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BIOLOGICAL CONTROL OF AVOCADO POSTHARVEST DISEASES

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ABSTRACT

Bacillus subtilis *was evaluated under commercial packinghouse conditions for the control of postharvest fruit diseases of naturally infected Hass fruit.* B subtilis, *applied in commercial Tag wax at various concentrations as well as in a water dip, significantly reduced anthracnose,* Dothiorella/Colletotrichum *fruit rot complex and stem-end rot. The antagonist water dip treatment was also equally or more effective than a prochloraz water dip in controlling the postharvest diseases.*

UITTREKSEL

Bacillus subtilis *is onder kommersiéle pakhuistoestande geévalueer vir die beheer van na-oessiektes op avokado. Die antagonis, toegedien in kommersiële Tagwaks teen verskillende selkonsentrasies, asook in 'n waterdoop, het antraknose,* Dothiorella/Colletotrichum *vrugvrot-kompleks en stingelendbederf suksesvol beheer. Die antagonis waterdoop was net so effektief en selfs beter as 'n prochloras waterdoop*

INTRODUCTION

One of the most important problems facing the South African avocado industry is postharvest diseases. Losses of 36% due to anthracnose and of 13% due to stem-end rot (SE), have been recorded on the overseas market (Bezuidenhout, 1983. Fungi most commonly associated with these diseases include *Colletotrichum gloeosporioides* (Penz) Sacc, *Thyronectria pseudotrichia* (Schw) Seeler, *Phomopsis perseae* Zerova, *Lasiodiplodia theobromae* (Pat) Griffon & Maubl and *Dothiorella aromática* (Sacc) Petr & Syd (Darvas, 1985). Reasonable control of the diseases has been achieved by preharvest sprays with copper oxychloride or benomyl (Darvas, 1982), or postharvest treatment with prochloraz. However, visible spray residues on harvested fruit and build-up of pathogen resistance preclude the continued use of these compounds. Investigation of alternative disease control measures is therefore urgently required. One such alternative is biological control, which has been applied successfully on several fruit and some vegetable crops (Wilson & Wisniewski, 1989). Biological control of avocado postharvest diseases has also been achieved with pre and postharvest applications of *Bacillus* spp (Korsten, Bezuidenhout & Kotzé, 1988; 1989).

In this paper, evidence is presented of the biological control of avocado postharvest diseases by *Bacillus subtilis,* applied in semi-commercial water dip experiments in the packinghouse. For the optimisation studies the antagonist was incorporated into Tag

wax at different cell concentrations.

MATERIALS AND METHODS

Bacillus subtilis isolate A6, originally isolated from the avocado phylloplane (Korsten *et al,* 1988), was selected for packinghouse treatments due to its strong inhibitory action against *C gloeosporioides, D aromatica, T pseudotrichia, P persea* and *L theobromae* (Korsten *et al,* 1989). Batches of antagonist were produced, harvested (Korsten *et al,* 1988), lyophilised and stored until required for packinghouse treatments.

Postharvest treatments

Packinghouse treatments were carried out at Westfalia Estate, north-eastern Transvaal on Fuerte avocado fruit. Three plastic packing crates containing between 100 — 160 hand culled fruit were dipped into the various treatments. Dipping was achieved by lowering each crate into a 100 fibre glass box containing 25 ℓ treatment solution and Agral 90, a commercial sticker, added at the registered rate. The treatments consisted of:

1 Untreated control.

2 Dipping fruit in tap water to represent the water dip control.

3 Dipping fruit into a prochloraz [Omega 45% ai EC, FBC (Pty) Ltd, Chloorkop *SA*] solution at the recommended rate (0,5 g ai/ℓ tap water).

4 Dipping fruit into a B *subtilis* suspension at 2,1 x 10^7 cells/mℓ.

5 Or dipping fruit into a half-strength (1 x 10^7 cells/mℓ) B *subtilis* solution.

Fruit from the treatments were packed separately and stored at 4°C for 28 days to simulate export conditions. It was ripened at ambient temperature (22°C) before being evaluated for anthracnose; *Dothiorella/Colletotrichum* fruit rot complex (DCC) and SE. Each fruit was assessed externally for anthracnose and DCC and internally for SE severity on a 0 to 10 scale, with 0 being healthy and 10 representing entire fruit decay. To avoid bias, these evaluations were conducted by three independent assessors. Data were analysed statistically by Duncan's new multiple range test.

For the optimisation experiment, 15 packing crates containing Hass avocado fruit, were randomly removed from the commercial packing line and transported to the laboratory, B *subtilis* was incorporated into Tag wax to final concentrations of 10^6, 10^6, 10^7 and 10^8 cells/mℓ. Three packing crates were used for each treatment. An untreated control served as means of comparison. The Tag wax was applied manually using a portable spray bottle, and spraying individual fruit until completely covered with the antagonist suspension. Fruit from the treatments were packed separately and stored at room temperature before being evaluated for postharvest diseases as described before.

Antagonist survival in Tag wax

Survival of B *subtilis* in Tag wax was determined by inoculating 1 mℓ of a suspension

containing either 7,05 x 10^7 freshly harvested vegetative cells, 1,35 x 10^8 freshly harvested spores or 3 x 10^7 lyophilised cells into a glass test tube containing 9 mℓ Tag wax. Three replicates of five tubes were used for each treatment. Tubes were incubated at 25°C and 1 mℓ of agitated Tag-antagonist suspension was diluted, using the standard dilution series technique of Herrigan & McCance (1966), to determine viable counts at 2 h intervals after inoculation. The Tag-antagonist suspension was also prepared for scanning electron microscopy according to the method described by Hayat (1981).

RESULTS

The two B *subtilis* dip treatments controlled anthracnose and DCC externally and internally, and reduced severity of SE internally (Table 1). Prochloraz was effective against anthracnose and DCC, but not against SE. However, the lower B *subtilis* concentration treatment (1 x 10^7 cells/mℓ) was significantly more effective than prochloraz in controlling external anthracnose (Table 1). The water dip control significantly increased external anthracnose above the level of the control (Table 1).

All antagonist concentrations in Tag wax effectively controlled external anthracnose, but only the higher two concentrations (10^7 and 10^8 cells/mℓ) reduced anthracnose severity internally (Table 2). The higher three concentrations (10^6, 10^7 and 10^8 cells/mℓ also controlled external SE.

Means within columns followed by the same letter do not differ significantly (P = 0,01) according to Duncan's new multiple range test. Values indicate mean disease severity. Fruit was evaluated on a 0 — 10 scale, 0 being healthy, and 10 representing entire fruit decay.

TABLE 1 Effect of *Bacillus subtilis* and prochloraz dip treatments on postharvest diseases of Fuerte avocado fruit

Treatment (a)	External evaluation			Internal evaluation		
	No of fruit	Anthracnose	DCC (b)	SE (b)	Anthracnose	DCC (b)
Control no dip	115	1,67b	2,17a	0,85a	1,21a	0,23a
Control water dip	114	2,27a	1,91a	0,73a	0,97a	0,17ab
Prochloraz	129	1,20bc	0,54b	0,66ab	0,34b	0,06b
B subtilis (c)	136	0,88cd	0,38b	0,21c	0,26b	0,04b
B subtilis (d)	130	0,44d	0,23b	0,35bc	0,23b	0,04b
Total no of fruit	**622**					
PR > F		0,0001	0,0001	0,0002	0,0001	0,0133

(a) After the dip treatments all fruit was commercially Tag waxed.
(b) DCC = *Dothiorella/Colletotrichum* fruit rot complex; SE = stem-end rot.
(c) *B subtilis* dip in 2,1 x 10^7 cells/mℓ.
(d) *B subtilis* dip in 1 x 10^7 cells/mℓ.

TABLE 2 Effect of *Bacillus subtilis* incorporated into Tag wax at various cell concentrations on postharvest diseases of Hass avocado fruit

Treatment	External evaluation			Internal evaluation		
	No of fruit	Anthracnose	DCC	SE (a)	Anthracnose (a)	DCC (a)
Control no treatment	134	1,02a	0,10a	1,43a	2,09a	0,36a
B subtilis 10^8 (b)	134	0,34bc	0,01b	1,39a	1,51b	0,17ab
B subtilis 10^7 (b)	139	0,40bc	0,01b	0,93b	1,41b	0,25ab
B subtilis 10^6 (b)	133	0,21c	0,01b	1,17ab	1,62ab	0,04b
B subtilis 10^5 (b)	135	0,61b	0,03ab	1,10ab	1,86ab	0,10b
Total no of fruit	**675**					
PR > F		0,0001	0,0480	0,0655	0,0702	0,0243

(a) SE = stem-end rot; DCC = *Dothiorella/Colletotrichum* fruit rot complex.
(b) *B subtilis* cell concentration/mℓ Tag commercial wax.

Means within columns followed by the same letter do not differ significantly (P = 0,01) according to Duncan's new multiple range test. Values indicate mean disease severity. Fruit was evaluated on a 0 — 10. Scale, 0 being healthy and 10 representing entire fruit decay.

The 10^7 cells/mℓ concentration reduced SE severity internally (Table 2). Internal DCC severity was reduced significantly only by the lower two concentrations of 10^5 and 10^6 cells/mℓ.

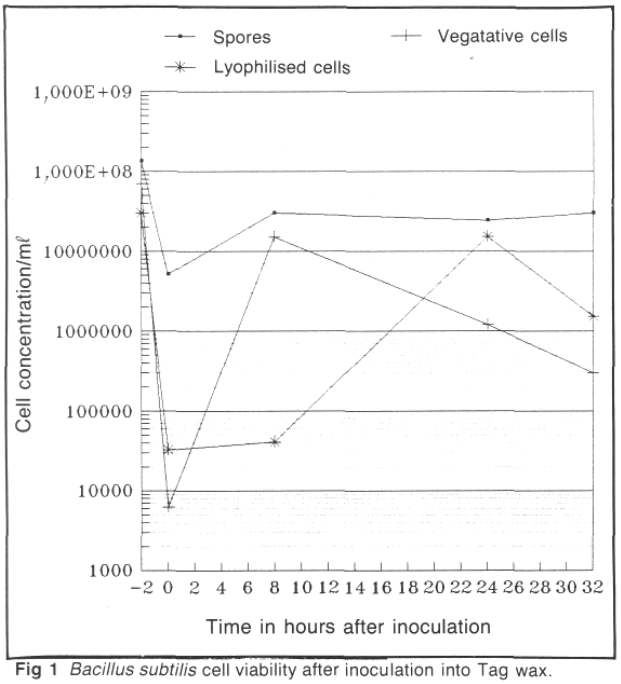

Fig 1 *Bacillus subtilis* cell viability after inoculation into Tag wax.

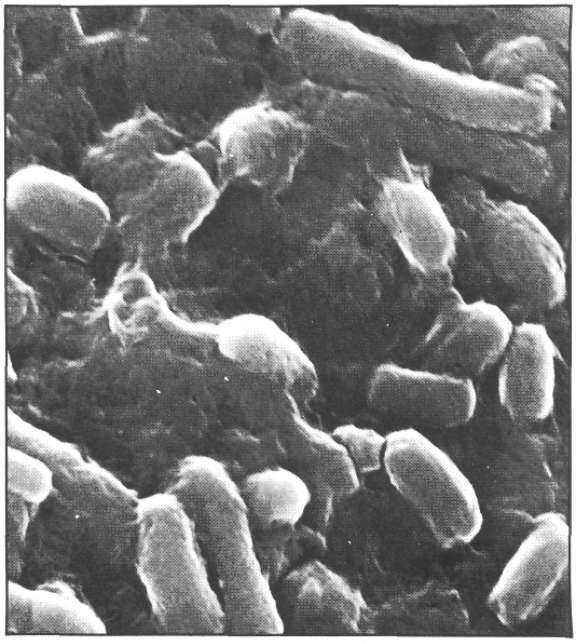

Fig 2 *Bacillus subtilis* vegetative cells in Tag wax as seen under the scanning electron microscope.

Antagonist survival in Tag wax

Although there was an initial drop in viable cell counts immediately after inoculation of Tag wax with the antagonists, cell numbers eventually increased to the same level as that of the original inoculum (Figure 1). The antagonist can therefore survive and multiply in the Tag wax (Figures 1 and 2).

DISCUSSION

For biological control to be accepted by industry, it has to be as effective as the best fungicide available (Baker and Cook, 1974). This investigation clearly showed that postharvest application of antagonistic *B subtilis* controlled anthracnose, DCC and SE on Fuerte avocado fruit as effectively, and even better than prochloraz. The latter is presently regarded as the best fungicide available for controlling some of these diseases (Darvas, 1985). Since most fruit have a number of important pathogens, controlling one only may merely favour another (Janisiewicz, 1988). The antagonist's wider spectrum of activity therefore enhances the superiority of biological control and establishes biocontrol as a viable alternative to the use of chemicals for the control of avocado postharvest diseases.

Furthermore, the South African avocado industry does not currently have a market-acceptable fungicide for postharvest applications at its disposal, since prochloraz has not been cleared for most export markets. However, registration and market acceptability of the biological control agents must still be established before the industry can commence evaluating the feasibility of this alternative control measure. As far as could be established none of the postharvest diseases evaluated in this investigation

have been successfully controlled in other crops with a similar biocontrol approach. Contrary to the work of Janisiewicz (1988), increasing antagonist concentrations did not necessarily improve disease control. Nevertheless the antagonist was capable of surviving in Tag wax. Packinghouses can thus mix the antagonists into wax in the morning and apply it throughout the day. The observation that dipping in water increased anthracnose severity is in accordance with the findings by Darvas (1982), who ascribed the phenomenon to a moisture effect. However, it should be remembered that "moist pockets" could also favour the antagonist, thereby enhancing disease control.

Several criteria such as market acceptability and registrability must still be met before biological control will be accepted for use on the postharvest level. Once this has been accomplished, environmentally friendly alternative disease control measures will be available for the industry.

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