South African Avocado Growers' Association Yearbook 1997. 20:109-112

Alternative Control of Avocado Post-Harvest Diseases

K van Dyk • E E de Villiers • L Korsten
Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, 0002

SUMMARY

Biological control agents and disinfectants, alone and in combination were evaluated for control of anthracnose, Dothiorella/Colletotrichum *fruit rot complex (DCC) and stem-end rot (SE).AII treatments were applied as post-harvest fruit dips.* Bacillus subtilis *was found to control anthracnose and SE as effectively as or better than thiabendazole. Disinfectants, ethanol and terminator were found to control anthracnose more readily than SE.*

Significant decreases in the severity of anthracnose and SE, when antagonist and disinfectant were used in combination compared to separately, were not consistent in this study. No control of anthracnose was obtained with thiabendazole treatment at Burpak, implicating possible resistant strains of C. gloeosporioides*. This issue should be immediately investigated.*

OPSOMMING

Biologiese beheeragente en oppervlak ontsmettingsmiddels, alleen en in kombinasie, is geévalueer vir beheer van antraknose, DothiorellalColletotrichum *vrugverottingskompleks (DCC) en stingel-end verotting (SE). Alle behandelings is aangewend as na-oes doopbehandelings.* Bacillus subtilis *het antraknose en SE het so effektief of beter as tiabendasool beheer Die ontsmettinsmiddels etanol en terminator het antraknose beter as SE beheer. Betekenisvolle afname in die intensiteit van antraknose en SE was nie konsekwent waar antagoniste en ontsmettingsmiddels in kombinasie gebruik is, teenoor elkeen apart.Tiabendasool het nie antraknose beheer by Burpak nie en dui op moontlike weerstandbiedende rasse van* C. gloeosporioides*. Hierdie aangeleentheid moet onmiddelik ondersoek word.*

INTRODUCTION

In South Africa, avocado is susceptible to various post-harvest diseases; the most important being anthracnose, stem-end rot (SE) and *Dothiorella/Colletotrichum* fruit rot complex (DCC) Darvas & Kotzé, 1978). Limited control of these diseases can be achieved by pre-harvest fungicide sprays, such as copper oxychloride or benomyl (Darvas & Kotzé, 1978), and by post-harvest applications of prochloraz (Darvas, 1985) or thiabendazole (Krause *et al,* 1996). However, copper oxychloride leaves visible residues on fruit, which have to be manually removed in the packhouse prior to packing (Lonsdale, 1992) while continuous use of benomyl can result in build-up of resistance in

target pathogens (Darvas & Kotzé, 1987). Furthermore, prochloraz has still not been given clearance for use on fruit exported to France (Boshoff *et al,* 1995). These considerations along with growing international concern over environmental pollution by pesticides and chemical residues on consumables make it desirable to seek alternative meaure to control post-harvest diseases.

Biological control of post-harvest fruit diseases has emerged in recent years as a promising alternative to synthetic chemicals (Wilson & Wisniewski, 1989). Effective biological control has been reported for post-harvest diseases of several crops including pome (Janisiewicz, 1987; Janisiewicz & March, 1992), stone (Pusey & Wilson), 1984), citrus (Singh & Deverall, 1984) and various subtropical fruits (Korsten *et al,* 1994). For avocado, successful biocontrol of post-harvest diseases has been achieved with both pre and post-harvest applications of *Bacillus subtilis* (Ehrenberg) Cohn (Korsten *et al,* 1994). However, more consistent control was obtained when the antagonist was applied post-harvestly as dip or wax applications (Korsten *et al,* 1994). The post-harvest environment seems to be especially favourable for biological control (Droby *et al,* 1992), since environmental conditions can be controlled and maintained, and the biocontrol agent can be directed precisely to the target site (Wilson & Pusey, 1985).

With the removal of several fungicides registered for use post-harvestly, and the decreasing residue tolerance for those that remain (Wisniewski & Wilson, 1992), sanitation of both fruit and packhouse surfaces could become important as a disease management tool (Roberts & Reymond, 1994). Detergents and/or sanitisers have been used on a routine basis in food processing and dairy industries to reduce inoculum of spoilage organisms (Park *et at,* 1991). Except for a recent study by Boshoff *et al* (1995) in which iodine and quaternary ammonium compounds were evaluated, disinfectants other than chlorine have not been tested for use on avocado fruit to control postharvest diseases.

The purpose of this study was to evaluate the effect of seven biocontrol agents and four disinfectants on post-harvest diseases of Fuerte avocado fruit. Because evidence suggests that biocontrol agents are able to occupy the biological vacuum created by disinfectants, treatments consisting of disinfectant applications followed by antagonist applications were also included.

MATERIALS AND METHODS

Six post-harvest dip experiments were conducted on Fuerte avocado fruit during the 1995/96 avocado season. Due to a shortage of export fruit, fruit intended for local markets had to be used for the first experiment. Harvested, untreated fruit were randomly collected from the receiving line at Burpak (Pty) Ltd. (Hazyview, RSA), packed into standard carton boxes and immediately transported to the laboratory at the University of Pretoria. Ten carton boxes each containing approximately 20 fruit were used for each treatment. Fruit was dipped in the respective solutions as described in table 1. Applications of *B. subtilis* isolate B246 and isolate B19 were prepared by mixing a lyophilised antagonist formulation (Korsten *et al,* 1989) in tap water to obtain a final concentration of 10^7 cells/m/. Thiabendazole (Tecto, 45% a.i., Logos Pharmaceuticals (Pty) Ltd., Halfway House, RSA) was applied at the registered rate (l,5g a.i./ℓ tap water),

while potassium hypochlorite (KOCl) (Hydrosan Technologies, Braamfontein, RSA) and dimenthyldidecyl-ammonium chloride (Terminator, Zeneca Agrochemicals, Paarl, RSA) were prepared according to manufacturers' details. Treatments consisting of ethanol (ethyl alcohol) (10%) and tea and coffee extracts (Alström, 1992) were also included. Fruit from each treatment was allowed to air dry before being packed into carton boxes, labelled and stored. Due to unavailability of cold storage facilities, export conditions could not be simulated and fruit was subsequently stored at ambient temperature until 'ready to eat' ripeness was reached. These experiments thus simulated local marketing conditions.

Table 1
Treatment descriptions for post-harvest dip applications of Fuerte avocado fruit obtained from Burpak and Westfalia Estate

Trreatment	*Concentration*	*Dip time*
Evaluating different antagonists to control avocado post-harvest diseases		
Untreated control	–	
Water	–	7 min
Water containing thiabendazole (TBZ)	150 m*l*/50*l*	20 sec
Water containing *Bacillus subtilis* (B246)	10^7 cells/m*l*	7 min
Water containing *B. subtilis* (B19)	10^7 cells/m*l*	7 min
Evaluation of different disinfectants and tea and coffee extracts to control avocado post-harvest diseases		
Untreated control	–	–
Water	–	7 min
Water containing thiabendazole	150 m*l*/50*l*	20 sec
Water containing terminator	40 m*l*/50*l*	7 min
Water containing ethanol	10%	7 min
Water containing potassium hypochlorite	716 m*l*/50*l*	7min
Water containing tea & coffee extracts	100g coffee + 100g tea/50	7 min
Evaluation of combinations of disinfectans and antagonists to control avocado post-harvest diseases		
Untreated control	–	–
Water	–	7 min
Water containing thiabendazole	150 m*l*/50*l*	20 sec
Water containing terminator followed	40 m*l*/50*l*	7 min
by water containing B246	10^7 cells/m*l*	7 min
Water containing ethanol followed by	10%	7 min
water containing B246	10^7 cells/m*l*	7 min
Water containing B246	10^7 cells/m*l*	7 min

In the second experiment, post-harvest dip treatments were conducted at Westfalia Estate commercial packhouse. Ten standard carton boxes containing approximately 15 untreated export quality fruit were randomly selected from the packing line. The treatments described before (table 1) were repeated. Dipped fruit was dried in an air tunnel, packed into carton boxes, labelled and stored at 5°C for 28 days to simulate export conditions. After removal from commercial cold storage, fruit was transported to the laboratory where it was ripened at ambient temperature to a 'ready to eat' stage. These experiments thus simulated export conditions.

Fruit from both experiments was evaluated externally and internally for anthracnose, SE and DCC, as well as for firmness. Evaluations were done on a 0 10 scale, where 0 represents healthy fruit and 10 totally affected fruit (Bexuidenhout & Kuschke, 1982). Firmness was rated on a similar scale, with 0 indicating soft fruit and firmness increases toward 10. Data was ranked and then analysed using Duncan's multiple range test (P = 0,05).

RESULTS

Due to a low incidence of DCC, no significant differences between treatments could be established and data is therefore not shown.

Effect of biological control agents on postharvest diseases of Fuerte avocado fruit

In the first experiment simulating local marketing conditions, both antagonists effectively controlled anthracnose, while only B246 could effectively control SE, when compared to the untreated control (table 2). However, when compared to the water dip control it had no significant effect on SE (table 2). Shelf life (expressed as fruit firmness) was significantly increased by the antagnoist treatments (B246 and B19) (table 2). The chemical treatment TBZ was not effective in controlling post-harvest diseases.

Table 2
Effect of biocontrol agents on post-harvest diseases and shelf life of Fuerte avocado fruit obtained from Burpak (Hazyview)

Treatment	*Fruit firmness*	*Stem-end rot*	*Anthrac-nose*
Control	17,05c	31,75b	31,90a
H_2O	14,85c	20,70bc	32,85a
TBZ	14,60c	25,35abc	37,15a
B. subtilis (B246)	35,65b	13,75c	15,80b
B. subtilis (B19)	45,35a	35,95a	9,80b
Pr > F	0,0001	0,0028	0,0001

Values in columns followed by the same letter do not differ significantly according to Duncan's multiple range test (P = 0,05).

In the second experiments simulating export conditions, both *B. subtilis* (B246 and B19) treatments reduced the incidence of SE, although not as effectively as TBZ (table 3). Both TBZ and *B. subtilis* (B246) effectively controlled anthracnose, while B19 was only effective against anthracnose when compared to the untreated control (table 3). TBZ and both antagonist treatments significantly increased shelf life (table 3).

Table 3

Effect of biocontrol agents on post-harvest diseases and shelf life of Fuerte avocado fruit obtained from Westfalia Estate (Tzaneen)

Treatment	*Fruit firmness*	*Stem-end rot*	*Anthrac-nose*
Control	16,90c	33,40a	36,60a
H_2O	17,70c	40,55a	34,65ab
TBZ	38,65a	8,65a	10,10d
B. *subtilis* (B246)	32,40ab	23,05b	20,20dc
B. *subtilis* (B19)	21,85a	21,85b	25,95bc
Pr > F	0,0005	0,0001	0,0001

Effect of disinfectants on post-harvest diseases of Fuerte avocado fruit

Terminator, ethanol and the tea and coffee extract effectively reduced anthracnose in the experiment simulating local marketing conditions, while none of the treatments had any significant effect on SE severity (table 4). The KOCL treatment significantly increased anthracnose when compared to the untreated control (table 4). Ethanol was effective in increasing shelf life (table 4).

Table 4

Effect of biocontrol agents on post-harvest diseases and shelf life of Fuerte avocado fruit obtained from Burpak (Hazyview)

Treatment	*Fruit firmness*	*Stem-end rot*	*Anthrac-nose*
Control	26,40c	41,10ab	40,15b
H_2O	22,35c	29,00bc	42,35ab
TBZ	24,20c	35,75c	47,ab
Terminator	46,86b	23,55c	26,15c
EtOH (10%)	65,50a	36,45abc	55,70a
KOCI	19,20c	53,80a	21,50c
Tea & Coff. extract	44,00b		
Pr > F	0,0001	0,0065	0,0001

Values in columns followed by the same letter do not differ significantly according to Duncan's multiple range test (P = 0,05).

In the experiments simulating export conditions, TBZ was effective in reducing both SE and anthracnose (table 5). The ethanol treatment significantly reduced SE when compared to the water dip control but not when compared to the untreated control, while it also effectively reduced anthracnose but only when compared to the untreated control (table 5). Interestingly, the KOCL treatment increased anthracnose above that of the control. Shelf life was significant increased by TBZ (table 5).

Table 5
Effect of biocontrol agents on post-harvest diseases and shelf life of Fuerte avocado fruit obtained from Westfalia Estate (Tzaneen)

Treatment	*Fruit firmness*	*Stem-end rot*	*Anthrac-nose*
Control	33,15bc	38,10ab	42,50b
H_2O	34,00bc	51,15a	40,30bc
TBZ	60,30a	7,95c	9,55d
Terminator	46,15b	36,05ab	31,80bc
EtOH (10%)	38,40bc	28,95b	27,45c
KOCI	11,50d	50,40a	59,95a
Tea & Coff. extract	25,00c	35,90ab	36,95bc
Pr > F	0,0001	0,00065	0,0001

Values in columns followed by the same letter do not differ significantly according to Duncan's multiple range test (P = 0,05).

Effect of combination treatments on postharvest diseases of Fuerte avocado fruit

In the experiment simulating local conditions, ethanol-B246 combination was the most effective treatment in reducing SE and anthracnose (table 6). All other treatments excluding TBZ, also reduced anthracnose when compared to the two control treatments (table 6). Terminator and the 10% ethanol treatment also effectively reduced SE but only when compared to the untreated control. Shelf life was significantly increased by terminator-anatagonist combination, ethanol alone and ethanol in combination with B246 (table 6).

TBZ and *B. subtilis* (B246) significantly reduced anthracnose and SE in the experiment simulating export conditions (table 7). However, TBZ was more effective than B246 in controlling SE. The ethanol on its own and in combination with B246 as well as the terminator combined with B246 effectively controlled SE but only when compared to the water dip control. These treatments all increased shelf life (table 7).

DISCUSSION

For biological control to be accepted by fruit industries, it has to be as effective as the best fungicides available (Baker & Cook, 1974). In this study, *B. subtilis* was found to control anthracnose and SE as effectively and in certain cases even more so than TBZ. Consistency of control measures remain an important objective for all disease control strategies. Korsten *et al.* (1993) previously showed that B246 could consistently control avocado post-harvest diseases. In this study B246 could control both anthracnose and SE with fruit simulating export conditions. However, only anthracnose could effectively be controlled with B246 simulating direct local marketing of fruit. This variation in effective control of SE could be attributed to various factors, since fruit were obtained from different geographical areas. It has also previously been shown that cold storage conditions can reduce certain postharvest diseases (Everett & Korsten, unpublished data).

Table 6

Effect of combinations of disinfectants and *B. subtilis* on post-harvest diseases and firmness of Fuerte avocado fruit obtained from Burpak (Hazyview)

Treatment	*Fruit firmness*	*Stem-end rot*	*Anthrac-nose*
Control	18,40d	53,70ab	56,90a
H_2O	16,05d	37,45bc	59,35a
TBZ	16,20d	45,04abc	64,55a
Terminator	31,65c	32,20cd	42,10b
Terminator + *B. subtilis* (B246)	60,80a	45,80abc	34,50b
EtOH (10%)	65,15a	33,95c	27,80b
EtOH (10%) + B246	66,05a	14,65d	7,30c
B246	49,70b	61,20a	31,50b
Pr > F	0,0001	0,0001	0,0001

Values in columns followed by the same letter do not differ significantly according to Duncan's multiple range test (P = 0,05).

Table 7

Effect of combinations of disinfectants and *Bacillus subtilis* on post-harvest diseases and firmness of Fuerte avocado fruit obtained from Westfalia Estate (Tzaneen)

Treatment	*Fruit firmness*	*Stem-end rot*	*Anthrac-nose*
Control	26,40c	50,30ab	57,45a
H_2O	28,80c	64,10a	54,05a
TBZ	58,55a	9,35d	14,10c
Terminator	40,00bc	46,95abc	44,80a
Terminator + B. subtilis (B246)	64,00a	43,75bc	46,50a
EtOH (10%)	30,35c	37,10bc	40,00ab
EtOH (10%) + B246	25,40c	41,65bc	41,70ab
B246	50,10ab	30,80c	25,40bc
Pr > F	0,0001	0,0001	0,0001

Values in columns followed by the same letter do not differ significantly according to Duncan's multiple range test (P = 0,05).

Boshoff *et al,* (1995) found ethanol to be effective against anthracnose. Similar results were obtained in this study using however a much lower concentration (10%). Cost would obviously not justify using high ethanol concentrations as post-harvest treatments. The use of lower ethanol concentrations as possible post-harvest applications should be further exploited. Terminator has been evaluated successfully for the decontamination of mainly pome and stone fruit surfaces (Zeneca Agrochemicals, users pamphlet). In this study, Terminator was found to control anthracnose more

readily than SE. This agrees with results of Boshoff *et al,* (1995) where anthracnose was generally more effectively controlled by quarternary ammonium compounds than SE.

Coates & Johnson (1993) suggested that biological control agents are able to occupy the biological vacuum created by disinfectants. Combination of these two agents in post-harvest treatments could therefore lead to synergistic disease control. The ethanol-antagonist combination was found the most effective treatment with fruit obtained from Burpak which were immediately ripened and evaluated. Results were however not consistent with similar experiments done at Westfalia Estate where fruit was first cold stored. No significant trend in reducing severity of anthracnose and SE could be established when disinfectants and antagonists were used in combination or on their own. Integration of these two control strategies hold great promise and should be further exploited.

Variability in the efficacy of TBZ to control anthracnose was observed between experiments done with Westfalia and Burgershall fruit. Anthracnose was much more readily controlled by TBZ at Westfalia than with fruit obtained from Burgershall. Build-up of pathogen resistance has been one of the key factors prompting investigation of alternative post-harvest disease control strategies (Delp, 1980), this aspect should be investigated further.

ACKNOWLEDGEMENTS

The authors wish to thank Burpak and Westafalia Estate for supplying fruit and SAAGA for financial support. Assistance of Elizabeth de Jager and Estelle Towsen during experiments is gratefully acknowledged as well as data analysis by Amanda Lourens.

REFERENCES

ALSTRÖM, S. 1992. Antibacterial activity of tea and coffee wastes against some plant pathogenic *Pseudomonas syringae strains. Journal of Phytopathology* 136: 329 - 334.

BAKER, K.F. & COOK, R.J. 1974. *Biological control of plant pathogens.* WH Freeman & Company, San Fransisco.

BEZUIDENHOUT, J.J. & KUSCHKE,' E. 1982. Die avokado ondersoek Rungis, Frankryk gedurende 1981. *South African Avocado Grower's Association Yearbook* 5: 18 - 24.

BOSHOFF, M., SLABBERT, M.J. & KORSTEN, L. 1995. Effect of detergent sanitisers on post-harvest diseases of avocado. *South African Avocado Grower's Association Yearbook* 18: 96 - 98.

COATES, L.M. & JOHNSON, G.I. 1993. Effective disease control in heat-disinfected fruit. *Postharvest News and Information* 4: 35N-40N.

DARVAS, J.M. 1985. ULV application of systemic fungicides for the control of post-harvest avocado diseases. *South African Avocado Grower's Association Yearbook* 8: 46 - 47.

DARVAS, J.M. & KOTZE, J.M. 1987. Avocado fruit diseases and their control in South

Africa. *South African Avocado Grower's Association Yearbook* 10: 117 - 119.
DELP, C.J. 1980. Coping with resistance to plant disease control agents. *Plant Disease* 64: 652 - 657.
DROBY, S., CHALUTZ, E., WILSON, C.L. & WISNIEWSKI, M.E. 1992. Biological control of post-harvest diseases: a promising alternative to the use of synthetic chemicals. *Phytoparasitica Supplement* 20 149S - 151S.
JANISIEWICZ, W.J. 1987. Post-harvest biological control of blue mold on apples. *Phytopathology* 77: 481 - 485.
JANISIEWICZ, W.J. & MARCH, A. 1992. Control of storage rots on various pear cultivars with a saprophytic strain of *Pseudomonas syringae. Plant Disease* 76: 555 - 560.
KORSTEN, L., BEZUIDENHOUT, J.J. & KOTZÉ, J.M. 1989. Biocontrol of avocado post-harvest diseases. *South African Avocado Growers' Association Yearbook* 12: 10 - 12.
KORSTEN, L., DE VILLIERS, E.E., ROWELL, A. & KOTZÉ, J.M. 1993. Post-harvest biological control of avocado fruit diseases. *South African Avocado Growers' Association Yearbook* 16: 65 - 69.
KORSTEN, L., DE VILLIERS, E.E., WEHNER, EC. & KOTZÉ, J.M. 1994. A review of biological control of postharvest diseases of subtropical fruits. Pages 170 - 185 In: *Post-harvest handling of tropical fruits.* Champ, B.R., Highley, E. & Johnson, G.I. (eds). The Australian Centre for International Agricultural Research, Brisbane, Australia.
KRAUSE, M., NEL, A. & VAN ZYL, K. 1996. A guide to the use of pesticides and fungicides in the Republic of South Africa. National Department of Agriculture, Pretoria.
LONSDALE, J.H. 1992. Evaluation of systemic fungicides as pre-harvest treatments of avocados. *South African Avocado Growers' Association Yearbook* 15: 35 - 38.
PARK, D.L., RUA, S.M. & ACKER, R.F. 1991. Direct application of a new hypochlorite sanitizer for reducing bacterial contamination on foods. *Journal of Food Protection* 54: 960 - 965.
PUSEY, PL. & WILSON, C.L. 1984. Post-harvest biological control of stone fruit brown rot by *Bacillus subtilis. Plant Disease* 68: 753 - 756.
ROBERTS, R.G. & REYMOND, J.T. 1994. Chlorine dioxide for reduction of post-harvest pathogen inoculum during handling of tree fruits. *Applied and Environmental Microbiology* 60: 2864 - 2868.
SINGH, V. & DEVERALL, BJ. 1984. *Bacillus subtilis* as a control agent against fungal pathogens of citrus fruit. *Transactions of the British Mycological Society* 66: 57 - 64.
WISNIEWSKI, M.E. & WILSON, C.L. 1992. Biological control of post-harvest diseases of fruits and vegetables: recent advances. *Hort Science* 27: 94 - 98.
WILSON, C.L. & WISNIEWSKI, M.E. 1989. Biological control of post-harvest diseases of fruits and vegetables: an emerging technology. *Annual Review of Phytopathology* 27: 425 - 441.