CHAPTER 1

GENERAL INTRODUCTION

The potato (*Solanum tuberosum* L.) is indigenous to the Peruvian and Bolivian Andes mountains in South America, where it was discovered by Spanish explorers in 1532 (Brown, 1993; Steyn, 1999). The potato was shipped to Europe and introduced into Spain in 1573. From Europe it was introduced to North America and the rest of the world, including South Africa (Aartappelreeks, 1974; Brown, 1993; Zuckerman, 1998). Potatoes are currently grown as a major food source in most countries with a temperate climate (Rich, 1983). Globally it is the fourth most important staple food after wheat, rice and maize (Hawkes, 1992).

South Africa is the largest potato producer in Africa, annually producing approximately 1.6 million tons (Potatoes South Africa, 1998/99). The country is divided into 14 production regions, stretching from coastal areas to 2000 m above sea level (Steyn, 1999). Because of the wide climatic spectrum of these regions, potatoes can be produced throughout the year. Tubers are mainly sold on local fresh produce markets and only 6.8% is exported to neighbouring countries such Namibia, Botswana, Mozambique, Angola and Swaziland (Potatoes South Africa, 1998/99). Some of the best known and widely cultivated potatoes in South Africa include cultivars such as Up-to-Date, BP1, Vanderplank and Buffelspoort (Potatoes South Africa, 1998/99). According to Nortje (1999), seed potatoes are produced in all provinces of South Africa with the Western Cape currently producing 35% of all certified tubers. More than 70% of South Africa's potatoes are produced from certified tubers.

One of the most important diseases of potatoes is bacterial wilt. This disease is caused by the bacterium, *Ralstonia solanacearum* (Smith) (Kelman, 1953; Yabuuchi *et al.*, 1995). It was first isolated in 1896 and identified and described by Erwin F. Smith. In 1914, it was reported for the first time on potatoes in South Africa, but it was not until 1978 that research on this disease gained momentum after its presence was established in a number of seed potato crops (Swanepoel and Young, 1988). The pathogen causes typical wilt symptoms in above ground plant parts (Harrison, 1961). The first symptoms usually appear during the warmer spells of the day with a slight drooping in the tip of one or two of the lower leaves. Within a few days infected plants will be completely wilted and eventually die (Kelman, 1953). Tubers may or may not show

external symptoms (Shekhawat *et al.*, 1992). When a diseased tuber is cut in half, distinct brown discoloration and localised decay can be seen in the vascular ring. If slight pressure is applied to the cut tuber, typical greyish-white bacterial slime will ooze from the vascular ring (Shekhawat *et al.*, 1992).

Currently bacterial wilt is not a threat for the South African potato industry and its presence is limited to isolated cases. This is mainly due to the certification scheme implemented on 1 June 1995 (Nortje, 1997). The scheme requires representative samples of all registered seed potatoes to be tested for various diseases. A zero tolerance for *R. solanacearum* is applied to ensure that no infected tubers are sold as seed potatoes.

Ralstonia solanacearum is also the causal agent of bacterial wilt of a number of other plant species (Buddenhagen and Kelman, 1964). Some of the most important agricultural crops affected by this pathogen include: tomato, potato, pepper, tobacco, eggplant, groundnuts and bananas. A number of ornamental plants, woody perennials as well as a very large group of weed species can also be infected (Kelman, 1953; Buddenhagen and Kelman, 1964; Hayward, 2000). The pathogen has a worldwide distribution and has been widely reported in all tropical, subtropical and warm temperate regions around the world (Kelman, 1953; Hayward, 2000).

Since the bacterium can infect various crops without inducing symptoms, tests are also required for detecting latent infection in plant material and in soil samples (Seal and Elphinstone, 1994). Many countries have implemented strict monitoring systems to avoid the introduction of infected material and various methods have been developed for detecting the pathogen *in situ* (Elphinstone *et al.*, 1996). The majority of these methods was primarily developed for detection of *R. solanacearum* in plant material and is subject to various difficulties and shortcomings when used for detection in soil (Jenkins *et al.*, 1967; Seal and Elphinstone, 1994). Most of the problems encountered with detection can be attributed to the heterogeneous nature of soil and the difficulty of adequate soil sampling. Selective media cannot effectively detect pathogen concentrations much lower than 10^3 cfu ml^{-1} of soil sample (Karganilla and Buddenhagen, 1972; Nesmith and Jenkins, 1979; Chen and Echandi, 1981). Using indicator plants to confirm the presence of *R. solanacearum* in suspect fields is effective but time consuming and laborious (Karganilla and Buddenhagen, 1972; Graham and Lloyd, 1978). Serological tests such as enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence antibody staining (IFAS) as well as DNA-based methods such as the polymerase chain reaction (PCR) is highly specific and

sensitive. They are, however rather expensive and not suitable for frequent use in developing countries with limited resources (Seal, 1994; Seal and Elphinstone, 1994).

Once the pathogen has been detected and isolated from soil its identity needs to be confirmed. This can play an important role in the development of control strategies since different strains have different abilities to survive (Elphinstone and Aley, 1993). The information may also be needed where crop rotation is used as control measure against bacterial wilt. Since different strains of the pathogen can infect different host plants (Walker and Stead, 1993; Hayward, 2000), knowing what strain is present can play an important role in deciding which plants to use for crop rotation. Traditionally, a binary system has been used for isolate classification where isolates were differentiated into races or biovars (Buddenhagen *et al.*, 1962; Hayward, 1964; Walker and Stead, 1993; Hayward, 1991). Molecular techniques such as PCR provide a fast alternative to distinguish between different organisms and have previously been used to illustrate the heterogenicity of different *R. solanacearum* isolates (Cook *et al.* 1989; Poussier *et al.* 1999).

Ralstonia solanacearum has an extremely wide host range (Kelman, 1953; Buddenhagen and Kelman, 1964; Hayward, 2000) and is able to survive for prolonged periods in the soil (McCarter, 1976; Graham *et al.*, 1979). Control of this pathogen is therefore difficult and several control methods such as crop rotation and soil amendments should be combined in an integrated control program to combat bacterial wilt. Due to the high costs involved as well as the detrimental effect that the excessive use of chemicals can have on human health and the environment, there has been a global interest in biological control as an alternative method for the control of weeds, insects and plant pathogens (Lydon and Duke, 1989; Lampkin, 1990). Akiew *et al.* (1996) and Kirkegraad *et al.* (1998) illustrated the potential of mustard (*Brassica juncea* L.) and other cruciferous species as biofumigation agents to suppress *R. solanacearum* populations in the soil.

The first objective of this study was to develop an effective detection method for *R. solanacearum* in infested soil. The technique should be easy to apply, affordable, fast and sensitive enough to detect low pathogen concentrations. This technique was compared in terms of sensitivity, affordability and applicability with existing methods. The second objective of this study was to characterise various South African *R. solanacearum* isolates using different molecular techniques. Polymerase chain reactions and restriction fragment length polymorphisms (RFLPs) were used to determine the homogeneity of a collection of *R.*

solanacearum isolates. To conclude this study, different herbal plant species were evaluated as possible biofumigation agents to suppress the pathogen in soil.

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