CHAPTER 1

GENERAL INTRODUCTION

The avocado, *Persea americana* Miller, also known as the "alligator pear", originates from South America. It has been an important constituent of the South Americans' diet for thousands of years (Snowdon, 1990). Currently, avocados are an important fruit crop in many parts of the world, including South Africa. Three main types of avocados can be distinguished, namely the Mexican (subtropical), Guatemalan (semitropical) and "West Indian" (tropical) (Biale & Young, 1971). The most important avocado cultivation regions in South Africa are the Lowveld of the Northern Province and Mpumalanga (Keevy, 1999). Other frost-free parts of the country are also used for cultivation. A number of cultivars, varying in fruit size, shape and colour, are currently cultivated in these regions of which the most important are Fuerte and Hass.

Like all tropical and subtropical fruit, both pre- and postharvest diseases are prevalent on avocados, which can result in major losses. Preharvest diseases include cercospora spot and anthracnose, while stem-end rot and anthracnose are diseases observed postharvestly (Snowdon, 1990; Hartill, 1991). The economic impact of postharvest diseases is difficult to assess, since losses during selective picking, sorting, re-packing and marketing are rarely included (Wilson & Wisniewski, 1989). It is estimated that up to 50 % of the total worldwide production of fruit crops can be wasted as a result of postharvest diseases (Wilson & Wisniewski, 1989).

The most common way to control plant diseases caused by fungi is by means of chemical fungicides. Fungicides are usually not easily biodegradable, since they must persist in the environment for optimal functionality (Campbell, 1989). This causes serious environmental problems due to pesticide build-up in soil or water ecosystems (Campbell, 1989). Pesticides accumulate in predators at the top of the food chain and also adversely affect non-target organisms. During the late 1980's it was estimated that annually 3000 hospitalisations, 200 fatalities and unexpected side effects, occurred annually in the USA alone due to the misuse of pesticides (Campbell, 1989). Incorrect use of fungicides can also lead to build up of pathogen resistance, resulting in reduced effectiveness of the product (Kotzé *et al.*, 1982; Darvas & Kotzé, 1987). Certain pesticides also leave a visible residue on the product that is not allowed for export. Such residues must be manually removed, thereby increasing production cost (Denner & Kotzé, 1986). Finally, small niche industries find it increasingly difficult to manage diseases since relatively few, if any new chemicals are registered. Major agrochemical companies are reluctant to invest in new products or re-register older products due to perceived lower profit margins (Denner & Kotzé, 1986). Alternative control measures and techniques must be developed to replace chemicals or to minimize their use.

Recently, the use of biological control agents has increased significantly as an alternative to pesticides. Biological control is the use of one organism to control another, especially pests or disease causing organisms (Atlas & Bartha, 1987). The most commonly known definition of biological control in plant pathology, is the reduction of inoculum density or disease-producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonist, or by mass introduction of one or more antagonists (Baker & Cook, 1974). Recently, numerous studies were aimed at the use of biological control agents to increase our understanding of the interactions between host, pathogen and antagonist (Andrews, 1992; Fiddaman & Rossall, 1993; Gilbert *et al.*, 1994; Milner *et al.*, 1997; Bellows, 1999; Lindow & Wilson, 1999; van Dijk & Nelson, 2000; Benhamou *et al.,* 2001; Helistö *et al.*, 2001).

In South Africa, biological control of postharvest diseases using natural antagonists has been demonstrated successfully (Korsten *et al.*, 1991). A bacterial antagonist, *Bacillus subtilis*, was isolated and successfully screened *in vitro* and *in vivo* to control postharvest diseases of subtropical crops. The use of *Bacillus* species in the biological control of plant pathogens is well documented (Korsten *et al.*, 1989; Korsten *et al.*, 1991; McKeen *et al.*, 1986). *Bacillus* species are very diverse and commercially useful and occur in almost all environments (Harwood, 1989). The Food and Drug Administration has placed *B. subtilis* under GRAS (Generally Regarded As Safe) status. This is mainly due to the global use of members of this species in several fermentation processes and also due to its general lack of pathogenicity (Harwood, 1989). The efficacy and consistency of *B. subtilis* to control avocado diseases received much attention (Korsten *et al.*, 1988; 1989; 1991; 1993; 1995; van Dyk *et al.*, 1997). However, commercialisation of biocontrol products requires not only proof of its efficacy and consistency, but also its mode of action.

Singular modes of action are rare in nature and often a range of synergistic interactions occurs. Competition for nutrients (Chalutz *et al.*, 1988), competitive colonization (Bhatt & Vaughan, 1962), site exclusion (Janisiewicz, 1988), antibiosis (Pusey & Wilson, 1984; Janisiewicz & Roitman, 1988), induction of host defence mechanisms (Janisiewicz, 1987; Chalutz *et al.*, 1988) and direct interaction with the pathogen (Dubos, 1984; Podile & Prakash, 1996) are some of the more familiar modes of action involved in antagonism. Several possible modes of action have been postulated for avocado pre- and postharvest diseases, namely competition for nutrients, competitive colonization and antibiosis (Korsten & de Jager, 1995).

The aim of this study was therefore to further investigate the mode of action of the antagonist, *B. subtilis*, and to determine the influence of nutrients and temperature on *in vitro* biocontrol activity. This information is critical for improvement of commercial product formulation. Finally, characterization of antifungal substance/s is required for product registration for commercial use and was therefore also investigated. In this study we hypothesize that antibiosis is the predominant mode of action.

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