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POSTHARVEST BIOLOGICAL CONTROL OF AVOCADO FRUIT DISEASES

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SUMMARY

Bacillus subtilis *on its own or integrated with prochloraz Tag-wax application was evaluated for control of avocado postharvest diseases anthracnose,* Dothiorella/ Colletotrichum *fruit rot complex (DCC) and stem-end rot (SE). Tag-waxed fruit and/ or fruit treated with prochloraz incorporated into Tag-wax, served as controls. The biological and integrated treatments were as effective as the prochloraz Tag-wax treatment in controlling DCC on Fuerte, and anthracnose and SE on Mass. In addition, the integrated treatment also effectively controlled SE on Fuerte and Ryan, at Westfalia Estate packhouse. Comparing a* B. subtilis *application at H.L. Hall & Sons, anthracnose and SE could effectively be controlled in two separate experiments.*

OPSOMMING

Bacillus subtilis *opsyeie ofge'inkorporeerin 'nprochloraz Tag-waks toediening was geëvalueer vir beheer van avokado na-oes siektes antraknose,* Dothiorella/ Colletotrichum *vrugte vrot kompleks (DCK) en stingelendbederf (SE). Tag-waks behandelde vrugte of vrugte behandel met prochloraz ge'inkorporeer in Tag-waks, net gedien as kontroles. Die biologiese en ge'integreerde behandelings was net so effektief as die prochloraz Tag-waks behandelings en kon DCK op Fuerte en antracnose en SE op Hass beheer. Verderhet die ge'integreerde behandeling ook SE by Fuerte en Ryan beheer soos geévalueer by Westfalia Landgoed pakhuis.*

INTRODUCTION

Avocado (*Persea americana* Mill.) is affected by various postharvest diseases, including anthracnose caused by *Colletotrichum gloeosporioides* (Penzig) Sacc., *Dothiorella/Colletotrichum fruit* rot complex (DCC) caused by C. gloeosporioides and *Dothiorella aromática* (Sacc.) Petr. & Syd., and stem-end rot (SE) caused mainly by *Thyronectria pseudotrichia* (Schw.) Seeler, *C. gloeosporioides, D. aromática* and *Phomopsis perseae* Zerova (Darvas & Kotzé, 1987). The incidence of anthracnose and SE in overseas consignments of fruit has been reported to be as high as 29% and 15% respectively (Bezuidenhout & Kuschke, 1983). Limited control of avocado postharvest diseases can be achieved by preharvest sprays with copper oxychloride or benomyl, and by postharvest applications of prochloraz (Darvas *et al.,* 1987) or thiabendazole (Vermeulen *et al.,* 1992). However, copper oxychloride leaves visible spray residues on fruit, which if not removed can result in rejection of export fruit (Pieterse, 1986). In addition, continuous use of benomyl may result in pathogen resistance (Darvas & Kotzé, 1987), while prochloraz has not been given product clearance on the French market (Anderson, 1986).

Fruit diseases have been successfully controlled with postharvest applications of *Bacillus* spp. eg. brown rot and Alternaria rot caused respectively by *Monilinia fructicola* (Wint.) Honey and *Alternaría alternata* (Fr.) Keissl. respectively on cherries (Utkhede & Scholberg, 1986), green mould, sour rot and Alternaría rot caused respectively by *Penicillium digitatum* (Pers. Fr.) Sacc., *Geotrichum condidum* Link ex Lemon and *Alternaría citri* Ellis & Pierce on citrus (Singh & Deverall, 1984), anthracnose caused by *C. gloeosporioides on* mango (Korsten *et al*., 1991b) and avocado (Korsten *et al*., 1988; 1989), DCC and SE on avocado (Korsten *et al.,* 1988; 1989), brown rot caused by *M. fructicola* on stone fruit and Rhizopus rot caused by *Rhizopus stolonifer* Ehrenb. ex Fr. Vuill on peach. (Pusey & Wilson, 1984), grey mould caused by *Botrytis cinérea* Pers ex Mocea & Balb on grape (Ferreira, 1990) and postharvest rot caused by *Phomopsis* sp., *Penicillium,* sp., *Pestalotiasp.* and *C. gloeosporioides* on litchi (Korsten *et al.,* 1993). However, only Droby *et al.* (1991) and Korsten *et al.* (1991 b; 1993) have succeeded in controlling natural infections of postharvest pathogens under simulated packinghouse conditions.

Although previous reports dealt with the successful control of avocado postharvest diseases (Korsten *et al.,* 1988; 1989) this paper describes the evaluation of the consistency of biocontrol using as antagonist of *Bacillus subtilis* (Ehrenberg) Cohn (isolate B246). In addition the effectivity in another packhouse and with various other cvs viz Fuerte, Ryan and Hass were also determined of *B. subtilis.*

MATERIALS AND METHODS

Preparation of bacterial antagonists for treatment of packhouse avocados

B. subtilis isolated from avocado leaf and fruit surfaces as described by Korsten *et al.,* (1988), was selected due to its strong inhibitory action against avocado postharvest pathogens and its effective control of avocado postharvest diseases anthracnose, DCC and SE (Korsten, 1993). Antagonists were maintained and stock cultures prepared for packhouse applications as described (Korsten *et al.,* 1988). However, antagonist pellets harvested after centrifugation were mixed with 15% Glycerol Ringers (Merck) mixture and were stored at 4 °C until required for packhouse treatments which was usually within a day or two.

Packhouse experiments

Treatment regimes are given in Table 1. One hundred ml antagonist Glycerol Ringers mixture was incorporated into 900 ml Tag-wax (polyethylene) (ICI, Johannesburg) to obtain a final concentration of 10^7 cells/ml. The antagonist Tag-wax mixture was applied by hand to the fruit with a sponge ensuring complete coverage of each fruit. Control fruit received only a Tag-wax application as described for the antagonist-Tag-wax mixture. In certain experiments prochloraz (Omega 45% a.i. E.G., FBC (Pty) Ltd Chloorkop, S.A.)

was added at 0,5 ml to 1C. Tag-wax to represent a chemical treatment. Prochloraz was also added at quarter strength to an antagonist Tag wax mixture to represent an integrated treatment. Treatments were done in a packhouse at Westfalia Estate using Fuerte fruit on the 30th April, Hass on the 9th July and Ryan on the 12th August. At H.L. Hall & Sons (Pty) Ltd packhouse, Fuerte fruit were used on the 25th June and 10th August. For these experiments approximately 140 fruit (depending on the size), were randomly selected from the packing line prior to commercial treatment.

Fruit from each treatment was randomly packed in 10 standard carton boxes, and the boxes stored at 5.5 °C for 28 days in rooms at either Westfalia Estate or H.L. Hall & Sons before being transported to the laboratory for ripening and evaluating. Ripening was at 22-28 °C for ca 7d, and postharvest disease severity was evaluated at ready-to-eat ripeness. Each fruit was evaluated for external and internal anthracnose, DCC and SE on a 0-10 scale, with 0 being healthy and 10 indicating entire fruit decay (Darvasef a/., 1987). Data ware ranked and subjected to Kruskal Wallis and subsequently Duncan's multiple range tests.

RESULTS

Effect of postharvest Tag-wax treatment with prochloraz, *Bacillus subtilis* on its own, or integrated with prochloraz on postharvest diseases of Fuerte, Mass and Ryan avocado fruit at Westfalia Estate.

With the first Fuerte experiment no significant difference between treatments were found for anthracnose (P=0,2120), while all treatments (T1.2; T1.3; T1.4) effectively controlled DCC compared to the control (Fig. 1). S.E. was effectively controlled with only the integrated treatment (Fig. 1).

All treatments (T1.2; T1.3; T1.4) significantly reduced anthracnose and SE of Hass avocado (Fig.2), while none of the treatments had any significant effect on DCC (p=1,0000). Only SE was effectively controlled with the integrated treatment (Fig. 3) on Ryan avocados, while none of the treatments (T1.2; T1.3; T1.4) had any significant effect on anthracnose (P=0,1524) or DCC (p=0,1285).

Effect of postharvest Tag-wax treatment with *Bacillus subtilis* on postharvest diseases of Fuerte avocado fruit at H.L. Hall & Sons

B. subtilis Tag-wax applications effectively controlled anthracnose and SE (Fig. 4) but had no significant effect on DCC (P=0,0994). Similar results were obtained when the experiment was repeated (Fig. 5), with only DCC not being effectively reduced (P=0,1160).

TABLE 1 Treatments regimes for various postharvest avocado fruit treatments with Bacillus subtilis on its own integrated on with quarter-strength prochloraz or prochloraz Tag-wax applications.

Code	Treatment	Concentration
Effect of postharvest Tag-wax treatments with prochloraz, *Bacillus subtilis* on its own, or integrated with prochloraz on postharvest disease of Fuerte, Hass and Ryan avocado fruit at Westfalia Estate		
T1.1	Control	Tag-wax only
T1.2	Prochloraz	0.5 ml /ℓ Tag-wax
T1.3	*B. subtilis*	101 cells/ml Tag-wax mixture
T1.4	Prochloraz +	0,1 ml/ ℓ Tag-wax
	B. subtilis	101 cells/ml Tag-wax
Effect of postharvest Tag-wax treatment with *Bacillus subtilis* on avocado postharvest diseases of Fuerte avocado fruit at H.L. Hall & Sons		
T2.1	Control	Tag-wax only
T2.2	B. subtilis	101 cells/ml Tag-wax

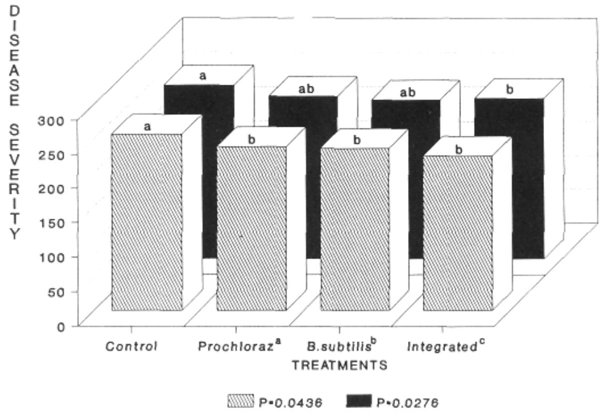
DISCUSSION

B. subtilis Tag-wax applications effectively controlled DCC, anthracnose and SE, on Fuerte avocado fruit. Korsten & Kotzé (1992) previously reported similar successful control of all three postharvest diseases on Fuerte avocado, using however dip applications, *B. subtilis* is also known to control several other fruit diseases e.g. brawn rot of apricot, nectarine, peach and plum (Pusey & Wilson, 1984), Rhizopus rot of peach (Pusey & Wilson, 1984), blackspot of mango (Pruvost & Luisetti, 1991), Alternaria rot, sour rot and green mould of citrus (Singh & Deverall, 1984) and Alternaría and brown rot of cherry (Utkhede & Scholberg, 1986). Evaluating the effectiveness of *B. subtilis* on other avocado cvs, less consistent results were obtained. In this study, *B. subtilis* Tag-wax applications effectively controlled anthracnose and SE on Hass, while in a previous study Korsten *et al.* (1991a), it effectively controlled all three postharvest diseases. It is however important to note that in the case of the latter study, the antagonist Tag-wax mixture was applied on the commercial packing line compared to this study where application was by hand. Furthermore, the incidence of DCC was very low during the 1992 season which could also explain the ineffectiveness of the treatment.

In contrast, hand applied *B. subtilis* Tagwax treatments were ineffective in controlling postharvest diseases on Ryan. Pusey & Wilson (1984) found similar variation in effectiveness when *B. subtilis was* sprayed onto different stonefruits. According to them, variation in effectiveness was possible due to differences in morphological features of the fruit surfaces such as trichomes, which could have improved adherence of bacteria on certain fruits. However, Janisiewicz & Marchi (1992) who reported on similar variation in effectiveness when different pear cvs were dipped in *Pseudomonas syringae* Van Hall, and attributed this difference to physiological processes, governing antagonism. Furthermore, its important to keep in mind that variation in susceptibility between cvs is a well-known phenomena with Fuerte being particularly susceptible to postharvest diseases (Jacobs, 1974). It is also known that variation in the incidence of avocado postharvest diseases occurs throughout the season. For instance, Darvas (1982) found a decrease in incidence of postharvest disease incited by *C. gloeosporioides* and *D. aromatica.* Thus a lower incidence of postharvest diseases can be expected with the late seasonal cvs Hass and Ryan compared to Fuerte. Furthermore, it has been shown that natural infections of avocado fruit pathogens were related to rainfall and in particular the duration of rainy periods (Peterson, 1978). Because of the drought in 1992 when tests were conducted, postharvest disease pressure was low as was evident in all tests, making it difficult to accurately evaluate biocontrol effectiveness. In addition, Darvas (1982) who evaluated chemical control of avocado postharvest diseases, over a range of experiments reported tremendous variation in development of these diseases, which were possibly influenced by a number of factors such as climate, area etc. These variations according to him often resulted in statistical non-significant readings of experiments, committing the author to use tendencies as guidelines.

Notwithstanding the drought conditions under which experiments were done, and the variation in effectiveness between cvs which could have been influenced by various aspects as mentioned before, biocontrol showed promise in particular when integrated with quarter strength prochloraz. This integrated approach could also effectively control SE on Fuerte and Ryan which neither the biological or chemical treatments could achieve. Similarly, Korsten *et al.* (1993) reported more effective control of anthracnose and DCC and equal effective control of SE when *Bacillus licheniformis* was integrated with quarter strength prochloraz in dip treatments. The integrated strategy therefore holds promise if use of the fungicide prochloraz is to be reduced, but not excluded.

In this study, *B. subtilis* proved equally effective to prochloraz Tag-wax applications, which is in agreement with previous reports by the authors (Korsten *et al*., 1991 a). Repeating the *B. subtilis* Tagwax applications at another packhouse under other circumstances, resulted in similar effective control of anthracnose and SE. The consistent performance of the antagonist on Fuerte over this and previous seasons (Korsten, 1993), makes biological control of avocado postharvest diseases a viable option for alternative disease control.



■ *Dothiorella/Colletorichum* fruit rot complex (p=0,0276)

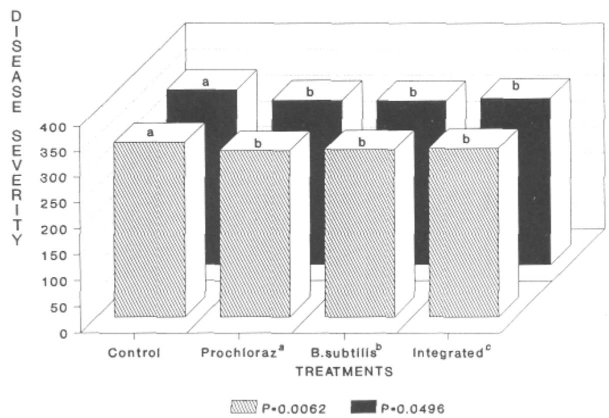
□ Stem-end rot (p=0,0436)

[a] Prochloraz at 0.5mℓ/ℓ Tag-wax

[b] *B. subtilis* at 10^7 cells/mℓ Tag-wax mixture

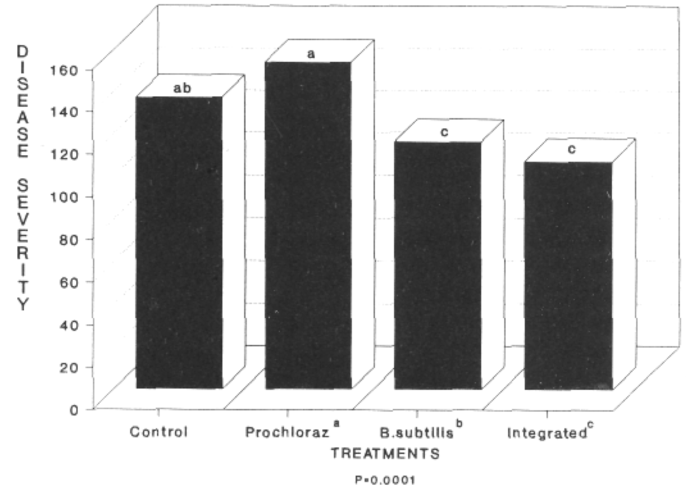
[c] Combination of prochloraz (0,1 mℓ/ℓ Tag-wax) with *B. subtilis* at 10^7cells/mℓ

FIG. 1 Effect of prochloraz, *Bacillus subtilis* on its own or integrated with prochloraz on postharvest diseases of Fuerte avocado, when fruit was Tag-waxed at Westfalia Estate.



■ Anthracnose (p=0,0062)
□ Stem-end rot (p=0,0496)
[a] Prochloraz at 0.5mℓ/ℓ Tag-wax
[b] *B. subtilis* at 10^7 cells/mℓ Tag-wax mixture
[c] Combination of prochloraz (0,1 mℓ/ℓ Tag-wax) with *B. subtilis* at 0^7 cells/mℓ

FIG. 2 Effect of prochloraz, *Bacillus subtilis* on its own or integrated with prochloraz on postharvest diseases of Hass avocado, when fruit was Tag-waxed at Westfalia Estate.

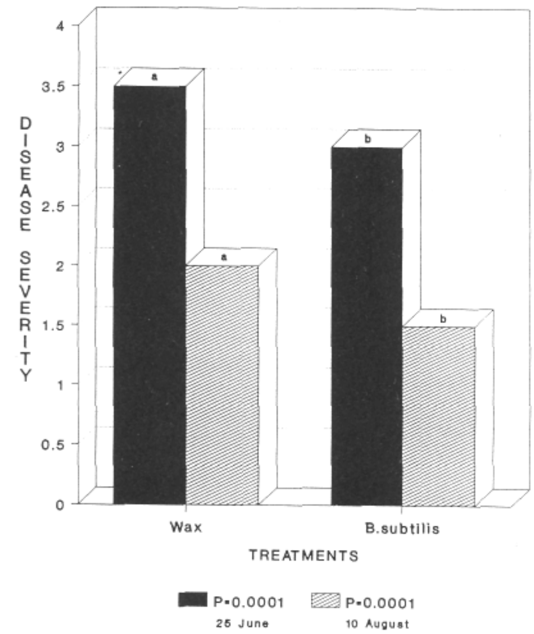


[a] Prochloraz at 0.5mℓ/ℓ Tag-wax

[b] *B. subtilis* at 10^7 cells/mℓ Tag-wax mixture

[c] Combination of prochloraz (0,1 mℓ/ℓ Tag-wax) with *B. subtilis* at 10^7 cells/mℓ

FIG. 3 Effect of prochloraz, *Bacillus subtilis* on its own or integrated with prochloraz on stem-end rot of Ryan avocado, when fruit was Tag-waxed at Westfalia Estate.



■ 25 June application (p=0,0001)

□ 10 August application (p=0,0001)

[a] *B. subtilis* at 10^7 cells/mℓ Tag-wax mixture

FIG.4 Effect of *Bacillus subtilis* Tag-wax application on anthracnose severity of Fuerte avocado fruit when evaluated at two different dates at H.L. Hall & Sons packhouse.

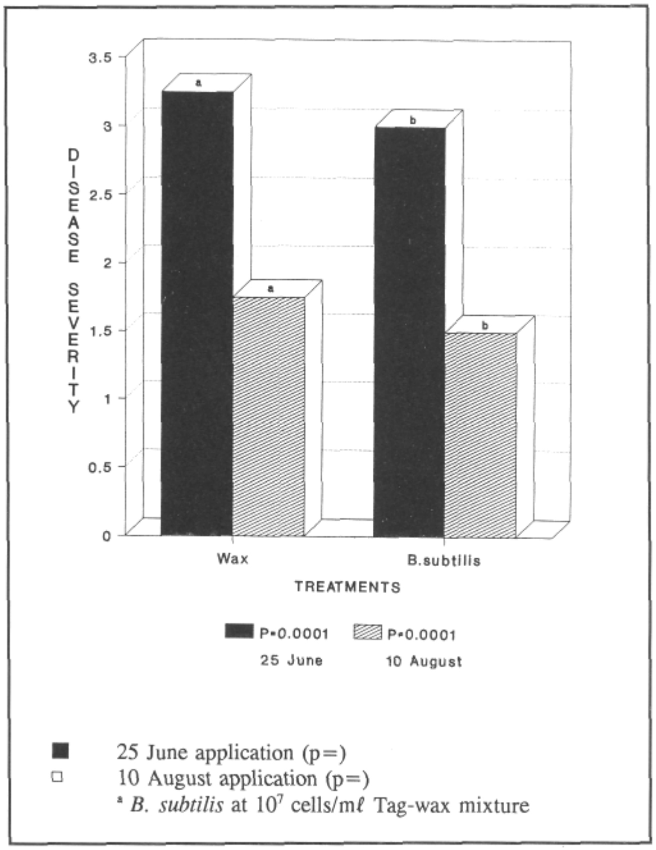


FIG.5 Effect of *Bacillus subtilis* Tag-wax application on anthracnose severity of fuerte avocado fruit when evaluated at two different dates at H.L. Hall & Sons packhouse.

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