Evaluation of Antioxidant Butylated Hydroxyanisole and Fungicide Prochloraz for Control of Post-Harvest Anthracnose of Avocado Fruit During Storage

D. Prusky, Department of Post-harvest Science of Fresh Produce, ARO, The Volcani Center, Bet Dagan 50250, Israel; **H. D. Ohr, N. Grech,** and **S. Campbell,** Department of Plant Pathology, University of California, Riverside 92521; and **I. Kobiler, G. Zauberman,** and **Y. Fuchs,** Department of Post-harvest Science of Fresh Produce, ARO, The Volcani Center, Bet Dagan 50250, Israel

ABSTRACT

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The incidence of post-harvest anthracnose of avocado caused by *Colletotrichum gloeosporioides* was reduced significantly after dip or spray treatment with the antioxidant butylated hydroxyanisole (BHA) or BHA + prochloraz. In small, medium, and semicommercial experiments in California on cv. Hass and in Israel on cv. Fuerte there was a significant reduction of decay by single treatments of 1,200 µg a.i. of BHA per ml or of 1,200 µg a.i. of BHA + 250 µg a.i. of prochloraz per ml. Prochloraz at 1,000 µg a.i./ml alone did not always reduce the incidence of decay while BHA or BHA + prochloraz reduced decay consistently. The effect of BHA + prochloraz lasted longer than that of BHA alone. It is suggested that the antioxidant might reduce post-harvest decay in avocado by modulating the natural fruit resistance.

Additional keywords: antifungal compound, post-harvest diseases, post-harvest treatments, quiescent infections

Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz., and stem end rot caused by *Diplodia natalensis* Pole-Evans, *Dothiorella gregaria* Sacc. or *Dothiorella aromatica* (Sacc.) Petr. & Syd. are the most important fruit rot diseases of avocado *Persea americana* Miller in the U.S., Israel, Australia, and South Africa (1,3,9,10,11). Anthracnose in its early stages in avocado is essentially a cosmetic disease. The peel of Hass avocados turns black as fruit ripen, masking early disease symptoms. When avocados are ripe enough to eat, the disease has not progressed beyond the peel into the fruit to any great degree and is removed with peeling. The Fuerte avocado is shipped from Israel to Europe where the blemishes of early disease development do not alarm the consumer. In California, post-harvest decay in avocado was not considered a significant problem until prolonged rain during the winter of 1992–93 resulted in an increase in its incidence. *Colletotrichum gloeosporioides* infects the avocado peel and *D. natalensis* infects the fruit pedicel during fruit development, but because avocados do not ripen on the tree the infections by both pathogens remain quiescent until after harvest, when the fruit ripens. Under commercial conditions, decay development can be delayed by storing fruit at 5 to 6°C during short-duration shipment or storage. When the fruit must be held for longer periods, (e.g., 20 to 24 days as with South African fruit), a combination of low temperature and fungicide treatments is needed (1). Fungicides are applied as pre-harvest sprays or, more efficiently, as post-harvest treatments (1,3).

Regulations limiting the residues of agrichemicals on fruit in local and export markets have stimulated the search for new alternatives to post-harvest fungicides. Biological control may be an alternative to fungicides but in spite of a significant amount of work invested in their development, there are no commercial products on the market for the biological control of post-harvest diseases (2). Prusky and Keen (5) have suggested a new approach for the prevention of post-harvest disease: the enhancement of the natural resistance of fruits. Prusky et al. (6,7) isolated an antifungal diene from the peel of unripe avocado fruit that prevents the development of *C. gloeosporioides* and *D. natalensis* beyond the initial infection in unripe avocados. The antifungal diene is apparently oxidized by lipoxygenase during fruit ripening, allowing the fungi to resume colonization of the fruit tissues (6). Lipoxygenase activity in the peel of ripening fruit is regulated by the concentration of the natural antioxidant epicatechin (4). Infiltration or dip treatment of avocado fruit with several antioxidants such as α-tocopherol, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and *tert*-butyl hydroquinone, that inhibited lipoxygenase in vitro, inhibited the decrease of the antifungal diene, and also inhibited development of anthracnose (4,8). Prusky (4) has described the possibility of using antioxidants to delay the onset of post-harvest diseases in avocado. Although BHA and BHT are common food additives, commercial formulations of these antioxidants suitable for agricultural use have only recently been developed. A commercial formulation of BHA was tested and found to be effective, in several trials, in preventing post-harvest diseases in avocado cv. Fuerte (9).

In this paper we report on the control of anthracnose in several small- and medium-scale experiments and semicommercial-level treatments with the antioxidant BHA. Experiments were carried out in Israel and the U.S., with two different avocado cultivars.

MATERIALS AND METHODS

General. Avocado fruit, cvs. Hass and Fuerte, were obtained from groves in California and Israel, respectively. All disease in these experiments resulted from natural infections. Chemicals used were prochloraz (Prochloraz 45 EC) and butylated hydroxyanisole (BHA) (Xedaphene-20, 20% a.i.). Data analyses was accomplished using the Waller-Duncan *k*-ratio *t* test.

Small- and medium-scale experiments with cv. Hass in California. Fruit were dip treated within 4 to 5 h of harvest or after 24 h at 5°C in a cold room. Fruit were dipped for 30 s in 1,200 µg a.i. of BHA per ml, 1,000 µg a.i. of prochloraz per ml, or 1,200 µg a.i. of BHA + 250 µg a.i. of prochloraz per ml. The low rate of 250 µg a.i. of prochloraz per ml was added to the 1,200 µg a.i. of BHA per ml to take advantage of any wetting agents present in the formulation that might enhance the effectiveness of the BHA. A 250 µg a.i. of prochloraz per ml control was not included because results in other studies indicated that concentrations at 300 µg a.i./ml or lower were ineffective (10). After treatment, fruit were either air dried at ambient temperature and then stored at 20°C for 11 days or stored at 5°C for 14 days and then transferred to 20°C for another 11 days. Symptoms of anthracnose exceeding 1 cm^2 total area of one or several spots in the

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Corresponding author: H. D. Ohr;
E-mail: hdohr@ucrac1.ucr.edu

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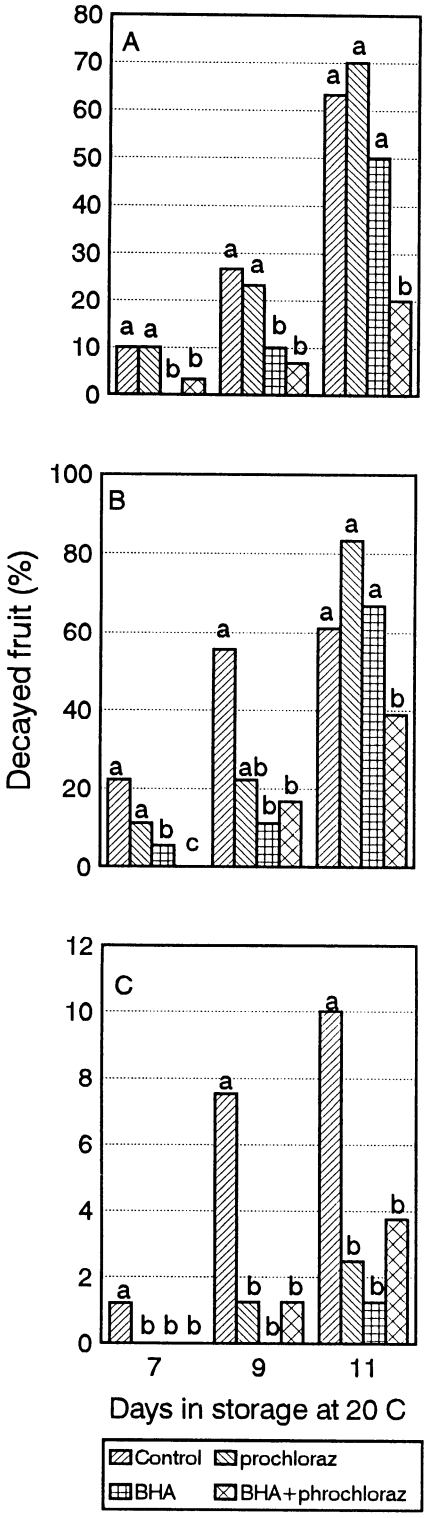

Fig. 1. Effect of post-harvest dip treatment of avocado cv. Hass on the percentage of decay development. Fruit from three groves in southern California (**A, B,** and **C**) were harvested and treated within 5 h in the laboratory with 1,000 µg a.i. of prochloraz per ml, 1,200 µg a.i. of butylated hydroxyanisole (BHA) per ml, or 1,200 µg a.i. of BHA + 250 µg a.i. of prochloraz per ml, and compared with untreated fruit. Fruit were stored at 20°C and scored for disease incidence at 7, 9, and 11 days. Bars with the same letter above them (within each observation day) are not significantly different using the Waller-Duncan *k*-ratio *t* test ($P < 0.05$).

fruit peel were considered positive for disease and not marketable. In small-scale experiments, each treatment was replicated four times with 10 fruit in each replicate. In medium-scale experiments, each treatment was replicated five times with 20 fruit in each replicate. Nontreated controls were included in each experiment. Experiments were conducted three times during the harvest season.

Semicommercial-scale experiments with cv. Fuerte in Israel. Experiments were carried out in three different packinghouses located at Mehadrin, Granot, and HaEmek, representing the main southern, central, and northern avocado-growing areas in Israel, respectively. All fruit were brought to the respective packinghouse on the day of harvest, cooled at 5°C overnight, and treated 1 day later. In Mehadrin the fruit were washed, brushed, and air dried before treatment. In Granot and HaEmek, fruit were only brushed before treatment. Materials were applied through a transverse pipe fitted with six conical nozzles placed 15 cm apart. The overlapping pattern of the individual nozzles completely covered the 1.5-m width of the packing line. Spray pressure was maintained at 405.3 kPa for a flow rate of 188 liters per h and an effective rate of 0.5 liter of solution per metric ton of fruit. Treated fruit were waxed and sorted in a continuous packing-line process. Each experiment included similar numbers of nontreated fruit.

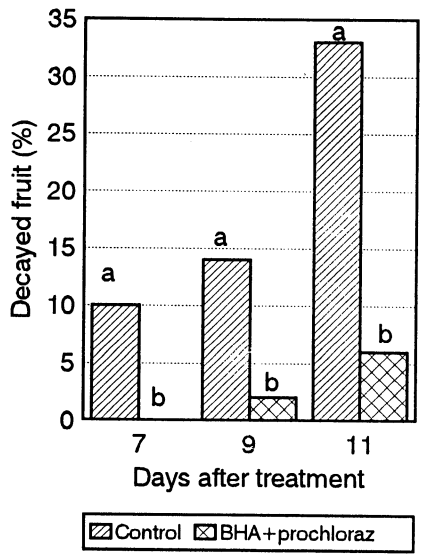

Fig. 2. Effect of post-harvest dip treatment of avocado cv. Hass on the percentage of decay development. Fruit from a grove in southern California were harvested and cooled at 5°C for 24 h before dip treatment in 1,200 µg a.i. of butylated hydroxyanisole + 250 µg a.i. of prochloraz per ml, and compared with untreated fruit. Fruit were stored at 20°C and scored for disease incidence at 7, 9 and 11 days. Bars with the same letter above them (within each observation day) are not significantly different using the Waller-Duncan *k*-ratio *t* test ($P < 0.05$).

Mehadrin, Israel. Fruit were treated with 1,200 µg a.i. of BHA per ml, 900 µg a.i. of prochloraz per ml, 1,200 µg a.i. of BHA + 225 µg a.i. of prochloraz per ml, or water for the control. Approximately 650 kg of fruit were treated, packed into cartons, stored for 14 days at 5°C, transferred to 20°C, and evaluated for disease after 8 days. This simulated a period for surface transportation by boat and then a period for marketing. There were six replicates of 20 fruit for each treatment. In the second test the fruit were treated and stored at 5°C for either 7 or 14 days, then transferred to 20°C and evaluated for disease after 4 and 6 days. There were 10 replicates of 14 fruit in each treatment.

Granot, Israel. Fruit were treated with 1,200 µg a.i. of BHA + 225 µg a.i. of prochloraz per ml, or water for the control, sorted according to weight, and packed 18 fruit (ca. 220 g each) per carton and 14 fruit (ca. 280 g each) per carton. There were 10 replicates (cartons) in each treatment. Treated fruit were stored at 20°C or at 5°C for 21 days and then at 20°C. Those fruit stored only at 20°C were evaluated after 9 and 12 days while those stored initially at 5°C were evaluated after they had been stored at 20°C for 7 and 9 days. The evaluation times were dictated by the disease progress.

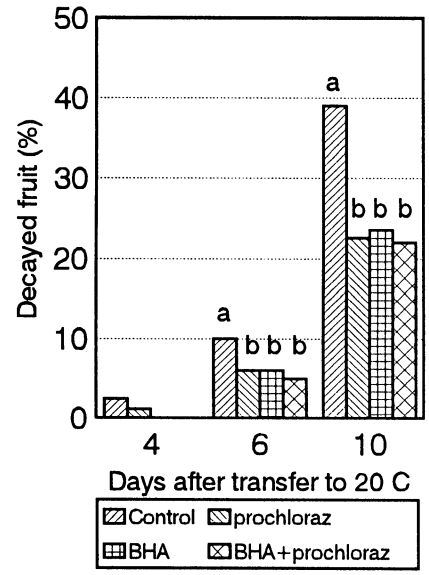

Fig. 3. Effect of post-harvest dip treatment of avocado cv. Hass on the percentage of decay development. Fruit from a grove in southern California were harvested, cooled for 24 h at 5°C and dip treated in the laboratory with 1,000 µg a.i. of prochloraz per ml, 1,200 µg a.i. of butylated hydroxyanisole (BHA) per ml, or 1,200 µg a.i. of BHA + 250 µg a.i. of prochloraz per ml, and compared with untreated fruit. Fruit were stored for 17 days at 5°C, then transferred to 20°C and scored for disease incidence at 4, 6, and 10 days. Bars with the same letter above them (within each observation day) are not significantly different using the Waller-Duncan *k*-ratio *t* test ($P < 0.05$).

HaEmek, Israel. Fruit were treated with 1,200 µg a.i. of BHA + 225 µg a.i. of prochloraz per ml. There were 10 replicates of 18 fruit each from each of two groves, Shaar Haamakim and Daliah. Treated fruit were stored at either 2 or 5°C for 14 days then transferred to 20°C and evaluated after 9 days. Control fruit were untreated.

RESULTS

Small-scale experiments. After 9 days of storage at 20°C the decay incidence among fruit treated with 1,200 µg a.i. of BHA per ml or with the mixture of 1,200 µg a.i. of BHA + 250 µg a.i. of prochloraz per ml was significantly lower than that among the control fruit (Fig. 1A,B). However, 2 days later, after 11 days at 20°C, decay incidence among untreated fruit averaged 62% (Fig. 1A,B) whereas the incidence among fruit treated with 1,000 µg a.i. of prochloraz per ml within 5 h of harvest averaged 76%. Fruit treated with BHA alone averaged 58.5% and only fruit treated with the mixture of BHA and prochloraz had a significant reduction of decay, averaging 22% in two groves (Fig. 1A,B). With fruit from a third grove, the decay incidence among the controls after 11 days at 20°C was only 10%; each of the treatments reduced the decay by 60 to 70% (Fig. 1C).

When fruit were precooled, disease incidence among untreated fruit averaged 10, 14, and 33% after 7, 9, and 11 days, respectively. By contrast, fruit treated with BHA + prochloraz averaged 0, 2, and 6%, respectively. The difference between the control and respective treatments was significant at $P = 0.05$ (Fig. 2).

Medium-scale experiments. Fruit precooled at 5°C for 24 h before treatment, followed by 17 days of post-treatment storage at 5°C, were free of symptoms when transferred to 20°C (Fig. 3). Six days later, however, decay incidence among the control fruit was nearly 10%, whereas that among treated fruit averaged 4 to 5%. After an additional 4 days at 20°C, no differences in decay were observed among fruit treated with BHA, prochloraz, or a mixture of both. All the treatments reduced the incidence of decay by about 45% compared with the control (Fig. 3).

Semicommercial experiments. *Mehadrin, Israel.* After storage for 14 days at 5°C, followed by transfer to 20°C, the incidence of decay among untreated fruit after 8 days at 20°C was 12±1% (data not shown). In contrast, fruit treated with 900 µg a.i. of prochloraz per ml or with 1,200 µg a.i. of BHA + 225 µg a.i. of prochloraz per ml had a decay incidence of 6±1%.

In experiment 2, as storage time at 5°C increased, the incidence of decay increased (Table 1). The treatment with BHA reduced the incidence of decay compared with the control; BHA + prochloraz showed the greatest reduction of decay but was not always significantly different from prochloraz alone.

Granot, Israel. After 9 days of storage of 220-g fruit at 20°C, disease incidence among the control was seven times that among treated fruit (Table 2). In 280-g fruit, the control had 1.6 times the decay of treated fruit after 9 days. Three days later, the incidence of decay among both fruit sizes had increased, but treated fruit still showed significantly lower decay incidence than the untreated controls. Among fruit stored 21 days at 5°C before storage at 20°C, decay in 220- and 280-g fruit used as controls was 2.2 and 3.2 times the decay of treated fruit, respectively. Two days later, the incidence of decay among all fruit had increased but treated fruit still had less decay than controls. Fruit of 280-g size showed a higher incidence of decay than fruit of 220-g size, but treated fruit always showed a significant decrease in decay symptoms (Table 2).

Shaar Haamakim and Daliah, Israel. Following 14 days storage at 2°C or 5°C followed by 9 days of storage at 20°C, the treated fruit showed significantly lower decay at both storage temperatures relative to the controls (Table 3).

DISCUSSION

A commercial formulation of BHA, used to inhibit lipid peroxidation in many foods, delayed quiescent infections of *C. gloeosporioides* in avocados from becoming active in semicommercial experiments. In the present study, disease development was delayed by a single post-harvest treatment of 1,200 µg a.i. of BHA per ml, or 1,200 µg a.i. of BHA + 225 or 250 µg a.i. of prochloraz per ml. Prochloraz alone has been shown to be effective in reducing post-harvest anthracnose, stem end rot, and Dothiorella rot in several countries (1,3,9). In Israel, prochloraz at 900 µg a.i./ml reduced decay but at 300 µg a.i./ml or less it had no effect (9,10). In the studies conducted in California, the efficacy of prochloraz at 1,000 µg a.i./ml was inconsistent, but the mixture of 1,200 µg a.i. of BHA per ml and 250 µg a.i. of prochloraz per ml significantly reduced the percentage

Table 1. Effect of butylated hydroxyanisole (BHA) and prochloraz on post-harvest decay by *Colletotrichum gloeosporioides* of Fuerte avocados stored 7 or 14 days post treatment at 5°C followed by transfer to 20°C for 6 days

	Storage at 20°C (days)			
Treatment	**4[x]**	**6[x]**	**4[y]**	**6[y]**
Control	16.7 a[z]	53.2 a	20.5 a	77.7 a
BHA, 1,200 µg a.i./ml	2.1 b	24.0 b	11.2 b	41.3 b
Prochloraz 900 µg a.i./ml	2.1 b	16.7 b	1.0 c	21.4 c
BHA, 1,200 µg a.i./ml + prochloraz 225 µg a.i./ml	0 b	6.3 c	3.1 c	18.8 c

[x] Means of 10 replications of 14 fruit each, storage at 5°C for 7 days.
[y] Means of 10 replications of 14 fruit each, storage at 5°C for 14 days.
[z] Numbers within columns followed by the same letter are not significantly different using the Waller-Duncan *k* ratio *t*-test ($P < 0.05$).

Table 2. Effect of butylated hydroxyanisole (BHA) + prochloraz on post-harvest decay by *Colletotrichum gloeosporioides* of 220-g or 280-g Fuerte avocados stored at 20°C and 5°C

	Percent decay after storage at 20°C (days)			
Treatment	**9[x]**	**12[x]**	**7[y]**	**9[y]**
220 g (ca.)				
Control	27.8 a[z]	71.2 a	24.9 a	87.8 a
BHA (1,200 µg a.i./ml) + prochloraz (225 µg a.i./ml)	3.7 b	58.8 b	11.1 b	44.5 b
280 g (ca.)				
Control	44.4 a	97.2 a	62.9 a	91.4 a
BHA (1,200 µg a.i./ml) + prochloraz (225 µg a.i./ml)	27.8 b	58.3 b	19.5 b	50.0 b

[x] Means of 10 replications of 14 fruit each, initial storage 20°C.
[y] Means of 10 replications of 14 fruit each, initial storage 5°C for 21 days.
[z] Numbers within columns, within fruit size, followed by the same letter are not significantly different using the Waller-Duncan *k* ratio *t*-test ($P < 0.05$).

Table 3. Effect of butylated hydroxyanisole (BHA) + prochloraz on post-harvest decay by *Colletotrichum gloeosporioides* (percent decay) of Fuerte avocados from two different growers stored 9 days at 20°C after 14 days at 5 or 2°C

	Shaar Haamakin		Daliah	
Treatment	**5 C[y]**	**2 C**	**5 C**	**2 C**
Control	48.8 a[z]	55.1 a	47.4 a	23.4 a
BHA (1,200 µg a.i./ml) + prochloraz (225 µg a.i./ml)	31.0 b	41.4 b	29.1 b	14.3 b

[y] Means of 10 replications of 18 fruit per replication.
[z] Numbers within columns followed by the same letter are not significantly different using the Waller-Duncan *k* ratio *t*-test ($P < 0.05$).

of unmarketable fruit. The effectiveness of low rates of prochloraz in combination with BHA may be the result of an additive or synergistic action that persists for more than 21 days in storage. Alternatively, the presence of a wetting agent in the prochloraz formulation may facilitate the entry of the antioxidant into the fruit.

Ever-increasing avocado yields and the search for new markets result in the need for fungicide treatments to preserve the fruit during long-term storage or shipping or to regulate marketing. These factors, accompanied by a mounting concern for chemical residues on the fruit, dictate the search for new options for the control of post-harvest diseases in avocado and other fruits. Our results and previous work by Prusky (4) indicate that it is feasible to reduce disease in avocado with low concentrations of fungicides and modulation of the natural resistance of the fruit.

In this study, the application of BHA prevented the conversion of quiescent infections into active ones, thereby delaying decay development sufficiently to permit normal marketing and consumption of infected fruit. A limitation of the use of the antioxidant is that the treatment needs to be applied within 24 h of harvest. Prusky and Prusky et al. (4,10) have reported that efficacy decreased if treatments were applied later than 24 h after harvest.

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