# Fungistatic Effects of Carbon Dioxide in a Package Environment on the Decay of Michigan Sweet Cherries by *Monilinia fructicola*

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**ABSTRACT**

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Fruits of five cultivars of sweet cherry were packaged in low-density polyethylene and stored in air or in carbon dioxide ($CO_2$) ranging from 12 to 50% at 20 C for 7 days, or 0 C for 14 days followed by 20 C for 7 days. Before packaging, one-half of the fruits were artificially wounded and inoculated with conidia of *Monilinia fructicola*. The incidence of brown rot was recorded daily, and lesion diameters were measured at the end of four of the six experiments. Elevated levels of $CO_2$ reduced the onset of lesion development by 1–7 days depending on the concentration and reduced the percentage of fruits with lesions, and at the end of the experiment, lesions were smaller on fruits held in $CO_2$ than on fruits held in air (controls). Control of brown rot improved as the concentration of $CO_2$ increased from 12 to 50%. At 50% $CO_2$, the development of brown rot on all fruits was completely inhibited for a 7-day period at 20 C. However, the fruits developed decay within 2–4 days after they were returned to air at 25 C, indicating that the $CO_2$ treatments were fungistatic not fungicidal.

Michigan ranks third in the United States after Washington and Oregon in the production of sweet cherries (*Prunus avium* (L.) L.), with about 4,000 ha of trees. Most of the sweet cherries in Michigan are grown for processing into maraschino cherries, whereas most of the cherries produced in the western United States are grown for the fresh market or for canning. In an attempt to diversify the sweet cherry industry, Michigan growers are expanding the production and marketing of fresh market cultivars. Currently, about 20% of Michigan's sweet cherry production is for the fresh market.

Brown rot, caused by *Monilinia fructicola* (G. Wint.) Honey, is a major disease in Michigan-grown sweet cherries. Disease development is encouraged by heavy rain and high humidity, which may occur during the preharvest period. Brown rot is a greater problem on fresh market than on processing sweet cherries because the fruit are harvested at a later stage of maturity and held for a few days or weeks before final consumption. Fungicides generally provide adequate protection against brown rot of processing cherries, but control of brown rot on fresh market cherries requires additional pre- and postharvest treatment to prevent decay during marketing. Because of concerns with food safety, there is renewed interest in nonchemical approaches to postharvest disease control.

The addition of carbon dioxide to the atmosphere surrounding sweet cherries is known to reduce losses from decay caused by *M. fructicola* and many other fungi (1,2,4,7). Concentrations of 15 and 25% $CO_2$ are generally considered adequate for the control of brown rot on sweet cherry fruits at 4.4 and 7.2 C, respectively (3). Sweet cherry is unique among the stone fruit crops because of its high tolerance to carbon dioxide (2). Before the advent of mechanical refrigeration, the use of carbon dioxide (dry ice) was an effective method for reducing brown rot in railroad cars carrying shipments of sweet cherries from production areas in the West to markets in the East. Low-density polyethylene (LDPE) films have shown promise as a packaging material for cherries chiefly through reducing moisture loss (8) and through reducing decay after the accumulation of carbon dioxide in sealed liners (4).

The objective of this research was to determine the level of carbon dioxide that is needed to reduce the incidence of brown rot in LDPE-packaged sweet cherries. This information is important for designing packages for extending the shelf life of the cultivars of sweet cherries grown in Michigan.

## MATERIALS AND METHODS

Fruits of sweet cherry cultivars Cavalier, Hedelfingen, Vega, Emperor Francis, and Gold were collected as they matured from trees located at the Department of Botany and Plant Pathology Research Farm, East Lansing, MI, and at the Northwest Michigan Horticultural Research Station, Traverse City, during June and July 1990. Cherries on trees at East Lansing (experiments 1–3) were sprayed twice with benomyl (Benlate 50WP, E. I. du Pont de Nemours & Co., Wilmington, DE) and those at Traverse City (experiments 4–6) twice with iprodione (Rovral 50WP, Rhone-Poulenc, Inc., Williamson, NC) during the preharvest period. On each harvest date, a minimum of 480 cherries were collected and, if necessary, stored at 0–4 C for a few days.

After bruised and cracked fruits were removed, the remaining fruits were sorted for uniformity of maturity and size. Twenty fruits with stems were placed suture-side up into each of four 15 × 23.5 cm polystyrene containers. Each container was made up of individual 3.5-$cm^2$ cells, which made it possible to keep each fruit separate. Each container of 20 fruit was considered a replication and all treatments were replicated four times. When variation in fruit maturity was present in the sorted fruit, as indicated by intensity of pigmentation, the treatments were blocked by level of maturity.

*M. fructicola* isolate CB-2, a virulent benomyl-resistant strain isolated from a sour cherry orchard near Hart, MI, in 1975 (5), was used for inoculating fruits in each experiment. The fungus was grown in an incubator in the dark at 20 C on potato-dextrose agar (Sigma Chemical Co., St. Louis, MO). Spore suspensions were prepared by adding 20 ml of sterile distilled water to 2-wk-old cultures, scraping the mycelium and spores from the agar surface with a sterile bent glass rod, and filtering the mixture through sterile cheesecloth to remove the mycelium. An equal volume of sterile Miller's solution containing 0.1% Tween 20 (Sigma Chemical Co., St. Louis, MO) was added to the suspension (6). Spore concentrations were determined with a hemacytometer. Suspensions were adjusted with equal volumes of sterile distilled water and of Miller's solution with Tween 20 to give a final concentration of $1 \times 10^5$ conidia per 30 $\mu$l of suspension.

All fruits were wounded along the suture to a depth of 4 mm with three pins held in place with a cork. In each experiment, one-half of the fruits were

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inoculated by placing a 30-μl droplet of conidial suspension onto each fruit at the site of wounding. The remaining half of the fruit were not inoculated. Because the incidence of brown rot and other diseases on the uninoculated fruit in each experiment was nil to very light, only results obtained with inoculated fruits are reported.

Atmospheres of $CO_2$ were maintained around each lot of 20 fruit by placing a LDPE bag with inlet and outlet ports (outer bag) over a perforated inner bag that contained the fruit. The polystyrene containers, each with 20 fruits, were placed into 22 × 34 cm bags of 0.00495-cm-thick (2 mil) LDPE film (Dow Chemical Co., Midland, MI). This inner bag was heat-sealed and perforated in 16 places with a 25-gauge needle to permit rapid gas exchange between the two bags. A 5-cm-high strip of rigid polyethylene was placed on each side of the polystyrene container to prevent the top of the bag from collapsing onto the fruit. Each bag was placed into a 34 × 40 cm bag of 0.00762-cm-thick (3 mil) LDPE film fitted with a silicon-sealed port on each end. The experimental units were placed in controlled temperature chambers, and the inlet port from each outer bag was connected to a supply of mixed gasses or to a supply of ambient air. To maintain high humidity in the inner bag, 20 ml of sterile distilled water was injected into each outer bag through the outlet port at the beginning of each experiment.

Six experiments (experiments 1–6) were conducted on five cultivars. Each experiment consisted of a control treatment (ambient air) plus two or three treatments of $CO_2$ gas. Air and gas mixtures were supplied to each bag at a continuous flow rate of 100 $ml^{-1}$ with a manifold and both Tygon and plastic tubing. The composition of $CO_2$ and $O_2$ in each gas mixture was verified daily by analyzing 0.5-ml gas samples from each outer bag with a type 255 infrared $CO_2$ gas analyzer (Analytical Development Co., Ltd., Hoddesdon, England) and an Ametek S-3A $O_2$ analyzer (Thermox Instruments Division, Pittsburgh, PA) connected in series with $N_2$ as the carrier gas (flow rate 200 $ml^{-1}$). The concentrations of $O_2$ were 2–6, 2.5–11.0, 11, 5, and 5–10% for $CO_2$ concentrations of 12, 25, 35, 45, and 50%, respectively. Atmospheric treatment durations were 7 days at 20 C in experiments 1–4 and 21 days (14 days at 0 C followed by 7 days at 20 C) in experiments 5 and 6.

Infection was evaluated daily by examining each fruit through the LDPE bags for the presence or absence of decay and sporulation of the brown rot fungus at the site of inoculation. In experiments 1–4, the diameter of lesions on 10 randomly selected fruit per replicate was measured after the 7-day treatment period at 20 C.

## RESULTS

Experiments 1–3 were conducted for 7 days at 20 C with sweet cherry fruits that had received preharvest treatments of benomyl. Inoculated fruits of Hedelfingen (first harvest, experiment 1) were subjected to concentrations of 12 and 25% $CO_2$. Compared with ambient air, the development of brown rot symptoms was initially delayed 1–2 days by the $CO_2$ treatments (Fig. 1A). All of the fruits in ambient air exhibited symptoms of brown rot on day 4, whereas most of the fruits in 12 and 25% $CO_2$ exhibited symptoms on days 5 and 6, respectively. All fruits, regardless of treatment, exhibited symptoms of brown rot on day 7.

When fruits of Cavalier (experiment 2) were subjected to concentrations of 12 and 50% $CO_2$, symptoms of brown rot were delayed 1–2 days in 12% $CO_2$ and 7 days in 50% $CO_2$ when compared with fruits in ambient air (Fig. 1B). None of the fruits held in 50% $CO_2$ exhibited symptoms of brown rot at the end of the experiment, but all the fruits exhibited symptoms of brown rot within 3 days after they were returned to ambient air.

When fruits of Hedelfingen (second harvest, experiment 3) were subjected to concentrations of 25, 35, and 45% $CO_2$, the onset of symptoms of brown rot was delayed about 2, 3, and 4 days, respectively (Fig. 1C). On day 7, all fruits in ambient air and in 25% $CO_2$, about half of the fruits in 35% $CO_2$, and only about 10% of the fruits in 45% $CO_2$ exhibited symptoms of brown rot. The experiment was continued to day 10, when 98% of the fruit in 35% $CO_2$ and 90% of the fruit in 45% $CO_2$ exhibited brown rot.

When fruits of Vega (experiment 4, preharvest treated with iprodione) were subjected to concentrations of 12, 25, and 50% $CO_2$ for 7 days at 20 C, the development of symptoms of brown rot was not delayed in 12% $CO_2$ when compared with fruits in ambient air (Fig. 1D). The onset of symptom development was delayed by 1 day on fruits held in 25% $CO_2$. None of the fruits held in 50% $CO_2$ exhibited symptoms of brown rot after 7 days.

In experiments 5 and 6, none of the fruits in any of the treatments exhibited symptoms of brown rot after 14 days at 0 C under ambient and modified atmospheres. When fruits of Emperor Francis were transferred to 20 C, symptom development on fruits in 12 and 25% $CO_2$ were delayed for 1 and 2 days, respectively, but the level of infection in these treatments after 7 days was not different

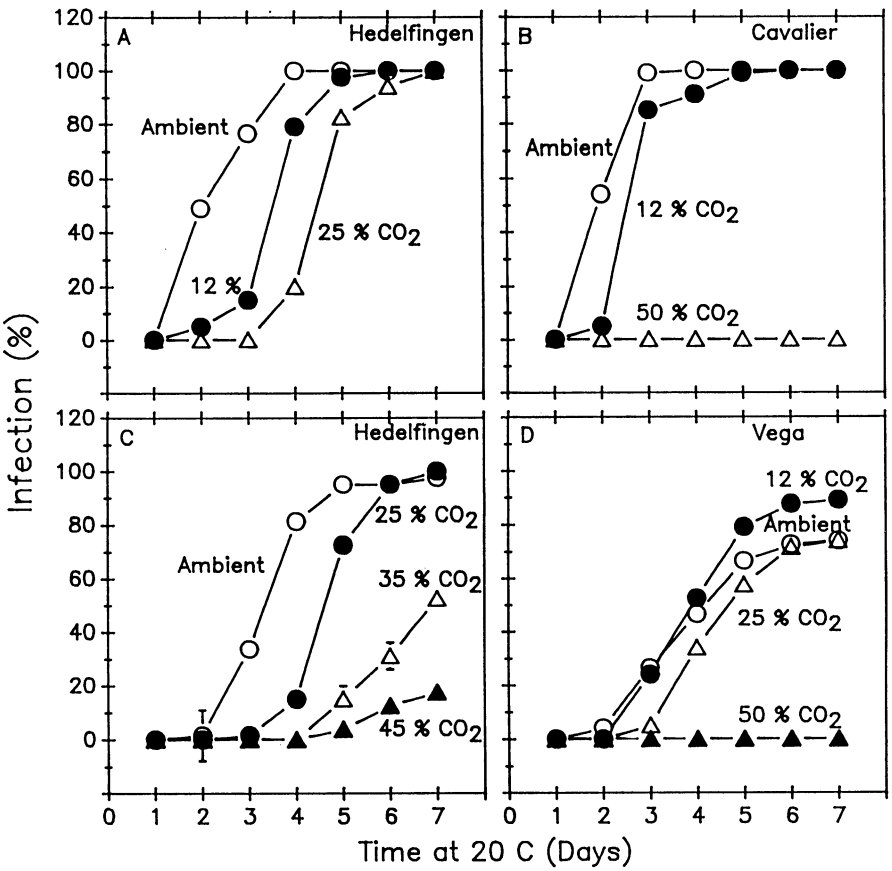


**Fig. 1.** Development of brown rot on inoculated fruits of the sweet cherry cultivars **(A)** Hedelfingen (first harvest), stored in 12 and 25% $CO_2$ or in ambient air; **(B)** Cavalier, stored in 12 and 50% $CO_2$ or in ambient air; **(C)** Hedelfingen (second harvest), stored in 25, 35, and 45% $CO_2$ or in ambient air; and **(D)** Vega, stored in 12, 25, and 50% $CO_2$ or in ambient air. All four experiments were conducted for 7 days at 20 C. Bars, which may be hidden by symbols, represent standard errors.

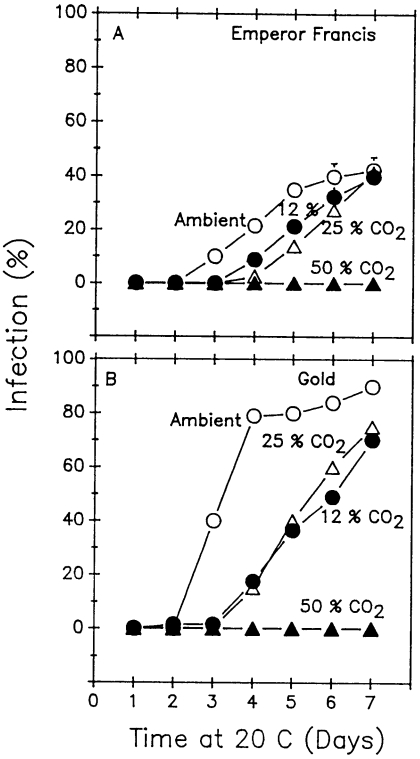


**Fig. 2.** Development of brown rot on inoculated fruits of **(A)** Emperor Francis, stored in 12, 25, and 50% $CO_2$ or in ambient air for 14 days at 0 C followed by 7 days at 20 C; and **(B)** Gold, stored at 12, 25, and 50% $CO_2$ or in ambient air for 14 days at 0 C followed by 7 days at 20 C. Bars, which may be hidden by symbols, represent standard errors.

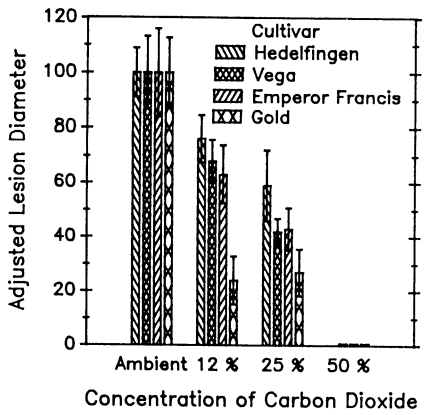


**Fig. 3.** Adjusted diameters of brown rot lesions measured at the end of four experiments on sweet cherry cultivars Emperor Francis, Gold, Hedelfingen, and Vega. Adjusted lesion diameters were calculated as follows: mean lesion diameters for control (ambient air) fruits in each experiment were equalized to 100% infection. Lesion diameters of fruit (40 per treatment) stored in $CO_2$ were divided by the mean lesion diameter for the control and multiplied by 100. The mean lesion diameters for controls were as follows: Hedelfingen, 44.5 cm; Vega, 16.0 cm; Emperor Francis, 14.7 cm; and Gold, 25.5 cm. Bars represent standard errors. The experiment with Hedelfingen sweet cherries did not include a 50% $CO_2$ treatment.

than in the control (Fig. 2A). None of the fruits held in 50% $CO_2$ exhibited symptoms of brown rot after 7 days or after 11 days (*data not shown*) in modified atmospheres. When fruits of Gold were transferred to 20 C but the modified atmospheres continued, symptoms of brown rot were delayed in each treatment compared with the ambient air treatment (Fig 2B). Symptom development was delayed by about 2 days in the 12 and 25% $CO_2$ treatments, and no symptoms of brown rot developed on the fruit in an atmosphere of 50% $CO_2$.

In experiments 1, 4, 5, and 6, the diameters of the brown rot lesions were measured at the end of the experiment. Lesion diameters were reduced as the concentration of $CO_2$ increased from 12 to 25% (except on Gold, experiment 6) and from 25 to 50% (Fig. 3). None of the fruit maintained in an atmosphere of 50% $CO_2$ exhibited brown rot after incubation of the fruit for 7 days at 20 C.

## DISCUSSION

Preharvest fungicide treatments were applied to the fruits to ensure that the cherries used in these experiments were relatively free of latent infections from *M. fructicola* and other fungal pathogens. To minimize possible interactions between the preharvest fungicide and the $CO_2$ treatments, fruits used in most of the experiments were treated with benomyl and then inoculated with a benomyl-resistant strain of *M. fructicola*. This procedure resulted in high levels of infection in control treatments (ambient air). Thus, reductions in the incidence of brown rot on fruits treated preharvest with benomyl were primarily attributable to the $CO_2$ and not to the fungicide. Infection developed much slower in experiments 4 and 5 where iprodione was the preharvest fungicide. Iprodione was also applied to the fruit used in experiment 6, but the interval between the last application and harvest was much longer.

Our results confirm that $CO_2$ supplementation during storage of sweet cherries is primarily fungistatic in nature, namely that decay attributable to a postharvest pathogen is delayed but not eliminated by $CO_2$ (2,7). In experiments 1–4, inoculated fruits without symptoms of brown rot after 7 days in atmospheres containing high levels of $CO_2$ developed brown rot lesions after they were returned to air at 25 C. Also, some uninoculated fruits developed decay when the fruits were returned to air at 25 C, because of natural infections with *Alternaria* spp., *M. fructicola*, and *Penicillium* spp. In experiments 5 and 6, some inoculated fruits without symptoms of brown rot after 21 days in atmospheres containing high levels of $CO_2$ developed brown rot after they were returned to 25 C.

Although no pitting of fruits was observed from any of the $CO_2$ treatments, fruits in treatments that lasted more than 7 days at 20 C had a noticeable off-flavor. Stems were often dried out despite the injection of water inside the packages at the outset of each experiment. However, significant moisture loss from stems was initially detectable when the fruits were sorted at the start of the experiments. Some fruit in experiment 6 become soft, but this probably occurred because the fruits were harvested at optimum maturity for immediate consumption rather than optimum maturity for storage.

Our results indicate that concentrations of 25–50% $CO_2$ are needed to effectively reduce the incidence of postharvest decay on sweet cherry fruits in a package environment. LDPE lug liners can only maintain atmospheres of 4–13% $CO_2$, and none of the films currently available have greater permeability to $O_2$ than $CO_2$ (4). In our study, concentrations of $CO_2$ greater than 12% were possible because an external source of $CO_2$ was used. In addition, high concentrations of $CO_2$ were present from the beginning of the storage period. The use of LDPE films for improving the storage life of sweet cherries is currently limited by the lack of appropriate films. In addition, a method is needed for increasing rapidly the concentration of $CO_2$ in the films.

In conclusion, a 1- to 7-day extension in shelf life was obtained at 20 C when fruits of sweet cherry were placed immediately after inoculation with the brown rot fungus into atmospheres containing 25–50% $CO_2$. As the concentration of $CO_2$ increased, the percentage of fruits with decay decreased. In a previous report (7), controlled atmosphere storage and storage in LDPE packages were only slightly more effective than cold storage in extending the storage life of sweet cherries. The advantage of the controlled atmospheres created in LDPE is that the high level of $CO_2$ helps to reduce brown rot while sweet cherries are being displayed in the market place without refrigeration. However, this technique is not practical with the LDPE films that are currently available.


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