Eur. J. Hortic. Sci. *84*(5), 274–281 | ISSN 1611-4426 print, 1611-4434 online | https://doi.org/10.17660/eJHS.2019/84.5.3 | © ISHS 2019

Original article

Postharvest fungal fruit decay in sweet cherry graded in water with low chlorine content

J. Børve[1] and A. Stensvand[1,2]

[1] Norwegian Institute of Bioeconomy Research, Ås, Norway
[2] Norwegian University of Life Sciences (NMBU), Ås, Norway

Summary

Sweet cherry fruit delivered at three packinghouses over two years in southern Norway was assessed for postharvest fungal decay after being graded in a line with water containing 2 ppm chlorine, in comparison with non-graded fruit. Assessment of decay was carried out after cold storage of the fruit for ten days at 2°C, followed by two days at 20°C. In mean of all assessments, there was no difference in total decay after storage between fruit graded in a water line or non-graded fruit, however, the first year there was a higher total incidence of fruit decay on water-graded fruit after storage. The grading-water was not changed during the day, but time of grading during the day did not seem to influence the amount of decay. Mucor rot and grey mould accounted for 80 and 19%, respectively, of the decay averaged for all assessments, and there was no significant difference in decay of the two diseases if graded in water or not. For blue mould and brown rot, the incidence was lower in water graded fruit, while it was the opposite for Cladosporium rot. On average, fruit decaying fungi developed on PDA from 57 and 17% of water samples from grading lines in the two years, respectively. On pieces with filter paper wetted in different locations of the grading line, 87% contained fruit decaying fungi when placed on PDA, and *Mucor* sp. was the most prevalent pathogen. Fruit cooled in a hydro-cooler containing either 2, 10 or 50 ppm chlorine, all reduced decay with about 75% compared to non-chlorinated water. Although the grading water may contain spores of pathogenic fungi, the present results indicated that water containing 2 ppm chlorine does not significantly increase fruit decay. Thus, only a slight chlorination of grading water may be sufficient to reduce postharvest contamination.

Keywords
Botrytis cinerea, chlorine, fruit rot, *Monilinia laxa*, *Mucor piriformis*, *Prunus avium*

Significance of this study

What is already known on this subject?

- Chlorine in grading line water may reduce viability of spores of fungal pathogens, but sensitivity varies among species.

What are the new findings?

- Although 2 ppm chlorine significantly reduced fungal fruit decay in water used to grade sweet cherry, it still contained viable spores of fruit decaying pathogens. However, chlorine levels up to 50 ppm in the hydro-cooler did not further reduce the decay.

What is the expected impact on horticulture?

- The research should encourage further investigations to reduce chlorine use in sweet cherry grading.

Introduction

In areas with frequent rainfall during the ripening period, sweet cherry trees are either covered with plastic roofs or kept in high plastic tunnels in order to avoid fruit cracking and fungal fruit decay. Even though the orchards are treated regularly with fungicides and covered with plastic, the fruit may develop fungal infections appearing pre- and postharvest (Børve and Stensvand, 2003; Børve et al., 2007; Palm and Kruse, 2008; Thomidis and Exadaktylou, 2013).

Cooling and grading of sweet cherry fruit in water lines is common worldwide, and such treatment is a conceivable point of fungal contamination of the fruit. From Washington USA, it was reported from sweet cherry packinghouses that the grading water may contain *Aureobasidium pullulans* and *Penicillium* spp. (Sanderson, 2003). In apple grading water, there have been reports of presence of *Penicillium* spp. (Spotts and Cervantes, 1986; Rosenberger et al., 1991; Sholberg and Haag, 1996), *Botrytis cinerea*, *Mucor piriformis* (Spotts and Cervantes, 1986), *Cladosporium* spp. and *Phialophora fastigiata* (Sholberg and Haag, 1996). In grading lines in Norway, the water is changed daily, but during the course of one day it is recycled continuously and has to satisfy a standard of maximum 2 ppm chlorine (present either as OCl^- or Cl_2; termed chlorine hereafter), as for drinking water. Higher levels of chlorine (between 30 and 150 ppm) in grading and hydro-cooler water is standard in some countries (Willett et al., 1989; Bahar and Dundar, 2001; Ritenour et al., 2014; Padilla-Zakour et al., 2007). The risk of contamination of fungal pathogens on healthy fruit has not been determined in low-chlorinated water, such as 2 ppm chlorine.

The objective with the present investigation was to evaluate the risk of development of fungal decay in commercially grown sweet cherry fruit after being sorted in a grading line with water of a low chlorine content (2 ppm) and followed through a simulated storage and sales period.

Materials and methods

Experiments were conducted in the growing seasons of 2007 and 2008, with fruit from commercial orchards.

Water grading

Fruit of 'Van' and 'Lapins' delivered from ten (2007) or five (2008) commercial orchards at each of three packinghouses located in Lærdal and Hardanger (both southwestern Norway), and Telemark (southeastern Norway) were assessed. In total, fruit of 26 'Van' and 30 'Lapins' orchards were assessed in 2007 and 15 'Van' and 15 'Lapins' orchards in 2008. The orchards delivering fruit to each packinghouse were all treated as commercial practice in Norway, with plastic roof covering over the trees from 3 to 4 weeks before harvest and throughout the harvest period to prevent cracking, and 3 to 5 fungicide applications from around bloom until time of plastic covering to prevent fruit decay. The number of fungicide applications and type of fungicides used differed from farm to farm. A typical fungicide spray schedule included 1–2 sprays during flowering, 1–2 on green fruit and 0–1 prior to harvest when fruit changed colour. Year of planting and tree size differed, and represented the range of orchards delivering fruit to the packinghouses. The grading lines were of different brands, but all consisted of a water dump, a singulator with water for separating fruit connected with stems, and no water on the sorting tables and conveyors. Temperature of the water was about 2°C. The length of time the fruit were in water differed but was from 1 to 5 minutes. The sources of chlorine for the grading line water were NaOCl in Lærdal and Hardanger and $Ca(OCl)_2$ in Telemark. The chlorine content was measured several times daily and adjusted to 2 ppm.

In 2007, deliveries from each orchard were sampled before and after grading. From each delivery 5 × 100 healthy appearing fruit suitable for marketing were sampled among fruit from unsorted 15 kg boxes prior to grading and among fruit in 5 kg boxes after grading in chlorinated water. The fruit were placed in open plastic baskets, with 50 fruit in each. Then the fruit were transported from the packinghouses to NIBIO Ullensvang (from 1.5 to 4 hours driving in a car without cooling). Immediately upon arrival, the boxes were placed in a ventilated cold store at 2°C. After 10 days at 2°C followed by 2 days at 20°C, decayed fruit were diagnosed and recorded.

In 2008, fruit from each orchard were subdivided in three parts. Before packing, 5 × 100 healthy appearing and marketable fruit were sampled from unsorted 15 kg boxes from each of the three parts. Then the remaining fruit of the three parts went through the water grading line at three different times during the day; early, mid-day and late, with about 2 hour intervals. After each of the grading times of fruit from each orchard, 5 × 100 healthy appearing and marketable fruit were sampled from 5 kg boxes at the end of the line. The samples were then stored and assessed as in 2007.

Hydrocooling at three chlorine levels

At the Lærdal packinghouse in 2008, an additional experiment with hydrocooling (2°C water temperature for 8 minutes) of the cherries was performed. Commercial deliveries of fruit were divided into five parts. One part was not cooled by the hydro-cooler, but placed directly into a cold store at 0.5 – 3°C. The other four parts were hydrocooled with either non-chlorinated water, or water containing 2, 10, or 50 ppm chlorine supplied as NaOCl, whereafter the fruit were placed in the cold store. From each part of each cultivar/orchard, 5 × 100 fruit were sampled in the cold store afterwards. The samples were stored and assessed as the samples from the grading line described above. The experiment was repeated with deliveries at three different days in August 2008 from five orchards of 'Van' (August 5), five of 'Lapins' (August 13), and two of 'Lapins' and one of 'Sweetheart' (August 21).

Fungal pathogens present

Disease identification was based on macroscopic appearance on the fruit and confirmed by microscopy if necessary. The following fungal pathogens (common disease names in parentheses) were observed: *Mucor* spp. (Mucor rot), *Botrytis* sp. (grey mould), *Penicillium* sp. (blue mould), *Colletotrichum* sp. (anthracnose), *Monilinia* spp. (brown rot) and *Cladosporium* sp. (Cladosporium rot). Fruit with symptoms and signs of decay similar to those of *Mucor* or *Rhizopus* rot was identified as *M. piriformis* by molecular methods (data not published). The other pathogens were identified morphologically.

Assessment of grading water

Both seasons, water was sampled in glass bottles from the dump tank of grading lines at the different fruit sampling times during the different packing days. All bottles were transported (1 to 4 hours) in a cooling box to the Ullensvang research center. In 2007, part of the samples were used to identify fungal spores by microscopy. Both years, water samples were plated on 9 cm Petri dishes containing potato dextrose agar (PDA) by pouring 1 mL (not diluted) with a pipette onto the plates in three or six replicates (plates) followed by incubation for 4 to 6 days at 20°C in continuous fluorescent light before assessment for visible fungal growth.

In 2008, pieces of sterile filter paper (about 16 cm^2) held by tweezers were wetted in different locations of the grading line and placed directly onto PDA plates followed by incubation as described above. The locations were: i) the conveyor belt transporting fruit from the water dump; ii) the conveyor belt after the singulator, which is the part where the fruit stems are cut to separate fruit (no more water in the machine after that point); iii) on different positions of the conveyors where decayed fruit were sorted out, and iv) where the fruit were transported to the packing boxes. At each location from two to six filter paper pieces (one on each plate) were wetted and incubated. To avoid contamination, the PDA plates were opened and closed quickly during the process of placing the filter papers on the agar and then immediately sealed with Parafilm. The plates were then transported to NIBIO Ullensvang in cooled boxes and assessed for fungal growth after 4 to 6 days at 20°C in continuous fluorescent light.

Climate data and statistical analysis

Data for daily precipitation (mm) and mean air temperature were obtained from weather stations in orchards located nearby the three packinghouses from 1 July until samples of fruit were collected at the three sites from end of July to mid-August.

Data were statistically analyzed by the GLM procedure of SAS (SAS Institute, Cary, NC, USA), and differences between means were separated by Student Newman Keuls method at $P = 0.05$. All data were analyzed both as pooled data and for each year, packinghouse, cultivar and orchard separately.

Results

Seasonal climate

The daily mean day temperature from 1 July until time of sampling was higher in 2008 than in 2007 in all the three areas were fruit were sampled, and in the same period of time number of days with precipitation was higher in 2007 than

Table 1. Number of days with more than 0.2 mm precipitation (Days) and mean daily temperatures (°C) from 1 July until sampling of fruit of sweet cherry 'Van' and 'Lapins' in the area of three sweet cherry packinghouses in southern Norway in 2007 and 2008.

Packinghouse	Cultivar	Sampling date		2007		2008	
		2007	2008	Days	°C	Days	°C
Lærdal	Van	7 Aug	5 Aug	24	15.5	13	18.2
	Lapins	14 Aug	13 Aug	28	16.0	20	17.7
Hardanger	Van	31 July	24 July	22	15.3	12	16.9
	Lapins	14 Aug	7 Aug	32	16.3	18	18.2
Telemark	Van	25 July	31 July	18	15.1	9	17.8
	Lapins	1 Aug	7 Aug	21	15.2	13	17.6

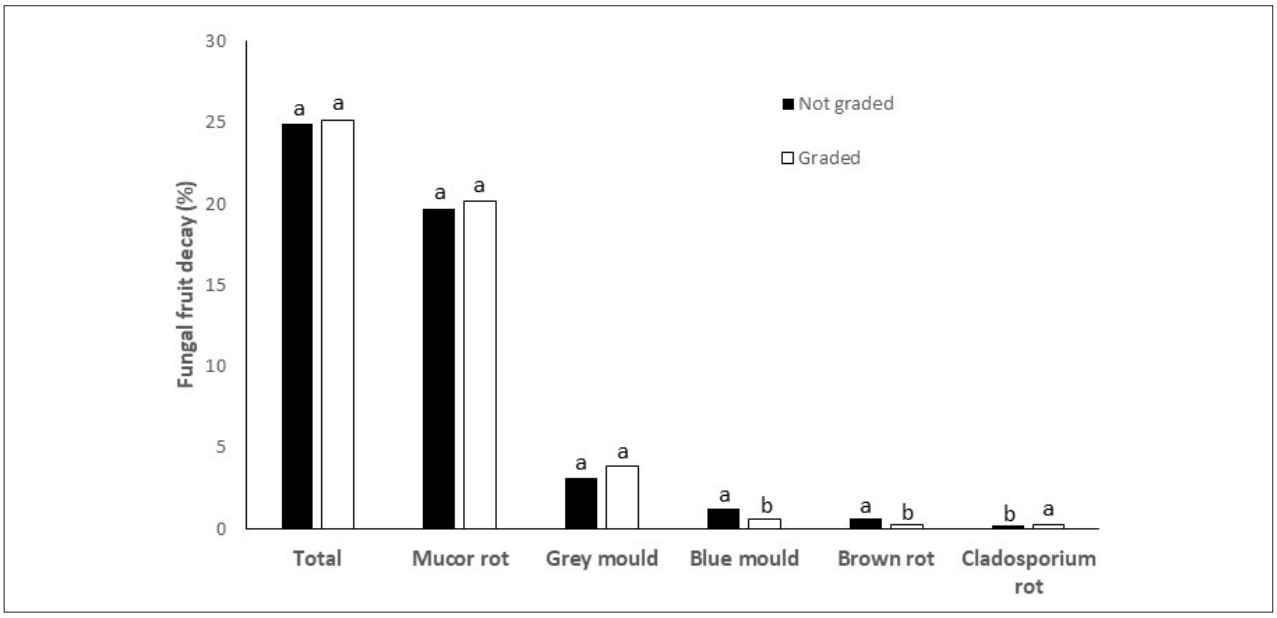

Figure 1. Fungal fruit decay (%) on sweet cherry fruit after storage for 10 days at 2°C and two days at 20°C in mean of two seasons for 'Van' and 'Lapins' from the three packinghouses; fruit were either graded in a line with slightly chlorinated water (2 ppm chlorine) or not graded. Mean of 430 non-graded and 730 graded fruit samples; each sample contained 100 fruit.

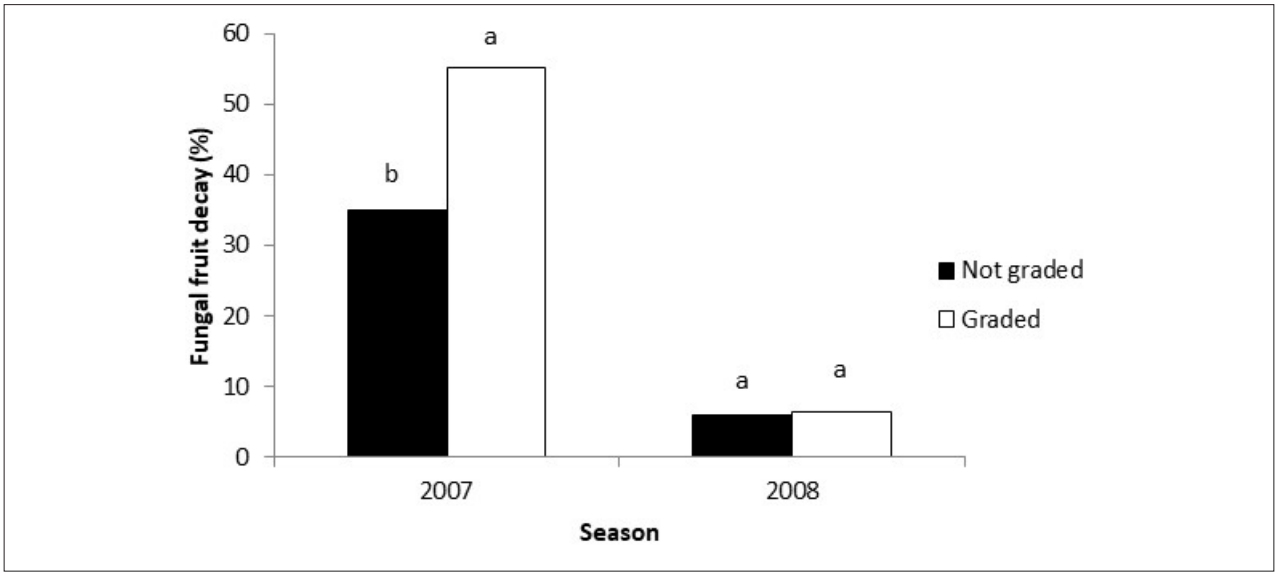

Figure 2. Fruit decay (%) on sweet cherry fruit after storage for 10 days at 2°C and two days at 20°C in mean of three packinghouses with 'Van' and 'Lapins'; fruit were either graded in a line with slightly chlorinated water or not graded in 2007 and 2008. Mean of of 430 non-graded (280 in 2007 and 150 in 2008) and 730 graded (280 in 2007 and 450 in 2008) fruit samples; each sample contained 100 fruit.

in 2008 (Table 1). Number of days with precipitation was lower in Telemark than at the other two locations both years.

Water grading vs. non-grading

When pooling all data (N = 1,160 observations) for total fungal decay, year (P = 0.0001) and cultivar (P = 0.0001) explained most of the differences. Fruit decay after 10 days in cold store was from 0 to 2% for all samples (data not shown). The total decay after another two days at 20°C increased to about 25% in mean of all assessments both years. There was no significant difference between fruit graded in water or non-graded fruit in incidence of total fruit decay, Mucor rot or grey mould. Incidence of Cladosporium rot was higher and brown rot and blue mould was lower on water graded fruit (Figure 1).

In 2007, the mean of total fungal fruit decay for all samples of 'Van' and 'Lapins' was significantly higher (P = 0.0001) for water graded (55.4%) than non-graded (35.2%) fruit after storage (Figure 2). Mucor rot was the dominating cause of decay both years (Tables 2 and 3), except for 'Van' at the Hardanger packinghouse in 2007, where grey mould was more prevalent (Table 2). Results from all three packinghouses showed difference in grey mould between water graded and non-graded fruit of 'Lapins', and there was less brown rot in graded compared to non-graded fruit in both cultivars. The incidence of blue mould was lower after grading at two of the

Table 2. Incidence (%) of fungal diseases on sweet cherry fruit of 'Van' and 'Lapins' graded in a line with water contact[a] or not water-graded, followed by storage for 10 days at 2°C and two days at 20°C in 2007.

Cultivar, packinghouse	Fungal fruit decay (%)						
	Total[b]	Range[c]	Mucor rot[d]	Grey mould	Blue mould	Brown rot	Cladosporium rot
'Van'							
Lærdal							
Not graded	22.5[e]	2.6–44	19.9	1.2	1.1	0	0.2
Graded	50.6	12–85	44.8	3.1	1.8	0	0.9
P-value	0.0001		0.0001	0.0582	0.1367	-	0.0202
Hardanger							
Not graded	70.7	32–100	40.5	24.6	4.2	1.2	0.2
Graded	99.8	98–100	44.3	53.4	1.7	0.4	0.1
P-value	0.0001		0.5114	0.0001	0.0001	0.0154	0.2091
Telemark							
Not graded	71.8	22–98	69.1	0.6	1.5	0.4	0.18
Graded	95.7	60–100	95.1	0.1	0.4	0.04	0.08
P-value	0.0001		0.0001	0.0362	0.0064	0.0132	0.2638
Mean for the three sites							
Not graded	60.0		46.7	10.0	2.4	0.6	0.20
Graded	86.9		64.0	21.3	1.2	0.2	0.27
P-value	0.0001		0.0001	0.0009	0.0011	0.0045	0.4256
'Lapins'							
Lærdal							
Not graded	17.8	5–34	16.2	0.52	1.1	0.06	0
Graded	25.1	5–35	23.0	0.32	1.8	0	0
P-value	0.0001		0.0001	0.2727	0.0271	0.0808	–
Hardanger							
Not graded	16.1	3–47	13.7	0	0.4	2.0	0
Graded	49.8	15–95	48.9	0	0.3	0.5	0
P-value	0.0001		0.0001	–	0.7135	0.0001	–
Telemark							
Not graded	6.9	2.6–29	3.0	0.2	2.2	1.2	0.14
Graded	9.1	1.6–29	6.3	0.1	2.3	0.3	0.26
P-value	0.1220		0.0074	0.1623	0.9184	0.0001	0.3067
Mean for the three sites							
Not graded	13.6		11.0	0.2	1.2	1.1	0.05
Graded	28.0		26.1	0.1	1.5	0.3	0.09
P-value	0.0001		0.0001	0.1346	0.5220	0.0001	0.3413

[a] For 1–5 minutes in water of 2°C and with 2 ppm chlorine.
[b] Mean of 5 × 100 fruit at each sampling point from 6 ('Van' in Lærdal) or 10 (all others) orchards.
[c] Range in orchard means of total decay.
[d] Causal pathogens: Mucor rot = *Mucor* sp., grey mould = *Botrytis* sp., blue mould = *Penicillium* sp., brown rot = *Monilinia* sp., Cladosporium rot = *Cladosporium* sp.
[e] Mean values denoted with different letters are different according to Student Newman Keuls method at P = 0.05.

Table 3. Incidence (%) of fungal diseases on sweet cherry fruit of 'Van' and 'Lapins' graded in a line with water contact[a] or not water-graded, followed by storage for 10 days at 2°C and two days at 20°C in 2008; water grading took place at 2-hour intervals early, mid-day or late during the packing day.

Packinghouse	Fungal decay (%) on 'Van'					Fungal decay (%) on 'Lapins'					
Time of grading	Total[b]	Range[c]	Mucor rot[d]	Blue mould	Cladosporium rot	Total	Range	Mucor rot	Blue mould	Brown rot	Cladosporium rot
Lærdal											
Not graded	1.4 b[e]	0.2–3.2	0.5 b	0.16	0.7	24.1	4–91	23.7	0.24	0	0.04 b
Early	2.8 a	0.6–7	1.4 a	0.16	1.2	26.4	4–20	26.4	0.12	0	0.04 b
Mid-day	2.0 ab	0.8–3.6	0.8 ab	0.08	1.1	21.2	4–31	20.3	0.24	0	0.7 b
Late	1.3 b	0.8–2.6	0.6 b	0.04	0.7	22.8	5–42	20.6	0.32	0	1.8 a
P-value	0.0186		0.0264	0.4363	0.4251	0.7052		0.5677	0.6982	-	0.0070
Hardanger											
Not graded	6.6 b	0.2–22	4.9 b	0.08	0.24	1.4	0.2–4	0.96 b	0.12	0.2	0.04
Early	7.8 b	0–27	6.6 b	0.04	0.48	2.9	0.6–5	2.6 a	0.08	0.1	0
Mid-day	13.0 a	2–38	11.0 a	0	0.48	1.4	0.6–4	1.2 b	0.04	0.04	0.04
Late	7.1 b	0.2–20	5.4 b	0.12	0.83	2.0	0–8	1.6 b	0.16	0.08	0.08
P-value	0.0001		0.0001	0.3028	0.2489	0.0630		0.0099	0.5505	0.5989	0.5490
Telemark											
Not graded	0.76 a	0.2–1.4	0.0	0.2 a	0.4	1.3	0–2.6	0.16	0.24	0.8 a	0
Early	0.28 b	0–1	0.0	0 b	0.2	1.1	0–2.6	0.48	0.12	0.5 ab	0
Mid-day	0.24 b	0–0.8	0.04	0.04b	0.1	0.7	0.2–1.4	0.16	0.12	0.3 b	0
Late	0.16 b	0–0.4	0.0	0 b	0.1	0.7	0.2–2.6	0.08	0.12	0.5 ab	0
P-value	0.0137		0.3968	0.0246	0.0763	0.0977		0.1480	0.7330	0.0536	–

[a] For 1–5 minutes in water of 2°C and with 2 ppm chlorine.

[b] Mean of 5 × 100 fruit at each sampling time and from five orchards of each cultivar.

[c] Range in orchard means of total decay.

[d] Causal pathogens: Mucor rot = *Mucor* sp., blue mould = *Penicillium* sp., Cladosporium rot = *Cladosporium* sp., brown rot = *Monilinia* sp.

[e] Values denoted with different letters are different according to Student Newman Keuls method at P = 0.05.

Table 4. Effect of chlorine level in hydrocooler water on total fruit decay on sweet cherry cultivars from different orchards at three days in 2008. Assessed after storage for 10 days at 2°C and two days simulated shelf life (20°C).

Treatment	Date of cooling and cultivar/orchard[a]										Mean
	August 5				August 13				August 21		
	Van[b]	Van	Van	Van	Lapins	Lapins	Lapins	Lapins	Lapins	Lapins	Sweetheart	
No water	10.3 b[c]	4.3 b	0.7 b	10.7 b	2.0	5.0	30.0 a	1.0 ab	0	21.7 a	1.7 a	7.7 a	8.0 b
Water	25.8 a	19.8 a	5.8 a	27.0 a	1.3	4.0	30.7 a	1.7 a	1.3	6.2 b	1.0 ab	7.2 a	11.6 a
2 ppm	5.0 b	0.6 b	3.6 ab	12.2 b	0.7	1.0	5.7 b	0 b	3.0	7.2 b	0.4 b	3.6 ab	3.9 c
10 ppm	2.8 b	1.4 b	2.0 b	7.6 b	1.7	4.7	6.0 b	0 b	0.3	8.0 b	0 b	1.0 b	3.0 c
50 ppm	1.6 b	1.0 b	3.4 ab	4.2 b	0.7	0	4.7 b	0.7 b	2.3	7.0 b	0.2 b	4.4 ab	2.7 c
P-value	0.0001	0.0001	0.0019	0.0001	0.6709	0.0951	0.0102	0.0044	0.0966	0.0003	0.0201	0.0039	0.0001

[a] Each column represents a delivery from one orchard at the specific date.
[b] Mean of 5 × 100 fruit at each sampling time and orchard.
[c] Values denoted with different letters are different according to Student Newman Keuls method at P = 0.05.

packinghouses on 'Van' but not on 'Lapins'. *Cladosporium* rot increased after grading of 'Van' at the Lærdal packinghouse, but there was no difference for 'Lapins' (Table 2). For 'Van' in 2007, total fruit decay was higher in water-graded fruit from all six orchards in Lærdal, 5 of 10 in Hardanger, and 7 of 10 in Telemark. For 'Lapins' in 2007 similar numbers for water-graded fruit were 5 of 10 in Lærdal, 10 of 10 in Hardanger, and 2 of 10 in Telemark. In mean for the 2008 season, there was no difference in decay between graded and non-graded fruit if pooling all data (P = 0.7247) (Figure 2).

Grading time during the day (2008 season)

When analyzed per cultivar, the packinghouse or the orchard explained more of the differences than time of grading. In mean of all samples, time of grading was significant for 'Van' (P = 0.0401) but not for 'Lapins' (P = 0.7423). For 'Van' in Lærdal, the mean incidence of fruit decay was significantly lower on late-graded and non-graded fruit compared to fruit graded either early in the day or during mid-day (Table 3). In Hardanger, fruit graded during mid-day developed more decay than the other two grading times and non-graded fruit in two of five orchards. In Telemark, fruit from all grading times had significantly less decay than non-graded fruit, and there were no differences in time of grading among individual orchards. For 'Lapins' there was no difference in fruit decay among the different grading times in total fruit decay at either of the packinghouses (Table 3).

Differences in major diseases between samples

Independent of water-grading or no grading in 2007, Mucor rot was the most frequently observed disease in six 'Van' and 10 'Lapins' orchards in Lærdal, in six of 10 'Van' and all 10 'Lapins' orchards in Hardanger, and in all 10 'Van' orchards and in five of 10 'Lapins' orchards in Telemark. In four 'Van' orchards in Hardanger, grey mould was most prevalent, and in five 'Lapins' orchards in Telemark, blue mould was most prevalent in three and brown rot in two.

Effect of chlorine level in hydrocooler water

In mean of 12 orchard deliveries, fruit cooled in chlorinated water developed significantly less decay than if cooled in water without chlorine or in non-hydrocooled fruit. There was no difference in fruit decay among the different chlorine levels of the cooling water (Table 4). Mucor rot accounted for 76 to 93% of the decay in the different treatments. The other decays present in low amounts were anthracnose, blue mould, brown rot, Cladosporium rot and grey mould.

Samples from water and filter papers

Fungal pathogens observed by microscopy in water from grading lines were *Monilinia* spp., *Colletotrichum* sp., *Penicillium* sp., *Mucor* sp. and *Cladosporium* sp. Plating of water from grading lines on PDA resulted in growth (incidence of the plates with fungal growth in parentheses) of *Penicillium* sp. (100%), *Cladosporium* sp. (22%) and *Mucor* sp. (12%), and in addition several unidentified fungi and bacteria. Fruit decaying fungal pathogens grew on PDA from 57 and 17% of the water samples in 2007 and 2008, respectively. Fungal growth was found at all times water were sampled during the day (Table 5). Fruit decaying fungi developed from 87% of the filter papers placed on PDA. All locations in grading lines assessed, developed fungal growth. *Mucor* sp. and *Penicillium* sp. were found in 100 and 93% of the samples with fungal growth, respectively.

Table 5. Fungal growth[a] of *Cladosporium* sp. (C), M = *Mucor* sp. (M) and P = *Penicillium* sp. (P) on PDA of water samples obtained from chlorinated water (2 ppm chlorine); sampling took place at different times during the day when grading sweet cherry fruit of 'Van' and 'Lapins' at three packinghouses in Norway in 2007 and 2008.

Packinghouse	Cultivar	Time of day, pathogen[a]									Total
		Early			Mid			Late			
		C	M	P	C	M	P	C	M	P	
Year 2007											
Lærdal	Van	0	0	1	0	0	1	0	0	1	15 of 15
	Lapins	0	0	0.67	0	0	0.83	0	0.17	0.17	8 of 15
Hardanger	Van	0.2	0.4	0.6	0	0	0.2	0	0	0	4 of 15
	Lapins	0	0.3	0.5	0	0	0	0	0	0	4 of 18
Telemark	Van	0.3	0	1	0.33	0	1	0	0.17	1	17 of 18
	Lapins	0.4	0	0.2	0	0	0	0	0	0	2 of 15
Year 2008											
Lærdal	Van	0	0	0	0	0	0	0	0	0	0 of 18
	Lapins	0	0	0	0	0	0	0	0	0	0 of 18
Hardanger	Van	0	0	0.33	0.33	0	1	0	0	0.33	5 of 9
	Lapins	0	0	0	0	0	0	0	0	0	0 of 9
Telemark	Van	0	0	0	0	0	0	0	0	0	0 of 18
	Lapins	0.17	0	0.67	0	0	0	0	0	0	4 of 18

[a] Assessed as incidence of the plates (0–1) with growth of the different pathogens and total number of plates with growth.

Discussion

As far as we are aware, this is the first report of contamination of fruit decaying fungal pathogens in sweet cherry in grading lines with water of low chlorine levels (2 ppm). Even though viable inoculum was documented in the grading water and from different locations in the packing machine in the present studies, total fungal fruit decay in mean of all assessments in two seasons was not different if fruit were water-graded or not (Figure 1). However, in one of the two years (2007), which was a very wet season with frequent rains, there was a higher incidence of decay in water-graded fruit (Figure 2). Although water in the grading lines was not changed during the day, time of the day for grading did not seem to have a clear influence on decay. It was documented that slightly chlorinated water in the grading lines as well as other locations in the lines may contain viable spores of *Penicillium* sp., *Mucor* sp. and *Cladosporium* sp. (Table 5). However, increasing the chlorine content in a hydro-cooler to up to 50 ppm did not reduce fruit decay compared to 2 ppm (Table 4). In total of all assessments both years, Mucor rot was the most commonly found cause of postharvest fruit decay, followed by grey mould, blue mould, brown rot and Cladosporium rot (Figure 1).

Preharvest decay of *M. piriformis* has been reported before in some orchards and years in Norway (Børve and Stensvand, 2003). There was an increase in incidence of Mucor rot after grading in water compared to non-grading in 36 of 56 orchards in 2007, and thus the present investigation clearly indicates that fruit may be contaminated in the grading line. Inoculum of *M. piriformis* may have come from infested fruit, from soil on delivery boxes or spores released in water from apparently healthy fruit. The packinghouses in the present investigation had identified some wasp-damage on the fruit during grading in 2007. *Mucor* spp. is primarily associated with wounds on stone fruits, and insects can disperse *Mucor* spp. from fruit on the orchard floor or from weeds to fruit in the trees (Michailides and Spotts, 1990).

Supplying the water with 2 ppm chlorine in the hydro-cooler significantly reduced the amount of decay after storage compared to water-grading without chlorine, but there was no further reduction in fruit decay by adding more chlorine (Table 4). In postharvest handling of tree fruits, *M. piriformis* was quite sensitive to chlorine dioxide and more so than *B. cinerea* and *Penicillium expansum* (Roberts and Reymond, 1994). If exposed to up to 100 ppm chlorine for two minutes, *B. cinerea* survived and developed mycelia on artificial media (PDA) (Ferreira et al., 1996), while spores of *M. piriformis* did not germinate after being exposed to 50 ppm chlorine (Spotts and Peters, 1980). In seasons with high incidence of Mucor rot and if low levels of chlorine are used in the grading water, careful handling of fruit from harvesting to packing will be very important to avoid wounds and thus avoid creating infection sites for *Mucor* sp. on the fruit.

Grey mould was the second most common disease in the present investigation (Figure 1). In mean of all assessments, there was no difference in incidence of grey mould if the sweet cherry fruit were water-graded or not. However, at the packinghouse with the highest incidence of *Botrytis* sp. (Hardanger), there was an increase in grey mould after water-grading in fruit from three of 10 orchards. Brown rot and grey mould are the most common fungal diseases found preharvest on sweet cherry in Norway (Børve and Stensvand, 2003), as well as in many other countries. If present in the current experiments, brown rot was less frequent after water-grading than in non-graded fruit both years. Previously, it was reported that brown rot may be controlled by relatively low chlorine levels, such as 3 to 5 ppm (Phillips and Grendahl, 1973). If exposed to 50 ppm chlorine dioxide in water, spores of *M. laxa* were effectively killed, but it did not control the growth of the fungus on the surface of plum and nectarine fruits (Mari et al., 1999).

Although present at a low level, Cladosporium rot in water-graded fruit increased slightly during the packing day (Table 3), and in mean of all observations Cladosporium rot was higher on water-graded than non-graded fruit (Figure 1). *Cladosporium* sp. was frequently observed on fruit with different wounds, e.g., small cracks at harvest. Avoid-

ance of such wounds on the fruit and careful sorting at harvest may thus reduce the risk of *Cladosporium* sp. entering the packinghouse. In a sweet cherry grading line in the state of Washington, USA, water with about 34 ppm chlorine contained viable spores of *Penicillium* spp. (Sanderson, 2003), however, in the present investigation incidence of blue mould decreased on water-graded compared to non-graded fruit. Blue mould was mainly found on fruit with the highest ripening degree, colour 7 (Planton, 1995). Although not assessed in the grading water, *Penicillium* spp., *Cladosporium* spp., *Rhizopus* spp., and *Mucor* spp. were the most prevalent fungal pathogens in stone fruit packinghouses in Spain (Bernat et al., 2017).

The differences between orchards was high in both years (Tables 3 and 4), and, e.g., at the Lærdal packinghouse the amount of fungal decay after storage differed from 4 to 91% on fruit harvested at the same day in 2008, the season with the lowest disease pressure (Table 3). Consequently, the packinghouses should demand improved orchard management and control regimes by the growers in order to improve and equalize the storage potential of the fruit from the different growers.

Conclusions

The results clearly indicate that there is a risk of contaminating sweet cherry fruit with fungal pathogens in grading lines in a season with high levels of fruit decay. In a season with weather less conducive to fruit decay, a slight chlorination of water was satisfactory to reduce the risk to an acceptable level. According to these results, packinghouses in drier regions with a climate less suitable for fruit decaying fungi, may consider reducing the chlorine level in the grading water.

Acknowledgments

Innovation Norway, Lærdal Grønt, Telefrukt AS and Hardanger Fjordfrukt BA supported this work financially. The authors thank technicians at NIBIO Ullensvang for invaluable help with the experiments.

References

Bahar, A., and Dundar, O. (2001). The effects of hydrocooling and modified atmosphere packaging system on storage period and quality criteria of sweet cherry cv. Aksenir Napolyonu. Acta Hortic. *553,* 615–616. https://doi.org/10.17660/ActaHortic.2001.553.147.

Bernat, M., Segarra, J., Casals, C., Torres, R., Teixido, N., and Usall, J. (2017). Identification of fungal populations in the environment and on surfaces of stone fruit packinghouses. Europ. J. Plant Pathol. *148*, 723–731. https://doi.org/10.1007/s10658-016-1120-6.

Børve, J., and Stensvand, A. (2003). Use of a plastic rain shield reduces fruit decay and need for fungicides in sweet cherry. Plant Dis. *87*, 523–528. https://doi.org/10.1094/PDIS.2003.87.5.523.

Børve, J., Meland, M., and Stensvand, A. (2007). The effect of combining rain protective covering and fungicide sprays against fruit decay in sweet cherry. Crop Prot. *26*, 1226–1233. https://doi.org/10.1016/j.cropro.2006.10.020.

Ferreira, M.D., Bartz, J.A., Sargent, S.A., and Brecht, J.K. (1996). An assessment of the decay hazard associated with hydrocooling of strawberries. Plant Dis. *80*, 1117–1122. https://doi.org/10.1094/PD-80-1117.

Mari, M., Cembali, T., Baralidi, E., and Casalini, L. (1999). Peracetic acid and chlorine dioxide for postharvest control of *Monilinia laxa* in stone fruit. Plant Dis. *83*, 773–776. https://doi.org/10.1094/PDIS.1999.83.8.773.

Michailides, T., and Spotts, R. (1990). Postharvest diseases of pome and stone fruits caused by *Mucor piriformis* in the Pacific Northwest and California. Plant Dis. *74*, 537–543. https://doi.org/10.1094/PD-74-0537.

Padilla-Zakour, O.I., Ruona, I., Cooley, H.J., Robinson, T.L., Osborne, J., and Freer, J. (2007). Shelf-life extension of sweet cherry by field management, post-harvest treatments and modified atmosphere packaging. New York Fruit Quarterly *15*(2), 3–6.

Palm, G., and Kruse, P. (2008). Einfluss der Überdachung von Süsskirschen auf das Aufplatzen der Fruchte und die Fruchtfäulnis. Mitt. des Obstbau Versuchringes *63*, 154–157.

Phillips, D.J., and Grendahl, J. (1973). The effect of chlorinating hydrocooling water on *Monilinia fructicola* conidia and brown rot. Plant Dis. Rep. *57*, 814–816.

Planton, G. (1995). Cerise: un code coleur pour améliorer la qualité à la récolte. [Cherries: a color code to improve crop quality.] Centre Techn. Interprofess. des Fruits et Legumes France. Infos No. *112*, 38–41.

Ritenour, M.A., Sargent, S.A., and Bartz, J.A. (2014). Chlorine use in produce packing lines. http://edis.ifas.ufl.edu/ch160. (accessed January 22, 2018).

Roberts, R.G., and Reymond, S.T. (1994). Chlorine dioxide for reduction of postharvest pathogen inoculum during handling of tree fruits. Appl. Environ. Microbiol. *60*, 2864–2868.

Rosenberger, D.A., Wicklow, D.T., Korjagin, V.A., and Roninarion, S.M. (1991). Pathogenicity and benzimidazole resistance in *Penicillium* species recovered from flotation tanks in apple packinghouses. Plant Dis. *75*, 712–715. https://doi.org/10.1094/PD-75-0712.

Sanderson, P.G. (2003). *Aureobasidium* rot in sweet cherry. http://jenny.tfrec.wsu.edu/wtfrc/PDFfinalReports/2004FinalReports/Cherry/SandersonAureobasidium.pdf. (accessed January 22, 2018).

Sholberg, P.L., and Haag, P.D. (1996). Incidence of postharvest pathogens of stored apples in British Colombia. Can. J. Plant Pathol. *18*, 81–85. https://doi.org/10.1080/07060669609500661.

Spotts, R.A., and Peters, B.B. (1980). Chlorine and chlorine dioxide for control of d'Anjou pear decay. Plant Dis. *64*, 1095–1097. https://doi.org/10.1094/PD-64-1095.

Spotts, R.A., and Cervantes, L. (1986). Populations, pathogenicity and Benomyl resistance of *Botrytis* spp., *Penicillium* spp. and *Mucor piriformis* in packinghouses. Plant Dis. *70*, 106–108. https://doi.org/10.1094/PD-70-106.

Thomidis, T., and Exadaktylou, E. (2013). Effect of a plastic rain shield on fruit cracking and cherry diseases in Greek orchards. Crop Prot. *52*, 125–129. https://doi.org/10.1016/j.cropro.2013.05.022.

Willett, M., Kupferman, E., Roberts, R., Spotts, R., Sugar, D., Apel, G., Ewart, H.W., and Bryant, B. (1989). Postharvest diseases of cherries. Posth. Pom. Newsletter *7*(3), 12–14.

Received: Feb. 21, 2018
Accepted: Mar. 18, 2019

Addresses of authors:
Jorunn Børve[1,*] and Arne Stensvand[1,2]
[1] Norwegian Institute of Bioeconomy Research, P.O. Box 115, 1432 Ås, Norway
[2] Norwegian University of Life Sciences (NMBU), 1432 Ås, Norway
* Corresponding author; E-mail: jorunn.borve@nibio.no
Tel.: +47 95878057