Effects of Several Postharvest Fungicide Treatments on the Quality and Ripeness of Cold-Stored Apples

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Benzimidazole fungicides and imazalil applied in postharvest treatments to apples (*Malus domestica* L., var. Starking and Golden Delicious) for the control of the most important postharvest diseases in pome fruit during cold storage caused small but statistically significant ($p = 0.01$) changes in total acidity and total sugar composition of both cultivars. No significant differences in firmness were observed in treated fruits relative to the untreated apples of the same cultivar, and an important improvement in their ripening was also detected. The ascorbic acid content of treated fruits was significantly higher than the corresponding values of untreated fruits during all storage times.

Benzimidazole fungicides, benomyl [methyl (1-(butylcarbamoyl)-1*H*-benzimidazol-2-yl)carbamate], carbendazim (methyl 1*H*-benzimidazol-2-ylcarbamate), methylthiophanate [dimethyl 4,4′-*o*-phenylenebis(3-thioallophanate)], and thiabendazole (2-thiazol-4-ylbenzimidazole), have been extensively used to control the most important diseases in pome fruit caused by *Penicillium expansum*, *Gloesporium sp.*, and *Botrytis cinerea* (Burchill and Edney, 1972; Bompeix and Morgat, 1970; Handenburg, 1974). Imazalil [allyl 1-(2,4-dichlorophenyl)-2-imidazol-1-ylethyl ether] is a relatively new systemic fungicide that has become necessary to control the storage diseases caused by *Penicillium* and *Alternaria sp.* resistant to the benzimidazole fungicides (Edgington et al., 1971).

A few papers have reported the influence of several pesticides on quality attributes of fruits during the growing season (Taylor and Mitchell, 1956; Riehl et al., 1957; Dean and Bailey, 1960; Rouchaud et al., 1984), but little is known about the effects of postharvest chemical treatments on the physiology and quality of fruit during the storage period.

In view of the large world production of apples and the improvement in the storage technology with extensive use of chemicals included, an investigation was made of the effects of these agents on the factors affecting the quality and ripening of cold-stored fruits. This work was included as part of an extensive study (Cano, 1985) on the penetration and persistence of these fungicides applied in postharvest treatments (Cano et al., 1987a,b) and their influence on the most important constituents of apples like carbohydrates (Cano et al., 1987c).

MATERIALS AND METHODS

Fungicide Treatments. Apples (*Pyrus malus* L.) cvs. Starking and Golden Delicious were grown in Málaga, Spain. Fruits were transported to the pilot plant of the Instituto del Frio, Madrid, where they were selected for uniformity of size and maturity. Only apples free of injury were treated.

The chemicals employed in this study were applied according to accepted postharvest practices to pome fruits. Twenty-four hours after harvest, treatments were carried out by immersion of fruits in aqueous solutions of each fungicide compound at the concentrations and times of immersion shown in Table I. Fruits were then placed in open plastic boxes and stored in 85–90% relative humidity and at 0 °C (cv. Starking) or +2 °C (cv. Golden Delicious).

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Table I. Program of Fungicide Treatments Applied to Apple Fruits

fungicide	formulation	concn a.i., mg/L	immersion time
benomyl	BENLATE	500	1 min
carbendazim	BAVISTIN	500	1 min
methylthiophanate	PELT	1000	1 min
thiabendazole	PECTO 60	1000	35 s
imazalil	DECCOZIL-S-7.5	1000	1 min

Sample Preparation. Apple puree was prepared for analysis as follows: The apples were hand-peeled, followed by removal of seeds and cores. The products were homogenized for 3 min in a Waring blender, and chemical analysis was carried out immediately.

Total Carbohydrates. Total carbohydrates (sucrose, fructose, glucose, sorbitol) were measured by the high-performance liquid chromatographic method reported by Cano et al. (1987c), using a Waters liquid chromatograph equipped with a Model R 401 differential RJ detector. Sample injection was made with a Model UGK injector, and a Model 6000A pump was used. The column utilized was a Sugar-pak (Waters) working at 90 °C. A Hewlett-Packard 3390A integrator was used to record and quantitate the peaks for comparison by the external standard method.

Standard mixtures of carbohydrates, obtained from commercial sources (Merck and Sigma), were prepared in deionized water filtered through a Millipore filter (0.45 μm) and degassed prior to the analysis. Flow rate was 0.8 mL/min.

pH Value and Titratable Acidity. A Radiometer Copenhagen pH meter 22 was used to determine the pH value. A 10-q sample of puree was titrated with 0.1 N NaOH to pH 8.1. The results were expressed as percent malic acid.

Ascorbic Acid. The AOAC (1984) 2,6-dichloroindophenol titrimetric method was used to determine the ascorbic acid. The results are expressed as milligrams per 100-g sample.

Soluble Solids. The soluble solid was measured with a digital refractometer ATAGO with automatic temperature correction.

Firmness. The fruits were peeled in three 10 × 10 mm areas. Firmness of individual fruit was determined with an INSTRON Food Testing Instrument, Model 1140, with a 11-mm plunger.

Statistical Analysis. Fruits were analyzed in duplicate each month during their storage time. Twenty-four apples were removed from each plot, and two samples of twelve fruits were used for analysis. Duncan's multiple-range test

0021-8561/89/1437-0330$01.50/0

Table II. Characteristics[a] of Apples before Fungicide Treatments

characteristic	cv. Golden Delicious	cv. Starking
size, mm	79.5 ± 0.5	72.1 ± 0.4
wt, g	222.2 ± 0.3	170.6 ± 0.3
firmness, kg	5.9 ± 0.7	4.0 ± 0.6
sol solids	12.9 ± 0.4	12.8 ± 0.7
total sugars, g/100 g FW	14.5 ± 0.6	12.5 ± 0.8
reducing sugars, g/100 g FW	13.0 ± 1.1	11.0 ± 0.9
sucrose, g/100 g FW	1.4 ± 0.4	1.4 ± 0.9
pH	3.8 ± 0.2	4.1 ± 0.1
titratable acidity, g malic acid/100 g FW	4.4 ± 3.0	4.9 ± 2.1
ascorbic acid, mg/100 g FW	0.62 ± 0.6	1.60 ± 0.9

[a] Values are the means of 10 fruits ± standard deviation.

was used to determine differences between treatments.

The statistical analysis was made with the data of each year separately, and only data of the last year (1983–1984) are reported in this paper.

RESULTS AND DISCUSSION

Total Carbohydrates. The initial composition of both apple cultivars is shown in Table II. Chemicals had a highly significant effect on total carbohydrate content in cold-stored fruits. Apples treated with benomyl and carbendazim showed lower total carbohydrate content than untreated fruits in the storage period. Methylthiophanate, thiabendazole, and imazalil produced a maximum increase in total carbohydrates at the second month after fungicide treatment, approximately 0.3 unit over those of controls on imazalil treated fruits. In the climateric stage (2–3 months of storage) there were significant differences (p = 0.05) between treated and untreated apples in both cultivars (Table III).

These chemical treatments may promote the hydrolysis of structural polysacharides of fruit, producing abnormal carbohydrate levels at the first stages of cold storage.

Titratable Acidity and pH. There was a decrease in total acid and rise of pH value during cold storage of fruits as reported other authors (Hulme, 1970). Apples treated with benzimidazole fungicides showed lower total acidity than untreated fruits (Table IV). In apples, cv. Starking, the fall in total acidity was significantly different between fruits treated with benomyl and thiabendazole and control. Moreover, methilthiophanate produced an early loss in acidity (second month of storage) in relation to untreated fruits in cv. Golden Delicious.

Ascorbic Acid. The chemical treatments produced an increase in ascorbic acid retention of fruits (Table V). The ascorbic acid content was slowly diminishing during cold storage. Untreated apples, cv. Starking, lost more that 60% of initial value (1.60 mg of ascorbic acid/100-g apple fresh weigh). At the end of storage time (about 5 or 6 months) the ascorbic acid content of treated fruits was greater than those of controls for the same date. These results were similar to those obtained in Golden Delicious apples; fruits treated with benomyl, carbendazim, and thiabendazole gave the most pronounced effects on ascorbic acid level (0.83, 0.83, and 1.24 mg of ascorbic

Table III. Effects of Postharvest Fungicides on Total Carbohydrate Content (g/100-g FW) of Stored Apples[b]

		months of storage						
cultivar	treatment	0	1	2	3	4	5	6
Golden Delicious	control[a]	14.54	14.95 a	16.62 b	14.38 a	12.04 b	10.18 a	10.17 a
	benomyl		14.01 b	12.73 a	11.31 b	14.53 a	8.24 b	4.63 b
	carbendazim		14.31 b	13.90 c	13.51 c	7.53 c	13.41 c	10.22 a
	imazalil		10.00 c	12.55 a	9.91 e	10.42 d	11.04 d	6.88 c
	methylthiophanate		14.84 a	10.68 d	14.99 d	13.62 e	10.98 d	4.65 b
	thiabendazole		12.51 d	13.66 c	14.07 a	6.73 c	9.11 b	10.68 a
significance[c]			**	*	**	**	*	*
Starking	control		17.84 a	16.66 b	13.18 ac	13.19 a	9.74 b	11.09 a
	benomyl		20.12 b	12.69 c	8.49 b	10.27 b	7.74 a	5.12 b
	carbendazim		16.47 a	10.25 a	9.49 b	10.88 b	10.02 bc	9.29 c
	imazalil		16.73 a	22.01 c	12.74 a	11.30 b	9.61 b	6.76 b
	methylthiophanate		17.99 a	19.91 d	8.93 b	9.88 c	11.08 c	5.93 b
	thiabendazole		20.90 b	11.56 c	14.84 c	4.64 d	7.63 c	10.02 a
significance[c]			*	*	**	**	*	*

[a] Control and treatment values of fruits harvested in October 1983. [b] Values followed by the same letter are not significantly different at the 5% level. [c] *, **, mean square values significant at the 5 and 1% levels, respectively.

Table IV. Effect of Postharvest Fungicides on Titratable Acidity (g of Malic Acid/100-g FW) of Stored Apples[b]

		months of storage						
cultivar	treatment	0	1	2	3	4	5	6
Golden Delicious	control[a]	0.44	0.34 a	0.33 b	0.32 a	0.27 ab	0.25 a	0.18
	benomyl		0.54 b	0.43 a	0.32 a	0.29 b	0.20 b	0.15
	carbendazim		0.39 a	0.37 d	0.35 c	0.26 a	0.17 c	0.17
	imazalil		0.57 b	0.32 b	0.29 b	0.26 a	0.21 b	0.17
	methylthiophanate		0.45 c	0.44 a	0.29 b	0.24 a	0.23 ab	0.19
	thiabendazole		0.69 d	0.49 c	0.31 a	0.26 a	0.21 b	0.16
significance[c]			**	**	*	*	*	NS
Starking	control	0.49	0.44 b	0.30 a	0.24 a	0.20	0.22 b	0.20 ab
	benomyl		0.27 a	0.30 a	0.26 a	0.20	0.19 a	0.19 a
	carbendazim		0.28 a	0.24 b	0.27 a	0.21	0.29 c	0.19 a
	imazalil		0.41 b	0.26 b	0.20 b	0.19	0.19 a	0.18 a
	methylthiophanate		0.31 c	0.32 a	0.23 ab	0.22	0.12 d	0.22 b
	thiabendazole		0.25 a	0.39 c	0.23 ab	0.20	0.20 b	0.20 ab
significance[c]			**	*	*	NS	*	*

[a] Control and treatment values of fruits harvested in October 1983. [b] Values followed by the same letter are not significantly different at the 5% level. [c] *, **, mean square values significant at the 5 and 1% levels, respectively; NS, not significant.

Table V. Effect of Postharvest Fungicides on Ascorbic Acid Content (mg/100-g FW) of Stored Apples[b]

		months of storage						
cultivar	treatment	0	1	2	3	4	5	6
Golden Delicious	control[a]	1.13	1.44 b	0.94 a	0.38 a	0.72 b	0.62 b	0.84 a
	benomyl		1.54 a	1.19 b	0.76 b	0.45 c	0.83 a	0.84 a
	carbendazim		1.23 c	1.05 c	0.76 b	0.72 b	0.83 a	1.06 b
	imazalil		1.33 d	0.94 a	0.76 b	0.54 a	1.14 c	0.63 c
	methylthiophanate		1.23 c	0.94 a	0.38 a	0.54 a	0.62 b	1.06 b
	thiabendazole		1.23 c	0.84 d	0.96 c	0.54 a	1.24 d	0.84 a
significance[c]			*	*	*	NS	*	*
Starking	control	1.60	1.33 a	1.05 b	0.38 a	0.91 b	0.62 a	0.64 a
	benomyl		1.23 b	1.19 c	0.86 b	0.63 c	0.83 b	0.84 b
	carbendazim		1.23 b	1.68 a	0.67 c	0.81 a	0.83 b	0.63 c
	imazalil		1.44 c	1.05 b	0.57 d	0.72 d	0.62 a	0.63 c
	methylthiophanate		1.33 a	0.94 d	0.48 e	0.54 c	0.41 c	0.84 b
	thiabendazole		1.23 b	0.84 e	0.76 c	0.72 d	0.83 b	0.63 a
significance[c]			*	**	*	NS	*	*

[a] Control and treatment values of fruits harvested in October 1983. [b] Values followed by the same letter are not significantly different at the 5% level. [c] *, **, mean square values significant at the 5 and 1% levels, respectively; NS, not significant.

Table VI. Effect of Postharvest Fungicides on Soluble Solids (°Brix at 20 °C) of Stored Apples[b]

		months of storage						
cultivar		0	1	2	3	4	5	6
Golden Delicious	control[a]	12.96	13.65 a	14.25 a	14.10 b	14.30 a	13.80 a	13.30 a
	benomyl		13.20 b	14.35 a	14.15 b	14.25 a	13.75 a	13.70 b
	carbendazim		14.70 c	14.20 b	13.85 a	14.30 a	14.50 b	14.80 b
	imazalil		13.55 a	13.95 c	14.25 b	13.55 b	14.15 c	14.25 c
	methylthiophanate		13.70 d	14.40 a	14.60 c	14.60 c	13.70 ad	14.25 c
	thiabendazole		14.50 e	14.60 d	13.65 d	14.40 d	13.60 d	14.20 c
significance[c]			**	*	*	*	NS	NS
Starking	control	12.80	13.54 a	13.25 b	13.60 a	13.45 b	13.90 a	14.00 a
	benomyl		12.05 b	12.75 a	11.15 b	11.30 a	11.00 b	12.50 b
	carbendazim		10.85 c	12.05 c	11.65 c	11.20 a	12.30 c	11.60 c
	imazalil		12.40 d	13.85 d	12.90 d	12.30 c	12.40 c	12.30 b
	methylthiophanate		12.30 d	12.45 e	12.50 e	12.50 c	12.90 d	12.00 c
	thiabendazole		13.10 e	12.45 e	13.50 a	13.45 b	13.45 e	12.50 b
significance[c]			**	*	*	NS	*	NS

[a] Control and treatment values of fruits harvested in October 1983. [b] Values followed by the same letter are not significantly different at the 5% level. [c] *, **, mean square values significant at the 5 and 1% levels, respectively; NS, not significant.

Table VII. Effect of Postharvest Fungicides on Firmness (Pulp Rupture Force, kg) of Stored Apples[b]

		months of storage						
cultivar		0	1	2	3	4	5	6
Golden Delicious	control[a]	5.95	4.27 a	4.04 b	3.93 a	3.70 a	3.63 a	3.42 b
	benomyl		4.30 a	4.19 a	3.89 ab	3.53 b	3.54 b	2.95 a
	carbendazim		4.25 a	3.98 b	3.93 a	3.64 c	3.44 c	3.05 ca
	imazalil		4.07 b	3.95 b	3.90 a	3.47 b	3.28 d	3.00 c
	methylthiophanate		4.30 a	4.42 c	3.89 a	3.45 b	3.36 cd	3.02 c
	thiabendazole		4.67 c	4.12 a	3.83 b	3.75 a	3.54 b	3.07 c
significance[c]			NS	*	NS	*	**	NS
Starking	control	4.34	4.43 a	4.16 b	4.03 a	4.10 a	3.71 a	4.02 a
	benomyl		4.40 a	4.11 b	3.96 a	4.11 a	4.16 b	3.55 b
	carbendazim		4.61 b	4.48 a	4.59 b	4.02 ab	3.76 a	3.70 c
	imazalil		4.20 c	4.10 b	3.99 a	3.93 b	3.91 c	3.75 c
	methylthiophanate		4.49 a	4.34 c	3.99 a	3.84 bc	4.07 d	3.84 c
	thiabendazole		3.93 d	4.26 c	3.84 c	3.75 c	3.85 a	3.83 c
significance[c]			*	NS	NS	*	**	NS

[a] Control and treatment values of fruits harvested in October 1983. [b] Values followed by the same letter are not significantly different at the 5% level. [c] *, **, mean square values significant at the 5 and 1% levels respectively; NS, not significant.

acid/100-g fruit weight respectively) versus 0.62 mg of ascorbic acid/100-g fresh weight of untreated apples at 5 months of cold storage.

Soluble Solids. Soluble solids were not clearly affected by treatment with these fungicides, opposite effects being observed in the two apple cultivars assayed (Table VI). Golden Delicious apples treated with thiabendazole, carbendazim, and methylthiophanate showed soluble solids values significantly different ($p > 0.05$) from the corresponding value of untreated fruits at 120 days of storage. On Starking cultivar these fungicides produced similar effects at all analysis dates during the experimental period, showing smaller soluble solids values than control.

Firmness. Postharvest fungicide effects on firmness were small in magnitude and appeared to be of little practical significance (Table VII). Treatments produced a slight decrease of pulp firmness on two cultivars after 6 months of storage. There were no statistically significant differences between treated and nontreated fruit during first stages of storage. Apples treated with imazalil showed greater dehydratation and excessive wrinkling of fruit peel at early storage time.

Fruits treated with benzimidazole fungicides maintained good quality in the first stages of cold storage although they showed faster ripening than untreated ones by postharvest treatments.

The different effects of postharvest chemical treatments on apples could be explained by interaction of chemical compound in the fruit metabolism.

There are two kinds of benzimidazole fungicides assayed according to their mode of action: Carbendazim and thiabendazole are systemic fungicides and fungitoxic per se, while benomyl and methylthiophanate must be altered in vivo to produce the fungistatic compound methyl 1*H*-benzimidazol-2-ylcarbamate (carbendazim) (Clemons and Sisler, 1969; Vonk and Sijpesteijn, 1971). The different biochemical mode of action of each product produced different effects in the apple quality and ripeness parameters (Brix degrees, acidity, total sugars, etc.), and they must be explained with a structure/activity studies of carbendazim analogues reported by Eckert and Rahm (1979). Imazalil has a different biochemical mode of action and structure (Eckert and Rahm, 1979), showing the most important effects on apple ripeness in both cultivars studied.

In general, postharvest chemical treatments of apples with fungicide products, benzimidazoles and imazalil, applied prior to their cold storage produce fast ripening and excessive wrinkling and peel dehydratation, but the fruits maintained good quality during the first stages of storage period.

Registry No. Benomyl, 17804-35-2; carbendazim, 10605-21-7; methylthiophanate, 23564-05-8; thiabendazole, 148-79-8; imazalil, 35554-44-0; ascorbic acid, 50-81-7.

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Received for review December 21, 1987. Revised manuscript received June 15, 1988. Accepted September 26, 1988.