

## Postharvest Biological Control of Avocado Postharvest Diseases

Lise Korsten and Jan M. Kotzé

Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002, Republic of South Africa

**Abstract.** *Bacillus* spp. were evaluated singly in dip treatments and in combination with the fungicide prochloraz under commercial packinghouse conditions for the control of postharvest fruit diseases of naturally infected 'Fuerte' avocado fruit. Untreated fruit, and fruit dipped in water or treated with prochloraz served as controls. *B. subtilis* and *B. licheniformis* significantly reduced anthracnose, *Dothiorella/Colletotrichum* fruit rot complex and stem-end rot and gave more effective control than prochloraz dip treatments. Combinations of antagonists or *B. cereus* on its own gave control comparable with that of a prochloraz ULV application.

One of the most important problems facing the South African avocado industry is postharvest diseases. Losses of 36% due to anthracnose and 13% due to stem-end rot (SE) have been recorded on the overseas market (Bezuidenhout, 1983). The most common fungi associated with these diseases include *Colletotrichum gloeosporioides* (Penz.) Sacc., *Thyronectria pseudotrichia* (Schw.) Seeler, *Phomopsis perseae* Zerova, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. and *Dothiorella aromatica* (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 (Darvas, 1985). 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 other fruit and some vegetable crops (Wilson and Wisniewski, 1989). Biological control of postharvest diseases have, thus far, been more effective when attempted as a postharvest treatment and mostly involved the use of *Bacillus* spp (Wilson and Wisniewski, 1989). Information concerning biological control of avocado fruit diseases is limited to two preliminary reports (Korsten *et al.*, 1988; 1989). In this paper, evidence is presented on biological control of avocado postharvest diseases by *Bacillus* spp. applied as dips and ultra low volume (ULV) sprays as compared to prochloraz dip and ULV treatments.

### Materials and Methods

*Bacillus subtilis* isolates A6 and B46, *B. cereus* and *B. licheniformis* originally isolated from the avocado phylloplane (Korsten *et al.*, 1988), were selected for packinghouse treatments due to their strong inhibitory action against *C. gloeosporioides*, *D. aromatica*,

*T. pseudotrichia*, *P. perseae* and *L. theobromae* (Korsten *et al.*, 1989). Batches of antagonists were produced, harvested (Korsten *et al.*, 1988), lyophilized and stored until required for packinghouse treatments. Plastic packing crates each containing between 100 and 160 avocado fruit were randomly removed from the commercial packing line at Westfalia Estate (South Africa) prior to processing. Three crates were used for each treatment (Table 1). For the dip treatment, each crate was lowered manually into a 100 liter dipping vessel containing 25 liter of the treatment solution or suspension. The commercial sticker Agral 90 (Agricura, Pretoria, South Africa) used to enhance bacterial attachment was included with each dip at the registered rate of 0.05%. Prochloraz (Omega 45% a.i. EC, FBC (Pty) Ltd, South Africa) was applied at a rate of 0.5 g a.i./L tap water for 5 min. Fruit for the other dip treatments were dipped for 7 min. Unless otherwise mentioned, fruit were air-dried at 50C in a commercial drying tunnel before being Tag-waxed (polyethylene wax) (ICI Agrochemicals South Africa (PTY) LTD., Johannesburg, South Africa) using 1 liter wax /ton fruit. For the ULV applications, fruit were sprayed with a hand held portable ULV applicator (Model ULVA8, Maryland, USA) at a rate of 1.6 liter spray mixture per ton fruit. Prochloraz was applied at a rate of 5 g a.i./L tap water. Fruit was turned manually halfway through the spraying to ensure complete coverage. Fruit from the ULV or dip treatments were packed separately and stored under commercial conditions of 4C for 28 days to simulate export conditions. Fruit were ripened at ambient temperature (22C) 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 was analyzed statistically using Duncan's new multiple range test.

## Results

With the first experiment, the two biocontrol treatments significantly reduced the severity of all three postharvest diseases (Table 2). Only anthracnose was controlled by the integrated treatment and by dipping in prochloraz, whereas ULV application of prochloraz controlled anthracnose and DCC (Table 2). With the second experiment, the two biocontrol treatments once again reduced anthracnose, DCC and SE (Table 3). Prochloraz dipping controlled DCC and the water dip control increased anthracnose above the level of that of the control (Table 3).

## Discussion

For biological control to be accepted by the avocado 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 *Bacillus* spp. reduces anthracnose, DCC and SE on avocado fruit as effectively as the prochloraz ULV application, and even to a greater extent than prochloraz applied as a dip, which is currently regarded as superior for controlling some of these diseases (Darvas, 1985). Since most fruits have a number of important pathogens, controlling only one may merely favor another

(Janisiewicz, 1988). Therefore, the antagonists which have a wider spectrum of activity than the fungicide can be more effective in controlling avocado postharvest diseases.

Biological control has been successfully used on fruit for the control of mostly wound pathogens (see review by Wilson and Wisniewski, 1989). Our data contrast with Janisiewicz (1988) where combinations of antagonists were effective in controlling postharvest diseases, but were not necessarily more effective than single applications in controlling all the diseases. Furthermore, he achieved improved disease control by increasing antagonist concentrations. In contrast, we found in our second experiment that doubling of the antagonist concentration did not necessarily improve disease control.

The fact that dipping in water increased anthracnose severity is in accordance with Darvas (1982) who reported that moisture on avocado fruit after harvest leads to an increase in postharvest diseases. Since moisture after dipping is retained in the lenticels, where many of the latent infections are located (Home and Palmer, 1935), a more favorable microclimate for the pathogen is created. However, it should be remembered that "moist pockets" could also favor the antagonist, thereby ensuring effective disease control.

Our results showed, that biological control is as efficient as the best fungicide available. Furthermore, the South African avocado industry currently does not have a market-acceptable postharvest fungicide since prochloraz has not been cleared for certain export markets. Biological control is therefore a viable alternative to the use of chemicals especially for the control of avocado postharvest diseases. However, registration and the market acceptability of the biological control agents must still be established before the industry can further evaluate the feasibility of this alternative control measure.

### **Literature Cited**

- Baker, K.F. and R.J. Cook. 1974. Biological Control of Plant Pathogens. W.H. Freeman and Company, San Francisco.
- Bezuidenhout, J.J. 1983. Die voorkoms van mesokarpverkleurings by 'Fuerte' avokados, op Rungis mark gedurende 1982. S. A. Avocado Growers' Assn. Yrbk. 6:24-27.
- Darvas, J.M.. 1982. Etiology and control of some fruit diseases of avocado (*Persea americana* Mill.) at Westfalia Estate. D.Sc. thesis. University of Pretoria, Pretoria, R.S.A.
- Darvas, J.M.. 1985. ULV application of systemic fungicides for the control of postharvest avocado diseases. S. A. Avocado Growers' Assn. Yrbk. 8:46-47.
- Home, W.T. and D.F. Palmer. 1935. The control of *Dothiorella* rot on avocado fruits. Univ. of California, Berkeley California Bull. 594. 16pp.
- Janisiewicz, W.J. 1988. Biocontrol of postharvest diseases of apples with antagonistic mixtures. Phytopathology 78:194-198.

- Korsten, L., J.J. Bezuidenhout, and J.M. Kotzé. 1988. Biological control of postharvest diseases of avocado. S. A. Avocado Growers' Assn. Yrbk. 11:75-78.
- Korsten, L., J.J. Bezuidenhout, and J.M. Kotzé. 1989. Biocontrol of avocado postharvest diseases. S. A. Avocado Growers' Assn. Yrbk. 12:10-12.
- Wilson, C.L. and M.E. Wisniewski. 1989. Biological control of postharvest diseases of fruits and vegetables: an emerging technology. Ann. Rev. Phytopathol. 27:425-441.

Table 1. Postharvest treatments with *Bacillus* spp. and prochloraz for control of avocado postharvest diseases.

Code	Treatments	Application	Concentration	
<b>EXPERIMENT 1: DIP AND ULV TREATMENTS</b>				
1	Control	Control	no dip or ULV <sup>z</sup>	
2	Chemical 1	Prochloraz	dip	commercial
3	Chemical 2	Prochloraz	ULV	Commercial
4	Biocontrol 1 (mixture)	<i>B. cereus</i> + <i>B. subtilis</i> (B46)	dip	10 <sup>4</sup> cells/mL
5	Biocontrol 2	<i>B. cereus</i>	dip	12x10 <sup>8</sup> cells/mL
6	Integrated	<i>B. cereus</i> + <i>B.</i> <i>lichineformis</i> + <i>B.</i> <i>subtilis</i> (B46 + A6)	Dip	10 <sup>4</sup> cells/mL
		Prochloraz	ULV	half strength
<b>EXPERIMENT 2: DIP TREATMENTS</b>				
1	Control	Control	no dip	
2	Water control	Control	dip	Water
3	Chemical 1	Prochloraz	dip	commercial
4	Biocontrol 1	<i>B. subtilis</i> (A6)	dip	2.1x10 <sup>7</sup> cells/mL
5	Biocontrol 2	<i>B. subtilis</i> (A6)	dip	1x10 <sup>7</sup> cells/mL

<sup>z</sup> ULV = ultra-low volume

Table 2. Effect of *Bacillus* spp. dip and prochloraz dip and ultra low volume applications on avocado postharvest diseases (Experiment 1).

Code	Treatments	# of fruit	Anthraco-nose	DCC <sup>z</sup>	SE <sup>z</sup>
1	Control	136	1.35 a <sup>y</sup>	0.54 a	0.35 a
2	Prochloraz dip	127	0.91 b	0.77 a	0.42 a
3	Prochloraz ULV	165	0.23 c	0.19 b	0.24 ab
4	<i>Bacillus</i> spp. dip <sup>x</sup>	123	0.07 c	0.12 b	0.08 b
5	<i>B. cereus</i> dip	115	0.21 c	0.13 b	0.11 b
6	<i>Bacillus</i> spp. dip <sup>w</sup> + prochloraz ULV	134	0.44 c	0.69 a	0.22 ab
	Total and PR > F	800	0.0001	0.0001	0.0001

<sup>z</sup> DCC = *Dothiorella/Colletotrichum* fruit rot complex, SE = stem-end rot

<sup>y</sup> 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.

<sup>x</sup> *B. cereus* ( $10^4$  cells/mL) + *B. subtilis* ( $10^4$  cells/mL)

<sup>w</sup> *B. subtilis* (A6 + B46) both at  $10^2$  cells/mL + *B. lichineformis* ( $10^2$  cells/mL) + *B. cereus* ( $10^4$  cells/mL)

Table 3. Effect of *Bacillus subtilis* and prochloraz dip treatments on avocado postharvest diseases (Experiment 2).

Code	Treatment	# of fruit	Anthraco	DCC <sup>z</sup>	SE <sup>z</sup>
1	Control no dip	115	1.67 b <sup>y</sup>	2.17 a	0.85 a
2	Control water	114	2.27 a	1.91 a	0.73 a
3	Prochloraz	129	1.20 be	0.54 b	0.66 ab
4	<i>B. subtilis</i> <sup>x</sup>	136	0.88 cd	0.38 b	0.21 c
5	<i>B. subtilis</i> <sup>w</sup>	130	0.44 d	0.23 b	0.35 be
Total + PR > F		622	0.0001	0.0001	0.0002

<sup>z</sup> DCC = *Dothiorella/Colletotrichum* fruit rot complex, SE = stem-end rot

<sup>y</sup> 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.

<sup>x</sup> concentration of  $2.1 \times 10^7$  cells/mL

<sup>w</sup> concentration of  $1 \times 10^7$  cells/mL