Influence of Cold-storage Temperatures on Strawberry Rot Caused by *Botrytis cinerea*

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Abstract

Thirteen fungal isolates from rotten strawberry fruits were collected from fields of Sharkia Governorate. The most frequent one was *Botrytis cinerea*. The susceptibility of Camarosa, Chandler, Kelia, and Sweet Charlie cultivars by *B. cinerea* showed that the Camarosa cultivar had the least incidence of total decay percentage at delayed cooling (4 h after inoculation), prompt cooling (1 h after inoculation), and accounted for 66.66% and 40.00% respectively. Other cultivars showed higher incidence of decay. The same pattern was observed in the severity of infection test. Prompt cooling was important for minimizing post postharvest decay of strawberries for both inoculated and non-inoculated fruits. Incidence and severity of decay were evaluated after storage for 7 d at 2˚C plus a simulated retail display period of 1 d at room temperature (20˚C).

Introduction

The increasing world population has led to increased demand for food and reduced per capita availability of arable land and irrigation water. Compounding this problem is the fact that most farmers in the developing world own only small plots of land that have the potential to feed one family and generate income (Salami *et al*., 2010). Small-scale farmers in developing countries are faced with many problems and constraints. Pre- and postharvest crop losses due to insect, diseases, weeds, and droughts in low and fluctuating yields, as well as risks and fluctuations in incomes and food availability (Tonukari, and Omotor, 2010).

Strawberry is an important small fruit, grown throughout the world. It is deep red in color with unique shape and flavor. Losses can be categorized on the basis of cause into three classes: Mechanical damage, physiological damage (storage disorders), and biological damage (insect and pathogen diseases)

(Ferguson *et al*., 1999). Biological damage is the most important portion.

The most common decay of strawberry is Botrytis rot, also called Gray Mold, caused by *Botrytis cinerea* (Ceponis *et al*., 1987). The disease can begin pre-harvest, remaining as latent infections, or begin postharvest.

Botrytis fruit rot is a universal problem wherever strawberries grow. The disease can develop before or after harvest. Postharvest incidences may range up to 30% to 50% of the crop (Borecka and Millikan, 1981; Dennis and Davis, 1977; Dennis and Mountford, 1975; Maas, 1978). Most fruit infection occurs in the field as latent infections of the stem attachment (Powelson, 1960), which usually remains quiescent until the fruit ripens. Additional inoculation and infection are associated with inadvertent wounds occurring during harvest. Increased postharvest strawberry losses have been associated with cooling delays of as little as 1 h after harvest (Mitchell, 1992; Mitchell *et al*., 1996). In fact, Kenny (1979) observed that delaying precooling from 3 to 8 h after harvest reduced strawberry storage life considerably. However, neither Mitchell *et al*. (1996) nor Kenny (1979) quantified the decay in their studies. Some delay from harvested fruit must be accumulated in the field, flats palletized, trucks loaded and driven to the cooling facility, and pallets of fruit finally staged for precooling. Since it is well known that lowering the temperature of fruits after harvest reduces the development of decays in general, it has been recommended that strawberry fruit should be cooled to 0 to 3˚C as soon as possible after harvest (Mitchell *et al*., 1996).

The objective of this work was to evaluate the effects of a short delay to cooling with fruit harvested during warm weather (35˚C), as might be encountered in normal commercial operations, on the development of decay in different cultivars of strawberries.

Material and methods

Isolation of the causal fungi:

Rotten strawberry fruits were collected from different fields (fresh planting system) at Sharkia Governorate. Diseased fruits were washed with tap water, cut into small pieces, each containing rotten tissues with adjacent healthy ones, surface was disinfected with 2% sodium hypochlorite for 2 min. and dried between sterilized filter paper. The cut pieces were cultured on potato dextrose agar (PDA) medium, and incubated at 20 ±1˚C. The growing fungi were sub cultured and purified by using hyphal tip technique and were identified according to their morphological features, using the description of Gilman (1957), Barnett and Hunter (1972) and Moubasher (1993).

Fruit inoculation:

Freshly harvested fruits were promptly moved from the field of Sharkia Governorate to the laboratory

within an average of 1 h at temperature of approximately 30˚C. Upon arrival, 120 strawberries of four strawberry cultivars namely; Camarosa, Chandler, Kelia, and Sweet Charlie were immediately selected for uniformity of ripeness (three-quarter to full red, but not overripe) and freedom from defects. Three replicates of five fruits (usually a mixture of the two maturities) each were placed in plastic clamshells and assigned randomly to each treatment or the control in order to simulate commercial harvest and inoculated. The remaining non-inoculated fruit were used as a control. Cultures of *B. cinerea* that had been isolated from diseased strawberries were grown on PDA for 5 d at 21˚C. The surfaces of the 5-d cultures were flooded with sterilized tap water containing 0.1% Tween 20 and then brushed with a bent glass spreader. The resulting suspension was filtered through cheesecloth and then diluted to 10^6 spores/ml for *B. cinerea*. Whole fruit were completely submerged in the spore suspensions or water plus surfactant alone (control) for 2 s.

Cooling delay, cooling, and storage conditions:

Following inoculation, the fruits were placed in a controlled temperature room at 35˚C ±1.0˚C and 70% to 80% relative humidity (RH) with no airflow in order to raise fruit temperatures uniformly to those of fruit harvested during a warm day. Fruit samples were removed from the 35˚C-storage after 4 h and immediately transferred to a room at 2˚C ±0.5˚C and 85% to 95% RH in the dark for 7 d. After 2˚C storage, the fruits were moved to 20˚C ±1.0˚C and 85% RH for 1 d to simulate retail market display.

Decay evaluation:

Decay incidence or number of fruit with distinctive lesions of gray mold was recorded after simulated retail display based on 1 to 5 scale, where 1= no visible changes in the tissues; 2= slight brown discoloration of the tissues; 3= slight to moderate mycelium growth; 4= moderate to heavy mycelium growth; 5= characteristic sporulation. The severity scale was converted to a percentage of full sporulation such that 1= 0%; 2= 25%; 3= 50%; 4= 75%; and 5= 100%.

Statistical analysis:

The obtained data were analysed for variance (Snedecor and Cochran, 1981), whereas the differences among treatments were tested by the calculated Least Significant Differences (L.S.D.) at 5% level.

Results

Frequency of fungi associated with fruit rots:

The fungi associated with strawberry fruit rots were isolated from samples showing the disease syndrome. The frequency of fungi associated with strawberry fruit rots was determined and the mean was calculated.

Results in Table (1) show that *B. cinerea* was the most frequently isolated fungus from the rotten strawberry fruits collected from Sharkia Governorate, while the other isolated fungi were found with low frequency. Moreover, Chandler and Sweet Charlie cultivars were free from *Aspergillus niger*, *Penicillium* spp. and *Sclerotinia sclerotiorum*, whereas *Colletotrichum* spp. isolated from all cultivars was observed except in case of Sweet Charlie cultivar.

Table 1: Frequency of occurrence of fungi associated with strawberry fruit rots collected from Sharkia Governorate during 2012.

Fungus	Frequency (%) of fungi isolated from Sharkia Governorate				
	Cultivars				
	Camarosa	Chandler	Kelia	Sweet Charlie	Mean
Alternaria alternata	10.06	9.54	8.76	9.37	9.43
Aspergillus niger	1.23	-	2.15	-	0.85
Botrytis cinerea	30.05	31.17	30.13	32.46	30.95
Colletotrichum spp.	4.08	5.21	2.72	-	3.00
Fusarium solani	5.31	6.34	5.43	6.31	5.85
Mucor hiemalis	2.34	1.01	2.21	2.31	1.97
Penicillium spp.	2.02	-	1.91	-	0.98
Phytophthora cactorum	6.25	7.33	6.31	10.20	7.52
Pythium ultimum	6.31	7.48	8.11	11.30	8.30
Rhizoctonia solani	12.08	11.31	9.75	11.94	11.27
Rhizopus stolonifer	4.81	5.40	5.44	5.90	5.39
Saccharomyces spp.	1.11	-	2.20	-	0.83
Sclerotinia sclerotiorum	14.35	15.21	14.88	10.21	13.66

The cooling treatment has significantly affected decay incidence and severity with less decay and reduced severity occurring in the promptly cooled fruit (Table 2 and 3). There was an average 34.79% less decay incidence in non-inoculated strawberries from the prompt cooling treatment than from the delayed cooling treatments after 7 d at 2˚C plus 1 d at 20˚C (Table 2). Fruits that were inoculated with *B. cinerea*, showed Botrytis rot incidence of total decay was 93.33% on Sweet Charlie while the Camarosa cultivar had the lowest rot incidence (66.66%). On the other hand Chandler and Kelia cultivars were 73.33% for delayed cooling (Table 2). Inoculated Strawberries that were cooled promptly had lower incidence of decay (Table 2). In fact, prompt cooling reduced the incidence decay of Botrytis rot in inoculated fruits of Camarosa, Chandler, Kelia and Sweet Charlie cultivars by 60.0,

63.6, 72.7 and 64.3%, respectively (Table 2).

Table 2: Effect of delayed cooling on the incidence of total postharvest decay of strawberries that had been dipped in a conidial suspension of *B. cinerea*.

Cultivar	Incidence of total decay (%)	
	delayed cooling (4 h after inoculation)	prompt cooling (1 h after inoculation)
Camarosa	66.66	40.00
Chandler	73.33	46.66
Kelia	73.33	53.33
Sweet Charlie	93.33	60.00
Control (water)	26.66	20.00
L.S.D. at 5%	4.64	

Data in Tables (2) and (3) revealed that the fruits of all cultivars tested were susceptible to different degrees to infection by *B. cinerea*. However, Camarosa was the least susceptible cultivar as it gave the lowest percentage of infection and infection severity. On the other hand Chandler, Kelia and Sweet Charlie were considered most susceptible, since they recorded the highest percentage of infection and severity. Delayed cooled, non-inoculated strawberries showed a higher incidence of decay than those from the promptly cooled treatment.

Table 3: Effects of delayed cooling on the severity of total postharvest decay of strawberries that had been dipped in a conidial suspension of *B. cinerea*.

Cultivar	severity of total decay (%)	
	delayed cooling (4 h after inoculation)	prompt cooling (1 h after inoculation)
Camarosa	25.00	16.66
Chandler	32.33	18.33
Kelia	35.00	25.00
Sweet Charlie	48.33	28.33
Control (water)	6.66	3.33
L.S.D. at 5%	1.96	

Discussion

Fungi isolated from naturally rotten strawberry fruits, collected from fields of Sharkia Governorate in Egypt belonged to different genera. Thirteen fungi were isolated from rotten fruits collected from fields. The isolated fungi were purified and identified as *Alternaria alternata*, *Aspergillus niger*, *Botrytis cinerea*, *Colletotrichum* spp., *Fusarium solani*, *Mucor hiemalis*, *Penicillium* spp., *Phytophthora cactorum*, *Pythium ultimum*,

Rhizoctonia solani, *Rhizopus stolonifer*, *Saccharomyces* spp. and *Sclerotinia sclerotiorum*. The most frequently occurring fungi, was *B. cinerea* which was the most abundant one. This result was in agreement with those reported by Khafagi, (1982); Mass, (1984); Legard *et al*., (2000); Younes (2002) and Saber *et al*., (2003).

Evaluation of the susceptibility of the four studied strawberry cultivars to fruit-rot infection by *B. cinerea* confirmed that all tested cultivars varied, to different degrees, in their susceptibility. Camarosa cultivar showed the least percentages of disease infection and severity. Opposite result was recorded for Sweet Charlie cultivar. The variation in susceptibility among cultivars may be attributed to firmness of the skin, liability to bruising or to physiological resistance, as reported by Khafagi (1982), El-Neshway (1988) and Tadrous (1991).

The incidences of Botrytis rot observed here were also higher than those reported by other authors (Ceponis *et al*., 1987; Sommer *et al*., 1973). For example, Sommer *et al*., (1973) observed a maximum of 12.9% gray mold rot in non-inoculated strawberries, which may be due to the handling procedures used here were more suitable to disease development than were those of Sommer *et al*., (1973) .

Temperatures between 20 and 35 ˚C generally promote rapid pathogen development (Sommer, 1992). This suggests that the temperature of the strawberries during the delay to cooling in our study, during which the fruit warmed from 20 to 30 ˚C, was quite conducive to decay establishment and probably favored greater decay development during subsequent storage. The additional day at 20 ˚C used in our study, which simulated a retail display, also contributed to the increased incidence and severity of decay in strawberries either from the prompt cooling or delayed cooling treatment.

Delaying the start of cooling greatly increased the severity of decay for both inoculated and non-inoculated strawberries. These results are in agreement with those reported by Nunes *et al*., (2005) and Siefkes-Boer *et al*., (2009).

دراسة تأثير التخزين بالتبريد على عفن الفراولة المتسبب عن فطر بوترايتس سينيريا

فرج على أبوشعالة

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الملخص

عزلت ثلاث عشرة عزلة من فطريات مختلفة من ثمار الفراولة التي جمعت من بعض حقول محافظة الشرقية بجمهورية مصر العربية، وكان الفطر بوترايتس سينيريا أكثرها شيوعاً على ثمار الفراولة. و كان الصنف كاماروزا أقل الأصناف المختبرة قابلية للإصابة بهذا الفطر مقارنة بالأصناف الأخرى (شاندلر، كيليا و سويت تشارلى). التأخير في التبريد (أربع ساعات بعد العدوى) أدى إلى زيادة نسبة الإصابة بهذا الفطر وكذلك زاد من شدة المرضية، حيث كانت نسبة الإصابة وشدة المرض مرتفعة مقارنة بمعاملة التبريد السريع (ساعة واحدة بعد العدوى). إن التبريد السريع قلل من الإصابة وشدة المرضية وفى كلا الحالتين المعاملة بالفطر والشاهد. أدى تخزين ثمار الفراولة لمدة سبعة أيام عند درجة الحرارة 2 °م إلى زيادة مدة العرض عند درجة حرارة الغرفة (20 °م).

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