



Occurrence of Gray Mold in Stored Strawberries as Affected by Ripeness, Temperature, and Atmosphere

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Development of postharvest decay in strawberries in general and gray mold caused by *Botrytis cinerea* in particular, was evaluated, specifically: 1) the effect of controlled atmosphere (CA; 5% O₂ + 15% CO₂) on different developmental stages of the pathogen; 2) the interaction of fruit ripeness and CA storage among fruit stored for 1 or 2 weeks at normal cold storage temperatures (4 or 10 °C) for commercial handling; and 3) the residual effects of CA storage after transfer to air on strawberry susceptibility to fruit rot. Delaying CA establishment for 12 or 24 h at 20 °C after wound inoculation resulted in progressively more disease development, suggesting that the CA had a greater inhibitory effect on *B. cinerea* spore germination vs. mycelial growth. The least decay occurred on three-quarter colored fruit stored in CA vs. air and at the lower temperature, and CA had a greater effect at 10 °C than at 4 °C. These effects persisted during a 24-h display treatment (fruit held in air at 20 °C after the main storage treatments). Additionally, inoculation following 1 or 2 weeks storage at 4 or 10 °C indicated that CA helped strawberry fruit maintain resistance to gray mold, especially at the higher storage temperature.

Postharvest decay of strawberries (*Fragaria xananassa* Duch.) causes major crop losses worldwide (Ceponis et al., 1987; Maas, 1978, 1992). Gray mold, caused by *Botrytis cinerea* P. Micheli ex Pers. is one of the most important postharvest strawberry fruit diseases. It may begin with infection of fruit before harvest, inoculation of wounds on fruit during harvest, or direct contact with diseased fruit. Senescing fruit become increasingly susceptible to various decay especially when stored at higher temperatures (Sommer, 1989). Postharvest decay of strawberries is generally controlled by maintenance of a “cold chain,” meaning fruit are cooled immediately after harvest and kept cold until the fruit are consumed (Mitcham and Mitchell, 2002; Nunes et al., 1995a; Sommer, 1989). The lowest decay incidence is invariably associated with prompt cooling after harvest, keeping the fruit cool throughout transit and marketing, and cooling to as near 0 °C as practical (Harvey et al., 1980; Nunes et al. 2005; Sommer et al., 1973).

Storage of strawberries in controlled atmospheres (CA) with low O₂ and high CO₂ concentrations has been used for controlling decay caused by *B. cinerea* and *Rhizopus stolonifer* as well as delaying fruit senescence and maintain quality (Couey and Wells, 1970; Nunes et al., 1995b, 2002; Smith, 1992; Sommer, 1989; Wells, 1970; Woodward and Topping, 1972). The effect of CA is fungistatic as pathogen activities resume once fruit are returned

to air (Littlefield et al., 1966). Some physiological effects of CA on strawberry fruit such as reduced respiration rate persist for a time after the atmosphere returns to normal (Li and Kader, 1989) and it has been speculated that fruit from CA storage have more resistance to decay than do those from air storage (Sommer, 1989). To our knowledge, this latter idea has not been directly tested by inoculating strawberries after CA vs. air storage.

The efficacy of CA storage for controlling postharvest decay in commercial handling where the cold chain has been maintained is unclear. Low O₂ or high CO₂ concentrations slow the growth of *B. cinerea* on media (Agar et al., 1990; Ceponis et al., 1987; Jerch et al., 1989; Smith and Worthington, 1965) and the development of the disease in strawberry fruit (Barkai-Golan, 1990; Borecka and Millikan, 1981; Brecht et al., 1992; Couey and Wells, 1970; Harris and Harvey, 1973; Smith, 1992; Sommer, 1989; Sommer et al., 1973; Wells, 1970; Woodward and Topping, 1972). However, Sommer et al. (1973) suggested that when strawberries were stored at the proper temperature (i.e., 0 °C), CA or modified atmosphere (MA) storage methods were of little benefit because the low temperature alone almost completely stops development of *B. cinerea*. A high CO₂/low O₂ environment also slows the senescence of strawberries (Larsen and Watkins, 1995; Nunes et al., 2002), which should indirectly slow the development of gray mold (Sommer, 1989).

Higher storage temperatures enhance the inhibitory effects of CA storage on *B. cinerea* (Woodward and Topping, 1972). Sommer et al. (1973) observed that high-CO₂ storage was more

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effective against *B. cinerea* at temperatures above 5 °C, since at lower temperatures, fungal growth was minimal regardless of the atmosphere composition. Moreover, development of decay from germinated spores placed in wounds was minimal below the 5 °C storage temperature limit. Transport temperatures of 2 to 10 °C are typical for strawberries (Harvey et al., 1980; Lai et al., 2011; Sommer et al., 1973), perhaps explaining the successful use of CA and modified atmosphere (MA) in commercial strawberry handling.

Harvesting less mature fruit may help control postharvest decays of strawberry. Pritts et al. (1987) observed less decay among fruit with a white tip at harvest as compared with those that were fully red. Immature strawberry fruit are more resistant to fungal attack than are more mature fruit (Jerch et al., 1989). The general progress of gray mold following an infection from senescing floral petals often results in the pathogen becoming quiescent until the fruit ripen (Braun and Sutton, 1987; Powelson, 1960). This progress suggests that conditions in fruit tissues become more favorable for fungal development as the fruit ripen. In our previous studies, three-quarter red as compared with fully red fruit developed less natural decay during storage and had a better retention of fruit quality over a longer storage interval (Nunes et al., 2002). The use of 4 vs. 10 °C and CA vs. air storage improved the quality retention by the three-quarter red fruit, which is the minimum acceptable harvest maturity.

The objectives of this study were to evaluate the development of postharvest decay in strawberries in general and gray mold, caused by *B. cinerea* in particular, specifically: 1) the effect of controlled atmosphere (CA; 5% O₂ + 15% CO₂) on different developmental stages of the pathogen; 2) the interaction of fruit ripeness and CA storage among fruit stored for 1 or 2 weeks at normal cold storage temperatures (4 or 10 °C) for commercial handling; and 3) the residual effects of CA storage after transfer to air on strawberry susceptibility to fruit rot. Incidence of gray mold was promoted by the intentional wound-inoculation of fruit prior to the onset of the storage treatments and after storage.

Material and Methods

PLANT MATERIAL. Strawberries were obtained from commercial operations in Florida and California. The fruit had been grown in double rows on raised beds covered with black plastic mulch, with drip irrigation and fertilization practices according to standard recommendations for strawberry production. To control fungal diseases including gray mold, the fungicide captan (Micro Flo, Memphis, TN) was applied on a 7-d schedule through the season along with up to four applications of iprodione (Rovral, Aventis CropScience, Research Triangle Park, NC) at peak blooming periods. Three sets of experiments with 'Chandler' strawberries were conducted during the Florida winter season and repeated with modifications during the California fall season using the same strawberry cultivar.

TREATMENT AND STORAGE CONDITIONS. Strawberries were commercially harvested in the morning with calyxes attached and packed into fiberboard flats containing 12 plastic mesh pint baskets. The flats of strawberries were removed from the field in Florida with minimal delay after harvest and transported to the laboratory in Gainesville within approximately 2 h after harvest. California fruit were shipped by air to Florida and held in refrigerated (1 °C) storage overnight prior to the start of the experiments. Fruit were selected for freedom from visible defects and sorted into two maturity stages: three-quarter red (TQR) and fully red (FR).

Three-quarter red fruit had red color on about 75% of the fruit surface, while FR fruit were light red over the entire fruit surface.

Air or CA (5% O₂ + 15% CO₂) was supplied through a mixture of air, nitrogen, or CO₂ at constant temperature and pressure regulated by flow boards with needle valve flow meters to six, 1-L jars per treatment each containing 20 strawberries. The CA gas mixture was selected because it reflected the composition of the MA found in commercially handled fruit (Mitcham and Mitchell, 2002; Nunes et al., 2002). Besides, in a previous study an atmosphere composed of 5% O₂ + 15% CO₂ was considered to be more beneficial in terms of maintaining fruit quality than 10% O₂ + 20% CO₂ (Nunes et al., 1995b). Gas mixtures were divided into replicate jars with flow boards consisting of a barostat and glass capillary tube for flow control. The total flow rate was calculated based on previously measured respiration rates for strawberries so that the CO₂ levels due to respiration would be maintained below 0.3%. Flow rate at 4 °C was maintained at about 30 mL·min⁻¹, while at 10 °C the flow was about 140 mL·min⁻¹ inside each jar. High humidity was maintained inside the jars by bubbling air or gas mixture through water. The storage rooms were set at 4 or 10 °C since these were the usual cold temperature and abuse temperature, respectively, for fruit in commercial shipments from California to the east coast of the USA (Harvey et al., 1980; Sommer et al., 1973). Gas composition was monitored every 4 d by sampling from the inlet ports of the containers and measured by gas chromatography (Nunes et al., 2002) but no adjustments were necessary.

INOCULUM PREPARATION. *B. cinerea* was isolated from diseased strawberries and grown on potato dextrose agar (Difco Laboratories, Detroit, MI). The fungus was grown at 24 to 26 °C under different light qualities. Plates were exposed to cool white light (F20T12-CW, Sylvania) at a distance of 44 to 46 cm. After 5 to 6 d, the plates were transferred to dark conditions for 24 or 30 h. Finally, the fungus was exposed to UV light (F20T12-BL 20 watt, General Electric) for an additional period of 4 to 6 d. The above regime was intended to cause physiological stress and, consequently, greater and more rapid spore production. The resulting spores produced in response to the UV light exposure were rinsed from the plates in 5 mL of sterile distilled water containing two drops of Tween 20. The suspension was filtered through a cotton cloth, and spore concentration was based on optical density at 490 nm as per a previously established plot of spore concentration vs. optical density. The spore suspension was diluted with sterile distilled water to a concentration of 1 × 10⁵ spores/mL.

INOCULATION PROCEDURE. The distal 3 mm of a metal transfer needle was bent to a 90° angle. The bent section of the needle was flamed and then thrust into a fruit near its equator to create a wound cavity that was 3 mm deep and 1.5 to 2 mm in diameter. Each fruit received one wound. The wounded fruit were separated into two sets. The wounds on one set were inoculated with a 10-mL sample of an aqueous spore suspension containing 10³ spores, whereas the second set was treated with a like amount of sterile distilled water.

DECAY EVALUATION. Incidence of decay was recorded by counting the number of fruit with symptoms of gray mold at the wound. Disease severity was estimated at the wound site on a 0 to 5 scale, where 0 = no visible changes in tissues at the wound-site (0% decay); 1 = slight brown discoloration around the wound (20% decays); 2 = moderate brown discoloration (40% decay); 3 = slight to moderate mycelial growth in and around the wound (60% decay); 4 = moderate to heavy mycelial growth (80% decay); and 5 = characteristic sporulation, heavy mycelium

growth (100% decay). The scale was converted to a percentage of full sporulation using the following formula: $\text{Arcsin} [\text{Sqrt} ((\% \text{ decay} - 1/4n)/100)]$; where n = number of replicates

EXPERIMENT 1: POSTHARVEST DECAY IN STRAWBERRIES STORED UNDER REFRIGERATION IN AIR OR CA. Immediately after harvest, strawberries of each stage of ripeness were wounded, inoculated or not, and then placed in the jars. Each jar contained 20 fruit. The jars were arranged in a completely randomized design with two atmosphere compositions (air and CA), two cold storage treatments (4 and 10 °C), and two storage times (1 or 2 weeks) applied to two stages of fruit ripeness (TQR or FR) with 10 fruit in each of three replicates. The fruit were kept in the dark and the relative humidity within each jar ranged from 90 to 95%. After 1 or 2 weeks storage, three replicate samples of 10 fruit from each temperature, atmosphere treatment, and ripeness stage combination were transferred to air at 20 °C for 24 h to simulate non-refrigerated consumer display. At transfer and after the 24-h-20 °C incubation period, fruit were examined for the incidence and severity of decay.

EXPERIMENT 2: SUSCEPTIBILITY OF STRAWBERRIES TO GRAY MOLD DEVELOPMENT AFTER REFRIGERATED STORAGE IN AIR OR CA. Fruit of each stage of ripeness were wounded-inoculated after a 1- or 2-week storage in air or CA at 4 or 10 °C as described above and incubated for 24 h at 20 °C. The stored fruit were examined carefully just before inoculation to ensure that they were relatively free of disease and that the inoculation point was free of symptoms.

EXPERIMENT 3: DEVELOPMENT OF GRAY MOLD IN STRAWBERRY FRUIT AFTER A 12- OR 24-H DELAY IN THE INITIATION OF CA STORAGE, Fruit were separated into ripeness stages immediately after harvest as described, wounded or wounded plus inoculated, stored in air at 20 °C for 0, 12, or 24 h, then placed into CA storage at 4 or 10 °C for 1 or 2 weeks as described above. The periods between wounding plus inoculation and cold storage were based on an evaluation of the time from the inoculation of PDA and germination of spores. Spore germination was observed by 12 h, whereas by 24 h mycelia were found.

STATISTICAL ANALYSIS. The percentage of full sporulation (severity of gray mold) at the wound sites and the incidence of disease were transformed by the arcsine square root method using radians. The transformed variables were analyzed with the Statistical Analysis System (SAS, Cary, NC) software by three-way ANOVAs, with atmospheric composition, maturity and storage time as factors. Fisher's protected LSD ($P \leq 0.05$) was calculated for the transformed disease severity values. The average LSD for each experiment was converted back to a percentage for ease of comparison among the percentage means. The Florida experiments were conducted sequentially with strawberries harvested from the same field on three different dates while the California experiments were conducted simultaneously using fruit from a single harvest, and storage was for 1 week at 10 °C only. The results of both the original (Florida) and repeated (California) experiments were in agreement, and thus in the interest of brevity, only the former are presented.

Results

EXPERIMENT 1: POSTHARVEST DECAY IN STRAWBERRIES STORED UNDER REFRIGERATION IN AIR OR CA. The signs and symptoms developing at diseased wound sites in the following experiments were consistent with gray mold. Rapid soft rot or heavy mycelial development that is typical of *Rhizopus* rot was not observed. The inoculation of strawberries with *B. cinerea* prior to storage led

to increased disease incidence and, depending on the duration of storage, up to a 2- to 10-fold increase in lesion development at both 4 and 10 °C (Figs. 1 and 2). More decay was observed among fruit that were FR at harvest than among fruit harvested TQR. Lower severity levels were recorded for TQR vs. FR fruit at both 4 and 10 °C for both inoculated and non-inoculated fruit. Significantly less disease was found among fruit stored in CA than in air for non-inoculated (Fig. 1) and inoculated (Fig. 2) fruit stored at 10 °C, and also for inoculated fruit stored at 4 °C, particularly after 1 week storage (Fig. 2). Overall, atmosphere, maturity and storage time had a significant effect ($P < 0.0001$) on the severity of decay in strawberry stored at 4 or 10 °C (Table 1).

An absence of decay was associated with shorter storage intervals and colder storage temperature, as well as with less ripe fruit, CA storage, and wounded but not inoculated fruit in both seasons. None of the non-inoculated fruit examined at transfer from storage at 4 °C for 1 week to a 24-h 20 °C-simulated consumer display had evidence of decay (Fig. 1). Only one of the non-inoculated 10 °C storage treatments had visible lesions, in which case browning around the wound occurred on 10% of the FR fruit stored in air. Lesions were not observed after 1 week on any of the inoculated TQR fruit stored at 4 °C in air or CA, or the FR fruit stored at 4 °C in CA. By contrast, 30% of the inoculated FR fruit stored in air at 4 °C had lesions. At 10 °C, both TQR and FR fruit stored in air had lesions by 1 week (50% and 100% incidence, respectively, in Florida strawberries, and 17% and 43% in California strawberries), whereas fruit of neither ripeness stage stored in CA had lesions. At the transfer after 2 weeks in cold storage to the simulated consumer display (20 °C), only the

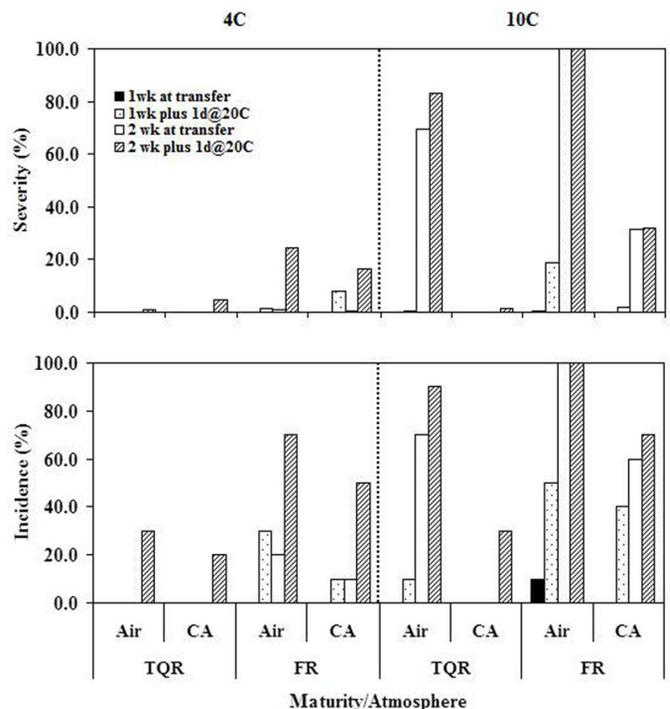


Fig. 1. Severity and incidence of postharvest decay of strawberries harvested at two stages of ripeness that had been wounded with a bent transfer needle but not inoculated, stored at 4 or 10 °C for 1 or 2 weeks in air or CA, and examined at transfer from cold storage or after 24 h in a simulated consumer display at 20 °C (Experiment 1). $\text{LSD}_{4^{\circ}\text{C}}$ (severity 1.0%, incidence 6.6%); $\text{LSD}_{10^{\circ}\text{C}}$ (severity 5.0%, incidence 7.9%). CA = controlled atmosphere; TQR = three-quarters-red; FR = fully red.

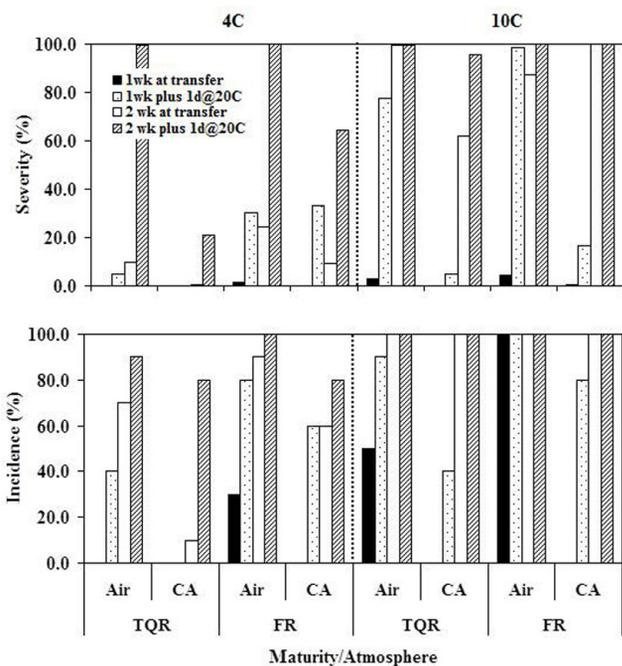


Fig. 2. Severity and incidence of postharvest decay of strawberries harvested at two stages of ripeness that had been wounded with a bent transfer needle, then wound inoculated with 103 spores of *Botrytis cinerea*, stored at 4 or 10 °C for 1 or 2 weeks in air or CA, and examined at transfer from cold storage or after 24 h in a simulated consumer display at 20 °C (Experiment 1). LSD_{4°C} (severity 4.2%, incidence 9.2%); LSD_{10°C} (severity 2.8%, incidence 8.3%). CA = controlled atmosphere; TQR = three-quarters-red; FR = fully red.

non-inoculated TQR fruit stored at 4 °C in air or CA, or at 10 °C in CA remained free of symptoms (Fig. 1).

Differences in disease among treatments observed at transfer from either the 1- or 2-week storage tended to persist during the subsequent 24-h display (Figs. 1 and 2). Disease severity after the display was significantly lower in most direct comparisons of TQR with FR fruit, fruit from CA vs. air storage, fruit from 4 °C compared with 10 °C, and in non-inoculated as compared with inoculated fruit. With non-inoculated fruit, decay severity at transfer from CA at 4 °C to the consumer display was similar to that in the air-stored treatment. However, after the 24-h display treatment, less disease was found among fruit from the CA storage.

EXPERIMENT 2: SUSCEPTIBILITY OF STRAWBERRIES TO GRAY MOLD DEVELOPMENT AFTER REFRIGERATED STORAGE IN AIR OR CA. Disease incidence and severity increased within 24 h of wound inoculation in most cases after 1 week of storage in comparison to non-inoculated controls (Fig. 3); the exception was TQR fruit stored 1 week in CA at 4 °C in which no decay developed in inoculated or non-inoculated fruit after storage. For 2-week-stored fruit, decay that developed during storage increased little or not at all during an additional 24 h at 20 °C, except for TQR and FR fruit stored in CA at 10 °C. Prior storage of TQR fruit for 1 or 2 weeks in CA vs. air at either 4 or 10 °C reduced both the incidence and severity of decay that developed after inoculation. For FR fruit, prior storage in CA at 4 °C reduced decay severity following inoculation, but only after 1 week of storage. In contrast, CA at 10 °C conferred greater resistance to decay (i.e., reduced decay severity after inoculation) for up to 2 weeks of storage for FR fruit. The severity of disease on the inoculated TQR fruit or on fruit that had been stored in CA at either storage temperature was usually significantly less than the respective control treat-

Table 1. Significance determined by ANOVA of the main effects and their interactions on severity of postharvest decay of strawberries harvested at two stages of ripeness that had been wounded with a bent transfer needle, inoculated or not inoculated, stored at 4 or 10 °C for 1 or 2 weeks in air or CA, and examined at transfer from cold storage or after 24 h in a simulated consumer display at 20 °C (Experiment 1).

Source of variation	Storage at 4 °C		Storage at 10 °C	
	Inoculated	Non inoculated	Inoculated	Non inoculated
Main effects				
Atmosphere (A) ^z	***	NS	***	***
Maturity (M) ^y	***	**	***	***
Storage time (S) ^x	***	***	***	***
Interactions				
A × M	NS	NS	***	NS
A × S	NS	NS	***	***
M × S	**	**	**	**
A × M × S	NS	***	**	NS

^zAir and CA Storage (5% O₂ + 15% CO₂).

^yThree-quarter red and full ripe.

^x1 and 2 weeks at 4 and 10 °C, and 1 and 2 weeks at 4 and 10 °C plus 1 d at 20 °C.

NS, *, **, ***Nonsignificant or significant at $P = 0.05, 0.01$ or 0.001 , respectively.

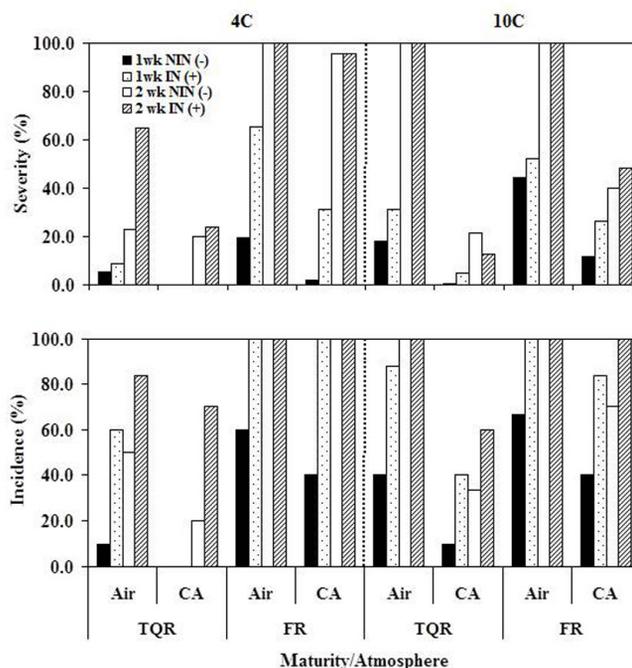


Fig. 3. Severity and incidence of postharvest decay in strawberries harvested at two stages of ripeness followed in sequence by storage at 4 or 10 °C for 1 or 2 weeks in air or CA, wounding with a bent transfer needle, the wound inoculated with 103 spores of *Botrytis cinerea* or water, and finally held for 24 h in a simulated consumer display at 20 °C (Experiment 2). LSD_{4°C} (severity 4.2%, incidence 5.5%); LSD_{10°C} (severity 4.9%, incidence 6.2%). NIN = non-inoculated; IN = inoculated; CA = controlled atmosphere; TQR = three-quarters-red; FR = fully red.

ments. Furthermore, the lowest severity or incidence within each combination of storage duration and temperature among either the inoculated or non-inoculated fruit was observed among TQR fruit that had been stored in CA. Overall, there was a significant effect of atmosphere, and fruit maturity on the severity of decay

of strawberry stored for 1 or 2 weeks; however temperature did not have a significant effect on the severity of decay in inoculated fruit (Table 2).

EXPERIMENT 3: DEVELOPMENT OF GRAY MOLD IN STRAWBERRY FRUIT AFTER A 12- OR 24-H DELAY IN THE INITIATION OF CA STORAGE. In both seasons, delays to initiation of CA treatment led to increased disease in both non-inoculated and inoculated strawberries of both ripeness stages (Tables 1 and 2). An increase in disease was also associated with inoculation, FR vs. TQR ripeness stage, and longer storage interval. Thus, the lowest disease incidence and severity were in non-inoculated TQR fruit that had been immediately placed in CA at 4 °C for which no decay developed

during 2 weeks of storage (Table 3); and disease development was greatest in inoculated FR fruit for which there was a delay of 24 h after inoculation before the fruit were placed in CA at 10 °C (Table 4). For fruit with inoculated wounds, immediate establishment of CA did not eliminate the incidence of disease, which was found on 30 to 40% of the fruit after 2 weeks storage at either 4 or 10 °C (vs. 0% for non-inoculated fruit), but disease severity was kept very low (<5%) on inoculated TQR fruit that were placed immediately into CA at 4 °C even after 2 weeks storage plus 1 d at 20 °C (Table 4). Increasing the delay before establishment of CA and lengthening the storage time led to progressively greater disease and this effect was greater among TQR than FR fruit.

Discussion

The incidence of decay reported in this study was in general much higher than the 2% allowed by grade standards and would not be consistent with commercially handled strawberries, which are typically handled for 1 week or less. However, the treatments that reduced decay incidence or severity would be expected to also delay decay onset and thereby prolong the postharvest life of the fruit in commercial handling. As expected, disease incidence and severity increased with inoculation and with storage at a warmer temperature (10 °C). Gray mold develops more rapidly at warmer storage temperatures (Nunes et al., 2002; Sommer et al., 1973; Woodward and Topping, 1972) particularly if the fruit have been inoculated (Sommer, 1989). The abuse temperature used in this study was the highest average recorded in truck shipments of California strawberries to the southeastern U.S. (Atlanta, Georgia) (Sommer et al., 1973). The 4 °C storage temperature used in this study is actually lower than the lowest temperature (5 °C) recorded in those shipments. Current recommendations for strawberries suggest cooling fruit to as close to 0 °C as possible (Mitcham and Mitchell, 2002). Whether 0 °C can be maintained consistently in truck shipments is unclear. In this study, storage at 4 °C instead of 10 °C delayed the onset of decay even when fruit were inoculated. In fact, the lower storage temperature delayed

Table 2. Significance determined by ANOVA of the main effects and their interactions on severity of gray mold in strawberries harvested at two stages of ripeness followed in sequence by storage at 4 or 10 °C for 1 or 2 weeks in air or CA, wounding with a bent transfer needle, the wound inoculated with 10³ spores of *Botrytis cinerea* or water, and finally held for 24 h in a simulated consumer display at 20 °C (Experiment 2).

Source of variation	Inoculated	Non inoculated
Main effects		
Atmosphere (A) ^z	***	*
Maturity (M) ^y	***	***
Temperature (T) ^x	NS	***
Interactions		
A × M	NS	NS
A × T	NS	NS
M × T	NS	NS
A × M × T	NS	NS

^zAir and CA storage (5% O₂ + 15% CO₂).

^yThree-quarter red and full ripe.

^x1 and 2 weeks at 4 and 10 °C, and 1 and 2 weeks at 4 and 10 °C plus 1 day at 20 °C.

NS, *, **, ***Nonsignificant or significant at $P = 0.05, 0.01$ or 0.001 , respectively.

Table 3. Severity and incidence of postharvest decay among strawberries harvested at two stages of ripeness that had been wounded^z but not inoculated, held for 0, 12 or 24 h in air at 20 °C, then stored in CA^y at 4 or 10 °C for 1 or 2 weeks plus 24 h simulated consumer display in air at 20 °C (Experiment 3).

Cold storage duration (wks)	Fruit ripeness stage	% Decay after	Severity (%)						Incidence (%)					
			Storage at 4 °C			Storage at 10 °C			Storage at 4 °C			Storage at 10 °C		
			0 h	12 h	24 h	0 h	12 h	24 h	0 h	12 h	24 h	0 h	12 h	24 h
1	TQR ^x	transfer	0.0 ^w	0.9	0.9	0.0	0.5	0.9	0	0	20	0	10	20
		display	0.0	0.5	1.4	0.9	1.9	5.1	0	10	30	20	30	30
	FR ^v	transfer	0.0	0.9	1.5	0.5	0.9	4.2	10	20	20	10	20	20
		display	4.2	4.7	4.7	4.2	5.7	8.9	30	30	40	20	40	50
2	TQR	transfer	0.0	0.5	0.9	0.0	0.5	0.9	0	10	20	0	10	20
		display	0.5	3.8	4.7	30.0	23.8	28.5	10	20	30	40	40	40
	FR	transfer	0.0	0.9	9.3	1.0	6.7	18.3	0	20	30	20	40	60
		display	5.1	8.0	9.5	26.6	52.4	62.6	30	40	40	40	60	80
			LSD _{4 °C} = 1.0%			LSD _{10 °C} = 2.0%			LSD _{4 °C} = 7.4%			LSD _{4 °C} = 9.8%		

^zWounded with a bent transfer needle to produce a wound that was approximately 3 mm deep × 2 mm diameter.

^yCA = controlled atmosphere; 5% O₂, 15% CO₂, 80% N₂.

^xTQR = three-quarters red.

^wEach value is the average of 30 fruit.

^vFR = fully red.

^wFisher's protected LSD at $P = 0.05$ calculated from the arcsin (square root of the percentage transformation) and then converted back to a percentage value.

Table 4. Severity and incidence of postharvest decay among strawberries harvested at two stages of ripeness that had been wounded and inoculated with spores of *Botrytis cinerea*, held for 0, 12, or 24 h in air at 20 °C, then stored in CA^v at 4 or 10 °C for 1 or 2 weeks plus 24 h simulated consumer display at 20 °C (Experiment 3).

Cold storage duration (wks)	Fruit ripeness stage	% Decay after	Severity (%)						Incidence (%)					
			Storage at 4 °C			Storage at 10 °C			Storage at 4 °C			Storage at 10 °C		
			0 h	12 h	24 h	0 h	12 h	24 h	0 h	12 h	24 h	0 h	12 h	24 h
1	TQR ^x	transfer	0.0 ^w	1.4	5.1	0.5	1.8	6.0	0	30	40	10	40	60
		display	0.9	24.8	28.9	8.9	24.2	29.3	20	40	80	50	50	90
	FR ^v	transfer	1.4	2.3	25.1	4.7	14.7	40.0	30	50	70	30	60	70
		display	16.6	24.8	51.4	7.7	43.2	69.9	60	80	80	60	90	100
2	TQR	transfer	0.5	9.9	54.3	12.5	27.9	45.6	30	50	70	30	60	70
		display	4.7	28.9	70.5	41.4	52.9	57.6	50	50	80	50	60	70
	FR	transfer	25.1	24.2	53.2	36.5	50.2	62.4	40	50	80	40	60	70
		display	43.6	28.3	67.6	41.1	64.5	91.5	60	80	100	80	100	100
			LSD _{4 °C} = 4.1%			LSD _{10 °C} = 6.5%			LSD _{4 °C} = 9.6%			LSD _{10 °C} = 10.0%		

^xWounded with a bent transfer needle and 10 µL containing 10³ spores placed in each wound that was approximately 3 mm deep × 2 mm diameter.

^v5% O₂, 15% CO₂, 80% N₂

^xTQR = three-quarters red.

^wEach value is the average of 30 fruit.

^vFR = fully red.

^uFisher's protected LSD at *P* = 0.05 calculated from the arcsin (square root of the percentage transformation) and then converted back to a percentage value.

the development of disease by about 1 week, which is consistent with the report of Sommer et al. (1973) who reported that after 7 d at 5 °C, fruit wound-inoculated with 10³ spores per wound (the same inoculum concentration used in this study) had a trace of rot development, whereas large lesions were found on inoculated fruit held at 10 °C for 7 d.

The storage of strawberries in an atmosphere containing high CO₂ or low O₂ slows the development of gray mold (Borecka and Millikan, 1981; Brecht et al., 1992; Couey and Wells, 1970; Harris and Harvey, 1973; Sommer, 1989; Wells and Uota, 1970). In this study, inoculated fruit were stored in an atmosphere containing 5% O₂ plus 15% CO₂ (balance N₂). This gas mixture reflects the usual concentrations of CO₂ and O₂ found in the modified atmosphere "Tectrol" process that is commonly used commercially (Mitcham and Mitchell, 2002). Fruit in our experiments that were stored for 1 week in CA at 4 or 10 °C were free from decay, whereas visible lesions were found on 15% and 75% of those stored in air at 4 or 10 °C, respectively. With storage at 4 °C, the apparently small effect of CA at 1 week was much larger after 2 weeks, where 80% of the fruit stored in air had symptoms vs. 35% of those stored in CA. With respect to disease severity, the effect of the CA vs. air storage on development of gray mold in wound-inoculated fruit was significant at both 4 and 10 °C. However, the incidence and severity of gray mold increased between 1 and 2 weeks for all storage treatments, including those with CA. At least two earlier reports, Wells (1970) and Smith (1992) concluded that the effect of CA was to delay disease development and not to prevent it completely.

Fruit ripeness at harvest, whether inoculated or not, significantly affected decay development in storage as previously reported (Sommer, 1989). The effect of fruit ripeness was significant for both inoculated and non-inoculated fruit at both storage temperatures. The increased susceptibility of the FR vs. TQR fruit to gray mold is consistent with previous reports. Pritts et al. (1987) observed that ripeness stage at harvest has a great effect on strawberry fruit decay. Fruit harvested with a white tip showed a lower rate of mold development when stored for 4 to

12 d at 1.6 or 7.2 °C than fully ripe fruit. Jerch et al. (1989) also reported that, when inoculated with conidial suspensions of *B. cinerea*, immature strawberry fruit were more resistant to fungal attack than more mature fruit.

Experiment 1 was intended to test how fruit ripeness and storage temperature affected the effectiveness of CA in controlling gray mold. The most rapid development of fruit rot occurred among wound-inoculated FR fruit stored at 10 °C in air where 100% of the fruit had symptoms within 1 week and all inoculation sites had sporulation of *B. cinerea* colonies within 2 weeks. By contrast, non-inoculated TQR fruit stored at 4 °C in CA for the same period were free of decay. Only 10% of the inoculated TQR fruit stored under the same conditions had evidence of decay with a severity rating of 0.5%. Thus, harvesting less than fully ripe fruit, by itself, helps to counter the predisposition to decay afforded by higher inoculum concentrations deposited in harvest-related wounds.

Most of the effects of the fruit ripeness, storage temperature and duration, and inoculation parameters on disease incidence and severity persisted during a 24-h simulated non-refrigerated consumer display. After 1 week of storage, the increase in disease severity during the display treatment ranged from an average of 5% for all non-inoculated treatments to 30% for all inoculated ones. After 2 weeks of storage, the respective increases were 12 and 36%. With respect to fruit previously stored in CA vs. air, the disease increase during the display of non-inoculated fruit stored for 1 week averaged over both storage temperatures was approximately 4% vs. 7%, respectively. When fruit were inoculated, the respective averages were 19% vs. 42%. These observations are consistent with previous reports where the effects of CA storage on decay persisted for a time after fruit were moved to air (Couey and Wells, 1970; Harris and Harvey, 1973; Wells, 1970). In this study, comparisons of the disease increase in the display for fruit previously stored for 2 weeks, however, were hampered by a number of 100% severity evaluations at transfer, which were more common among fruit stored at 10 °C. Larger increases in disease during the display occurred among

fruit stored for 2 weeks vs. 1 week, but only at the 4 °C storage temperature. Thus, a rapid reduction in fruit quality associated with temperature abuse prevented accurate estimation of the disease increase over 24 h at 20 °C.

Fruit harvested at the TQR stage of ripeness with or without inoculation were less apt to develop decay during the display than those inoculated at the FR stage. After 1 week at 4 °C, the disease increase during the display for the TQR fruit averaged approximately 1% and 21% for the non-inoculated and inoculated fruit, respectively, whereas corresponding values for the FR fruit were 10% and 40%, respectively. If the effects of the CA were on fruit ripeness or pathogen growth, then non-inoculated TQR fruit stored for 1 week in CA should develop lesions more slowly in the non-refrigerated consumer display than inoculated FR fruit stored for 2 weeks in air.

There is evidence that increased susceptibility to gray mold is associated with senescence of strawberry fruit. Storage and handling treatments that inhibit the development of decay also inhibit senescence, which indirectly leads to a reduction in fruit rot. Quiescent infections in green fruit become active as the fruit ripens and after harvest, diseased fruit may contaminate sound fruit. For example, chlorinated clean water used to hydrocool good quality strawberries reduced the naturally occurring incidence of gray mold as compared with clean water without chlorine, evidence that spores of *B. cinerea* can exist on fruit surfaces and can infect those fruit during storage (Ferreira et al., 1996). In addition, a delay in cooling fruit from 30 to 35 °C to 1 to 2 °C has been associated with both reduced fruit firmness and increased decay (Nunes et al., 1995a, 2005). Maas (1978) noted that strawberry cultivars remaining firm over a longer period were less susceptible to *B. cinerea* than were those that softened more quickly. As strawberry fruit ripen, softening occurs and the cell walls become thinner leading to easier fungal penetration in the cells (Szczeniak and Smith, 1969). Sommer (1989) also noted that fruit become softer and more susceptible to disease as they senesce. The softness was associated with changes in the cell walls that gave decay pathogens more opportunities to penetrate. Experiment 2 was intended to determine if fruit stored in CA have greater resistance to decay than fruit stored in air. In this study, fruit harvested at the TQR instead of the FR stage or that were stored in CA as compared with air both had less gray mold developing at a wound site that was or was not inoculated after storage. However, CA at 10 °C appeared to maintain resistance to *B. cinerea* as well as or better than CA at 4 °C, which seems to be inconsistent with the increased susceptibility accompanying increased senescence hypothesis since fruit should senesce more rapidly at the higher temperature. Since the control of gray mold by CA appeared to be associated with an effect on pathogen development, fruit in Experiment 3 were wounded, inoculated, and then placed into CA storage after various intervals at 20 °C to determine if particular stages of pathogen development were more sensitive to CA at either 4 or 10 °C. Based on the literature (Ke et al., 1993; Pesis and Avissar, 1990; Vaughn et al., 1993) and preliminary observations, spores were considered germinated by 12 h after inoculation, whereas colonization would have begun within 24 h. The TQR fruit were included in the tests to minimize the effect of senescence. The TQR fruit did not become fully red within 24 h at 20 °C and, as a result, would not have senesced as rapidly as FR fruit during the 12- or 24-h delay between inoculation and CA treatment.

A delay between inoculation and CA treatment led to increased fruit rot, during either the storage or post-storage consumer

display for either FR or TQR fruit. Previously, Mitchell et al. (1964) reported that cooling California strawberries from 29 to 4 °C within an hour of harvest yielded the highest fruit quality and lowest incidence of disease during storage. In contrast, delays in cooling of more than 2 h led to increased decay and reduced fruit quality. Similarly, Nunes et al. (2005) showed that the incidence of gray mold averaged 60% in fruit cooled 1 h after harvest and 85% in fruit from a 6-h delay treatment. In this study, the inhibitory effect of CA storage on the development of gray mold decreased as infections developed during the 24-h delay at 20 °C. The effect of a delay in air at 20 °C before establishment of CA storage in allowing greater disease development during and after storage was greater for FR vs. TQR fruit, but showed relatively little difference for 4 and 10 °C storage temperatures. Since lesion development would be expected to occur faster in tissue from riper fruit, this implies that the initialization of a decay lesion prior to storage is more important for the development of disease than the conditions during storage. Thus, the greatest effect of CA would be on freshly inoculated fruit, whereas the least would be on colonized fruit.

Based on evidence presented in this study, prediction of how much disease will develop when packaged fruit are held in a consumer display cannot be based on visible signs and symptoms observed at transfer from cold storage. Previous handling and exposure to inoculum must be considered in determining how consumer packs are displayed. The value of culling diseased fruit and reworking packs vs. discarding those with visible disease prior to setting up the displays is unclear. For example, in this study, 5% to 60% of the fruit that were free of disease when transferred from cold storage developed symptoms during the 24-h display at 20 °C. The lower value was obtained for TQR and FR fruit stored for 1 week in CA at 4 °C, whereas the higher value was obtained for fruit stored for 1 week in CA at 10 °C, regardless the maturity at harvest. The larger disease increase in the display for fruit from 4% vs. 10 °C or those stored for 1 vs. 2 weeks appears inconsistent with a persistence of control of gray mold for at least 24 h after removal from the treatments as noted for CA. This inconsistency, however, is likely due to the high disease severity observed at transfer for fruit from 10 °C or from a 2-week storage period.

In this study, it was shown that the interaction of strawberry fruit ripeness, storage temperature, and storage atmosphere on control of gray mold is such that CA is more effective when applied to riper fruit and in conjunction with higher storage temperature, but maximum postharvest life for strawberries are achieved by selecting less ripe fruit for harvest and by using lower storage temperatures. Our results also show that the effectiveness of CA in reducing gray mold of strawberries is greatest when CA is applied quickly after harvest, before spore germination occurs in fresh harvest wounds. The use of a commercial CA as simulated in this study reduces decay development, especially when fruit are fully ripe or storage temperatures are abusive, but cannot be relied upon to protect fruit as well as cold storage.

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