compare such strains to those associated with center rot of onions in the USA. Nutrient agar is the common growth medium used to isolate *P. ananatis* from plant material and seed. Nutrient agar, however, is non-selective and many other organisms present as saprophytes or endophytes in plant material and in seed may hamper the detection of the target pathogen. In Chapter 2, a semi-selective medium, PA 20, is described, which suppresses growth of many saprophytic microorganisms and serves as a suitable medium for growth and enumeration of *P. ananatis*. The medium was specifically developed to detect this pathogen on onion seed. Chapter 3 describes the isolation and identification of *P. ananatis* from South African onion seed.

In 2004 and 2005, a new disease was observed in commercial maize fields in the Northwest and Mpumalanga Provinces. Diseased plants were scattered throughout the fields and 10-70% of the crop was affected. Gram-negative bacteria producing yellow colonies were consistently isolated from diseased tissues and these were tentatively identified as belonging to the genus *Pantoea.* Two *Pantoea* species have been reported to cause diseases on maize worldwide. *P. stewartii* subsp. *stewartii* causes Stewart's wilt in Europe, Asia and the Americas. *P. ananatis* was described as an agent of leaf spot on maize in Brazil.

The second goal of this study was to identify and characterise the causal agent of brown stalk rot of maize observed in South Africa. Chapter 4 describes the identification of the bacteria causing this new disease of maize.

The third goal of this study was to characterise a collection of *Pantoea* strains from onion and maize using a polyphasic approach based on analyses of carbon source utilisation, physiological characteristics, fluorescent amplified fragment length polymorphism (F-AFLP), repetitive sequence based PCR (rep-PCR) genomic fingerprinting, 16S rRNA gene sequence analysis and DNA-DNA hybridisations. The results are presented in Chapter 5.