CHAPTER 6

GENERAL DISCUSSION

Ralstonia solanacearum is a diverse pathogen, capable of causing significant crop losses throughout the world on a diverse range of crops (Hayward, 2000). On potatoes, the pathogen affects both above and belowground plant parts and damage can occur in two ways; premature wilting of top growth, and rotting of tubers either in the soil or in storage (Shekhawat *et al.*, 1992). Bacterial wilt is currently not a threat for the South African potato industry due to an effective certification program (Nortje, 1997). However, due to its wide host range and ability to survive for long periods in the soil, its presence needs to be continuously monitored to prevent serious future outbreaks. It is therefore important to have techniques available for its detection, both in plant material and in soil. Various techniques such as enzyme-linked immunosorbent assay (ELISA) and immunofluorescence antibody staining have been developed for the detection of *R. solanacearum* in plant material (Bellstedt and van der Merwe, 1989; Elphinstone *et al.*, 1998). However, none of these techniques were developed for soil systems. The primary objective of this study was therefore to development an alternative technique for the detection of *R. solanacearum* in soil systems and to compare it with techniques currently used in South Africa.

In South Africa, techniques that are commercially used for the detection of *R. solanacearum* in plant material are ELISA and selective media (Pers. Comm. N.J.J. Mienie, Potatoes South Africa, Seekoegat). These techniques were primarily developed for detection of the pathogen in plant material and not in soil systems. If used for detection of *R. solanacearum* in soil samples, the techniques are not sensitive enough to detect low pathogen concentrations (Graham and Lloyd, 1978; Nesmith and Jenkins, 1979; Seal, 1994; Seal and Elphinstone, 1994). The technique developed in this study was based on using an indicator plant system. Tissue culture potato plants were successfully used to trap the pathogen in artificially inoculated soil suspensions. This trapping technique was found to be sensitive and could detect pathogen concentrations as low as 10^1 cfu ml^{-1} soil suspension within ten days. Although the other techniques only require three days to produce results, it could only detect the pathogen at concentrations higher than 10^3 or 10^4 cfu ml^{-1} depending on the technique used. The trapping technique used in this study proved to be

a successful alternative method for the detection of *R. solanacearum*, was practical and did not require specialised equipment. It was however found to be not rapid enough for commercial use and can therefore not replace the existing ELISA and selective media diagnostic methods.

Once the pathogen has been isolated, identity needs to be confirmed. Traditionally, a binary system has been used for isolate classification (Hayward, 1991). Isolates could either be differentiated into five races according to hosts primarily affected (Buddenhagen *et al.*, 1962; Walker and Stead, 1993) or more commonly into five biovars (Hayward, 1964). Biovars can be distinguished based on the isolates' ability to utilise different carbon sources. Molecular techniques such as polymerase chain reaction (PCR) provide a fast alternative to distinguish between different organisms and it was decided to evaluate the enterobacterial repetitive intergenic consensus (ERIC)-PCRs' ability to distinguish between biovar 2 and 3 isolates. Eight South African isolates were selected and the technique was successfully used to distinguish between these two groups.

Isolates can also be characterised using molecular techniques such as restriction fragment length polymorphism (Gillings and Fahy, 1993). This classification can play an important role in the development of new control strategies since different strains have different abilities to survive (Elphinstone and Aley, 1993). Different strains also have the ability to infect different plant species and care should be taken to choose non-host plants for crop rotation (Walker and Stead, 1993; Hayward, 2000). Integrated control programs should therefore not only be adapted to suit certain climatic conditions, soil type, farming system and the socio economic situation of a specific area, but also the pathogen strain (Elphinstone and Aley, 1993; Mienie, 1997). The ribosomal intergenic spacer analysis-PCR and RFLP with *Sau*3A were used to characterise and evaluate possible variation between 44 *R. solanacearum* isolates collected throughout the potato growing areas of South Africa. These techniques could also distinguish between biovar 2 and 3 isolates but no correlation could be drawn between the different isolates and the regions from which they were isolated.

Once the pathogen has been detected and identity confirmed, a suitable control program could be implemented. Since there is no chemical registered for the control of *R. solanacearum*, herbal plant material was evaluated for its potential use in biofumigation to suppress the pathogen in soil. This biofumigation was based on the method described by Kirkegraad *et al.* (1998) but proved unsuccessful in our study. Although the pathogen could not be suppressed successfully,

new hosts for *R. solanacearum* were determined. Biovar 3 was able to infect marjoram, nasturtium, parsley, coriander, chive and mustard and biovar 2, marjoram, nasturtium and parsley.

For this study it was found that *R. solanacearum* could successfully be detected from artificially inoculated soil systems using the trapping technique. Molecular techniques such as the ERIC-PCR and RFLP with *Sau*3A could be used to distinguish between biovar 2 and 3 isolates, but could not be used to draw a correlation between the different isolates and the regions from which they were isolated. Biofumigation could not be applied successfully.

Future studies should focus on the detection of *R. solanacearum* in naturally infested soil using the trapping technique. The molecular techniques used in this study should be tested against biovars 1, 4 and 5 to determine whether or not they can distinguish between all five *R. solanacearum* biovars. Biofumigation should be re-evaluated under different glasshouse conditions to determine if herbal plant material could be used to suppress *R. solanacearum* in soil.

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