

ISOLATION AND CHARACTERIZATION OF FUNGAL CONTAMINANTS ASSOCIATED WITH FIVE SELECTED FRUITS OFFERED FOR SALE IN LAPAI, NIGER STATE NIGERIA

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ABSTRACT

The alarming rate of fruit poisoning caused by microbial contaminant is enormous. In view of this, mycotic contaminants of banana, orange, mango, garden egg and apple on sale in Lapai, Niger State, Nigeria were investigated. Fruit samples were collected weekly for a period of five months from vendors. Samples collected were cut into pieces and stock dilutions of 1g of sample per 9ml of sterile distilled water were prepared. Serial dilution of samples (10^{-2}) were inoculated on Potato Dextrose Agar (PDA) and incubated for 72 hours at $28 \pm 2^{\circ}\text{C}$. Pure culture of the fungi isolated were obtained and identified. Percentage rate of occurrence of isolated fungi was determined for each fruit. A total of ten (10) fruits spoilage fungi (*Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Mucor hiemalis*, *Candida tropicalis*, *Penicillium digitatum*, *Alternaria* sp., *Fusarium notatum*, *Rhizopus stolonifer* and *Colletotrichum* sp.) belonging to seven (7) different genera were isolated and characterized. *Rhizopus stolonifer* has the highest average frequencies of occurrence in orange (18.72%), banana (17.81%), garden egg (19.16%) and apple (25.03%) which were significantly different ($P < 0.05$) from other isolates. However, in mango, *A. niger* has the highest percentage frequency of 21.64%. In all the fruits *A. fumigatus* has the least percentage of occurrence except in garden egg. From this study, the presence of fungi contaminants some of which are known to produce mycotoxin that cause serious effects to human health if consumed was established. Therefore, an enforceable safety standard for the production, harvesting and distribution of fruits generally is suggested.

Key words: Fruit, Contaminant, mycoflora, occurrence

INTRODUCTION .

Fruits, including grains, occupy a central role in world agriculture and play a vital role

in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet

and help to keep a good and normal health (Abhinaba, 2009).

One of the limiting factors that influence the fruits economic value is the relatively short shelf-life period caused by microbial attack. Fruits are affected by a wide array of microorganisms causing their decay. These microorganisms, under the influence of environmental factors, pose a serious threat to fruits production, causing changes in their condition, making them less palatable or even toxic and often accompanied by alterations in taste, smell, appearance or texture (Akinmusire, 2011). Fruits contain high levels of sugars and nutrient elements and their low pH value make them particularly desirable to fungal decayed (Barth *et al.*, 2009). Fungal fruits infection may occur during growing season, harvesting, handling, transport and post-harvest storage and marketing condition or after purchasing by the consumers. Most of the fruits are transported in contaminated packing materials in bulks by trailers to the market. This infringe injuries to the fruits and thus leads to their contamination. It was reported that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling and losses are often more severe due to inadequate storage and transportation facilities (Droby, 2006).

Fruits spoilage is a complex event in which a combination of microbial and biochemical activities may interact. Microbial of fruits spoilage has over the years received considerable attention, and the

characterization of typical micro flora which develops on different types of fruits during storage has been well documented (Mossel *et al.*, 1995).

Generally, fungi associated with fruit spoilage are considered toxigenic or pathogenic. Toxigenic fungi that produce mycotoxins especially during storage have been isolated from fruits by Tournas and Stack, (2001). One of these fungi, *Aspergillus* spp. are known to produce several toxic metabolites, such as malformins, naphthopyrones and ochratoxin (Uzuegbu and Smith, 2006). Pathogenic fungi, on the other hand, could cause infections or allergies in animals including man (Manso, 2004; Al-Hindi *et al.*, 2011). Some fungi responsible for fruits spoilage as reported by Dave, (2005) include, *Penicillium* spp., *Botrytis cinera.*, *Rhizopus stolonifer*, *Aspergillus niger* *Cladosporium* spp. In addition to these, some variety of yeast such as *Kloeckera anapiculis*, *Saccharomyces cerevisiae*, *Torulopsis stellate*, *Rhodotenulaglutin varglutins* and *Babaryomyces nansenii* which generally bring about fermentation in fruit leading to the production of alcohol making them unsuitable to consume have been isolated from fruits as reported by Davenport and Beech (2001).

Lapai is an agricultural dominated town where most of the farmers pay more attention to fruit crops production. Endowed with very large market where farmers bring their fruit crops for sale from different parts of the Local Government Area. Therefore, this study investigated the

Fungi contaminants associated with some selected fruits offered for sale in Lapai, Niger State, Nigeria

MATERIALS AND METHODS

Study Area

This study was carried out in three different areas (Badegi market, Soje Garage and Central market) where fruits are popularly offered for sale in Lapai, Lapai Local Government Area Niger State, Nigeria.

Collection of Samples

Fresh apparently healthy samples of apple, banana, garden egg, mango and orange were randomly selected before they were displayed for sale and another samples that have been on displayed for at least three days and had been infected were obtained from fruit vendors at three different areas (Badegi market, Soje Garage and Central market Lapai) in Lapai on weekly basis for 5 months. The samples were collected in sterilized polythene bags and brought to laboratory of Biology Department Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria for analysis.

Isolation and Identification of the Fungi

The method of Burkar *et al.* (2009) was adopted and used. The samples which were apparently infected were cut from the advancing edges of lesion with a sterilized scalpel. The cut portion of the lesion was disinfected with 85% ethyl alcohol (BDH chemicals Ltd Poole England) for 2 minutes and then in 1% sodium hypochlorite for 3 mins and rinsed in three different changes of sterile distilled water. Segment (3 mm)

pieces were cut using sterile razor blade. Each segment was then homogenized using a sterile glass rod and a test tube in 10ml of the homogenate (1g +9 ml) (10^1) was made and serially diluted down to 10^{-4} . Prepared Potato Dextrose Agar (PDA) containing Chloramphenicol (30 mg/l) to prevent the growth of bacteria were poured into plates, inoculated with 0.1ml aliquots of the sample (10^{-2}) and incubated at room temperature ($28 \pm 1^\circ \text{C}$) for 7 days under 12 h photoperiod. The same operation was repeated for the apparently healthy ones, which served as controls. Three replicates of each experiment were prepared.

Fungal growths were monitored, the colonies counted and recorded as spore forming unit per millilitre (sfu/ml) during incubation. Pure culture of each colony type was prepared and maintained by sub-culturing. Isolated fungi were identified on the basis of their colonial morphology and microscopic examination as described by Samson and Varga (2007); Akintobi, *et al.* (2011).

Pathogenicity Tests

Pathogenicity test was carried out as described by Akintobi *et al.* (2011). Apparently healthy fruits of orange, banana, garden egg, mango and apple were washed with tap water and rinsed with sterile distilled water and surface sterilized with 75% ethanol. A sterile 4mm cork borer was used to make holes in each of the fruits. A colony of fungi isolate (from each pure culture) was used to inoculate the fruits and the core of the fruits were replaced. The point of

inoculation was sealed with petroleum jelly to prevent contamination. Controls of each was set up as described above but not inoculated. Three replicates of each were made. The inoculated fruits and the controls were placed in clean polyethylene bag (one fruit per bag) each moistened with wet balls of absorbent cotton wool to create a humid environment and incubated at $28 \pm 1^\circ\text{C}$ for 5 days. After 72 h, the inoculated fruits were observed for symptom development. The causal agents were re-isolated from the infected fruits and compared with the original isolates.

Data Analysis

Percentage frequency of isolated fungi in each of the fruit were calculated and data generated were subjected to Analysis of Variance (ANOVA) to test for the significant difference in fungi prevalence and Duncan's New Multiple Range Test was employed to separate the means of the different groups at $P < 0.05$ level of significance.

RESULTS

A total of ten (10) fruits spoilage fungi (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Mucor hiemalis*, *Alternaria* sp., *Candida tropicalis*, *Penicillium digitatum*, *Fusarium notatum*, *Rhizopus stolonifer* and *Colletotrichum* sp.) belonging to seven (7) different genera were isolated and characterized from infected and fresh healthy oranges, bananas, mangoes, garden eggs and watermelons offered for sales in

Badegi market, Soje garage and Central market in Lapai, Niger State, Nigeria (Table 1). Pathogenicity tests conducted authenticated the spoilage abilities of these fungi on fruits.

From Badegi market (Table 2), a total of eight (8) fungal species (*Aspergillus niger*, *Mucor hiemalis*, *Candida tropicalis*, *Penicillium digitatum*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Rhizopus stolonifer* and *Colletotrichum* sp.) were isolated from all the samples. However, *M. hiemalis* was not isolated from garden egg and apple while *A. fumigatus* was not isolated from apple. In oranges, *Aspergillus niger* has highest percentage frequency of occurrence (20.56%) and was significantly different ($P > 0.05$) from other isolates. Followed closely, was *R. stolonifer* (21.37%), *Colletotrichum* sp. (14.31%), *M. hiemalis* (14.30%) and *P. digitatum* (14.26%). Percentage frequency of *M. hiemalis* (14.30%) *Colletotrichum* sp. (14.31%) and *P. digitatum* (14.26%) were not significantly different ($P > 0.05$). From bananas, *A. niger* has the highest percentage frequency of occurrence (15.00%). The frequency of occurrence of *M. hiemalis* (12.56%), *P. digitatum* (12.39%), *A. flavus* (12.50%) and *R. stolonifer* (12.74%) were not significantly different ($P > 0.05$). *A. niger* has the highest percentage frequency of occurrence (18.53%) in spoiled mangoes. Percentage frequencies of occurrence of *P. digitatum* (7.10%), *A. fumigatus* (7.11%) and *Colletotrichum* sp. were not significantly different ($P > 0.05$).

In Garden eggs, *M. hermalis* was not isolated while *C. tropicalis* has the highest percentage frequency of occurrence (18.34%). *Rhizopus stolonifer* and *P. digitatum* have percentage frequency of 16.78% and 16.57% and respectively. The percentage frequencies of *A. niger* and *A. flavus* in apple were 18.53% and 18.36% respectively and were not significantly different ($P>0.05$). *Aspergillus fumigatus*

From Soje garage (Table 2), a total of eight (8) fungi species (*Aspergillus niger*, *Mucor hiemalis*, *Candida tropicalis*, *Penicillium digitatum*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Rhizopus stolonifer* and *Colletotrichum* sp.) were isolated from fruits sampled. *Mucor hiemalis* was not isolated from oranges, garden eggs and apple while *P. digitatum* was not isolated from mangoes and apples, and *A. fumigatus* was not isolated from

Table 1: Percentage frequencies of fungal isolates from apparently healthy fruits samples obtained from Soje Garage Lapai, Niger state Nigeria.

Samples	Orange	Banana	Mango	Garden egg	Apples
<i>Aspergillus niger</i>	1(12.5)b	1(11.11)b	1(16.67)b	1(16.67)b	0(0.00)a
<i>Alternaria</i> sp.	1(12.5)b	2(22.22)c	0(0.00)a	0(0.00)a	0(0.00)a
<i>P. digitatum</i>	0(0.00)a	1(11.11)b	0(0.00)a	1(16.67)b	0(0.00)a
<i>A. flavus</i>	1(12.50)b	0(0.00)a	2(33.33)c	2(33.33)c	0(0.00)a
<i>R. stolonifer</i>	4(50.00)c	5(55.55)d	3(50.00)d	2(33.33)c	1(100.00)b
<i>Fusarium</i> sp.	1(12.50)b	0(0.00)a	0(0.00)a	0(0.00)a	0(0.00)a
TOTAL	100	100	100	100	100

Values followed by the same alphabet along the column are not significantly different at $P>0.05$ level of significant from Duncan Multiple Range Test (DUMRT)

oranges, banana and mango. In mangoes and garden eggs, *A. niger* has the highest percentage frequency of 23.00% and 30.70% respectively. The percentage frequencies of *R. stolonifer* were generally high in all the fruits samples collected from Soje garage. Only five fungal species (*Aspergillus niger*, *Mucor hiemalis*, *Aspergillus flavus*, *Aspergillus fumigatus*, and *Rhizopus stolonifer*) were

isolated from apples in this study. A total of eight (8) fungal species (*Aspergillus niger*, *Mucor hiemalis*, *Candida tropicalis*, *Penicillium digitatum*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Rhizopus stolonifer* and *Colletotrichum* sp.) were isolated from all infected fruits sampled obtained from central market (Table 3) except *A. fumigatus* that was not isolated from

Table 2: Percentage frequencies of fungal isolated from apparently fruits samples obtained from Badegi market Lapai, Niger state, Nigeria.

Samples	Orange	Banana	Mango	Garden egg	Apples
<i>Aspergillus niger</i>	20.56 ±0.02d	15.00 ±0.17c	18.53 ±0.06c	15.03 ±0.15c	18.53 ±0.39c
<i>Mucor hiemalis</i>	14.30 ±0.06c	12.56 ±0.03b	21.39 ±0.02d	0.00 ±0.00a	0.00 ±0.00a
<i>Candida tropicalis</i>	8.00 ±0.00a	18.63 ±0.03d	10.00 ±0.00b	18.34 ±0.06d	14.33 ±0.03b
<i>Penicillium digitatum</i>	14.26 ±0.09c	12.39 ±0.05b	7.10 ±0.03a	16.57 ±0.03cd	14.23 ±0.23b
<i>Aspergillus flavus</i>	0.00 ±0.00a	12.50 ±0.00b	18.31 ±0.17c	14.98 ±0.07c	18.36 ±1.04c
<i>Aspergillus fumigatus</i>	7.20 ±0.06b	6.18 ±0.06a	7.11 ±0.03a	8.30 ±0.02b	0.00 ±0.00a
<i>Rhizopus stolonifer</i>	21.37 ±0.02d	12.74 ±0.05b	10.39 ±0.00b	16.78 ±0.03c	14.55 ±0.40b
<i>Colletotrichum sp.</i>	14.31 ±0.03c	10.00 ±0.00b	7.17 ±0.03a	10.00 ±0.00b	20.00 ±0.00c

Values are Mean±Standard Error of mean of four replicate. Values followed by the same alphabet along the column are not significantly different at $P>0.05$ level of significant from Duncan Multiple Range Test (DMRT)

mangoes and apples. *C. parapsilosis* has the highest percentage frequency (18.30%) from spoilt orange, *Rhizoctonia stolonifer* in banana (22.5%), *A. niger* in mango (23.40%) and *P. digitatum* in garden eggs and apples with 21.40% and 25.30% respectively. The least isolated fungal species was with 6.5%, 7.15%, 0.00%, 6.30% and 0.00% in oranges, bananas, mangoes, garden eggs and apples respectively. Table 4 shows the summary of the mean percentage frequencies of fungal species isolated from various fruits offered for sales in three marketing areas in

Lapai. *Rhizoctonia stolonifer* has the highest mean percentage frequencies in all the five types of fruits sampled. In oranges it was 18.72%, 17.81% in bananas, mangoes (21.64%), garden eggs (19.16%) and apples (25.03%). *Aspergillus fumigatus* had the least mean percentage frequencies in all the fruits sampled with 2.37% in mango, 4.57% in orange, 4.17%, 4.90% and 4.17 in apple.

DISCUSSION

Protecting fruits from disease does not end with harvesting. Fruits and vegetables remain susceptible to disease even after they have been picked and stored. Some decay-causing organisms are essentially wound pathogens (Akinmusire, 2011). Damage on the fruit skin such as stem punctures, limb rubs and bruises may be created at harvest or during the fruit handling process. Decay-causing pathogens, particularly those airborne pathogens such as gray mould fungus may contaminate wounds and later cause decay

symptoms in storage. The results from this study showed that both healthy and infected fruits samples obtained from Badegi market, Soje Garage and Central market of Lapai Local Government Area of Niger State, Nigeria were contaminated with relatively high fungi pathogen from five genera (*Aspergillus* sp., *Candida* sp., *Penicillium* sp., *Rhizopus* sp., *Mucor* sp.) This was similar to the finding of Bukar *et al.* (2009), who isolated six genera of fungi namely *Aspergillus* sp., *Candida* sp., *Penicillium* sp., *Rhizopus* sp., *Mucor* sp., and *Alternaria* sp. from fruits sampled obtained from Na'ibawa

Table3: Percentage frequencies of fungal isolates from infected fruits samples obtained from Soje Garage Lapai, Niger state Nigeria.

Samples	Orange	Banana	Mango	Garden egg	Apples
<i>Aspergillus niger</i>	16.46 ±0.29c	13.00 ±1.44c	23.00 ±1.73d	30.70 ±0.64e	26.66 ±1.61c
<i>Mucor hiemalis</i>	20.00 ±0.28d	18.00 ±0.00d	14.30 ±0.86b	7.60 ±0.28b	12.50 ±0.40b
<i>Candida tropicalis</i>	0.00 ±0.00a	12.00 ±1.56c	15.40 ±2.42b	0.00 ±0.00a	0.00 ±0.00a
<i>Penicillium digitatum</i>	18.20 ±0.69cd	6.29 ±0.01b	0.00 ±0.00a	8.07 ±0.23b	0.00 ±0.00a
<i>Aspergillus flavus</i>	9.00 ±0.86b	12.01 ±0.06c	13.0 ±1.90b	13.03 ±0.89c	12.50 ±0.69b
<i>Aspergillus fumigatus</i>	0.00 ±0.00a	0.00 ±0.00a	0.00 ±0.00a	7.60 ±1.44b	12.50 ±1.15b
<i>Rhizopus stolonifer</i>	18.20 ±0.86cd	18.20 ±3.11d	16.70 ±1.15b	25.40 ±0.02d	35.84 ±0.00d
<i>Colletotrichum</i> sp.	18.14 ±0.86cd	20.50 ±1.84d	17.60 ±0.29c	7.60 ±0.00b	0.00 ±0.00a

Values are Mean±Standard Error of mean of four replicate. Values followed by the same alphabet along the column are not significantly different at P>0.05 level of significant from Duncan Multiple Range Test (DUMRT)

yanlemu market in Kano, Nigeria. It was observed from this study that only five of these genera with very low frequencies were isolated from fresh uninfected fruits suggesting probably that since these fungi are saprotrophs they infect fruits when display for sales as earlier reported by Baiyewu *et al.* (2007) and Akintobi *et al.* (2011). They reported that microorganisms contamination of fruits can be brought in by outside elements such as wind, soil, water, insects, animals or human handling. Contamination may also probably be from farm during harvest or transportation. Bukar

et al. (2009) and Jay (2003) attributed contamination of postharvest produce to the presence of the fungi or their resistant spores from the farms during harvest and some from the stores due to horizontal contamination by the already spoiled fruits.

As earlier reported by Baiyewu *et al.* (2007), pathogenicity test carried out, especially inoculation done through wounds showed that all the fungi isolated were responsible for the deterioration of these fruits and also confirmed the fact that fungi cause deterioration of the fruits when they gained entrance into them through

Table 4: Percentage frequencies of fungal isolates from infected fruits samples obtained from central market in Lapai, Niger State Nigeria.

Fungi	Fruit samples				
	Orange	Banana	Mango	Garden egg	Apple
<i>Aspergillus niger</i>	13.10 ±0.15b	18.70 ±0.20d	23.40 ±0.05d	11.40 ±0.10b	10.02 ±0.58c
<i>Mucor hiemalis</i>	16.60 ±0.20c	12.40 ±0.10b	15.00 ±1.00c	7.10 ±0.10a	6.00 ±0.00b
<i>Candida tropicalis</i>	12.30 ±0.20b	15.00 ±1.00c	17.00 ±0.00c	10.00 ±0.00b	12.50 ±0.30c
<i>Penicillium digitatum</i>	8.30 ±0.20a	11.65 ±0.10b	8.30 ±0.30b	21.40 ±0.30d	25.30 ±0.50e
<i>Aspergillus flavus</i>	8.30 ±0.20a	6.30 ±0.30a	8.00 ±0.60b	8.49 ±0.00ab	6.30 ±0.00b
<i>Aspergillus fumigatus</i>	6.50 ±0.20a	7.15 ±0.05a	0.00 ±0.00a	6.30 ±0.10a	0.00 ±0.00a
<i>Rhizopus stolonifer</i>	16.60 ±0.10c	22.50 ±0.10e	10.00 ±0.00b	15.31 ±0.20c	24.70 ±1.00c
<i>Colletotrichum sp.</i>	18.30 ±0.20c	6.30 ±0.10a	18.30 ±0.20c	20.00 ±0.00d	15.00 ±0.00e

Values are Mean ± Standard Error of mean of four replicate Values followed by the same alphabet along the column are not significantly different at P > 0.05 level of significant from Duncan Multiple Range Test (DUMRT)

Table 5: Mean percentage frequencies of fungal isolates from infected fruits samples obtained from Lapai, Niger State Nigeria.

Fungi	Fruit samples (%)				
	Orange	Banana	Mango	Garden egg	Apple
<i>Aspergillus niger</i>	16.71 ±0.29c	15.57 ±0.20c	21.64 ±0.07d	19.04 ±0.00d	18.04 ±0.86c
<i>Mucor hiemalis</i>	16.97 ±0.20c	14.32 ±0.11c	16.90 ±0.06c	4.90 ±0.10a	6.17 ±0.03a
<i>Candida tropicalis</i>	6.77 ±0.17a	15.21 ±0.60c	14.13 ±0.04b	9.48 ±0.10b	9.45 ±0.31b
<i>Penicillium digitatum</i>	13.56 ±0.30b	10.08 ±0.08b	5.13 ±0.25a	15.35 ±0.30c	13.08 ±0.30b
<i>Aspergillus flavus</i>	5.77 ±0.20a	10.27 ±0.25b	13.10 ±0.60b	12.17 ±0.01d	12.39 ±0.09b
<i>Aspergillus fumigatus</i>	4.57 ±0.18a	4.47 ±0.10a	2.37 ±0.20a	7.40 ±0.04b	4.17 ±0.08a
<i>Rhizopus stolonifer</i>	18.72 ±0.13c	17.81 ±0.14c	12.36 ±0.10b	19.16 ±0.10d	25.03 ±1.04d
<i>Colletotrichum sp.</i>	16.93 ±0.12c	12.27 ±0.11b	14.37 ±0.10b	12.50 ±0.20c	11.67 ±0.10b

Values are Mean±Standard Error of mean of four replicate values followed by the same alphabet along the column are not significantly different at P>0.05 level of significant from Duncan Multiple Range Test (DUMRT)

mechanical injuries such as bruises and wounds. These may be inflicted by the way these fruits are being forcibly packed in the boxes and sacs, transportation in trucks and carts by farmers and the fruit vendors which adds to the issues of bruising from where the pathogen of post harvest rots easily enter and spoil the whole lot of fruits. According to Hamd Meer *et al.* (2013) the post harvest rots aggravate due to unavailability of optimum temperature as well as dumping

of the wooden boxes irregularly in the market. Post Harvest Information Network (2014), ascertained that decay caused by *Penicillium* is initiated at wound sites, such as cuts, stem punctures and through lenticels on unbroken skin, particularly at bruise sites.

The most dominant species isolated from spoilt orange were *Aspergillus niger*, *Rhizopus stolonifer* and *M. hiemalis*; from spoilt banana, *A. niger*, *Candida tropicalis* and

A. niger were obtained from spoil mango, *Aspergillus niger*, *Colletotrichum* spp and *M. niemaiis*, while from spoil garden eggs and apple, the same trends of contaminations were obtained with *R. stolonifer* and *Aspergillus niger*. Prevalence of these genera including *Candida* confirmed the earlier reports of Al-Hindi *et al.* (2011) and Akinjobi *et al.* (2011) who separately reported the preponderance of these fungi with deterioration of orange. Odeboke and Samusi (1999) also isolated *Botryodiplodia theobromae*, *Rhizopus oryzae*, *Aspergillus niger*, *A. flavus* and *Fusarium equiseti* from ripped banana. Generally, spoilage was observed to increase with period of storage probably as a result of physiological changes and senescence which favours pathogen development as earlier separately reported by Eckert *et al.* (1996) and Prusky (1996). The findings from this study showed that fruits on sale in Lapai are contaminated with fungi. Most of them are known saprophytes that produce mycotoxins causing greater deterioration of fruits which affects the quality of their juice and make it dangerous for human consumption. Therefore, control of postharvest decay should start in the orchard and continue until the fruit are sold.

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