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Effect of *Bacillus subtilis* and fungicide sprays for control of preharvest diseases of avocado

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ABSTRACT

Preharvest sprays with Bacillus subtilis, *on its own and integrated with copper oxychloride, reduced the severity of avocado black spot, caused by* Pseudocercospora purpurea *in three field experiments. However, control was not as effective as with copper oxychloride spraying only, or as with copper oxychloride and benomyl. Sooty blotch, caused by* Akaropeltopsis *sp was reduced by* B subtilis *integrated with copper oxychloride in one experiment only.*

INTRODUCTION

Black spot (BS), caused by *Pseudocercospora purpurea* (Cke) Deighton (Darvas, 1982) and sooty blotch (SB) caused by *Akaropeltopsis* sp (Smith *et al,* 1987), are the two most important preharvest fruit diseases of avocado *(Persea americana* Mill) in South Africa.

BS can cause losses of up to 69% in untreated orchards (Darvas & Kotzé, 1987), whereas SB lowers the market value of fruit due to unsightly discolouration of the skin (Pieterse, 1986). Chemical control of these diseases has been attempted (Darvas & Kotzé, 1987), with the accompanying disadvantages such as spray residues on harvested fruit (Denner & Kotzé, 1986), build up of pathogen resistance (Darvas & Kotzé, 1987) and growing international concern about environmental pollution.

The authors recently reported on the successful control of postharvest diseases of avocado such as anthracnose, *Dothiorella/Colletotrichum* fruit rot complex and stem-end rot with pre-harvest *Bacillus subtilis* (Ehrenberg) Cohn sprays (Korsten *et al,* 1989). Various reports (Knudsen & Spurr, 1987; Baker *etal,* 1985; Gullino & Garibal di, 1988) indicate that pre-harvest diseases can also be controlled biologically. This paper, therefore, presents evidence on the effect of *B subtilis,* either on its own or integrated with fungicide applications, on BS and SB.

MATERIALS AND METHODS

B subtilis (isolate B246), isolated from the avocado phylloplane and with *in vitro* inhibiting action against various avocado postharvest pathogens, was cultured, harvested, lyophilised and stored as described previously (Korsten *et al,* 1988). Experiments were conducted at three localities, viz Waterval (block 4) and block 34B at

Westfalia Estate, and Omega near Burgershall. At each site, 20 mature Fuerte trees were randomly selected and treated as described in Table 1. Five single tree replicates were used per treatment, and 100 ℓ of each treatment solution were sprayed with high volume ground sprayers, to full coverage per tree at each application date. Nu-Film 17 (Hygrotech Seed) was added to all treatment solutions at the registered rate of 0,02% (v/v). At least 30 fruit were harvested at random from each tree in April 1991, pooled and evaluated for BS on a 0-3 scale and for SB on a 0-4 scale as described by Lonsdale (1991). Data were subjected to analysis of variance and treatment comparisons were made using Duncan's multiple range test.

RESULTS

B subtilis, as well as the integrated treatment significantly reduced BS at all three localities (Tables 2, 3 and 4). The integrated programme was superior at block 34B and at Omega. However, the best control was achieved with fungicides only. The severity of SB was reduced by copper oxychloride and the integrated treatment at block 34B, and by both chemical treatments at Omega. At the latter locality, treating trees with benomyl followed by *B subtilis* resulted in a significant increase in SB.

DISCUSSION

This investigation showed that the pre harvest application of *B subtilis* on it's own and integrated with fungicide spraying, effectively reduces the severity of BS, but that control was not comparable to that achieved by fungicide programmes without the antagonist. The better results obtained with the integrated programme than with the antagonist, only underlines the efficacy of chemical control, and corresponds with previous findings where bean rust and Cercospora spot of peanut were controlled biologically, but less effectively than by commercially applied fungicides (Baker *et al,* 1985; Knudsen & Spurr, 1987). How ever, considering the exceptional performance of *B subtilis* applied pre-harvestly against postharvest avocado diseases (Korsten *et al,* 1988), it is anticipated that the use of this antagonist against both pre- and postharvest diseases could be beneficial from a cost-effective viewpoint.

TABLE 1 Treatment regimes for pre-harvest application of *Bacillus subtilis* (on it's own and integrated with chemical sprays)

Area Treatment	Composition	Concentration	Oct	Nov	Dec	Jan	March
WESTFALIA							
Block 34B							
1 Control	–	–	–	–	–	–	–
2 Chemical	Copper-oxychloride	255 g ai/100 ℓ	–	+	–	+	–
3 Integrated	Copper-oxychloride	255 g ai/100 ℓ	–	+	–	–	–
followed by	*B subtilis*	1×10^7 cells/mℓ	–	–	+	+	+
4 Biological	*B subtilis*	1×10^7 cells/mℓ	–	–	+	+	+
Waterval (block 4)							
1 Control	–	–	–	–	–	–	–
2 Chemical	Copper-oxychloride	255 g ai/100 ℓ	+	+	–	+	–
3 Integrated	Copper-oxychloride	255 g ai/100 ℓ	+	–	–	–	–
followed by	*B subtilis*	1×10^7 cells/mℓ	–	–	+	+	–
4 Biological	*B subtilis*	1×10^7 cells/mℓ	–	+	+	+	–
OMEGA							
1 Control	–	–	–	–	–	–	–
2 Chemical 1	Copper-oxychloride	255 g ai/100 ℓ	+	+	–	+	–
3 Chemical 2	Benomyl	50 g ai/100 ℓ	+	–	–	–	–
followed by	Copper-oxychloride	255 g ai/100 ℓ	–	+	–	+	–
Integrated	Copper-oxychloride	255 g ai/100 ℓ	+	–	–	–	–
4 followed by	*B subtilis*	1×10^7 cells/mℓ	–	–	+	+	+

a) *Bacillus* spray treatments were prepared by suspending lyophilised bacteria in 1 ℓ Standard 1 nutrient broth (STD) (Biolab), before mixing into a spray tank containing 1 500 ℓ tap water to a final concentration of 10^7 cells/mℓ. Copper-oxychloride 85% WP (Demildex) and benomyl 50% WG (Benlate) suspensions were prepared at the registered rate in separate spray tanks.

TABLE 2 Effect of *Bacillus subtilis* applied on it's own or integrated with copper-oxychloride pre-harvest sprays, on pre-harvest diseases of avocado at block 34B, Westfalia Estate

Treatment	No of fruit evaluated	Black spot	Sooty blotch
Control	150	1,25 a	0,69 a
Chemical [b]	150	0,18 d	0,44 b
Integrated [b]	151	0,64 c	0,51 b
B Subtilis	150	0,93 b	0,67 a
Total no fruit	601		
PR > F		0,0001	0,0019

a Copper-oxychloride
b Copper-oxychloride followed by *B subtilis* application

Means within columns followed by the same letter do not differ signficatnly (P = 0,05), according to Duncan's multiple range test. Values represent mean disease severity. Fruit was evaluated on a 0–3 scale for black spot and on a 0–4 scale for sooty blotch (Lonsdale, 1991).

Furthermore, it should be kept in mind that timing of application is of crucial importance in biological Control programmes (Bhatt & Vaughan, 1962). Application dates in the present investigation were mainly based on epidemiological data for *P purpurea,* as determined at Westfalia Estate (Darvas, 1982). Subsequently Lonsdale and Scott (1991) showed that spore release by the pathogen at Burgershall occurs earlier than at Westfalia. Optimised application aimed at establishing the antagonist before arrival *oí P purpurea* inoculum, could in future experiments result in enhanced control.

In contrast to it's effect on BS, *B subtilis* appeared to be ineffective against SB. Also the integrated treatment produced variable results, reducing 'SB in one experiment, giving no control in a second, and actually in creased disease severity in a third. At present the removal of epiphytic growth of *Akaropeltopsis* from fruit by chlorine rinsing in the packhouse (Bezuidenhout, 1991) thus remains the most effective means of controlling SB. Nevertheless, the results obtained at Omega suggest that SB could be an iatrogenic disease, i.e. one which in creases in severity as other diseases are controlled (Griffiths, 1981). This is an aspect which should be monitored in future control experiments.

TABLE 3 Effect of *Bucillus subtilis* applied on it's own or integrated with copper-oxychloride pre-harvest sprays, on pre-harvest diseases of avocado at Waterval (block 4), Westfalia Estate

Treatment	No of fruit evaluated	Black spot	Sooty blotch
Control	150	1,77 a	1,01 a
Chemical	150	0,42 c	1,01 a
Integrated [a]	150	1,10 b	1,17 a
Biological [b]	150	1,08 b	1,21 a
Total no fruit	600		
PR > F		0,0001	0,0578

a Copper-oxychloride
b Copper-oxychloride followed by *B subtilis* application

Means within columns followed by the same letter do not differ significatnly (P = 0,05), according to Duncan's multipĺe range test. Values represent mean disease severity. Fruit was evaluated on a 0–3 scale for black spot and on a 0–4 scale for sooty blotch (Lonsdale, 1991).

TABLE 4 Effect of *Bacillus subtilis* integrated with copper-oxychloride pre-harvest sprays on pre-harvest diseases of avocado at Omega

Treatment	No of fruit evaluated	Black spot	Sooty blotch
Control 1	155	1,89 a	1,27 b
Chemical 1[a]	152	0,36 c	0,42 c
Chemical 2[b]	152	0,44 c	0,58 c
Integrated [c]	152	0,72 b	1,85 a
Total no fruit	611	0,0001	0,0001
PR > F			

a Copper-oxychloride
b Benomyl followed by copper-oxychloride application
c Copper-oxychloride followed by *B subtilis* application

Means within columns followed by the same letter do not differ significantly (P = 0,05), according to Duncan's multiple range test. Values represent mean disease severity. Fruit was evaluated on a 0–3 scale for black spot and on a 0–4 scale for sooty blotch (Lonsdale, 1991).

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