

Status of Research on Biological Control of Avocado Pre- and Post-harvest Diseases: An Overview

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The South African avocado growers was the first industry to financially support a biological control programme with the aim of reducing its dependency on fungicides. The biocontrol programme commenced in 1985, starting with an ecological study of the avocado phyllo-and fructoplane (leaf and fruit surfaces) microflora (Figure 1). With this attempt at understanding the natural microbial diversity, its interactions, and the various aspects which can influence the climax microbial community, it became evident that this neglected milieu can be exploited to achieve natural disease control. This can be achieved by re-establishing the original microbial ecological balance. After the initial ecological study, we embarked on a two-pronged approach, viz. bio-augmentation (Jeffries & Jeger, 1990) utilising one organism (the antagonist) to reduce disease and secondly, biomanagement (Figure 1). The latter involves studying the naturally-occurring epiphytes on the plant surfaces and the eventual aim of manipulating them to achieve disease control. The first approach delivered results and a solution within a short period of time, whereas the second approach is of longer duration, and less likely to be funded, but may provide the ultimate objective of natural control.

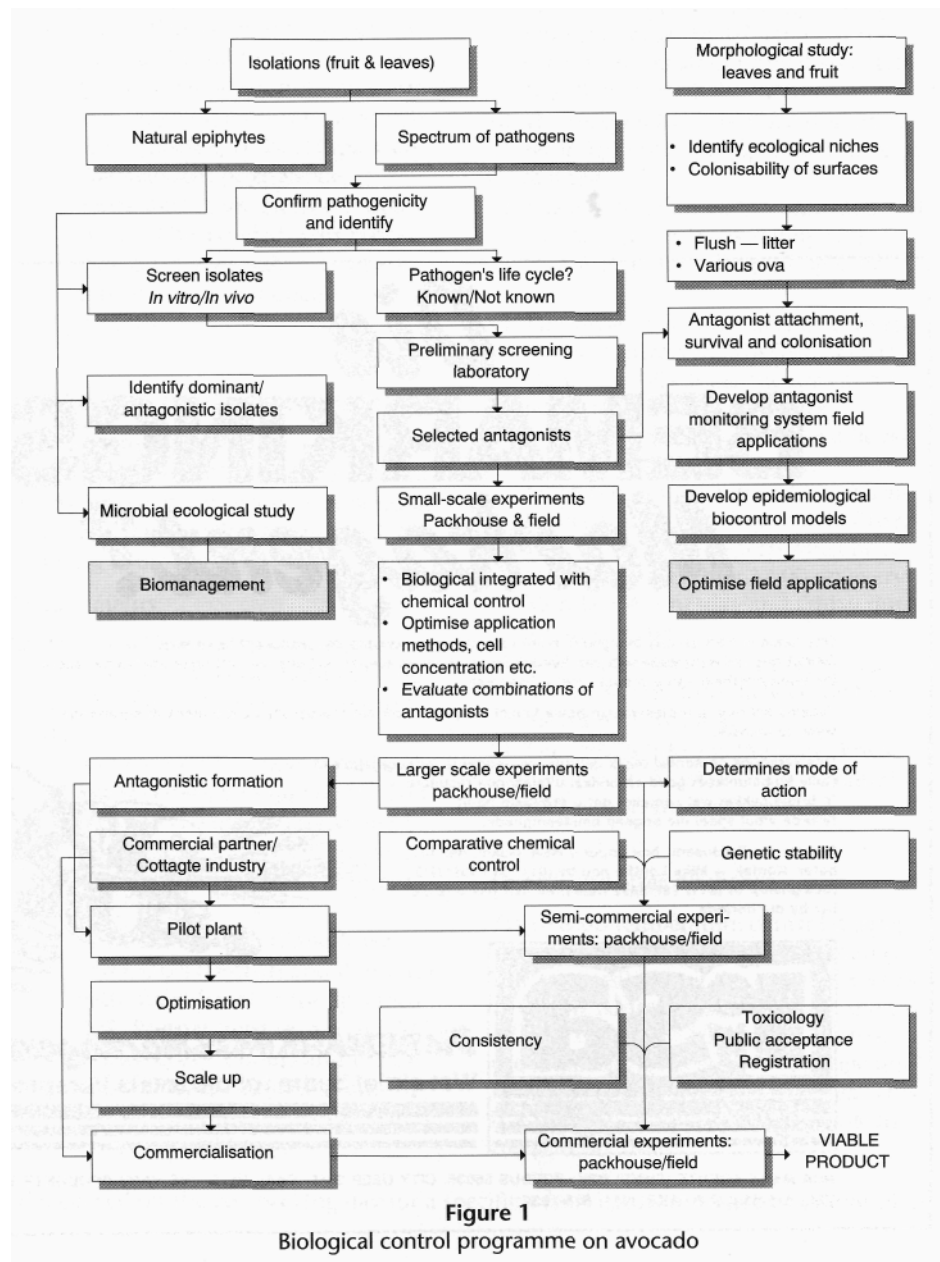


Figure 1
Biological control programme on avocado

According to Blakeman (1985) it is essential to first obtain fundamental information on the nature of the relationship between host, pathogen and associated microflora, as well as the character of the plant surface and environment (Figure 2), before initiating a biocontrol programme. Due to the complex and time-consuming nature of this approach, few researchers have opted for it. Since the advent of fungicides, producers relied heavily on chemicals to control diseases and subsequently neglected more natural ways of disease control. With the growing interest in safer and environmentally-friendly control measures, researchers are focusing more and more on existing natural disease control options (Figure 1). Due to the complexity of stem-end rot (SE) and its reported 14 pathogens which can play a role in the disease syndrome (Darvas, 1982; Darvas &

Kotzé, 1987), we decided to follow a holistic approach to selecting antagonists. Such antagonists had to be effective against the full spectrum of pathogens associated with avocado fruit diseases (Figure 2).

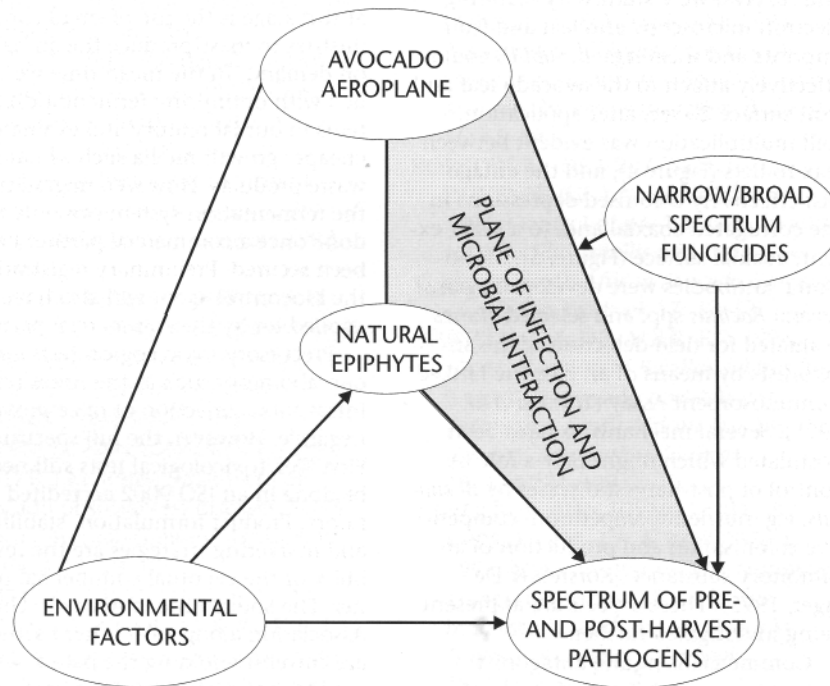


Figure 2
The disease triangle with the neglected fourth dimension representing natural epiphytes that can be manipulated to achieve natural disease control (Arrows indicate direction of intervention to obtain disease control).

The host provides the surface on which interactions between epiphytes and pathogens can occur and it also contains numerous attachment sites and micro-ecological niches for microbial colonisation (Figure 2). The avocado phylloplane represents a typical water-repellent surface, more so abaxially than adaxially due to differences in corrugation and wax rodlet composition. Especially the abaxial side provides an ideal surface for microbial colonisation due to its highly corrugated configuration with depressions and wax rodlets serving as micro niches (Figure 3).

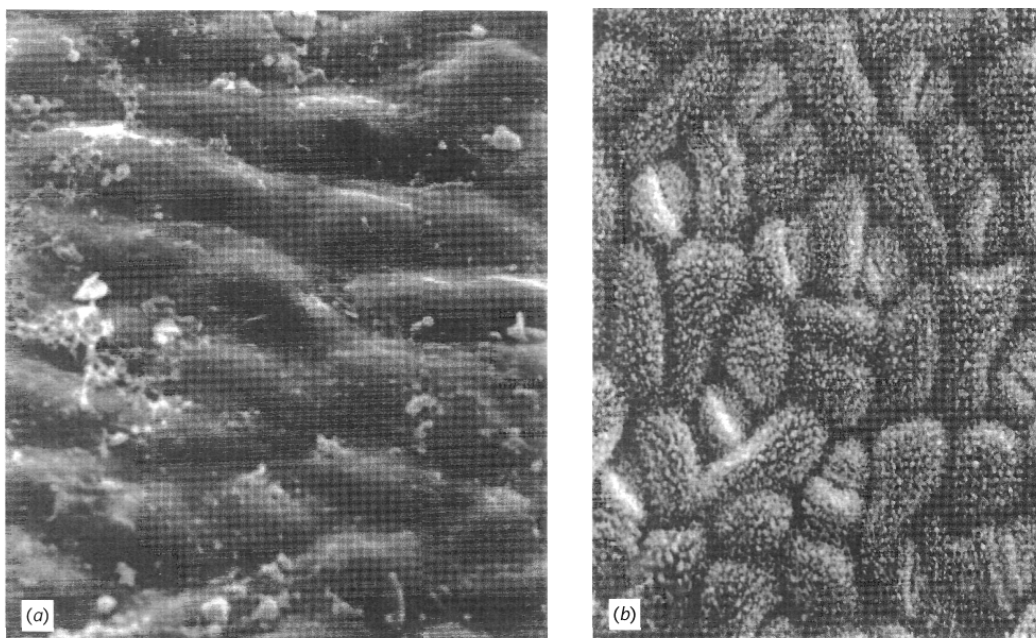


Figure 3

Scanning electron micrographs showing (a) adaxial (1 151 × mag. specimen viewed at a 45° angle) leaf surface of Fuerte avocado leaves and (b) the abaxial surface (1 151 × mag.)

Various bacteria, filamentous fungi and yeasts have been isolated from avocado surfaces using leaf imprint and leaf and fruit washing techniques (Korsten, 1993). From the 106 representative fungal and 176 dominant bacterial isolates, 36 fungi and 48 bacteria were identified to genus or species level. All isolates were screened *in vitro* against *Dothiorella aromática* (Sacc.) Petrak & Sydow according to the dual culture technique, and *in vivo* on avocado fruit. Promising isolates were further screened against the other avocado post-harvest pathogens by means of direct and indirect *in vitro* assays (Korsten, 1993). Several promising isolates were evaluated as pre-harvest field sprays and post-harvest dip applications. The most promising antagonist *Bacillus subtilis* was selected for large-scale field spray applications at Westfalia Estate and Omega. One of the most important prerequisites for commercial acceptance of a biocontrol agent is consistency and comparability with existing chemical treatments. We were able to show that a single early seasonal copper oxychloride spray followed by *B. subtilis* sprays later in the season consistently controlled cercospora spot as effectively as commercial chemical spray programmes (Korsten *et al*, 1992; 1994). Decreased spraying of copper fungicides in the integrated programme reduced build-up of visible chemical residues on fruit which would otherwise have to be removed manually in the packinghouse. In the long term, copper build-up in soils under these sprayed trees should also be less.

Post-harvestly, *B. subtilis* dip and wax applications proved effective in reducing anthracnose, *Dothiorella/Colletotrichum* fruit rot complex (DCC) and SE (Korsten, 1993). Once again consistency was evaluated over several years (Korsten *et al*, 1988; 1989; 1991; 1992; 1993; Korsten, 1993). Although control was achieved in most instances, the level of effectivity varied. It should however be kept in mind that extreme

drought conditions prevailed for several years in the major avocado-producing areas of South Africa and that those conditions influenced disease severity, which in turn affected the level of significance of treatments. In various experiments the fungicide included for comparative purposes (prochloraz), also proved ineffective (Korsten & Kotzé, 1992; Korsten *et al*, 1991; 1993; Korsten, 1993).

The antagonist was tested in various packinghouses of viz. Westfalia, Omega and Everdon, as well as in Australia and New Zealand and proved effective in controlling post-harvest diseases on Fuerte, Edranol, Hass and Ryan (Korsten, 1993; Korsten & Kotzé, 1992; Korsten *et al*, 1991; 1993 and unpublished data).

Antagonist attachment, colonisation and survival were studied by scanning electron microscopy and leaf and fruit imprints and washings. *B. subtilis* could effectively attach to the avocado leaf and fruit surface 20 sec. after application. Cell multiplication was evident between wax rodlets (Figure 4), and the antagonist effectively colonised depressions in the corrugated abaxial and, to a lesser extent, adaxial surface (Figure. 5). Monoclonal antibodies were developed against several *Bacillus* spp. and selected clones evaluated for field detection of the antagonists by means of an Enzyme Linked Immunosorbent Assay (Towsen *et al.*, 1995). Several mechanisms have been postulated which might play a role in control of post-harvest diseases by *B. subtilis*, e.g. nutrient competition, competitive colonisation and production of an inhibitory substance (Korsten & De Jager, 1995). These aspects are at present being investigated further.

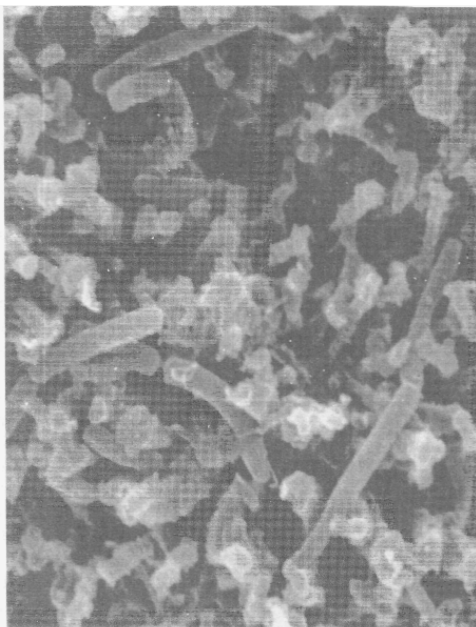


Figure 4
Bacillus subtilis colonisation of the avocado phylloplane growing between wax rodlets protruding from the abaxial surface (10 908 × mag.).



Figure 5
Bacillus subtilis colonise corrugated depressions between epidermal cells on the adaxial surface (4 040 × mag.).

Commercialising the antagonist proved the most difficult obstacle. Local agrochemical companies were not interested, mainly due to the low profit margins anticipated in local

sales and the possible difficulties in registering a natural antagonist which might produce antibiotics in or on fruit. The whole issue surrounding the possible role of antibiotics must therefore be cleared as a matter of urgent priority. We must also not forget that the Japanese daily consume *B. subtilis* in the form of a fermented soybean product called Natto. The only alternative for a commercial partner envisaged at this stage is the use of small cottage industries to mass-produce the antagonist on demand. In the mean time we continued with optimising fermentation systems in our laboratory and evaluated cheaper growth media such as natural waste products. However, upgrading of the fermentation system can only be done once a commercial partner has been secured. Preliminary registration of the biocontrol agent will also have to be applied for by the commercial partner.

Precursory toxicological tests done in our laboratory such as the Ames test and intravenous injection of mice proved negative. However, the full spectrum of First Tier Toxicological tests still need to be done in an ISO 9002 accredited laboratory. Product formulation, stability, and marketing strategies are the responsibility of the eventual commercial partner. The South African Avocado Growers' Association along with other partners is currently holding the patent with regard to formulation and application of the biocontrol agents.

During 1994 the author was invited to visit New Zealand (February) and Australia (September) with the purpose of evaluating our biocontrol agent. In both countries our *B. subtilis* antagonist could successfully control anthracnose and SE (unpublished data). Through this visit we have established a research agreement and anticipate to collaborate on biocontrol issues of mutual interest to the advantage of all three avocado industries. Future research will include continuous screening of new antagonists and determining the interaction between pathogens and natural antagonists. Understanding and eventually manipulating the natural avocado microflora remains a top priority in our biocontrol agenda. Our knowledge of the importance of various SE pathogens and their respective life cycles is still lacking. In order to optimise our biocontrol strategies, we need to understand the interactions between these pathogens, when they infect, how they are disseminated and where they survive. Once we understand the complex interactions between the pathogens and natural epiphytes, as well as their importance in the different avocado-growing areas of South Africa, we can concentrate on designing more tailor-made environmentally friendly disease control packages for individual avocado producing areas and farmers.

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