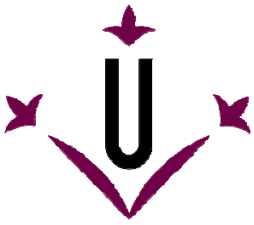

Identification of fungal population in the environment and on surfaces of stone fruit packinghouses

Maria Bernat,[a] Joan Segarra,[b] Carla Casals,[a] Rosario Torres [a], Neus Teixidó,[a] and Josep Usall [a*]

[1]IRTA, XaRTA-Postharvest, Edifici Fruitcentre, Parc Científic i Tecnològic Agroalimentari de Lleida, Parc de Gardeny, 25003 Lleida, Catalonia.

[2]Department of Crop and Forest Sciences, University of Lleida, Avda. Rovira Roure, 191, 25198, Lleida, Catalonia.

Abstract

In the present work, the fungal population present in the environment and on surfaces of equipment and facilities was determined and quantified in two stone fruit packinghouses during 2012 and 2013. The fungi present in the environment were sampled according to the gravimetric method. The fungi present on the surfaces of floors, walls, containers and lines were sampled with Rodac plates. Dirty zones (reception of fruits and first selection) were more contaminated than clean zones (washing of fruits, lines and containers), even though in the shipping room the presence of different fungi was high. The most prevalent genera recovered in both packinghouses and in all zones were *Penicillium* spp followed by *Cladosporium* spp. The presence of *Rhizopus* spp. was also highly detected in all zones, which could result in new postharvest infections. Moreover, *Monilinia* spp., the most important postharvest disease on stone fruit, was poorly detected, indicating the low risk of fruit infection in packinghouses.

Keywords: Brown rot, Rhizopus rot, *Cladosporium* spp., *Penicillium* spp., infection risk.

1. Introduction

The main worldwide postharvest diseases caused by fungi both in peach and nectarine fruits are brown rot caused by *Monilinia fructicola* or *M. laxa*, *Rhizopus*, rot caused by *Rhizopus stolonifer*, and grey mould caused by *Botrytis cinerea* (Crisosto and Kader, 2014). Brown rot is by far the most important disease on stone fruit in Europe, with direct losses from fruit rot at preharvest and postharvest. Other fungi diseases such as those caused by *Penicillium* spp., *Cladosporium* spp., *Alternaria* spp. and *Aspergillus* spp. on stone fruit are casual and losses associated with them are minor (Usall et al., 2013).

Fruit infection probability is directly related to the amount of conidia on the fruit surface with respect to appropriated pathogen, and environmental conditions such as temperature and RH. This dependence has been demonstrated by *Penicillium* spp. (Bancroft et al., 1984) and *Monilinia* spp. (Villarino et al., 2012), as well as by other pathogens. The conidia from the fields and conidia produced in chambers due to developing of latent infection or recent infections can lead to secondary infections that spread in packinghouses. Therefore, the measures that are adopted to reduce the level of inoculum present on the fruit surface and on different zones in packinghouses can contribute to reducing the disease.

To design effective methods of cleaning and disinfection, and reduction of new infections at postharvest, it is necessary to identify and evaluate the critical points of packinghouses where there are more risks of infections. The objective of the present investigation was to determinate and quantify the fungal population present in the environment, on surfaces of facilities and grading lines in stone fruit packinghouses during the harvest season.

2. Material and Methods

Fungal populations were sampled in two packinghouses located in the Lleida area (Catalonia, Spain) which the main activity is the sorting of stone fruit for the international market. During two seasons, sampling was carried out at 7-day intervals during 2012 and at 15-day intervals during 2013, from August to October in both periods. In packinghouse A, a total of 5 samples in 2012 and 4 samples in 2013 were taken, and in packinghouse B, a total of 6 samplings in 2012 and 4 samplings in 2013 were taken.

The environmental fungal populations were sampled at the following zones for packinghouse A: fruit reception from the field; the hydrocooling room where fruit is

quickly chilled by a hydrocooling tunnel; the cold chamber with fruit bins; the dirty zone where all fruit are selected by hand in searching for rot and submerged into a water tank; the clean zone where fruits are handled; and shipping room within which fruits are stored until their transportation out of the facility. The temperatures in the hydrocooling room, the cold chamber and the shipping room was controlled at 0-3 ºC, but the temperatures within the other zones were higher than 15 ºC, although temperature is not controlled and it largely depends on the weather (ambient temperature). Walls and floor surface of the aforementioned zones were sampled, as well as the surfaces of the sorting lines and fruit containers. Dirty containers were considered as being used to keep discarded fruits, and clean containers where considered as being used to keep fruits stored in cold chambers.

For packinghouse B, fungal populations of environment were sampled at: the precooling room where fruit is stored during 24 hours; the cold chamber where fruit is stored for longer periods; the waiting room within which fruit is stored for a short period of time before being sorted; the dirty zone where all fruit are selected by hand for in searching for rot; the clean zone within which fruits are sorted; the shelf-life room where one is able to determine how infected fruit came from orchard; and the shipping room within which fruits are stored until their transportation out of the facility. The temperature in the precooling room, cold chamber, waiting room and shipping room was controlled at 0-3 ºC, but the other zone temperatures were higher than 15 ºC, although temperatures were not controlled and they depend on weather conditions (ambient temperatures). Wall and floor surfaces were sampled from the previously described zones, as well as sorting lines (wet and dry lines, depending on whether it have water dump), and dirty and clean containers that were previously described in the explanation of the A packinghouse.

The environmental fungal population was determined using the gravimetric method. Three Petri dishes of 9 cm diameter, containing potato dextrose agar (PDA) medium (Biokar Diagnostic, 39 gL^{-1}), were equidistantly distributed throughout each zone and were left open for 3 min to allow fungal spores to fall down via gravity onto the Petri dishes. Surfaces were sampled with 5.5 cm diameter Replicate Organism Direct Agar Contact (Rodac) plates (containing PDA medium with contact between the culture medium and the surface, with slight pressure applied to keep spores adhering to the medium. Three Rodac plates were used for each selected zone: floor, walls, containers and sorting lines.

All dishes were incubated at 20±1 ºC for 5 days, then examination and counting the fungal colonies were undertaken. In order to identify the fungal colonies, a

relevant taxonomic keys (Samson et al., 1981) were used and observations were made both visually and microscopically.

For statistical analysis, a sampled unit was considered as the average of three plates that were used to sample one zone, all sampling days, and two years for each packinghouse. The fungal population of each sample unit was expressed by the number of colony-forming units per plate (cfu/plate).

Two statistical analyses were undertaken, depending on the experimental data belonging to each recovered fungus. The first statistical analysis was Pearson's Chi-squared test through contingency tables for each fungus, and it was used to compare the presence and absence of the fungal population at each packinghouse zone. This analysis was necessary for *Rhizopus* spp., because it was only possible to count the presence or absence of the fungus by considering the growth that was invading all of the Petri dishes, and for other fungi where their presence at packinghouses was very low. The Welch test was used to compare the average of colony-forming units (cfu) per plate (whenever this was possible), because the data did not follow a normal distribution and equal variance could not be assumed. When the Welch test was significant, the differences of the individual fungi that were recovered between each zones were compared via the Tukey test ($P<0.05$), using the R statistical software package (R version 3. 2. 3, 2015).

3. Results

The results of the sampling showed an overall number of 7 relevant genera of filamentous fungi that were recovered from the environment and the surfaces of the packinghouses. The main genera identified were, *Penicillium* spp., *Cladosporium* spp., *Fusarium* spp., *Aspergillus* spp., *Rhizopus* spp. (this includes *Mucor* spp.) and *Alternaria* spp. Minor species included *Monilinia* spp and those classified as *others* comprised of *Geotrichum* spp., *Botrytis* spp., and other fungi which could not be classified. Although sample of *Monilinia* spp. conidia were rarely recovered, the results are shown in the figures because of the importance of the pathogen on stone fruits.

Among the colonies that were recovered from the Petri dishes in the environment of packinghouse A, there were: 50.7% *Penicillium*, 16.9% *Cladosporium*, 8.9% *Alternaria*, 4.5% *Fusarium*, 2.3% *Aspergillus*, and 0.1% *Monilinia*. The remaining 16.6% genera belonged to *Geotrichum*, *Botrytis*, and other fungi genera. *Rhizopus* was also identified in all zones sampled, but was recovered as a presence or absence, hence it is not included in the percentages. The most contaminated zones were the

fruit reception, the dirty zone, and the clean zone (Table 1). *Cladosporium* was the fungus sampled in a greater number at the fruit reception zone, followed by *Alternaria* and *Penicillium*. *Penicillium* was the main genera identified at the dirty zone, followed by *Cladosporium*. The main genera recovered from the environment of the clean zone and chamber was P*enicillium*. The presence of *Rhizopus* was homogeneous throughout the packinghouse, although the lowest presence was at the fruit reception. Only in the 2013 season, was one *Monilinia* conidia that was detected in the environment of the hydrocooling room.

Among the colonies that were recovered from the Rodac dishes on the surfaces of packinghouse A, the percentages were: 27.7% *Penicillium*, 18.9% *Cladosporium*, 7.9% *Fusarium*, 6.7% *Alternaria*, and 3% *Aspergillus*. The remaining 35.8% genera belonged to *Geotrichum*, *Botrytis*, and other fungi genera. The zones with higher fungal population were the chamber, the clean container of the dirty zone, the fruit reception, the shipping room, the hydrocooling room and line 1 of the clean zone (Table 2). *Penicillium* was the main genera recovered at the chamber, although *Cladosporium* and *Alternaria* were also abundantly recovered. In 2013, only one colony was identified as *Monilinia* on the chamber. In the hydrocooling room, the shipping room and the fruit reception, the main genera recovered were *Penicillium*. On the clean container surface of the dirty zone, *Cladosporium* and *Alternaria* were the main genera identified. There were significant differences between the presence and absence of *Rhizopus* with respect to the packinghouse zones. The dirty zone, the clean zone and the dirty container of the dirty zone had the most surfaces that were contaminated by *Rhizopus*. In the shipping room zone, eight *Monilinia* conidia were recovered during 2013.

Among the colonies that were recovered from the Petri dishes in the environment of packinghouse B, the percentages were: 47.6% *Penicillium*, 20.3% *Cladosporium*, 10% *Alternaria*, 5.6% *Fusarium*, 2.7% *Aspergillus* and 0.85% *Monilinia*. The remaining 13% of genera belonged to *Geotrichum*, *Botrytis*, and other fungi genera. The total environmental fungal population recovered had no significant differences in their distributions from each of the zones of packinghouse B (Table 3). *Cladosporium* and *Penicillium* were the most prevalent genus in all of the sampled zones. *Rhizopus* was isolated in all of the studied packinghouse zones, although a lower presence was detected in the precooling, storage, waiting and shipping rooms. The clean and dirty zones and the shelf life room were zones with the most contamination by *Rhizopus*. A total of 13 *Monilinia* conidia were detected in 2013 at the precooling, storage, waiting and shelf life rooms and the dirty zone. In contrast, *Monilinia* was not detected during 2012.

Table 1. Fungal population (cfu/plate) in the environment of different zones at the packinghouse A.

Zones	Fungal population								
	***Monilinia* spp.**	***Penicillium* spp.**	***Fusarium* spp.**	***Cladosporium* spp.**	***Aspergillus* spp.**	***Alternaria* spp.**	**Others**	**Total**	***Rhizopus* spp.** [x]
Fruit reception	0.00	1.33 c	0.73 a	4.47 a	0.00	2.40 a	1.80 a	10.73 ab	0.07
Hydrocooling room	0.04	0.67 c	0.11 b	0.56 c	0.07	0.81 b	1.00 abc	3.26 c	0.11
Chamber	0.00	2.85 abc	0.12 b	0.63 c	0.05	0.63 b	0.63 bc	4.78 bc	0.24
Dirty zone	0.00	7.19 a	0.41 ab	2.11 b	0.85	0.22 b	1.33 ab	12.11 a	0.30
Clean zone	0.00	6.69 ab	0.33 ab	0.59 c	0.07	0.11 b	0.44 c	8.00 abc	0.52
Shipping room	0.00	1.96 bc	0.56 a	0.30 c	0.07	0.37 b	0.74 abc	4.00 bc	0.30
Welch Test[1]	nd	< 0.0001	0.030	0.001	nd	0.003	0.017	< 0.0001	nd
Chi-Square Test[2]	0.403	0.137	0.047	<0.001	< 0.0001	< 0.0001	0.014	nd	0.115

Each value is the mean of six Rodac plates of each zone, all sampling days and two years.

[1]*Welch Test is a nonparametric test to compare quantitatively the fungal population between zones. Means of each fungus followed by the same letter are not significantly different (Post Hoc: Tukey test < 0.05).*

[2]*Chi-Square Test compares the presence and absence of fungal population between zones.*

nd : not determined because experimental data did not allowed it.

[x] *Only was possible to recover presence or absence of Rhizopus spp. fungus in each Petri plate. Data shown the average* Rhizopus *spp. presence per plate.*

Table 2 Fungal population (cfu/plate) on the surface of different zones at the packinghouse A.

Zones	Fungal population								
	***Monilinia* spp.**	***Penicillium* spp.**	***Fusarium* spp.**	***Cladosporium* spp.**	***Aspergillus* spp.**	***Alternaria* spp.**	**Others**	**Total**	***Rhizopus* spp.[x]**
Fruit reception	0.00	1.10 bc	0.55	1.50	0.57	0.17	2.00	5.89 ab	0.43
Hydrocooling room	0.00	1.31 bc	0.24	0.80	0.06	0.35	1.69	4.44 ab	0.44
Chamber	0.01	3.25 a	0.61	1.81	0.07	1.51	1.22	8.50 a	0.39
Dirty zone	0.00	1.13 bc	0.15	1.07	0.07	0.09	1.09	3.61 b	0.83
Clean container	0.00	1.00 bc	0.48	2.00	0.22	1.26	1.74	6.70 ab	0.52
Dirty container	0.00	0.59 c	0.04	0.00	0.19	0.07	1.81	2.70 b	0.63
Line 1	0.00	0.13 c	0.20	0.67	0.48	0.00	1.10	2.58 b	0.48
Line 2	0.00	0.56 c	0.29	0.90	0.10	0.05	0.76	2.65 b	0.41
Clean zone	0.00	0.64 c	0.00	0.02	0.08	0.00	1.08	1.81 b	0.62
Line 1	0.00	0.33 c	0.93	0.70	0.04	0.00	1.96	3.96 ab	0.44
Line 2	0.00	0.30 c	1.30	0.00	0.11	0.00	0.93	2.63 b	0.37
Shipping room	0.15	2.76 ab	0.15	0.54	0.09	0.13	1.56	5.37 ab	0.26
Welch Test[1]	nd	< 0.0001	nd	nd	0.754	nd	0.146	< 0.0001	nd
Chi-Square Test[2]	0.831	< 0.0001	0.024	< 0.0001	0.279	< 0.0001	0.061	nd	< 0.0001

Each value is the mean of six Rodac plates of each zone, all sampling days and two years.

[1]Welch Test is a nonparametric test to compare quantitatively the fungal population between zones. Means of each fungus followed by the same letter are not significantly different (Post Hoc: Tukey test < 0.05).

[2]Chi-Square Test compares the presence and absence of fungal population between zones.

nd : not determined because experimental data did not allowed it.

[x] Only was possible to recover presence or absence of Rhizopus spp. fungus in each Petri plate. Data shown the average Rhizopus *spp. presence per plate.*

Table 3. Fungal population (cfu/plate) in the environment of different zones at the packinghouse B.

Zones	Fungal population								
	***Monilinia* spp.**	***Penicillium* spp.**	***Fusarium* spp.**	***Cladosporium* spp.**	***Aspergillus* spp.**	***Alternaria* spp.**	**Others**	**Total**	***Rhizopus* spp.**[x]
Precooling room	0.05	5.60	0.70	2.85 ab	0.71	2.50 a	0.60	12.35	0.15
Chamber	0.29	3.47	0.48	1.62 ab	0.49	1.14 ab	0.57	7.43	0.24
Waiting room	0.17	3.72	0.27	1.63 ab	0.24	0.90 b	0.40	7.03	0.07
Dirty zone	0.07	3.00	0.37	4.27 a	0.59	1.03 ab	1.57	10.77	0.40
Clean zone	0.00	5.27	0.13	0.93 b	0.11	0.07 b	0.77	7.40	0.67
Self life room	0.03	3.69	0.14	1.52 ab	0.14	0.34 b	1.55	7.45	0.59
Shipping room	0.00	4.97	0.31	0.24 b	0.29	0.07 b	0.97	6.55	0.24
Welch Test[1]	nd	0.527	0.083	< 0.0001	nd	< 0.0001	0.101	0.683	nd
Chi-Square Test[2]	0.453	0.849	0.008	0.002	0.002	< 0.0001	0.462	nd	< 0.0001

Each value is the mean of six Rodac plates of each zone, all sampling days and two years.

[1] *Welch Test is a nonparametric test to compare quantitatively the fungal population between zones. Means of each fungus followed by the same letter are not significantly different (Post Hoc: Tukey test < 0.05).*

[2] *Chi-Square Test compares the presence and absence of fungal population between zones.*

nd : not determined because experimental data did not allowed it.

[x] *Only was possible to recover presence or absence of Rhizopus spp. fungus in each Petri plate. Data shown the average* Rhizopus *spp. presence per plate.*

Table 4. Fungal population (cfu/plate) on the surface of different zones at the packinghouse B.

Zones	Fungal population								
	***Monilinia* spp.**	***Penicillium* spp.**	***Fusarium* spp.**	***Cladosporium* spp.**	***Aspergillus* spp.**	***Alternaria* spp.**	**Others**	**Total**	***Rhizopus* spp.**[x]
Precooling room	0.00	0.90 b	0.38 ab	0.97 ab	0.39	0.35	0.73 b	3.33	0.33
Chamber	0.00	0.71 a	0.36 ab	0.81 ab	0.37	0.57	1.17 ab	3.64	0.36
Waiting room	0.00	3.07 ab	0.67 a	0.97 ab	0.59	0.45	1.03 ab	6.28	0.30
Dirty zone	0.00	2.43 ab	0.32 ab	1.43 ab	0.33	0.17	1.63 ab	6.07	0.45
Clean container	0.00	2.34 ab	0.10 ab	2.92 a	0.09	0.41	1.83 ab	7.48	0.55
Dirty container	0.00	1.14 ab	0.34 ab	0.19 ab	0.29	0.17	0.59 b	2.86	0.76
Dry line	0.03	0.79 b	0.42 ab	2.46 a	0.34	0.10	1.35 ab	5.42	0.54
Wet line	0.00	0.95 b	0.05 ab	1.64 ab	0.06	0.33	0.60 b	3.64	0.52
Clean zone	0.00	3.97 ab	0.05 ab	0.00 c	0.04	0.02	1.25 ab	5.33	0.68
Dry line	0.00	0.36 b	0.05 b	0.31 ab	0.03	0.55	2.64 a	4.02	0.48
Wet line	0.00	0.51 b	0.03 b	0.51 ab	0.02	0.00	2.13 ab	3.59	0.56
Self life room	0.00	4.00 ab	0.05 ab	0.10 c	0.05	0.03	0.43 b	4.65	0.65
Shipping room	0.00	5.92 b	0.03 ab	0.05 c	0.04	0.05	1.28 ab	7.43	0.32
Welch Test[1]	nd	< 0.0001	0.034	0.026	0.053	nd	< 0.0001	0.105	nd
Chi-Square Test[2]	0.466	< 0.0001	0.047	< 0.001	0.010	< 0.001	< 0.001	nd	< 0.0001

Each value is the mean of six Rodac plates of each zone, all sampling days and two years.

[1]*Welch Test is a nonparametric test to compare quantitatively the fungal population between zones. Means of each fungus followed by the same letter are not significantly different (Post Hoc: Tukey test < 0.05).*

[2]*Chi-Square Test compares the presence and absence of fungal population between zones.*

nd : not determined because experimental data did not allowed it.

[x] *Only was possible to recover presence or absence of Rhizopus spp. fungus in each Petri plate. Data shown the average* Rhizopus *spp. presence per plate.*

Among the colonies that were recovered from the Rodac dishes on the surfaces of packinghouse B, the percentages were: 42.8% *Penicillium*, 15.1% *Cladosporium*, 4.1% *Alternaria*, 4% *Fusarium* and 2.5% *Aspergillus*. The remaining 31.5% of genera belonged to *Monilinia*, *Geotrichum*, *Botrytis*, and other fungi genera. No differences were found between the total fungi recovered and each zone sampled (Table 4). The main pathogens that were present on the surfaces the packinghouse zones were *Penicillium*, followed by *Cladosporium*, and *Alternaria*. Significant differences were found in the presence or absence of *Rhizopus* throughout packinghouse B. One example of *Monilinia* conidia was detected on the surface of the dry line during 2013. In contrast, *Monilinia* was not detected during 2012.

4. Discussion

To the best of our knowledge, this study is the first attempt to determinate the fungal population in the environment and on the surfaces of stone fruit packinghouses. Other studies have been reported in citrus packinghouses (Palou et al., 2001, Fischer, 2008). There is no general criterion that enables us to distinguish the critical limits of fungal amount from which there is an inadmissible high risk of infection. However, in a study carried out in packinghouses by Orihuel et al. (1996), it was proposed that the maximum concentration of 0.7 cfu cm^{-2} was in evidence, following sanitation processes. The averages of fungal population on the surface were 0.30 ufc cm^{-2} for packinghouse A, and 0.20 ufc cm^{-2} for packinghouse B, which is lower than the proposed critical limit. Also, this is lower than the average of the fungal population recovered on the surfaces of citrus packinghouses that were sampled in Spain (Palou et al., 2001) with 1.7 cfu cm^{-2}, and that were sampled in Brazil (Fischer et al., 2008) with 1.9 cfu cm^{-2}. Environment sanitation procedures of packinghouses from the Lleida area, are usually undertaken before and after the season; however, the surfaces of sorting lines or containers are cleaned more frequently, at a rate of at least once per week.

In the environment of packinghouse A, the dirty zone was statistically more polluted than other zones, and on their surfaces the average number of colonies on the clean zone, line 1 and line 2 were less polluted than other zones. The same trend was observed for packinghouse B, although nonparametric statistics found no significance differences. It has been recommended that the citrus industry should aim at designing facilities in a manner that would maintain separate clean zones (fruit after-washing or packaging) and dirty zones (fruit reception or fist manual selection) (Palou, 2011). The selected stone fruit packinghouses have separated zones; however, fungal contamination on surfaces of packinghouses was higher in the shipping room than in

the dirty room and the clean room, which likely aids the development of infections among the stored fruit.

Penicillium spp. and *Cladosporium* spp. were the most frequent genera that were consistently present in the environment and on surfaces that were sampled in the packinghouses. This result agrees with previous studies in citrus packinghouses (Palou et al., 2001; Fischer et al., 2008), on stone fruit mummies (Hong et al., 2000), on commercial fruit surfaces (Watanabe et al., 2011), on sweet cherry grading lines (Borve, 2014), and within the interiors of food production facilities of such as yogurt, canned or sweet products, among others (Şimşekli et al., 1999). Although *Penicillium* and *Cladosporium* were the genus that were abundantly recovered, their postharvest disease incidence on stone fruit was usually low (Borve, 2014), because only *P. expansum* and *C. herbarum* species are responsible for postharvest decay on stone fruit (Sommer, 1989).

Rhizopus spp. (note that we also include *Mucor* spp.) was widely detected over time on surfaces of both packinghouses A and B in 60% and 50% of Rodac plates, respectively. *Rhizopus* is a genus that is abundantly recovered from other sampling studies in citrus packinghouses in Spain (Palou et al., 2001). Rhizopus rot, caused by *Rhizopus stolonifer*, is one of the most destructive postharvest diseases of stone fruits. The high presence of *Rhizopus* spp. could result in new postharvest risks of infection, and important stone fruit losses due to its fast growth.

The most important postharvest disease affecting stone fruit in the Ebro Valley area of Spain, and in many other production areas around the world is brown rot, caused by *M. fructicola* and *M. laxa* (Villarino et al., 2013). In the present study, *Monilinia* spp. was rarely recovered in all of the sampling zones, and this was an unexpected result. For the period from June to September of 2012, the ambient conditions were dry and warm and for the same period of 2013 season the ambient conditions were much wet. The higher number of *Monilinia* colonies during 2013 can be attributed to those weather conditions, although its presence was very low in any case. Our results suggest that the risk of fruit infection by *Monilinia* spp. inside packinghouses is low and, therefore, the great majority of infected fruit in packinghouses comes from the orchards.

5. Acknowledgements

This study was supported by the Ministry of Economy and Competitiveness (Government of Spain) with project AGL2011-30472-C02-02 and by a PhD grant BES-2012-059949 for Maria Bernat. The authors are also grateful to both

packinghouses where the study was carried out. We thank Marta Sisteré for her help at the packinghouses and Jorge R. Sanchez for his statistical support.

6. References

Borve, J., 2014. Fungal contamination of fruit in sweet cherry grading lines. In: Acta Horticulturae. International Society for Horticultural Science (ISHS), Leuven, Belgium, 127-130.

Crisosto, C. H., Kader, A. A., 2014. Peach. In: The commercial storage of fruit, vegetables, and florist and nursery stocks. http://www.ba.ars.usda.gov/hb2066/peach.pdf. Acceded 5 October 2015.

Fischer, I. H., Lourenço, S. A., Spósito, M. B., Amorim, L., 2008. Characterisation of the fungal population in citrus packing houses. European Journal of Plant Pathology, **123**, 449-460.

Hong, C. X., Michailides T. J., Holtz, B. A., 2000. Mycoflora of stone fruit mummies in California orchards. Plant Disease, **84**, 417-422.

Orihuel, B., Canet J. J., Bertó, R., 1996. Límites críticos y contaminación fúngica de superficies en una central hortofrutícola. Fruticultura profesional, **83**, 114-118.

Palou, L., 2011. Control integrado no contaminante de enfermedades de poscosecha (CINCEP): Nuevo paradigma para el sector español de los cítricos Levante Agrícola: Revista internacional de cítricos, **406**, 173-183.

Palou, L., Usall, J., Pons, J., Cerdà, M. C., Viñas, I., 2001. Micoflora en centrales citrícolas de Tarragona. Revista investigación agraria. Producción protección vegetal., **16**, 447-462.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Samson, R. A., Hoekstra, E. S., Frisvad, J. C., Filtenborg, O., 1981. Introduction to food-borne fungi. Centraalbureau voor Schimmelcultures, Institute of the Royal Netherlands, Academy of Arts and Sciences.

Şimşekli, Y., Gücin, F., Asan, A., 1999. Isolation and identification of indoor airborne fungal contaminants of food production facilities and warehouses in Bursa, Turkey. Aerobiologia, **15**, 225-231.

Usall, J., Torres, R., Viñas, I., Abadias, M., Teixidó, N., 2013. Principales enfermedades de postcosecha y su control. In: Poscosecha de pera, manzana y melocotón. Mundiprensa, Madrid, Spain, 247-280.

Villarino, M., Eguen, B., Lamarca, N., Segarra, J., Usall, J., Melgarejo, P., De Cal, A., 2013. Occurrence of *Monilinia laxa* and *M. fructigena* after introduction of *M. fructicola* in peach orchards in Spain. European Journal of Plant Pathology, **137**, 835-845.

Villarino, M., Melgarejo, P., Usall, J., Segarra, J., Lamarca, N., De Cal, A., 2012. Secondary inoculum dynamics of *Monilinia* spp. and relationship to the incidence of postharvest brown rot in peaches and the weather conditions during the growing season. European Journal of Plant Pathology, **133**, 585-598.

Watanabe, M., Tsutsumi, F., Konuma, R., Lee, K. I., Kawarada, K., Sugita-Konishi, Y., Kumagai, S., Takatori, K., Konuma, H., Hara-Kudo, Y., 2011. Quantitative analysis of mycoflora on commercial domestic fruits in Japan. Journal of Food Protection, **74**, 1488-1499.

Wild, B. L., Eckert, J. W., 1982. Synergy between a benzimidanole-sensitive isolate and benzimidazole-resistant isolates of *Penicillium digitatum*. Phytopathology, **72**, 1329-1332.