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Characterization and Quantification of Postharvest Losses of Apple Fruit Stored under Commercial Conditions

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Abstract. **The objectives of this study were to characterize and quantify postharvest losses of apples under commercial conditions in Santa Catarina state, Brazil. Two experiments were conducted using 'Gala' and 'Fuji' apples. The first experiment was to characterize and quantify the most important causes of loss of fruit treated or not treated with 1-methylcyclopropene (1-MCP) then held in controlled atmosphere (CA) storage. This experiment was conducted in commercial storage facilities from 2007 to 2010. In each year, 10 samples of ≈380 kg each for 'Gala' and 400 kg each for 'Fuji' were collected from bins of commercially harvested fruit from each of 15 'Gala' and 17 'Fuji' orchards. Half of the samples from each orchard were treated with 1-MCP at harvest. Fruit were stored in CA, at 0.7 °C, for 150 to 300 days. After storage, one subsample of 100 disorder-free apples were selected from each sample and held at 22 °C for 7 days to simulate shelf-life conditions. The fruit were analyzed after CA storage and shelf life for the incidence of disorders. The second experiment was conducted in 2011 to identify the main fungi causing decay during storage. In this study, apples were stored in 10 commercial CA storage rooms at 0.7 °C for 180 to 240 days. After storage, fruit with decay symptoms were collected at the commercial sorting line. A total of 10 samples of 100 decayed apples were taken throughout the sorting period for each cultivar and storage room. The fungal decays were identified by visual symptoms on each fruit. Total apple losses during storage varied from 3.9% to 12.1% for 'Gala' and 6.6% to 8.4% for 'Fuji', depending on the year and 1-MCP treatment. During storage, deterioration caused by fungal decay was ≈60% and 80% of total losses for 'Gala' and 'Fuji', respectively. During shelf life, additional losses caused by fungal decay ranged from 8.4% to 17.6% for 'Gala' and 12.4% to 27.2% for 'Fuji', depending on the year. Senescent breakdown and superficial scald were the major physiological disorders. 1-MCP treatment had no effect on losses due to decay. Bull's-eye rot, blue mold, gray mold, and alternaria rot were the most prevalent fungal decay symptoms, accounting for 52%, 27%, 9% and 10% of 'Gala' losses and 42%, 25%, 18% and 5% of 'Fuji' losses, respectively. Sources of variability for losses among years and orchards is discussed.**

Although the efficiency of food production worldwide has increased over the years, studies estimate approximately one-third of all food and 45% to 55% of all fruit and vegetables produced worldwide are lost or wasted, corresponding to 1.2 to 2 billion tons of food loss every year (Porat et al., 2018). For apple fruit, estimates include 8.6% fresh apples lost at retail and 20% lost at the consumer level in the United States (Buzby et al., 2011). These estimates suggest a highly inefficient use of natural resources such as land, water, and energy for apple production (Buzby et al., 2011). Therefore, minimizing postharvest apple fruit losses is more sustainable than increasing production to compensate for these losses (Kader, 2005).

Factors contributing to loss and waste of fruit after harvest include development of fungal decay, physiological disorders, mechanical injuries, as well as deterioration of appearance, texture, and flavor that lead to consumer dissatisfaction (Kader, 2005).

Studies have shown that many fungal species can cause decay in apple fruit after harvest (Sutton et al., 2014), although only a few lead to fruit losses under commercial conditions (Sugar, 2002). The likelihood of fungal decay depends on apple genotype, pre- and postharvest management practices, and environmental conditions (Cameldi et al., 2016; Spotts et al., 2009; Sugar, 2002; Sutton et al., 2014). Postharvest fungal decay results mainly from preharvest latent infection or wound infection that can occur either before or after harvest (Wenneker and Thomma, 2020). Latent infections occur through the intact fruit tissue or natural skin openings such as lenticels, whereas wound infection occurs through wounds caused by insects, hail, physiological disorders, and mechanical damage (Prusky et al., 2013; Sugar, 2002). Some of the most important fungi causing postharvest apple decay, such as *Botrytis* spp. *Penicillium* spp., *Mucor* spp., and *Monilia* spp., infect the fruit through wounded tissues, whereas others such as *Neofabraea* spp., *Colletotrichum* spp., *Alternaria* spp., *Fusarium* spp., *Sphaeropsis pyriputrescens*, and *Phacidiopycnis washingtonensis* can infect the fruit by latent infections (Kim and Xiao, 2006, 2008; Sugar, 2002; Wenneker and Thomma, 2020). Identification of the most important fungi causing decay can help develop effective control approaches considering that each pathogen has a different response to environmental and crop management conditions (Sugar, 2002). Apple postharvest losses caused by fungi have been reported to range from 0.25% to 25%, depending on orchard and postharvest fungicide treatments (Kim and Xiao, 2008); 2% to 40%, depending on the storage duration and year (Neri et al., 2009; Neuwald and Kittemann, 2016); 0% to 70%, depending on harvest maturity, year, and growing region (Breeyen et al., 2020; Cameldi et al., 2016).

Apples can develop many physiological disorders after harvest that are expressed by skin and or flesh browning, blackening or cracking, mealy texture, corking, shriveling, and other symptoms. The incidence of physiological disorders in apples is highly regulated by the genotype and environmental conditions before and after harvest (Watkins and Mattheis, 2019). These disorders have been reported to potentially cause losses higher than 80% of total stored fruit (DeEll and Ehsani-Moghaddam, 2013; DeLong et al., 2004; Koushesh Saba and Watkins, 2020; Lee et al., 2016; Mattheis et al., 2017). Although studies have attempted to elucidate the factors and mechanisms regulating physiological disorders in apples, there is still limited knowledge to develop efficient prediction approaches as a means to reduce losses caused by these disorders (Watkins and Mattheis, 2019).

Sanitation and storage technologies have been greatly improved to reduce postharvest apple losses due to disorders (Adaskaveg et al., 2002; Bai et al., 2009; Watkins, 2008). These technologies have also been used to extend the duration apples are available at retail throughout the year, stimulating fruit consumption and production. However, longer storage periods potentially result in higher incidence of decay and physiological disorders (DeEll et al., 2007; DeLong et al., 2004; Neri et al., 2009). Although effective for slowing fruit ripening and preventing development of some physiological disorders, the use of CA storage with low pO_2 and high pCO_2 and the inhibition of ethylene responses by 1-methylcyclopropene (1-MCP) may not be efficient to prevent fungal decay as these technologies have no direct effect on fungi that cause decay (Adaskaveg et al., 2002; Sugar, 2002; Watkins, 2008).

1-MCP inhibits apple fruit ripening by competing with ethylene in the ethylene response pathway (Mattheis, 2008; Watkins, 2008). The impact 1-MCP has on the fungal decay process have been variable. Studies have shown that 1-MCP may decrease (Cameldi et al., 2016; DeEll and Ehsani-Moghaddam, 2013; Gago et al., 2015; Li et al., 2017; Saftner et al., 2003; Zhou et al., 2016) or increase (Janisiewicz et al., 2003; Leverentz et al., 2003) decay incidence in apples, whereas other studies have shown no effect on decay (DeEll et al., 2007; DeLong et al., 2004; Errampalli et al., 2012). Similarly, 1-MCP can inhibit physiological disorders such as superficial scald, senescent breakdown, and bitter pit while exacerbating others such as carbon dioxide injury, leather blotch, and diffuse skin browning (DeEll et al., 2003; Mattheis, 2008; Watkins, 2008; Watkins and Mattheis, 2019). Considering that 1-MCP is routinely applied by apple storage operators worldwide, further investigation is important to develop information regarding its effect on fungal decay and physiological disorders, as well as postharvest apple losses under commercial conditions.

Although numerous studies conducted under controlled laboratory conditions have shown the potential amount of apple losses during and after storage, studies carried out to characterize and quantify apple losses under large-scale commercial storage conditions are lacking. These studies can help guide future research to control losses and ultimately improve efficiency of the apple fruit industry. The objectives of this study were to characterize and quantify sources of postharvest losses of apples stored under commercial conditions with and without the use of 1-MCP.

Material and Methods

Two experiments were conducted using 'Gala' and 'Fuji' apples grown in Santa Catarina State, Brazil. The first experiment was performed from 2007 through 2010 to characterize and quantify apple losses during long-term CA storage and shelf life periods. The second experiment was conducted in 2011 to identify the main pathogenic fungi responsible for apple fruit decay.

Expt. 1 Characterization and quantification of apple losses

Fruit samples. Twenty samples of apples were collected from harvested bins from each of 15 'Gala' and 17 'Fuji' orchards (grower lots) within 24 h after harvest each year from 2007 to 2010. These orchards represent apple-producing areas in southern Brazil. Commercial harvest was accomplished during a period of 15 d within the commercial harvest window of fruit intended for long-term storage. Fruit were not treated with fungicide or sanitizers after harvest. Each sample had ≈380 kg (≈3140 fruit) of 'Gala' or ≈400 kg (≈2963 fruit) of 'Fuji'. One subsample of 10 apples from each bin (200 apples per cultivar and orchard) was assessed for fruit firmness at harvest. The remaining fruit from each sample (≈3130 'Gala' or 2953 'Fuji') were assessed 24 h after long-term CA storage. One subsample of 100 apples without visual symptoms of fungal decay or physiological disorders with medium size representative of each bin was taken from each bin 24 h after long-term CA storage for assessment after 7-d shelf-life simulation. Therefore, 2560 bin-samples (4 years × 32 orchards per year × 20 bin-samples per orchard) were analyzed after CA storage and 2560 samples with 100 apples were analyzed after exposing the fruit to shelf life conditions.

1-MCP treatment. For each year, orchard and cultivar, half of the 20 bin-samples were exposed to 1-MCP for 24 h, within 60 h after harvest, in commercial storage rooms following commercial recommendation for the SmartFresh System (AgroFresh Inc., Spring House, PA). The amount of SmartFresh used was proportional to each storage room volume, targeting the label rate in Brazil for apples between 25 and 50 $\mu mol \cdot m^{-3}$. The untreated control fruit were held for 24 h in a separate storage room filled or partially loaded with apple fruit. Temperature in storage rooms for controls and 1-MCP treatments were identical. After the 1-MCP treatment, each room was opened and vented for at least 2 h. Then the control fruit were moved to the same room containing the 1-MCP treated fruit.

Fruit storage and shelf-life conditions. Fruit cooling was initiated within 24 h after harvest and the fruit temperature reached ≈4 °C within 36 h and 0.5 °C to 0.8 °C within 96 h after harvest. Fruit from each orchard were held in separate commercial CA rooms at 0.5 °C to 0.8 °C, for 150 to 270 d for 'Gala' and 170 to 300 d for 'Fuji'. Therefore, a total of 32 commercial storage rooms were used each year, and each storage room held fruit from one orchard. Each storage room was loaded with the 20 samples for this study plus 500 to 800 tons of apples for marketing. Concentration of O_2 was maintained at 1.5 ± 0.2 kPa for both cultivars, whereas CO_2 concentration was 2.5 ± 0.2 kPa for 'Gala' and <0.5 kPa for 'Fuji'. pO_2 was reduced to 4kPa by N_2 injection in ≈20 h and to 1.5 kPa by fruit respiration in 4 to 5 d for both cultivars. The injection of N_2 in 'Gala' storage rooms was within 12 h after 1-MCP treatment. Establishment of CA was delayed 3 weeks for 'Fuji' to avoid CO_2 injury. After CA storage, fruit were held in air (≈1 °C) for 24 h before analyses. For shelf-life simulation, fruit were held 7 d in a 100 m^3 room at 22 ± 1 °C.

Assessment of fruit losses during storage and shelf life. After CA storage, all apples from each bin-sample were manually segregated by presence or absence of any visual disorder. Disorders were identified as fungal decay or physiological disorders such as shrivel, superficial scald, low calcium disorders (bitter pit and blotch pit), or senescent breakdown (skin cracking and or skin discoloration). Only the most prominent disorder was recorded for each fruit. For instance, if superficial scald and fungal decay were both present, the disorder affecting the largest amount of skin area was recorded. This approach allowed calculating the total amount (%) of apple loss by adding the percentage of fruit affected by each disorder. The percentage of apples affected by each disorder in each bin-sample was calculated by multiplying the total weight of apples with a disorder by 100 and dividing by the total fruit weight of each respective bin-sample. Severely decayed fruit were replaced by a healthy fruit with similar size for weighing.

After shelf life, apples were visually assessed for external disorders as described earlier as well as for flesh firmness and internal disorders including flesh browning (diffuse light browning in the cortex), CO_2 injury (well-defined, dark, brown areas in the flesh), core browning and moldy core rot. After shelf life, all disorders present were recorded for each fruit. This approach does not allow calculating the total amount (%) of apple loss by adding the percentage of fruit affected by each disorder. The percentage of apples with each disorder was calculated by multiplying the number of fruit for each disorder by 100 and dividing by the total number of fruit in each sample (100).

Flesh firmness was determined on a peeled equatorial region, between exposed and shaded sides, using a penetrometer with a standard Effegi 11-mm diameter probe mounted on a motorized drive (Güss, Strand, South Africa). The physiological disorders superficial scald, flesh browning, bitter pit, blotch pit, core browning, and CO_2 injury were identified by visual symptoms as previously described (Pierson et al., 1971; Watkins and Mattheis, 2019).

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Expt. 2: Identification of apple fruit decay symptoms

'Gala' and 'Fuji' apples harvested in 2011 were stored in commercial CA conditions as described for Expt. 1. Fruit from 10 commercial storage rooms per cultivar were collected after 180 to 240 d in storage. In each storage room, 10 samples of 100 apples with visible decay symptoms were randomly picked at the pre–size sorting line throughout the sorting period for the whole room. Apples were then grouped by the most prominent decay-causing pathogen, identified by the visual symptoms, following previous descriptions (Pierson et al., 1971; Snowdon, 1990; Sutton et al., 2014). Pathogen incidence was calculated by multiplying the number of fruit with each pathogen symptom by 100 and dividing by the total number of fruit per sample (100).

Weather data

Temperature, relative humidity, and rainfall data were obtained from a weather station of Santa Catarina Agricultural Research and Extension Corporation located in the midwest of Santa Catarina State. Temperature (°C) and relative humidity (%) were measured hourly using a chart recorder thermo-hygrograph (Wilh. Lambrecht, Einbeck, Germany). Temperatures were also recorded daily at 9:00 AM, 3:00 PM, and 9:00 PM using a mercury thermometer (Incoterm, Porto Alegre, RS, Brazil). The rainfall (millimeters) was recorded daily at 9:00 AM, 3:00 PM, and 9:00 PM using Ville de Paris rain gauge (Hidromec, RJ, Brazil). Accumulated rainfall was obtained by adding daily rainfall for the fruit growing period (from full bloom to the harvest window) and for the 2 months before the harvest (including the harvest window) of each year and cultivar. Daily mean temperatures were used to obtain the mean temperature for each fruit production year. The number of hours below 7.2 °C was computed for the winter period (from June to September) of each preceding harvest year.

Statistical analysis

Expt. 1 was conducted as a 4 × 2 factorial (production year × 1-MCP treatment) for each cultivar. Expt. 2 was conducted as a 2 × 8 factorial (cultivar × pathogen). Both experiments were carried out in randomized complete block design with 10 bin-samples or ten 100-fruit samples per replication. Data without normal distribution were transformed by arc.sin$\sqrt{Y/100}$ before analysis of variance using SAS software (SAS Institute Inc., Cary, NC). Mean values were compared using Tukey's test ($P < 0.05$). The variability of apple losses among orchards (storage rooms) was described by percentiles of orchards in classes (%) of apple loss presented as boxplots. Correlation analysis of apple losses and flesh firmness after shelf life was accomplished using Pearson product–moment.

Results

Losses during CA storage

There was no interaction between production year and 1-MCP treatment on disorder incidence after CA storage for either cultivar (Tables 1 and 2). Production year had a significant effect on decay incidence, senescent breakdown, shrivel, and total losses for 'Gala' and on the incidence of bitter pit and blotch pit for 'Fuji'.

The highest total loss of 'Gala' was observed in 2007, reaching 12.1%, whereas losses between 3.9% to 5.6% were observed in the other years. In 3 of 4 years, the incidence of decay was the major cause of 'Gala' loses, representing 56% to 81% of total loses followed by senescent breakdown, shrivel and low Ca disorders. In 2008, the incidence of decay was low, and senescent breakdown was the major cause of 'Gala' loss. Treatment with 1-MCP did not affect 'Gala' losses during CA storage. Apple losses due to shrivel and low Ca disorders were below 1% for both cultivars.

The total 'Fuji' loss was similar in all 4 years and varied from 8.4% in controls to 6.6% in 1-MCP-treated fruit. This reduction of 'Fuji' losses for 1-MCP-treated fruit was associated with reduced superficial scald incidence. In all 4 years, the incidence of decay was the major cause of 'Fuji' losses, representing 75% to 85% of total losses, followed by superficial scald, shrivel, and low Ca disorders.

Apple losses during and after CA storage varied among orchards within each year in both cultivars (Fig. 1). The variability among orchards was higher in 2007 than 2008–10 for 'Gala' and was slightly higher in 2007 and 2009 compared with 2008 and 2010 for 'Fuji'. 'Gala' total loss in 2007 ranged from 6.1% to 18% for 50% of the orchards, and between 2.2% and 26.5% for 90% of the orchards, whereas in 2009, total 'Gala' losses ranged from 1.6% to 5.3% for 50% of the orchards, and from 1.2% to 7.1% for 90% of the orchards (Fig. 1A). In 2007 the total losses of 'Fuji' ranged from 4.1% to 10.3% for 50% of the orchards, and between 2.4% and 15% for 90% of the orchards, while in 2009, the total losses ranged from 5.2% to 9.3% for 50% of the orchards, and between 4.7% and 11.6% for 90% of the orchards (Fig. 1B).

Losses during shelf life

After the shelf-life period, there was no interaction between production year and 1-MCP treatment for either cultivar (Tables 3 and 4). Decay was the major cause of losses during shelf life. Treatment with 1-MCP did not affect development of decay in either cultivar but reduced the second major cause of loss for each cultivar with 'Gala' flesh browning and 'Fuji' superficial scald.

The highest incidence of decay in 'Gala' apples was observed in 2007 and 2010 with losses of 17.6% and 17.1%, whereas the lowest incidence was in 2008 and 2009 with losses of 8.4% and 9.4%, respectively. The incidence of 'Fuji' decay was highest (27.2%) in 2010 and ranged from 12.4% to 17.5% in the other years.

The incidence of flesh browning in 'Gala' was highest in 2007 and 2010 and lowest in 2009, ranging from 4.8% to 15% depending on year and 1-MCP treatment. Shrivel in 'Gala' apples was unaffected by production year or 1-MCP treatment, ranging from 0.0% to 1.7%. Low-Ca disorder (bitter pit and blotch pit) incidence was highest in 2009, reaching 0.5%, and not detected in 2007.

Superficial scald incidence for 'Fuji' was similar in all years and was reduced by 1-MCP treatment from 12.7% in nontreated fruit to 0.7% in treated fruit. Core rot incidence was unaffected by production year or 1-MCP treatment, ranging from 1.6% to 3.0%. Shrivel and core browning incidence in 'Fuji' was highest in 2010 and was reduced by 1-MCP treatment. The incidence of Ca-related disorders was highest in 2010, reaching 1.4% and was unaffected by 1-MCP treatment, whereas

Table 1. Incidence (%) of fungal decay and physiological disorders on 'Gala' apple fruit after controlled atmosphere storage. Fruit were assessed 24 h after removal from storage.

	Fungal decay			Senescent breakdown			Shrivel			Ca-deficiency disorders		Total		
Yr	Control	1-MCP	Avg	Control	1-MCP	Avg	Control	1-MCP	Avg	Control	1-MCP	Control	1-MCP	Avg
2007	7.4	6.1	6.8 a	4.8	3.4	4.1 a	1.12	0.55	0.83 a	0.35	0.36	13.7	10.5	12.1 a
2008	1.4	1.7	1.6 b	4.1	3.3	3.7 a	0.07	0.01	0.04 b	0.22	0.38	5.8	5.4	5.6 b
2009	3.2	3.2	3.2 b	0.3	0.3	0.3 b	0.10	0.03	0.06 b	0.40	0.25	4.0	3.9	3.9 b
2010	3.2	3.0	3.1 b	1.0	0.6	0.8 b	0.02	0.01	0.02 b	0.25	0.18	4.5	3.8	4.2 b
Average	3.8	3.5		2.6	1.8		0.33	0.15		0.30	0.29	7.1	6.0	
Year	***			***			**			NS		***		
Treatment	NS			NS			NS			NS		NS		
Year × treatment	NS			NS			NS			NS		NS		

Data are mean of 15 orchards each held in separate storage rooms each year. There were ten ≈380-kg samples for each treatment [control, 1-methylcyclopropene (1-MCP)] for each orchard. The total number of fruit for each treatment in each year was ≈469,500.

Average followed by same letter in each column are not different by Tukey test ($P < 0.05$).

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

Table 2. Incidence (%) of fungal decay and physiological disorders on 'Fuji' apple fruit after controlled atmosphere storage. Fruit were assessed 24 h after removal from storage.

	Fungal decay		Superficial Scald		Shrivel		Ca-deficiency disorders			Total	
Yr	Control	1-MCP	Control	1-MCP	Control	1-MCP	Control	1-MCP	Avg	Control	1-MCP
2007	7.2	6.2	1.25	0.02	0.5	0.4	0.68	0.37	0.53 b	9.7	7.0
2008	5.2	5.3	0.92	0.03	0.6	0.4	0.49	0.41	0.45 b	7.3	6.2
2009	6.8	6.0	0.15	0.02	0.2	0.2	0.88	1.17	1.02 a	8.0	7.4
2010	6.0	5.0	1.71	0.23	0.4	0.2	0.38	0.33	0.35 b	8.4	5.8
Average	6.3	5.6	1.01	0.07	0.4	0.3	0.61	0.57		8.4	6.6
Year	NS		NS		NS		***			NS	
Treatment	NS		***		NS		NS			**	
Year × treatment	NS		NS		NS		NS			NS	

Data are mean of 17 orchards each held in separate storage rooms each year. There were ten ≈400-kg samples for each treatment [control, 1-methylcyclopropene (1-MCP)] for each orchard. The total number of fruit for each treatment in each year was ≈502,010.
Average followed by same letter in each column are not different by Tukey test ($P < 0.05$).
NS, *, **, ***Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

Table 3. Incidence (%) of fungal decay and physiological disorders on 'Gala' apple fruit after 7 d at 22 °C following controlled atmosphere storage.

	Fungal decay			Ca-deficiency disorders			Shrivel		Flesh browning		
Yr	Control	1-MCP	Avg	Control	1-MCP	Avg	Control	1-MCP	Control	1-MCP	Avg
2007	19.0	16.3	17.6 a	0.00	0.00	0.00 b	0.20	0.02	14.2	12.3	13.3 a
2008	8.1	8.7	8.4 b	0.10	0.20	0.15 ab	0.00	0.12	8.6	8.0	8.3 ab
2009	10.2	8.5	9.4 b	0.60	0.40	0.50 a	0.36	0.07	8.2	4.8	6.5 b
2010	18.6	15.7	17.1 a	0.40	0.50	0.44 ab	1.72	1.04	15.0	11.5	13.3 a
Average	14.0	12.3		0.28	0.28		0.57	0.31	11.5	9.2	
Year	***			*			NS		***		
Treatment	NS			NS			NS		*		
Year × treatment	NS			NS			NS		NS		

Data are mean of 15 orchards each held in separate storage rooms each year. There were ten 100-fruit samples for each treatment [control, 1-methylcyclopropene (1-MCP)] for each orchard. The total number of fruit for each treatment in each year was 15,000.
Average followed by same letter in each column are not different by Tukey test ($P < 0.05$).
NS, *, **, ***Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

CO_2 injury incidence was highest in 2007 and was enhanced by 1-MCP treatment.

The relative loss (%) of apple fruit by decay and physiological disorders increased during the shelf-life period compared with during CA storage for both cultivars, regardless of 1-MCP treatment.

In addition to the observed higher incidence of decay after shelf life, the variability among orchards due to decay was greater after shelf life (Fig. 1E and F) than after CA storage (Fig. 1C and D). In 2007, 'Gala' losses due to decay during shelf life varied from 6.3% to 24% for 50% of the orchards, and between 2.8% and 39.7% for 90% of the orchards, whereas in 2009, 'Gala' losses due to decay during shelf life varied from 1.6% to 5.3% for 50% of the orchards, and between 0.6% and 30.5% for 90% of the orchards (Fig. 1E). In 2010, 'Fuji' losses due to decay varied from 16% to 35.6% for 50% of the orchards, and between 15% and 46.4% for 90% of the orchards, whereas in 2009, 'Fuji' losses due to fungal decay varied from 6.6% to 16.8% for 50% of the orchards and between 2.6% and 22.2% for 90% of the orchards (Fig. 1F).

Flesh firmness

Flesh firmness of 'Gala' at harvest and after storage was lowest in 2007 and was equal in fruit harvested in 2008, 2009 and 2010 (Table 5). Flesh firmness of 'Fuji' at harvest and after storage was lowest in 2010 and was equal in fruit harvested in 2007, 2008 and 2009. Fruit treated with 1-MCP had higher flesh firmness for both cultivars after CA storage plus shelf life compared with untreated fruit.

A significant negative correlation existed between flesh firmness and the incidence of flesh browning in 'Gala', regardless of year, and between flesh firmness and fungal decay in 3 of 4 years for 'Gala' and in 2 of 4 years for 'Fuji' (Table 6). Flesh firmness after storage was positively correlated with incidence of superficial scald in 1 of 4 years.

Weather data

Accumulated rainfall was highest from bloom to harvest of 'Fuji' of 2010 (Table 7). During the last 2 months of fruit growth, the rainfall was 53% to 84% higher in 2010 than in the other 3 harvest years for 'Fuji' and 47% to 33% higher in 2010 than in 2007 and 2008 for 'Gala'. The relative humidity was slightly lower (2% to 6%) from bloom to harvest of 2007 and 2008 in comparison with the same period of 2010 harvest. The relative humidity during last 2 months of 'Fuji' fruit growth was 7% lower in 2009 than in 2010. The mean temperature from bloom to harvest was 1.2 to 1.6 °C higher in 2007 and 2010 than in the 2008 and 2009. The number of hours with temperatures below 7.2 °C in the winters preceding growing seasons in 2008, 2009, and 2010 were similarly higher (≈20%) than in the winter preceding the growing season in 2007.

Fungi associated with decay symptoms in apples

There was a significant interaction between cultivar and symptom-based identification of pathogens (Table 8). Major diseases in order of decreasing prevalence were bull's-eye rot (*Neofabraea* sp.), blue mold (*Penicillium* sp.), gray mold (*Botrytis* sp.), alternaria rot (*Alternaria* sp.), core rot (*Alternaria* sp., *Fusarium* sp.), bitter rot (*Glomerella cingulata*), white rot (*Botryosphaeria* sp.), and rhizopus rot (*Rhizopus* sp.). The incidence of gray mold and moldy core rot were higher in 'Fuji' compared with 'Gala'.

Discussion

Postharvest losses of 'Gala' and 'Fuji' apples during storage and shelf life

Food losses have been estimated to reach ≈ 3%, 1%, and 12%, during storage, packing, and retail, respectively (Porat et al., 2018). However, the current study shows that apple losses can be higher at these steps along a simulated supply chain in Brazil. The apple losses ranged from 18.3% to 26.6%, considering the 4-year mean for all physiological disorders and fungal decay during CA storage, plus the fungal decay during shelf life. In Brazil, ≈1 million cubic tons of 'Gala' and 'Fuji' apples are harvested annually, and

≈50% of this production is stored for periods longer than 150 d. On the basis of the results of this study, ≈91,500 to 133,000 cubic tons of long-term storage apples could be lost annually during storage and shelf life. The results highlight the potential economic impact of postharvest apple fruit losses.

Few estimates of postharvest apple losses in large-scale trials under commercial condition have been published. A study conducted in Australia showed average losses during storage of 7.5% due to postharvest injuries and 6.2% from fungal decay, although the magnitude of the loss varied with apple variety, orchard, time in storage and handling method (Holmes, 1990). In Washington State (USA), the average apple losses by decay during storage was estimated at 1.9% and 5.1% for fruit treated and untreated with postharvest fungicide, respectively (Kim and Xiao, 2008). A survey of refrigerated stores with 'Cox's Orange Pippin' apples in England during four seasons showed that about 6% of the stored crop was lost during storage and about half of the loss was due to fungal rots (Preece, 1967). On the other hand, many studies conducted under controlled laboratory conditions have shown potential losses of 0% to 20% (Colgan and Johnson, 1998; DeEll and Ehsani-Moghaddam, 2013; Gago et al., 2015; Neuwald and Kittemann, 2016) and 0% to more than 50% (Breeyen et al., 2020; Cameldi et al., 2016; Neri et al., 2009) due to fungal decay, and from 0% to more than 50% by physiological disorders such as superficial scald (DeEll and Ehsani-Moghaddam, 2013; DeLong et al., 2004), bitter pit (Gago et al., 2015; Mattheis et al., 2017), and senescence disorders (Lee et al., 2016), depending on pre- and postharvest experimental treatment.

Although in our study retail conditions were simulated by exposing apples to 22 °C for 7 d after CA storage, a previous study conducted under commercial conditions showed 'Gala' and 'Fuji' apples have an average shelf-life period of 17 and 28 d, respectively (Argenta et al., 2015). According to this study, after CA storage, apples are held in cold rooms at packing houses and distribution centers, as well as in trucks during transportation, and eventually in market displays (Argenta et al., 2015). Additionally, apples at retail in Brazil are displayed at a wide range of temperatures (Argenta et al., 2015). Therefore, the incidence of apple losses in our study could be an underestimate, considering the possible combinations of time and temperature that apples are exposed to after long-term storage under commercial conditions. Increasing incidence of decay (Neri et al., 2009) and physiological disorders (Koushesh Saba and Watkins, 2020; Lee et al., 2016) during shelf life after cold storage is presumably due to increased temperature and advanced fruit ripening. Relatively warm temperature during shelf life (15 to 25 °C) following cold storage favor fungal growth (Adaskaveg et al., 2002) and enhance ethylene

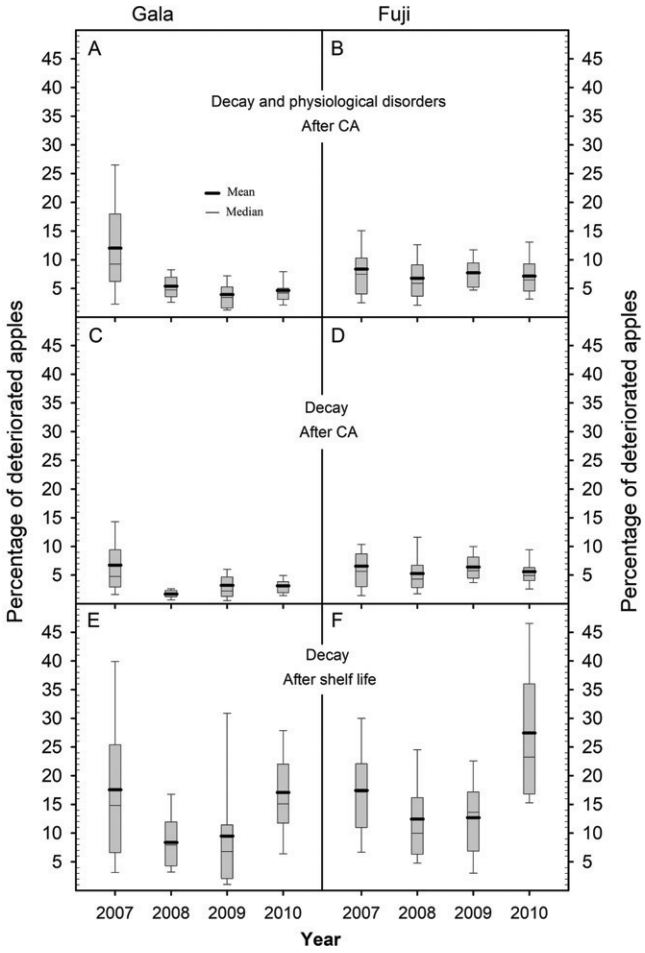

Fig. 1. Variability of deteriorated apple incidence (%) among orchards (storage rooms) for each cultivar and year. Fruit were assessed for fungal decay and physiological disorders after removal from controlled atmosphere (CA) storage rooms (**A–D**) and after 7 additional days of shelf life at 22 °C (**E, F**). Fruit loss by fungal decay plus physiological disorders during CA storage (**A, B**), for fungal decay during CA storage (**C, D**) and for fungal decay after 7 d of shelf life at 22 °C following CA storage (**E, F**). For 50% of orchards (storage rooms) the incidence (%) of deteriorated apples is in the range of vertical box (25th and 75th percentile of orchards). For 90% of orchards, the incidence of deteriorated apples is between the down and upper boundary of error bars (10th and 90th percentiles). The thick and thin cross line in the box are the means and medians (%) of deteriorated apples, respectively. Data of treated and untreated fruit with 1-MCP were averaged for these analyses.

Table 4. Incidence (%) of fungal decay and physiological disorders on 'Fuji' apple fruit after 7 d at 22 °C following controlled atmosphere storage.

	Fungal decay			Superficial scald		Core rot		Shrivel			Ca deficiency disorders			Core Browning			CO_2 Injury		
Yr	C	M	Avg	C	M	C	M	C	M	Avg	C	M	Avg	C	M	Avg	C	M	Avg
2007	16.1	18.8	17.5 b	9.3	0.6	1.7	1.9	0.6	0.3	0.4 b	0.0	0.0	0.0 b	0.4	0.2	0.3 b	1.9	3.0	2.4 a
2008	10.7	14.1	12.4 b	15.8	0.8	1.8	1.8	0.4	0.2	0.3 b	0.3	0.4	0.3 b	1.0	0.3	0.7 b	0.5	1.6	1.1 b
2009	12.0	13.5	12.7 b	8.4	0.5	1.6	1.8	0.3	0.2	0.2 b	0.6	0.3	0.4 b	3.6	0.3	2.0 b	0.4	0.3	0.4 b
2010	27.3	27.1	27.2 a	17.4	0.9	2.2	3.0	3.8	1.3	2.6 a	1.6	1.1	1.4 a	8.6	4.9	6.7 a	0.2	1.1	0.6 b
Average	16.5	18.4		12.7	0.7	1.8	2.1	1.3	0.5		0.6	0.4		3.4	1.4		0.8	1.5	
Year	***			NS		NS		***			***			***			***		
Treatment	NS			***		NS		*			NS			*			*		
Year × treatment	NS			NS		NS		NS			NS			NS			NS		

Data are mean of 17 orchards each held in separate storage rooms each year. There were ten 100-fruit samples for each treatment [control (C), 1-methylcyclopropene (M)] for each orchard. The total number of fruit for each treatment in each year was 17,000.

Average followed by same letter in each column are not different by Tukey test ($P < 0.05$).

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

Table 5. Flesh firmness of 'Gala' and 'Fuji' apples at harvest and after controlled atmosphere storage plus 7 d at 22 °C.

		Firmness of 'Gala' (N)					Firmness of 'Fuji' (N)			
			After storage					After storage		
Yr	Days of storage	At harvest	Control	1-MCP	Avg	Days of storage	At harvest	Control	1-MCP	Avg
2007	188 ± 31	68.0 b	52.8	57.7	55.2 b	229 ± 28	72.9 a	68.7	71.6	70.1 a
2008	229 ± 21	77.8 a	58.2	61.0	59.6 ab	250 ± 38	74.7 a	68.7	71.2	70.0 a
2009	190 ± 34	76.9 a	59.2	62.2	60.7 a	223 ± 35	72.9 a	67.0	72.1	69.6 a
2010	222 ± 23	76.4 a	60.1	63.2	61.7 a	235 ± 30	69.8 b	62.4	66.4	64.4 b
Average	207 ± 39	74.5	57.7	61.2		234 ± 33	72.5	66.8	70.3	
Year		***	**				***	***		
Treatment			***					***		
Year × treatment			NS					NS		

Data are mean of 15 (for 'Gala') and 17 (for 'Fuji') orchards each year. There were twenty 10-fruit samples for each orchard at harvest and ten 100-fruit samples for each treatment [control, 1-methylcyclopropene (1-MCP)] for each orchard after storage.
Average followed by same letter in each column are not different by Tukey test ($P < 0.05$).
NS, *, **, ***Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

Table 6. Pearson correlation coefficient among flesh firmness and incidence of fruit affected by major disorders for each cultivar and year, after shelf life. Data of treated and untreated fruit with 1-methylcyclopropene were averaged for these analyses.

	Gala		Fuji	
Harvest season	Fungal decay	Flesh browning	Fungal decay	Superficial scald
2007	–0.43**	–0.71***	–0.15 NS	0.12 NS
2008	–0.39*	–0.77***	–0.34*	0.09 NS
2009	–0.29 NS	–0.42**	–0.11 NS	0.13 NS
2010	–0.64***	–0.42**	–0.47*	0.40*

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

Table 7. Rainfall, relative humidity, and temperature along the fruit growing season and cumulated hours with temperatures below 7.2 °C along the winter, before the fruit growing season.

		Harvest yr			
Variable	Season period	2007	2008	2009	2010
Accumulated rainfall (mm)[z]	During 2 mo. before harvest of Gala	261	209	367	390
	During 2 mo. before harvest of Fuji	222	226	76	481
	From bloom to harvest of Gala	742	766	896	844
	From bloom to harvest of Fuji	964	991	972	1325
Relative humidity (%)[z]	During two mo. before harvest of Gala	77.0	81.0	83.0	82.5
	During two mo. before harvest of Fuji	82.0	82.0	78.5	83.0
	From bloom to harvest of Gala	76.0	77.2	80.6	78.4
	From bloom to harvest of Fuji	76.8	77.7	80.7	79.3
Mean temperature (°C)[z]	From bloom to harvest of Gala	20.3	19.1	18.8	20.4
	From bloom to harvest of Fuji	20.5	19.1	19.0	20.2
Hours below 7.2 °C	Winter	446	564	572	539

[z]Including the harvest window period.
The full bloom was early October for both cultivars and the harvest windows were in February for 'Gala' and late March and early April for 'Fuji'.

production, ripening and eventually senescence of apples (DeEll et al., 2007; Lee et al., 2016), while resistance to fungal decay decreases as fruit begin to ripen and senesce (Neri et al., 2019; Nybom et al., 2020; Prusky et al., 2013).

Fungal decay was the major cause of postharvest losses and the incidence increased during shelf life as reported previously (Neri et al., 2009). This result reinforces the importance of maintaining the cold chain to prevent qualitative and quantitative losses of apples after CA storage and agrees with previous studies suggesting that apple deterioration from packing to supermarket display is mainly due to decay and bruising (Argenta et al., 2015).

In our study, apple losses were highly variable across years, especially for 'Gala', as well as among orchards (storage rooms) in each year, as previously reported for decay incidence in apples and pears (Breeyen et al., 2020; Kim and Xiao, 2008; Lennox et al., 2003). The variability of apple loss among years may be in part associated with seasonal variability in environmental conditions that can impact pathogen infection in the field (Adaskaveg et al., 2002; Sholberg and Conway, 2016; Spotts et al., 2009; Sugar, 2002). According to these previous reports, winter temperatures can affect the amount of inoculum during the following growing season; high rainfall favors sporulation and dispersion of fungal propagules to the fruit; fungal propagules adhere more strongly to wet surfaces of flowers and fruit, increasing probability of fruit infection; rainfall can affect fruit growth and the integrity of the cuticle as a barrier to fungal infection, as well as the amount of fungicide residue that remains on the fruit surface; fungus survival on fruit surfaces is reduced by higher solar radiation; and most pathogens generally grow best at 20 to 25 °C. Therefore, the higher fungal decay incidence observed after shelf life of both apple cultivars in 2010 is consistent with the higher rainfall observed during fruit growth and development in the same year (Table 8). In addition, the higher incidence of decay observed in 'Gala' apples in 2007 and 2010, after shelf life, could be associated with higher temperatures during the growing season. However, the highest incidence of fungal decay observed in 'Gala' apples in 2007 was possibly not related to rainfall, but could be associated with lower flesh firmness at harvest and after storage as well as with less chilling temperatures during the winter. The observed higher incidence of senescent breakdown and flesh browning in 'Gala' in 2007 could also be associated with advanced maturity, shown by the lower flesh firmness at harvest.

The observed high variability in fruit losses among orchards each year is likely the result of combined effects due to pre- and postharvest factors such as harvest maturity, orchard management practices and storage duration. Postharvest conditions such as storage temperature and atmosphere (pO_2 and pCO_2) were equal for all fruit of the same cultivar each year. In addition, no fungicide or sanitizer treatments were applied after harvest. Therefore, the main postharvest factor that may have accounted for the observed high variability in fruit losses among orchards was possibly the storage duration as increased storage duration can increase the opportunity for postharvest infection and fungal development in the fruit (Kim and Xiao, 2006; Neri et al., 2009).

The correlation analyses also suggest that the variability among orchards regarding the incidence of major disorders was associated with fruit firmness after storage, particularly for 'Gala'. Considering that fruit from different orchards were stored under the same conditions, the flesh firmness observed after storage is the result of combined effects of harvest maturity, storage duration, and preharvest factors that can affect fruit softening such as size and mineral content (DeEll et al., 2001). Higher incidence of decay in fruit samples with lower firmness (advanced

Table 8. Relative incidence (%) of fungus causing decay symptom in 'Gala' and 'Fuji' apples after controlled atmosphere storage.

Decay	Fungus	Gala	Fuji	Cultivar
Bull's-eye rot	*Neofabraea* sp.	51.6 a	42.1 a	NS
Blue mold	*Penicillium* sp.	27.5 b	24.6 b	NS
Gray mold	*Botrytis* sp.	9.1 c	18.3 bc	*
Alternaria rot	*Alternaria* sp.	10.2 c	5.2 de	NS
Moldy core rot	*Alternaria* sp. *Fusarium* sp.	0.1 d	8.7 cd	***
Bitter rot	*Glomerella cingulata*	0.8 d	1.0 ef	NS
White rot	*Botryosphaeria* sp.	0.7 d	0.2 ef	NS
Rhizopus rot	*Rhizopus* sp.	0.1 d	0.1 f	NS
Cultivar			*	
Decay			***	
Cultivar × decay			***	

Average followed by same letter in each column are not different by Tukey test ($P < 0.05$).
NS, *, **, ***Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

ripening) is possibly due to the reduction of fruit phenolic compounds and increasing cell wall breakdown that make the fruit more susceptible to pathogen growth and development (Nybom et al., 2020; Prusky et al., 2013; Sugar, 2002).

The variability in apple losses due to decay among orchards is possibly related to preharvest crop management and environmental conditions. Postharvest decay susceptibility is determined by a number of factors including tree age, preharvest disease control, pruning, nutrient management, weed control, fruit thinning, handling during harvest, and orchard sanitation (Cameldi et al., 2016; Lennox et al., 2003; Spotts et al., 2009; Sugar et al., 2003; Valdebenito-Sanhueza et al., 2010). Preharvest calcium sprays can decrease, whereas excessive nitrogen fertilization can increase susceptibility of apple fruit to fungal decay (Fallahi et al., 1997; Sugar et al., 2003). Similarly, tree and fruit nutrition in the field as well as cultural practices are potential causes of seasonal and orchard variability on fruit losses by physiological disorders (Watkins and Mattheis, 2019).

Fruit treated with 1-MCP maintained higher flesh firmness and had reduced incidence of superficial scald, flesh browning, and shrivel during and after long-term storage. However, 1-MCP had no effect on pathogen decay incidence after storage and shelf life. Indeed, the role of 1-MCP in inhibiting decay incidence has been variable. Studies have shown that 1-MCP can reduce decay caused by gray mold, bull's eye rot, and blue mold after apple storage and shelf life (Cameldi et al., 2016; Saftner et al., 2003). Other studies have also shown reduction in the incidence of gray mold, bull's eye and phacidiopycnins rots when Anjou pear fruit were treated with 1-MCP (Spotts et al., 2007). In addition, 'Red Fuji' apples treated with 1-MCP showed lower incidence and severity of blue mold, which was suggested to be the result of ethylene biosynthesis inhibition, leading to higher reactive oxygen species production that can inhibit the growth of *Penicilium expansum* (Li et al., 2017). However, other studies show that 1-MCP increased bitter rot and blue mold decay, which was attributed to the reduction in the activity of phenylalanine ammonia-lyase, the enzyme involved in many defense mechanisms (Janisiewicz et al., 2003).

Fungi causing apple fruit decay

The most important diseases leading to postharvest losses in both 'Gala' and 'Fuji' apples grown in southern Brazil were bull's-eye rot and blue mold, which together reached 79% and 67% of the total decayed apples, respectively. However, bull's-eye rot was the most frequent cause of decay incidence, reaching 51.6% and 42.1% of total decayed fruit in 'Gala' and 'Fuji', respectively. Indeed, bull's eye rot has been reported to be a major disease in apples around the world (Breeyen et al., 2020; Cameldi et al., 2016; Nybom et al., 2016; Valdebenito-Sanhueza et al., 2010; Wenneker et al., 2016). The incidence of blue mold was higher in 'Fuji' than in 'Gala', whereas the incidence of alternaria rot was higher in 'Gala' than in 'Fuji', showing that depending on the disease, the resistance response of each cultivar can be different. Accordingly, studies have shown that a cultivar highly resistant to one pathogen can be highly susceptible to other pathogens (Spotts et al., 1999).

Although total fungal decay incidence results are similar to those previously reported from other apple production regions, the results for individual pathogens are different from other studies possibly due to different interactions among pathogen, host, and environmental conditions, which determines pathogen infection before harvest. *Penicillium* spp., *Botrytis cinerea*, *Sphaeropsis pyriputrescens*, *and Neofabraea* spp. accounted for 32%, 28%, 17%, and 13.4% of decayed apples, respectively, and *Alternaria* spp., *Mucor piriformis*, and *Phacidiopycnis washingtonensis* were less frequent causes of postharvest decay in Washington State (Kim and Xiao, 2008). The most common pathogens causing postharvest decay in apples have been reported to be *Penicillium* spp. (29% incidence), *Botrytis cinera* (25%), *Mucor* spp. (22%), and *Neofabraea* spp. (10.5%) in Canada (Sholberg and Haag, 1996) and *Neofabraea* spp. (62%), *Botrytis cinerea* (30%), and *Colletotrichum acutatum* (8%) in the Nordic region of Europe (Nybom et al., 2016). In northern Greece, the most important pathogens leading to postharvest losses in 'Fuji' apples were *Penicillium expansum*, *Alternaria* sp., and *Botrytis cinerea* (Konstantinou et al., 2011). In the Netherlands, the most important apple pathogen has been reported to be *Neofabraea alba*, followed by *Botrytis* spp., *Pencillium* spp., *Fusarium* spp., *Alternaria* spp., and *Cladosporium* spp. (Wenneker et al., 2016). The most abundant fungi causing decay in 'Nicoter' apples grown in Belgium was *Penicillium* spp., followed by *Fusarium* spp., *Botrytis* spp., *Neonectria* spp., and *Monilinia* spp. (Naets et al., 2020).

In summary, these results identify some limiting factors to long-term storage of apples and suggest future research on developing efficient approaches to reduce losses and improve the efficiency of the whole apple industry. Apple losses during CA storage and shelf life were from 18.3% to 26.6% and were highly variable among orchards and production years. Decay accounted for ≈60% to 80% of total losses of 'Gala' and 'Fuji' apples during storage, respectively. It is known that most decays are triggered by preharvest pathogen infection, which develops later during and after storage when the fruit becomes more susceptible to pathogen growth (Prusky et al., 2013; Wenneker and Thomma, 2020). Hence, although new apple cultivars genetically tolerant or resistant to these pathogens are not available, approaches to reduce apple losses must consider the improvement and adoption of orchard management practices as an integrated system that includes proper orchard management to reduce pathogen reproduction, preharvest treatment with fungicides, orchard sanitation, development of predictive models for determining the incidence of decay that can help in segregating orchards for different storage rooms and storage duration, prevention of fruit wounding, rapid postharvest cooling, and postharvest sanitation treatments (Adaskaveg et al., 2002; Sholberg and Conway, 2016; Spotts et al., 2009). In addition, the observed higher fruit loses due to fungal infection during shelf life highlights the importance of the cold chain following CA storage for reducing fruit losses.

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