

# Control of Brown Rot Decay of Nectarines with 15% Carbon Dioxide Atmospheres

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**ABSTRACT.** Effects of short-term exposure to a 15% CO<sub>2</sub> atmosphere on nectarines [*Prunus persica* (L.) Batsch (Nectarine Group) ‘Summer Red’] inoculated with *Monilinia fructicola* (Wint.) Honey (causal agent of brown rot) were investigated. Nectarines were inoculated with spores of *M. fructicola* and incubated at 20 °C for 24, 48 or 72 hours and then transferred to storage in either air or air enriched with 15% CO<sub>2</sub> at 5 °C. Fruit were removed from storage after 5 and 16 days and were examined for brown rot decay immediately and after ripening in air for 3 days at 20 °C. Noninoculated nectarines were stored and treated likewise for evaluation of postharvest fruit attributes to determine their tolerance to 15% CO<sub>2</sub>. Incubation period after inoculation, storage duration, and storage atmosphere had highly significant effects on fruit decay. ‘Summer Red’ nectarines tolerated a 15% CO<sub>2</sub> atmosphere for 16 days at 5 °C. Development of brown rot decay in fruit inoculated 24 hours before 5 or 16 days storage in 15% CO<sub>2</sub> at 5 °C was arrested. After 3 days ripening in air at 20 °C, the progression of brown rot disease was rapid in all inoculated nectarines, demonstrating the fungistatic effect of 15% CO<sub>2</sub>. The quantity of fungal cell wall materials (estimated by glucosamine concentration) was compared to visual estimation of decayed area and visual rating of fungal sporulation. The glucosamine assay defined the onset and progress of brown rot infection more precisely than either of the two visual tests.

*Monilinia fructicola*, the causal agent of brown rot, together with *M. laxa* (Adreh. and Ruhl.) Honey (the cause of blossom blight as well as brown rot) are the major pre- and postharvest diseases of stone fruit in California and elsewhere (Northover and Cerkauska, 1992; Ogawa et al., 1985). The incidence of brown rot decay is usually increased by preharvest rains, accumulation of excess moisture on the fruit, wind, and movement of insect vectors. Application of fungicides and insecticides at proper intervals, careful handling of harvested fruit to avoid wounding, proper sanitation pre- and postharvest, rapid cooling after harvest, and storage at 0 °C are methods used to decrease disease incidence, increase the lag period of the germination of brown rot spores, and postpone the onset of disease (Osorio et al., 1993; Sommer, 1985). Despite these efforts, postharvest decay can result in significant crop losses (MacNab, 1975; Ogawa et al., 1985).

Development of pathogen resistance to chemical sprays, strong public opinion against the use of synthetic fungicides and insecticides, and health risks involved in their application make other solutions for control of the brown rot disease more desirable. Controlled atmospheres (CAs) enriched with higher than 12% CO<sub>2</sub> have been shown to have fungistatic properties (Kader et al., 1982; Sommer, 1985). DeVries-Paterson et al (1991) found that CO<sub>2</sub> inhibited growth of *Monilinia fructicola* during modified atmosphere packaging of sweet cherries (*Prunus avium* L.), however, there was no residual effect on decay growth upon opening of the bags. A combination of careful handling, sanitation, and temperature management, with application of high CO<sub>2</sub> regimes during shipment, is an attractive management strategy at the present time. Many stone fruit, with the exception of sweet cherries (DeVries-Paterson et al. 1991; English and Gerhardt, 1942), cannot tolerate elevated concentrations of CO<sub>2</sub> for extended storage periods (Allen and Smock, 1937; Sommer, 1985). However, their short-term tolerance to CA treatments of 15% CO<sub>2</sub> is unknown.

The objectives of the present study were to examine the tolerance of nectarines to 5 and 16 d exposures to 15% CO<sub>2</sub> at 5 °C and to describe the effects on brown rot decay in various stages of development. In addition, a comparison was made between the precision with which a glucosamine assay could describe the onset and development of the brown rot infection and that of the conventional measurements of the size of the damaged area and the degree of sporulation.

## Materials and Methods

**PREPARATION AND TEST OF FUNGAL ISOLATE.** The *M. fructicola* strain used in this study was a single spore isolate, recovered from decayed nectarines, cultured, and maintained on potato-dextrose agar (PDA) slants at 4 °C (Sommer et al., 1981). Spores of the isolate were grown on PDA medium for 10 d under a 12 h photoperiod [20 mmol·m<sup>-2</sup>·s<sup>-1</sup> from a 30-W, cool-white fluorescent lamp as measured at the level of the cultures by a quantum sensor (LI-190S-1; LI-COR, Lincoln, Neb.)] at 22 °C. The pathogenicity of the spores was confirmed on peaches [*Prunus persica* L. Batsch (Peach Group)] and nectarines at 20 °C and various inoculum strengths were tested to determine the spore number which resulted in minimal visible decay (1 to 2 mm diameter) after incubation at 20 °C for 72 h. Conidia were harvested from 10-d-old cultures in sterile distilled water containing one drop of Tween 80 per 100 mL of water, centrifuged at 2000 g<sub>n</sub>, and resuspended in sterile distilled water without Tween 80. The spore suspension was filtered through four layers of sterile cheese cloth and the spore concentration determined using a hemocytometer. The spore concentration was adjusted with sterile distilled water to 5.4 × 10<sup>4</sup> spores per milliliter of inoculum. Fruits were inoculated immediately after preparation of the spore suspension.

**PREPARATION AND CHARACTERIZATION OF FRUIT SAMPLES.** ‘Summer Red’ nectarines were obtained at harvest on 18 Aug. 1994 without any postharvest treatments from the Central Valley of California, and transported to Davis, Calif. Fruit were sorted carefully for uniform maturity, size, and absence of defects and decay. Nine nectarines were evaluated for initial fruit maturity and quality including firmness, soluble solids, titratable acidity, internal condi-

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tion and decay. Fruit tissues were frozen at  $-30^{\circ}\text{C}$  for later determination of the background concentration of glucosamine. Remaining fruit were stored at  $0^{\circ}\text{C}$  until used.

**FRUIT INOCULATION.** Seventy-two nectarines were injured, each at three positions (the stem end, equator on cheek, and blossom end), to a depth of  $\approx 2$  mm with a sterile 200-mL plastic pipette tip and 10 mL of inoculum (540 spores) were injected into each wound. For each injury and inoculation, a fresh pipette tip was used and the inoculum was stirred frequently. Inoculations were performed under sterile conditions. The inoculated fruit were then placed in a closed container on a metal screen over water to maintain high humidity, covered, and incubated for 72 h at  $20^{\circ}\text{C}$ . After 24 h, the second lot of 72 nectarines were removed from  $0^{\circ}\text{C}$  storage and inoculated in the same fashion, and were incubated at  $20^{\circ}\text{C}$  for 48 h. On day 3, the third lot of fruit samples were injured, inoculated as described previously, and were incubated for 24 h at  $20^{\circ}\text{C}$  to develop three groups of fruit with varying levels of brown rot decay development.

**STORAGE TREATMENTS.** After the incubation period, all inoculated fruit were transferred to storage at  $5^{\circ}\text{C}$ . Two atmospheres (air and air enriched with 15%  $\text{CO}_2$ ), humidified by bubbling through a water reservoir, were provided to 9.5 L glass jars with a flow-through system ( $37.5\text{ mL}\cdot\text{min}^{-1}$ ). Nectarines with different incubation times after inoculation (24, 48, and 72 h) and different atmospheres were placed in separate jars with 12 fruit per jar. There were three replications for a total of 18 jars.

The remaining (noninoculated) nectarines (in  $0^{\circ}\text{C}$  storage) were also transferred to storage at  $5^{\circ}\text{C}$  in air or air plus 15%  $\text{CO}_2$  for fruit tolerance studies. There were three replications for each atmosphere for a total of six jars with 12 fruit per jar. Six fruit were randomly selected and removed from each jar after 5 d treatment and the jars were immediately resealed. The remaining six fruit were removed from the jars after 16 d.

**EVALUATION OF BROWN ROT DECAY.** Following removal of nectarine samples from storage at  $5^{\circ}\text{C}$  for 5 or 16 d, three fruit per jar were evaluated immediately and three fruit were evaluated after 3 d of ripening at  $20^{\circ}\text{C}$ . The progress of brown rot decay was recorded as the percentage of affected epidermis tissue on each nectarine. Average diameter of decayed tissue at each inoculation area (three areas/fruit) was measured and the total decayed surface area was calculated for each fruit. Total decayed surface area was divided by the surface area of the fruit (calculated from the average diameter, assuming a spherical fruit), and multiplied by 100, defining the percentage of affected surface area of the fruit. For a few samples, the decayed area could not be defined precisely (the boundaries of the decayed area had merged because of the progress of disease); therefore, a visual approximation of the affected area was recorded.

Sporulation of brown rot decay was scored for each decayed area, using a subjective scale of 0 to 4 (none, slight, moderate, severe, and very severe sporulation, respectively). After visual analysis of disease progression, one fruit from each replication was randomly selected, weighed, chopped, and frozen at  $-30^{\circ}\text{C}$  for analysis of glucosamine content at a later date.

**QUANTIFICATION OF FUNGAL CELL WALL (GLUCOSAMINE ASSAY).** Frozen nectarines were thawed rapidly, weighed, and homogenized with a Polytron (Brinkmann Instruments, Westburg, N.Y.) on ice; small volumes of distilled water were added as necessary. The equivalent of 0.5 to 4 g (generally 2 g) of homogenized fruit tissue was transferred to a 15 mL graduated conical, glass centrifuge tube resting on ice.

The glucosamine assay used to quantify fungal cell wall materials in fruit tissues was first described by Ride and Drysdale (1972), modified by Jarvis (1977) and Bishop et al. (1982) and also modified slightly in our laboratory. Fruit samples were diluted with 10 mL of 1

methanol : 1 chloroform (v/v), stirred with a glass rod for five min, and centrifuged ( $1.5 \times 10^3 g_n$ , 10 min). Pellets were diluted with nine volumes of anhydrous acetone, mixed for 5 min and centrifuged as described above. Pellets were resuspended in acetone and centrifuged again. The pellets were dried overnight in a vacuum oven at  $40^{\circ}\text{C}$ , suspended in 4 mL of concentrated KOH (120 g KOH/100 mL distilled water) and autoclaved at  $121^{\circ}\text{C}$  for 15 min. Chitosan was precipitated by adding 10 mL (2.5 volumes) of 75% ethanol at  $0^{\circ}\text{C}$ . A 0.9 mL celite (Johns-Manville, Denver, Colo.) suspension (1 g in 20 mL of 75% ethanol) was layered on top of each sample. Tubes were centrifuged for 10 min as described above. Pellets were washed with 10 mL of 40% ethanol ( $0^{\circ}\text{C}$ ) and recentrifuged for 10 min ( $1.5 \times 10^3 g_n$ ). Pellets were washed twice with distilled water ( $0^{\circ}\text{C}$ ) and recentrifuged. Pellets in each tube were adjusted to 1.5 mL with distilled water, then 1.5 mL of 5% (w/v)  $\text{NaNO}_2$  and 1.5 mL of 5%  $\text{KHSO}_4$  were added to each centrifuge tube. Tubes were stirred for 15 min and centrifuged for 5 min at  $1.5 \times 10^3 g_n$ . Two samples (replications) of 1.5 mL of the supernatant were removed. To each sample, 0.5 mL of 12.5% (w/v)  $\text{NH}_4\text{SO}_3\text{NH}_2$  was added and after 5 min of stirring, 0.5 mL of 0.5% MBTH (3-methyl-2-benzothiazolone hydrozon) was added. The mixture was heated in a boiling water bath for 3 min, cooled, and 0.5 mL of  $\text{FeCl}_3$  (0.5% w/v) was added and mixed well for 30 min at  $20^{\circ}\text{C}$ . Absorbance of the samples at 650 nm was recorded (Gilford response TM spectrophotometer, Obelin, Ohio).

Standard solutions of glucosamine-HCl were prepared using the method described by Bishop et al. (1982). The glucosamine concentration of each inoculated fruit sample was calculated from the linear region of the standard curve (8 point linear standard curve: 0, 0.5, 1.0, 2.0, 3.0, 5.0, 7.0, and 10.0 mg) and expressed as micrograms glucosamine per gram of fruit sample. The glucosamine content of six initial, noninoculated control fruit were calculated, and the average glucosamine concentration of these samples was subtracted as background from those of inoculated fruit (adjusted glucosamine concentration).

**FRUIT QUALITY AND CONDITION.** After 5 and 16 d storage, six noninoculated nectarines were removed from each jar. Three fruit were evaluated immediately and three were evaluated after ripening 3 d at  $20^{\circ}\text{C}$ . Firmness of each fruit was measured on each cheek using an Ametek penetrometer (J. Chatillon & Sons, Greensboro, N.C.) fitted with an 8-mm-diameter probe. Nectarine juice was extracted from three slices (wedge shape cut from stem to blossom end), one slice from each of the three fruit in each replication (jar). Juice was titrated to pH 8.2 with NaOH and percent titratable acidity (TA) was calculated based on malic acid equivalents. Total soluble solids (TSS) was measured with a temperature-compensated refractometer (American Optical Corp., Buffalo, N.Y.).

All noninoculated fruit samples were examined carefully for external discoloration and decay. Fruit were cut and their internal tissues were also examined for discoloration, injuries, and mealiness. Tissues of six fruit from the noninoculated 5-d  $\text{CO}_2$  treatment and three fruit from the initial lot at harvest which showed no visual signs of decay were frozen for fungal cell wall analysis as controls (background).

**EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS.** A completely randomized design with a factorial arrangement of treatments was used for all experiments. Data were subjected to analysis of variance (ANOVA) using SAS General Linear Model procedures (SAS Inst. Inc., Cary, N.C.). All interactions were also included and compared.

For all quality parameters tested, means were compared among storage treatments, storage durations, and jar replication. Scores of brown rot decay were handled in the same fashion across storage treatments, storage durations, periods of inoculation, and jar replication. Means of the adjusted glucosamine concentration of inoculated fruit samples were tested among storage treatments and their durations and periods of inoculation. Means of the above visual tests of the brown rot

Table 1. Analysis of variance for area of infected epidermis, sporulation score, and fungal cell wall material (glucosamine) of 'Summer Red' nectarines inoculated with *Monilinia fructicola*, and postharvest quality attributes [firmness, total soluble solids (TSS), and titratable acidity (TA)] of noninoculated fruit.

Source	df	Glucosamine					
		Infected skin area (%)	Sporulation score	content (mg·g <sup>-1</sup> fresh wt)	Firmness (N)	TSS (%)	TA (%)
Atmosphere (A)	1	****	****	****	*	NS	NS
Days in storage (D)	4	****	****	****	****	****	****
A × D	4	****	****	****	NS	NS	NS
Inoculation time (I)	2	****	****	****	---	---	---
I × A	2	**	NS	**	---	---	---
I × D	8	****	****	****	---	---	---
A × D × I	8	****	****	***	---	---	---

NS,\*,\*\*,\*\*\*,\*\*\*\* Nonsignificant or significant at  $P < 0.05, 0.01, 0.001, \text{ or } 0.0001$ , respectively.

lesion were nonsignificant among jar replications. Means of the main effects were partitioned using Duncan's multiple range test.

### Results

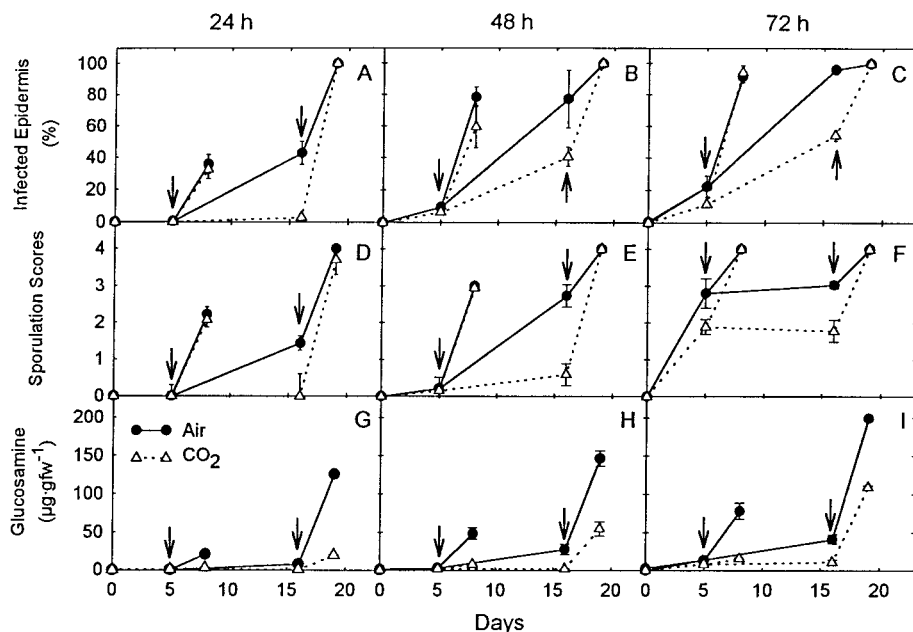
**EFFECT OF 15% CO<sub>2</sub> ON BROWN ROT DECAY DEVELOPMENT.** Analysis of variance of percent infected epidermis area and sporulation score data for *M. fructicola* revealed that the two atmosphere treatments (air and air plus 15% CO<sub>2</sub>), days in storage (0, 5, 16, 5+3, and 16+3), three inoculation times (24, 48, and 72 h), and their interactions were highly significant (Table 1). The one exception was the inoculation time and atmosphere interaction for sporulation score. The three jar replication environments were not significant. Analysis of variance of glucosamine content released from fungal cell wall chitin of inoculated fruit revealed that the storage atmosphere, days in storage plus ripening, inoculation time, and their interactions were highly significant (Table 1).

Decay development during storage in fruit inoculated and held 72 h at 20 °C before storage at 5 °C was excessive, but was much lower in fruit stored in air enriched with 15% CO<sub>2</sub> than air alone (Fig. 1). After 5 or 16 d at 5 °C, the size of lesions on the epidermis of inoculated fruit stored in 15% CO<sub>2</sub> was almost half the size of lesions on fruit stored in air. At the same time, sporulation of *M. fructicola* was slight to moderate and limited to the immediate area surrounding the original inoculation wound. The boundaries of the three lesions on each fruit were distinct and did not overlap. Fruit released 2- to 4-fold less glucosamine from fungal chitin when stored in 15% CO<sub>2</sub> versus air (Fig. 1). However, the glucosamine content of inoculated fruit stored for 5 or 16 d in 15% CO<sub>2</sub> was 3-fold higher than that of those only incubated 72 h at 20 °C after inoculation. After 3 d ripening at 20 °C, the lesions overlapped completely, sporulation was occurring over the entire fruit, and it was impossible to distinguish a difference between fruit stored previously in 15% CO<sub>2</sub> or air. However, the glucosamine content still showed a 2- to 3-fold lower level of infection in fruit stored previously in 15% CO<sub>2</sub> as compared to air stored fruit (Fig. 1).

Brown rot decay development in fruit inoculated 48 h before storage at 5 °C was less than on those fruit inoculated 72 h before storage at 5 °C, but these nectarines were also

commercially unacceptable (Fig. 1). After 5 d cold storage, 9.5% and 6.2% of the epidermis of inoculated nectarines stored in air or air enriched with 15% CO<sub>2</sub>, respectively, were infected. The extent of sporulation of *M. fructicola* was minute and sporulation was confined to the original area of inoculation. After 16 d storage at 5 °C, the size of the infected area on inoculated fruit stored in 15% CO<sub>2</sub> was half the size of the lesions, and the extent of sporulation was 4-fold less than the amount of sporulation on fruit stored in air at 5 °C. After 3 d ripening at 20 °C, decay lesions covered 80% and 60% of the epidermis of fruit stored for 5 d in air or air with 15% CO<sub>2</sub>, respectively (Fig. 1). The entire surface of fruit stored for 16 d at 5 °C plus 3 d at 20 °C was infected, regardless of the storage atmosphere. *Monilinia fructicola* sporulated rapidly at 20 °C and there was no difference between air and CO<sub>2</sub> treatments. In contrast to the infection data, fruit stored for 5 or 16 d in CO<sub>2</sub> released the same amount of glucosamine as those incubated for 48 h at 20 °C

Fig. 1. (A–C) percent infected epidermis, (D–F) sporulation score, and (G–I) glucosamine released from the hydrolysis of cell walls of *M. fructicola* of nectarines inoculated (A, D, G) 24, (B, E, H) 48, and (C, F, I) 72 h before storage for 5 or 16 d at 5 °C in air or air enriched with 15% CO<sub>2</sub>, and after an additional 3 d of ripening at 20 °C. Arrows denote the point of transfer to 20 °C. Sporulation score: 0 = none, 1 = slight, 2 = moderate, 3 = severe, and 4 = very severe. Vertical bars represent SD values.



after inoculation. However, the glucosamine content of fruit stored in air for 5 or 16 d at 5 °C was 2- and 10-fold greater than that of fruit stored in 15% CO<sub>2</sub>, respectively. After 3 days ripening, the glucosamine content remained different between air and CO<sub>2</sub>-stored fruit, with 5- and 3-fold more glucosamine released from fruit previously stored for 5 or 16 d in air than in CO<sub>2</sub>, respectively.

There was very little visible decay development on the epidermis of fruit inoculated 24 h before 5 d storage in 15% CO<sub>2</sub> at 5 °C (Fig. 1). Size of the lesions were the same as the original inoculation wounds and only 0.6 mg of fungal cell wall glucosamine was released per gram of homogenized fruit tissue. Decayed area on fruit stored 5 d in air at 5 °C was 5- to 6-fold larger than on those stored in air enriched with 15% CO<sub>2</sub>. *Monilina fructicola* did not sporulate during 5 d storage in air or air plus 15% CO<sub>2</sub> at 5 °C. The size of the decay lesions on inoculated fruit stored 16 d in air at 5 °C was 14-fold greater than on inoculated nectarines stored 16 d in air enriched with 15% CO<sub>2</sub>. Sporulation of *M. fructicola* was arrested completely during 16 d storage in 15% CO<sub>2</sub>; however, it was slight to moderate on inoculated nectarines stored 16 d in air only. There was no change in glucosamine content in fruit stored for 5 d. After 16 d storage at 5 °C, the glucosamine content increased ≈10-fold in fruit stored in air, but did not increase in fruit stored in 15% CO<sub>2</sub>. After 3 d ripening at 20 °C, the size of the infected area and the amount of sporulation on fruit stored for 5 d were moderate and disease development rendered them unacceptable, regardless of the atmosphere treatment (Fig. 1). For fruit stored for 16 d, decay lesions and sporulation developed rapidly and covered the entire fruit during the 3 d at 20 °C, regardless of the storage atmosphere (Fig. 1). Glucosamine content increased greatly at 20 °C, and the differences between fruit stored previously in air versus 15% CO<sub>2</sub> were greater than after cold storage. Amount of glucosamine released from fungal cell walls was 4- and 6-fold greater in fruit stored previously in air versus CO<sub>2</sub> following 3 d ripening in air (Fig. 1).

**EFFECT OF 15% CO<sub>2</sub> ON FRUIT QUALITY.** Firmness of nectarines stored in air or air enriched with 15% CO<sub>2</sub> at 5 °C for 5 d was not significantly different from firmness at harvest (Table 2). After 16 d storage, firmness of fruit stored in air decreased almost 8 N while firmness of nectarines stored in 15% CO<sub>2</sub> remained unchanged; however, neither difference was significant. Nectarines stored 16 d

in air at 5 °C and ripened for 3 d at 20 °C were firmer than those stored 5 d at 5 °C and ripened for 3 d at 20 °C (Table 2). There was a similar but insignificant trend for fruit stored in CO<sub>2</sub>. Mean firmness of fruit stored in air enriched with 15% CO<sub>2</sub> was significantly greater than that of fruit treated with air (Table 1).

TSS and TA were not affected significantly by storage atmosphere (Tables 1 and 2). However, TSS increased and TA decreased during fruit ripening. No external or internal injuries, decay, discoloration or changes in the internal texture of fruit tissue were observed.

## Discussion

Brown rot is a significant postharvest decay of stone fruit grown in California and results in considerable product losses. Stone fruit are often treated pre- and postharvest with registered fungicides to control *M. fructicola*. However, these chemicals often elicit development of resistance among pathogens after a period of application and may leave behind harmful residues (Adaskaveg and Ogawa, 1994). Ogawa et al. (1985) reported that the annual cost of preharvest fungicide applications and the estimated 1% loss of stone fruits before marketing from brown rot decay exceeded \$50 million in the six major California stone fruit-producing regions. Without fungicide sprays, stone fruit loss to brown rot may exceed 60% (MacNab, 1975). CA regimes of low oxygen (2.2%) enriched with 15% to 20% CO<sub>2</sub> at low temperature (0 to 2 °C) usually reduce growth of *M. fructicola* (Sommer, 1985), but 15% CO<sub>2</sub> may cause internal damage to stone fruit after 2 to 6 weeks cold storage (Allen and Smock, 1937), with the exception of sweet cherries (DeVries-Paterson, et al., 1991).

In this study, a 16 d storage regime of air enriched with 15% CO<sub>2</sub> at 5 °C delayed the progression of disease in nectarines inoculated 24 or 48 h before CA storage, as judged by results of an accurate biochemical assay and visual methods of measuring brown rot decay development. There was no evidence of CO<sub>2</sub>-induced fruit injury, after 16 d plus 3 d ripening.

Results indicate that an atmosphere of air enriched with 15% CO<sub>2</sub> prolonged the lag period of infection and postponed the onset of disease in infected nectarines. In this study, each inoculated fruit

Table 2. Firmness, total soluble solids (TSS) and titratable acidity (TA) of 'Summer Red' nectarines stored in air or 15% CO<sub>2</sub> at 5 °C.

Days of storage and ripening	Firmness (N)		TSS (%)		TA (%)	
	Air	15% CO <sub>2</sub>	Air	15% CO <sub>2</sub>	Air	15% CO <sub>2</sub>
0	63.8 a <sup>z</sup>	---	12.03 a	---	0.90 a	---
5	62.6 a	59.9 a	11.13 a	10.66 a	0.78 a	0.83 a
5 + 3	26.3 c	38.1 b	11.30 a	11.77 a	0.79 a	0.77 a
16	55.9 a	62.2 a	11.87 a	10.90 a	0.66 a	0.74 a
16 + 3	39.0 b	43.9 b	11.63 a	11.70 a	0.59 a	0.68 a
		Mean		Mean		Mean
15% CO <sub>2</sub>		55.6 a		11.59 a		0.79 a
Air		49.5 b		11.41 a		0.74 a
Storage and ripening						
0		63.8 a		12.03 a		0.90 a
5		61.2 a		10.90 c		0.81 ab
5+3		32.2 c		11.53 b		0.78 b
16		59.0 a		11.38 b		0.70 bc
16+3		41.4 b		11.67 ab		0.63 c

<sup>z</sup>Mean separation within columns by Duncan's multiple range test  $P \leq 0.05$

received over 500 spores per wound at three different locations on the fruit and at a depth of 2 to 3 mm under the epidermis. These injuries, for all practical purposes, are highly exaggerated and similarly injured fruit would usually be detected and discarded during sorting and packing. The conidia concentration of the inoculum ( $5.4 \times 10^4$  spores/mL) was also higher than natural inoculum of *M. fructicola* on unripe or ripe fruit in typical orchards. However, in an unsprayed orchard, a mummy or an infected fruit peduncle has the potential of producing  $4 \times 10^6$  and  $10^4$  conidia with 80% to 90% viability, respectively (Byrde and Willetts, 1977).

Sizes of the brown rot lesions on the epidermis of fruit samples incubated for 24 h before storage in 15% CO<sub>2</sub> for 5 d were generally comparable with the sizes of lesions at the beginning of cold storage. Size of the lesions for those fruit incubated for 48 or 72 h were progressively greater. At 16 d, size of the brown rot lesions was much greater in fruit incubated 48 or 72 h before cold storage as compared with 24 h. However, as defined by the glucosamine assay, infection development in nectarines inoculated 24 or 48 h before 5 or 16 d storage in 15% CO<sub>2</sub> was insignificant during cold storage, but infection did develop in nectarines inoculated 72 h before 5 or 16 d storage in CO<sub>2</sub>. The discrepancy in the data for lesion size and glucosamine content may be due to the fact that one measures fungal growth only at the surface of the fruit while the other measures fungal chitin throughout the fruit volume.

The fungistatic effect of 15% CO<sub>2</sub> atmospheres inhibited or slowed disease development and sporulation of *M. fructicola* during cold storage, depending on the incubation period before storage in CO<sub>2</sub> at 5 °C. However, after 3 d ripening in air at 20 °C, progression of the brown rot decay rendered all inoculated fruit commercially unacceptable, as also reported for sweet cherries (De Vries-Paterson et al. 1991).

Development of decay during ripening indicated that other measures, such as application of a reduced level of fungicides or biological control agents, would be needed to completely prevent decay development and extend the shelf life of peaches and nectarines in seasons with high disease pressure. The CO<sub>2</sub> atmospheres may arrest sporulation and prevent spread of decay from infected to healthy fruit during transportation and distribution. However, a second sorting of fruit for brown rot decay upon transfer to ripening temperatures may be needed to prevent the spread of brown rot to healthy fruit during ripening.

None of the noninoculated fruit stored for 5 or 16 d at 5 °C in air enriched with 15% CO<sub>2</sub> and subsequently ripened, exhibited any external or internal injuries, mealiness or decay lesions. Their postharvest fruit quality attributes were comparable to nectarines stored in air at 5 °C and subsequently ripened at 20 °C for 3 d, except that nectarines stored in 15% CO<sub>2</sub> were firmer. These results agree with those of Youssef and Mitcham (1997) who found that 'Sparkle' peaches tolerated a 15 d exposure to 15% CO<sub>2</sub> at 5 °C.

Measurement of glucosamine released from fungal chitin of inoculated nectarines described the progression of brown rot infection (fungal hyphae advancement) more accurately than visual measurement of the decayed area on the fruit epidermis at the onset of disease and its early progression. Fungal chitin was detected after only 24 h incubation at 20 °C in inoculated fruit. Decay lesions could be detected visually only after 72 h incubation at 20 °C. The glucosamine assay also described the extent of brown rot decay development in inoculated fruit stored 5 d in CO<sub>2</sub> at 5 °C and ripened 3 d at 20 °C more accurately than size measurement of decay lesions. A more accurate means of determining fungal growth in the fruit may provide a useful tool for researchers studying the impact of fungicidal or fungistatic treatments. Perhaps this precise and accu-

rate assay could be automated for rapid determination of brown rot inoculum in orchards and the extent of infection in harvested fruit. It may guide growers and packinghouse operators to pre- or postharvest control measures.

Results demonstrate that 'Summer Red' nectarines tolerated a 16 d exposure to 15% CO<sub>2</sub> at 5 °C. The CO<sub>2</sub> atmosphere reduced development of brown rot decay and sporulation of *M. fructicola* during CA storage. However, because the CO<sub>2</sub> atmospheres are fungistatic and decay development is rapid during the ripening period, this technique cannot be used as a single method for decay control in years with high disease pressure. Fruit inoculated 24 h before storage would be similar to nectarines and peaches packed commercially with careful handling and sorting by experienced packinghouse operators. To optimize the benefit of CA, packers are advised to harvest their fruit carefully, hydrocool them using cold, chlorinated water, sort out injured and decayed fruit during packing, and place them under CO<sub>2</sub> atmospheres at 0 to 5 °C within 24 h of harvest for short term storage, transportation, and distribution.

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