Effect of Intermittent Warming and Modified Atmosphere Packaging on Fungal Growth in Peaches

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ABSTRACT

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Firm-breaker peaches (*Prunus persica* cv. Paraguay), treated with 0.25 g · liter⁻¹ iprodione, were inoculated with 10⁶ spores per ml of *Alternaria tenuis* and stored for up to 3 weeks at 0.5°C. Three intermittent warming (IW) cycles of 1 day at 20°C every 6 days at 0.5°C and modified atmosphere packaging (MAP) were applied. Fungicide treatment was ineffective in controlling growth of *A. tenuis* and *Cladosporium* spp. during storage. MAP prevented the growth of both fungal genera. IW alone or combined with fungicide seemed to increase total CFU counts and did not control decay (mainly due to exacerbated *Cladosporium* spp. development). CFU counts and losses through fungal attack were only occasionally significantly correlated.

Additional keywords: cold storage, postharvest, Rovral, weight losses

Susceptibility to chilling injury, weight loss, and decay permit only a brief cold storage of peaches. At 0°C, their shelf-life is about 3 weeks (20,24). Intermittent warming (IW) and modified atmosphere packaging (MAP) have been applied to extend the shelf life of peaches because of the resulting reduction in chilling injuries (2,4,20).

Because of concerns with food safety, there has been a renewal of interest in nonchemical approaches to postharvest disease control (14). For stone fruits, several types of physical treatments as alternatives to fungicides, such as chilling inactivation (27), controlled atmosphere or MAP (7,22), pre-storage heat treatments, individual seal-packaging (5), and combinations of the above, have been applied. Levels of CO₂ above 20% (16) and postharvest chemical treatment are the main strategies for controlling postharvest decay in stone fruits. In particular, iprodione at 1 g · liter⁻¹ was effective against species of *Botrytis*, *Monilinia*, *Rhizopus*, and *Penicillium* in harvested peaches (9,10,19). A gas composition of 10 to 15% O₂ and 15 to 25% CO₂ within the packages, as generated by conventional MAP, provides a satisfactory retention of quality attributes in peaches (15). However, exposure of fresh produce to levels of O₂ or CO₂ outside

their limits of tolerance (depending on peach cultivars, less than 2% for O₂ and up to 15 to 25% for CO₂) (15,28) can increase anaerobic respiration and the development of off flavors and other physiological disorders (15).

The present work was conducted to examine the effectiveness of IW and MAP individually or combined on the reduction of postharvest decay in cold stored peaches treated with iprodione and inoculated with *Alternaria tenuis*.

MATERIALS AND METHODS

Firm-breaker peaches (*Prunus persica* (L.) Batsch 'Paraguay') were harvested from a commercial orchard in Cieza (Murcia) and transported by an air-conditioned car to a packinghouse (Cofrucieza Soc. Coop., Cieza) where they were sorted and selected for uniform size and appearance and freedom from defects. The mean and standard deviation measurements of the fruit were as follows: axial diameter 43.2 ± 1.3 mm; longitudinal diameter 74.2 ± 4.3 mm; and weight 137.4 ± 14.4 g. Boxes of 5 kg each were quickly transported by an air-conditioned car to the laboratory at Murcia (35 km from Cieza) where they were immediately precooled in forced air to reach 5°C next to the stone 5 h later and 0.5°C 12 h later. Temperatures were recorded at 15-min intervals with a LCD portable thermometer (Digi-Thermo; Tecnoquim, Murcia, Spain) with an external sensing probe inserted at the pit of each of 5 fruit. The following morning, the peaches were arbitrarily divided into batches of 54 fruit, representing three replicates of 18 fruit each. Other fruit were sealed in oriented

polypropylene film (30 fruit representing 5 replicates of 6 fruit each).

The mean and standard deviation specifications of the polymeric film (Derfilm 40 DF 300), as provided by the supplier (Derprosa, Madrid, Spain), were 42.5 ± 3.8 µm thickness and permeabilities of 0.090 ± 0.001 g · m⁻² · day⁻¹ to water vapor, 1,112 ± 23 g · m⁻² · day⁻¹ to CO₂ and 290 ± 14 g · m⁻² · day⁻¹ to O₂ (all values at 83% relative humidity [RH] and 5°C).

A postharvest dip treatment with iprodione (Rovral 50WP, Rhône-Poulenc, Paris, France) at 0.25 g · liter⁻¹ (pH = 7 and 18°C for 5 min) was used to study its efficacy when combined with IW and MAP techniques. All fruit used for MAP storage were fungicide treated. Peaches selected for continuous cold storage were placed inside clean plastic boxes with the fruit touching and the boxes then placed inside 360-liter, gas-tight, stainless steel chambers in a cold room at 0.5 ± 0.5°C and 95% RH. The chambers were equipped with a renewal humidification system with an airflow of 360 liters · h⁻¹ (3). During IW storage, three warmings were applied by removing the corresponding boxes and bags from the cold room to another room at 20°C and 95% RH. After 1 day of warming, the fruit were re-transferred to 0.5°C (3). The batches of peaches undergoing MAP were placed in the same cold room at 0.5°C.

Weight loss was determined with a scale with an accuracy of 0.1 g (Mettler, PJ1220, Madrid, Spain). Changes in O₂ and CO₂ concentration within the bags were monitored in a gas chromatograph (Perkin Elmer, Autosystem 1020, Madrid, Spain) equipped with a thermal conductivity detector.

A. tenuis was isolated from a rotted Paraguay peach. A conidial suspension was prepared in sterile, distilled water (10 g · liter⁻¹) from a 7-day-old, single-conidium culture grown on Rose Bengal Agar (Adsa-Micro SA, Barcelona, Spain) at 30°C. The suspension was stirred before and during inoculation to maintain uniform conidial distribution (17). Iprodione-treated fruit were inoculated 5 h after harvest with a spore suspension of 10⁶ spores ml⁻¹ of *A. tenuis*. Initial counts of microorganisms were made 24 h later, and results were expressed as CFU per ml.

For the sampling procedure, 6 fruit from each replicate and treatment were aseptically removed, put into a bath of 2.5 liters

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of sterile, distilled water, and shaken vigorously for 30 s. From each replicate, 0.2 ml of a 10^{-1} dilution of this water was transferred to a petri dish (three dishes per replicate). Microflora counts were made after 3 days at 30°C and then microscopically examined. In order to identify fungal groups, the macroscopic characteristics of the colonies were observed (color, shape, and firmness) as well as some microscopic characteristics (fruiting type, shape and color of the spores), following the procedure of Barnett and Hunter (8) and Artés et al. (6). Fruit were examined for rot incidence at the beginning of storage and at weekly intervals until 3 weeks of storage.

The experimental design was completely randomized. Each plastic box or each plastic bag constituted a replicate. The percentage of total losses and CFU per ml were statistically analyzed by converting values to their respective λ root transformation (11) or arcsine transformation (25), respectively. For the statistical analysis of CFU per ml, the λ values were between 0.25 and 0.1 for *A. tenuis* and between 0.5 and 0.1 for *Cladosporium* spp. Given the factorial structure of the treatments (conventional cold storage [CS] with or without fungicide [F], IW with or without fungicide, MAP, and IW+F+MAP), the analysis of variance and residual analysis (21,25) were divided in two sections (I and II). Thus, the statistical model was as follows: $Y_{ijk} = \mu + T_i + IW_j + F_k + T_i \cdot IW_j + IW_j \cdot F_k + T_i \cdot F_k + IW_j \cdot T_i \cdot F_k + \epsilon_{(ijk)}$, where Y_{ijk} is the l th replicate ($l = 1, 2, 3$) of the i th time (T) ($i = 0, 1, 2, 3$, in weeks) and j th IW ($j = 0, 1$) and k th fungicide or MAP ($k = 0, 1$). If significant differences in time were found, least significant difference or orthogonal polynomial contrasts were performed (3,21,25).

RESULTS AND DISCUSSION

Identification of microorganisms. Except in MAP-treated fruit, all treatments exhibited symptoms of *A. tenuis* after 1 week of storage. After 2 weeks of storage, *Cladosporium* spp. appeared. *Cladosporium* is a weak parasite that rarely causes loss in cold stored peaches (13,26). The rotted tissue affected by this fungus is confined to the surface of peaches (12), and in our experiment, infections by *Cladosporium* spp. were superficial, associated mainly with overripe fruit (showing excessive softness and shriveling). The fungus's high rate of growth was probably related to a disruption of its balance with *A. tenuis*. Other ubiquitous fungal pathogenic species (*Rhizopus*, *Penicillium*, and *Aspergillus*) and yeasts also appeared occasionally but to a lesser extent. In accordance with the terminology used to describe fungal and bacterial growth by sigmoid curves (27), the lag phase of *A. tenuis* growth was similar in all treatments. However, a lag and log phase of *Cladosporium* spp.

Fig. 1. Means of decay and CFU during storage of peach cv. Paraguay at 0.5°C (no CFU counts and no decay of both fungi genera were observed on modified atmosphere packaging (MAP) plus fungicide treatment at any time; no CFU of *Cladosporium* spp. at harvest and after 1 week of storage were observed). CS = conventional storage at 0.5°C; CS + F = CS with a previous fungicide dip (iprodione, 0.25 g · liter⁻¹). IW = intermittent warming, 3 cycles of 1 day at 20°C every 6 days at 0.5°C. Bars are least significant difference at $P = 0.05$.

Table 1. Analysis of variance of the CFU and total decay incidence on peaches inoculated with *Alternaria tenuis* and stored in air

Source	df ^w	<i>Cladosporium</i> spp. (CFU per ml)		Total decay (%)	
		SS ^x	P ^y	SS	P
Intermittent warming (IW)	1	731.2	***	0.010	NS
Fungicide (F)	1	15.4	NS	0.012	NS
Time	3	1263.3	***	0.653	***
Linear effect	1	1042.0	***	0.293	***
Quadratic effect	1	1.4	NS	0.307	***
Cubic effect	1	219.8	***	0.053	NS
IW × F	1	1.5	NS	0.000	NS
Time × IW ^z	3	751.8	***	0.035	NS
Linear × IW	1	517.6	***	0.005	NS
Cubic × IW	1	223.7	***	0.011	NS
Time × F ^z	3	16.2	NS	0.064	NS
Time × IW × F ^z	3	2.6	NS	0.157	*
Cubic × IW × F	1	0.02	NS	0.130	**
Residual	32	457.2		0.502	
% variance explained			85.9		65.0

^w Degrees of freedom.

^x Sum of squares.

^y Probability: NS = not significant; *, **, *** significant at $P = 0.05$, 0.01, or 0.001, respectively.

^z Only significant terms of the polynomial trend partition of time factor are shown.

growth was promoted by IW storage, possibly because spore germination was induced by the temperatures applied in the warming phase, which were near the optimum temperature for fungal growth.

Effect on CFU counts. Fungicide treatment reduced the initial CFU counts on the fruit surface (Fig. 1), although during the experiment as a whole, such treatment, with or without IW, had no significant effect on total CFU counts (Table 1). This was probably due to the low concentration of iprodione used, since a concentration of 2.4 g · liter⁻¹ of iprodione has been reported as being effective in controlling *A. alternata* and *A. mali* in apples (18,23).

IW had no effect on the CFU counts of *A. tenuis* (Fig. 1). After the first warming period, total CFU counts increased to reach a constant level because development of *Cladosporium* spp. probably was exacerbated by IW. Following the analy-

sis of variance model (Tables 1 and 2), only the linear effect of time was significant ($P = 0.01$) for CFU counts of *A. tenuis* with a low explained variance (35.6 to 41.4%). High CFU counts of *C. herbarum* have been reported in apricots with a maximum level after 10 days of storage at 0°C (5).

In contrast, MAP alone resulted in no CFU counts and no decay for both *A. tenuis* and *Cladosporium* spp. (Table 2). Its efficacy was similar when combined with IW (IW+F+MAP treatment), although particularly effective in controlling *Cladosporium* spp. (Fig. 1 and Table 2).

Effect on weight losses and decay. A high correlation ($R^2 = 0.88$ to 0.99 , $P \leq 0.001$) was found from the linear regression model used to predict weight losses during cold storage (Fig. 2). MAP drastically reduced total weight losses during storage, while IW and IW+F+MAP almost

doubled the values obtained with conventional storage and MAP, respectively (Figs. 1 and 2; Table 3). The effect of IW in air confirms our previous results in other cultivars (2,4). However, in MAP fruit a quadratic trend was detected (Figs. 1 and 2; Table 3). Differences observed between MAP and IW+F+MAP were probably related to the linear effect of increasing weight losses due to IW. The quadratic trend in the MAP fruit response may be related to water vapor accumulation within the unperforated bags during the first week of storage, practically without weight loss.

No significant effect on the efficacy of iprodione dip alone or combined with IW in reducing decay caused by both *A. tenuis* and *Cladosporium* spp. was observed. IW had no effect in reducing decay by *A. tenuis* or *Cladosporium* spp. (Fig. 1). Total losses were similar to those observed in conventionally stored fruit due to overripeness (less than 2 and 4% fruit affected after 2 or 3 weeks of storage, respectively, in IW or IW plus iprodione). In IW fruit, *A. tenuis* and *Cladosporium* spp. attacked overripe fruit to a greater extent. Iprodione showed no synergistic effect with IW, refuting in part our previous hypothesis (4). It is possible that this synergistic effect might occur at higher concentrations and/or in more mature fruit, because of different susceptibilities to fungal attack and/or a different degree of resistance to gas diffusion (3,27,28).

MAP prevented the growth of both *A. tenuis* and *Cladosporium* spp., confirming previous results (5,15). This was probably due to the fungistatic effect of high CO₂ generated by passive MAP ($19.5 \pm 2.5\%$ CO₂ and $5.1 \pm 1.6\%$ O₂ means and standard errors, respectively) or IW+F+MAP (where CO₂ levels increase temporarily by about 5% CO₂ after every IW period). A synergistic effect between O₂ and CO₂ in reducing fungal attack is very improbable (1). However, when this effect is combined with IW, a slight growth of both fungal genera was observed, probably due to water condensation within the plastic bags.

According to the hypothesis of Barkai-Golan (7), antifungal compounds that were active in the immature Paraguayo peaches and that decrease with ripening (enhanced by IW in the present work) could be related to changes in fruit resistance against fungal development during IW and MAP. Fungicide treatment, IW, and MAP would only modulate the development of this disruption after inoculation with *A. tenuis*. This disruption of the balance was not found in MAP fruit, probably because of pathogen growth inhibition by high CO₂ concentrations.

In conclusion, MAP retarded the onset of senescence, confirming previous results of Barkai-Golan (7), who showed that MAP also inhibited ripening and senescence and probably suppressed the progress of deep-seated infections. This fung-

Table 2. Mean squares from the analysis of variance of the CFU and total decay incidence in peaches inoculated with *Alternaria tenuis* and treated with 0.25 g · liter⁻¹ iprodione

Source	df ^w	<i>Cladosporium</i> spp. (CFU per ml)		Total decay (%)	
		SS ^x	P ^y	SS	P
Intermittent warming (IW)	1	2.215	***	0.003	NS
MAP	1	2.307	***	0.087	**
Time		3.787	***	0.205	***
Linear effect	1	3.011	***	0.111	**
Quadratic effect	1	0.000	NS	0.087	**
Cubic effect	1	0.753	*	0.007	NS
IW × MAP		1.127	**	0.003	NS
Time × IW ^z	3	2.650	***	0.052	NS
Linear × IW	1	1.262	**	0.019	NS
Cubic × IW	1	1.176	**	0.035	NS
Time × MAP ^z	3	2.658	***	0.064	NS
Linear × MAP	1	2.388	***	0.013	NS
Quadratic × MAP	1	0.180	NS	0.040	*
Time × IW × MAP ^z	3	1.130	*	0.033	NS
Linear × IW × MAP	1	0.868	**	0.002	NS
Residual	32	3.225		0.274	
% variance explained			83.1		62.0

^w Degrees of freedom.

^x Sum of squares.

^y Probability: NS = not significant; *, **, ***, significant at $P = 0.05$, 0.01, or 0.001, respectively.

^z Only significant terms of the polynomial trend partition of time factor are shown.

Table 3. Analysis of variance of weight losses under conventional storage and modified atmosphere packaging (MAP) of peaches^v

Source	df ^w	Conventional		MAP	
		SS ^x	P ^y	SS	P
Time	3	28.46	***	0.469	***
Linear effect	1	28.30	***	0.462	***
Quadratic effect	1	0.08	NS	0.006	**
Cubic effect	1	0.08	NS	0.005	*
Intermittent warming (IW)	1	3.48	***	0.056	***
Time × IW ^z	3	3.69	***	0.024	***
Linear × IW	1	3.36	***	0.023	*
Residual	56	9.69		0.041	
% variance explained			78.6		93.2

^v Weight losses as percent on a fresh weight basis (wt/wt).

^w Degrees of freedom.

^x Sum of squares.

^y Probability: NS = not significant; *, **, ***, significant at $P = 0.05$, 0.01, or 0.001, respectively.

^z Quadratic and cubic interactions time × IW were not significant.

istic effect on CFU counts and decay was probably due to high CO₂ levels in MAP. However, the application of IW, which produced a slight and short-term increase in CO₂ in the atmosphere, was less efficient in controlling decay than MAP.

The effect of IW in increasing CFU of *Cladosporium* spp. in air-stored fruit

(which has not previously been reported) was well correlated with an increase in decay. However, the growth of *A. tenuis* was limited. Moreover, low concentrations of iprodione dip had no effect on CFU counts and total losses. High CFU counts and losses by fungal attacks were only occasionally correlated significantly, which is

in agreement with previous findings (6). Further studies are required to clarify possible synergistic effects among IW, fungicide treatments, and peach maturity stage at harvest.

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Fig. 2. Prediction of weight losses (percent on a fresh weight basis at harvest) during different peach storage conditions at 0.5°C by linear regression. Continuous storage with or without fungicide (CS and CS+F, respectively) treatment (open circle); 3 cycles of intermittent warming (IW) of 1 day at 20°C every 6 days at 0.5°C, with or without fungicide (IW and IW+F, respectively) treatment (solid triangle); modified atmosphere packaging (MAP), fruit treated with fungicide, (open triangle), and IW+F+MAP (solid circle). Bars are standard errors of means and arrows below the x axis indicate IW. All equations were significant at $P \leq 0.001$.

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