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Assessment of *Penicillium expansum* and *Escherichia coli* transfer from poplar crates to apples

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ABSTRACT

Poplar crates are widely used in contact with foodstuffs. One of the major markets for poplar crates is apple packaging. As a direct food contact material, wood is subject to European regulation n° 1935/2004, which requires no negative effect of material or packaging on foodstuffs. This study aims to quantify the transfer of *Penicillium expansum* conidia and *Escherichia coli* from poplar specimens to Golden apples. This [microorganisms–poplar–Golden apple] model was developed taking into account realistic parameters with extreme and usual conditions. Poplar specimens were artificially contaminated with *P. expansum* and *E. coli*. They were then put in contact with apples and stored in a climatic room for up to one week. Both wood specimens and apples were analyzed to assess the survival of microorganisms on wood specimens and the proportion of microorganism transfer to apples. Whereas *P. expansum* conidia survived but did not grow on wood specimens, *E. coli* mortality was observed after one hour of contact with wood. The proportion of both microorganisms transferred to apples was less than 0.25%. The impact of wood moisture content, contact time between wood and apples, and apple supply variability on the transfer levels were studied. The hygroscopic properties of wood were discussed. The low and reproducible transfer levels observed demonstrate that poplar is a safe packaging material for apples.

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1. Introduction

As a traditional and natural material, wood is widely used in the food industry and in home kitchens. Wooden utensils, packaging and working surfaces, such as cutting boards and ripening shelves, are used for their “technological”, mechanical or hygroscopic properties. Thus, some hardwood and softwood species, like oak, cherry tree and spruce, are considered as “technological tools”. They contribute to the organoleptic qualities of wine (Gougeon et al., 2009), vinegars (Callejón, Torijab, Masb, Moralesa, & Troncosoa, 2010) and cheese ripening due to their hygroscopic and microbiological properties (Mariani et al., 2011; Richard, 1997).

Moreover, Filip et al. carried out microbiological analysis by swabbing 105 samples of wooden trays (*Abies* spp.) and 105 of plastic trays (PET) used for drying fresh pasta (Filip, Fink, Oder, & Jevšnik, 2012). The authors showed that the total number of microorganisms (CFU/20 cm²) was significantly lower on wooden frames compared to plastic frames and that 30% of swabs sampled from plastic frames exceeded 200 CFU/20 cm², whereas the value for wooden frames was only 3%. Secondly, *Escherichia coli* was not detected among the 210 samples, *Staphylococcus aureus* was significantly lower on wooden (3% positive swabs) than on plastic frames (54% positive swabs). The levels of moulds and yeasts were significantly lower on wooden frames compared to plastic frames. With these results the authors concluded that wood is appropriate in the food industry from a hygienic and technological point of view.

Because of their lightness, their mechanical resistance to shocks and their high tolerance to moisture, hardwood and sapwood species are chosen by manufacturers. They are selected to make

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crates, boxes and pallets for packaging foodstuffs (such as fruits, vegetables, fish, shellfish and cheeses). In France, more than 20 wood species are authorized by the French ministerial order of November 1945 (Anonymous, 1945) and the information note of the Economy and Finance French Ministry (Anonymous, 2012) for use in direct contact with solid or liquid food products.

In France, fruit and vegetables is the main food sector using wooden packaging, generally made from poplar, maritime pine, scots pine, spruce and douglas pine. Poplar is particularly common for manufacturing light packaging such as crates intended for fruits and vegetables.

As a direct food contact material, wood can be subject to specific regulations. For example, in Europe, regulation ECR 1935/2004 (1935/2004, 2004) specifies that materials intended for safe food contact must not modify foodstuff characteristics.

In order to check the ability of surfaces to be in direct contact with food, microbiological tools are needed. Analyses of packaging material can be used as quality control procedures thus preventing the contamination of foods (Kusumaningrum, Riboldi, Hazeleger, & Beumer, 2003) by cross-contamination events. In fact, food contact surfaces have been shown to be responsible for such cross-contamination (Sanborn, 1963). Other studies have described the survival of *Listeria monocytogenes* (S. A. Wilks, Michels, & Keevil, 2006) and *E. coli* O157 (S. Wilks, Michels, & Keevil, 2005) on stainless steel surfaces. In 2011, Pui et al. reported the transfer of *Salmonella* spp. from plastic surfaces to dragon fruits (Pui et al., 2011). However, studies on wooden packaging are lacking.

The aim of this study was to assess the microbiological transfer from wooden packaging to food in an accurate “food-wood” contact model. For this purpose, we studied and quantified transfer levels from inoculated poplar crates, as contact surfaces, to Golden apples. We chose two relevant hygienic risk models. Firstly, *Penicillium expansum*, is an important spoilage Eurotiomycetes in the fruit sector, particularly for apples and pears (Amiri, Cholodowski, & Bompeix, 2005). The mold *P. expansum* is known to be responsible for one of the major postharvest decay of apples called blue mold (Conway et al., 2004; Ikeura, Somsak, Kobayashi, Kanlayanarat, & Hayata, 2011; Xiao, Kim, & Boal, 2014) and of several fresh fruits like tomatoes (Chatterton, Wylie, & Punja, 2012), peaches (Liu, Jiang, Bi, & Luo, 2005; Yu et al., 2012), winter pear (Sanderson & Spotts, 1995), jujube fruits (Cao, Li, Tian, & Qin, 2012; Liting Wang, Wu, Qin, & Meng, 2014), date palm fruits (Palou, Montesinos-Herrero, Taberner, & Vilella-Esplá, 2013) and sweet cherries (Lei Wang et al., 2015). Secondly, *E. coli*, a gram-negative bacterium, is often used as an indicator of general hygienic practices in the food industry. *coli* 157:H7 was found to be responsible for causing diarrhea in people having ingested freshly-pressed apple cider (Richard, 1997). Several studies have demonstrated that *E. coli*, and mostly serotype O157:H7, can colonize apple surfaces after aqueous contamination (Buchanan, Edelson, Miller, & Sapers, 1999) or when contaminated water is used in washing or processing fruits (Vojdani, Beuchat, & Tauxe, 2008). Many scientific studies have demonstrated that *E. coli* can survive and/or grow in several minimally processed fruits, such as peaches (Alegre, Abadias, Anguera, Usall, & Viñas, 2010), pineapples (Sivapalasingam, Friedman, Cohen, & Tauxe, 2004), honeydew melons (Leverentz, Conway, Janisiewicz, & Camp, 2004), apples (Alegre et al., 2010; Janisiewicz, Conway, & Leverentz, 1999) and bruised apples (Dingman, 2000).

To study the microorganism transfer from wood to apples, we used a new method that was developed to quantify microorganisms in the wood matrix (Ismail et al., 2014). The influence of different parameters was explored by their impact on the transfer rate of *P. expansum* spores and non-pathogenic *E. coli*. Different contact times between inoculated poplar samples and apples were

tested. Different wood moisture contents were compared.

2. Materials and methods

The [microorganisms-poplar-Golden apple] model was developed taking into account realistic parameters of the use of packaging crates for Golden apples. The usual conditions and duration of storage were used for this model. An “extreme scenario” was considered by selecting a high level of microorganism inoculation on wood, a high level of wood moisture content (37%) to increase the survival of microorganisms, and a long time of contact between wood and apples to obtain optimal conditions for the transfer of microorganisms to apples.

2.1. Wood specimens and apples

Populus x euramericana (I214 clone) poplar sapwood strips were freshly rotary cut to a thickness of 0.4 cm (Bois Diffusion, Valanjou, France). Wood specimens measuring 60 × 40 mm (corresponding to a surface area of 24 cm²) were cut from these strips. After 14 days of conditioning in a climatic chamber at 20 °C ± 2 °C and 65% ± 5% relative humidity (RH), wood specimens were packed in sealed bags and sterilized by ionizing radiation (Gamma rays, 25 kGy) prior to use (Ionisos, Dagneux, France).

Golden Delicious apples from two French suppliers were used in this study. They were stored at 4 °C ± 2 °C and disinfected with 70% ethanol prior to use. As a control, contact plates of PCA medium (tryptone 5 g/L, yeast extract 2.5 g/L, glucose 1 g/L) were applied for 10 s to the surface of apples and incubated at 22 °C ± 2 °C for 48 h to ensure that disinfection had been fully carried out.

2.2. Determination of contact surface area between wood specimens and apples

Apples (Golden, diameter 70) were stained in a black painting bath for 30 s. After 15 min of draining, 8 kg of apples was placed in a poplar crate, randomly, to reproduce “food-packaging” contacts in real conditions. Apples remained in contact with the wooden packaging for 5 min. Then, they were carefully removed in order to have no impact on the apple contact mark. This labeling step was repeated on 3 different wooden crates in order to obtain an average contact area. Moreover, an isolated apple was stained as described above and put in contact with a poplar strip to define the area of a single contact point.

After drying for 24 h, black and white pictures of marked packaging were taken using a CANON EOS 550D camera on a tripod. Pictures of labeled packaging were taken on a white background while those of the control packaging (unmarked) were taken on a black background. In both cases, the packaging was disassembled to obtain plane images and to quantify the contact surface area between the apples and the packaging.

The pictures were analyzed using the free ImageJ® software (<http://imagej.nih.gov/ij/>) to quantify the “apples-packaging” contact area. A double-decimeter was photographed in order to calibrate the images analyzed with ImageJ®. A color threshold was applied to the pictures so that ImageJ® only took into account the black surfaces of the labeled packaging to quantify the real contact area. However, ImageJ® took into account all the white surface of the control packaging to quantify the total internal surface area of a wooden crate.

2.3. Microorganism strains and inoculum preparation

E. coli strain (ATCC-700926) was stored in beads containing cryotubes at −80 °C. To prepare the inoculum solution, two beads

of a cryotube were suspended in 10 mL of PCA medium for 24 h at 37 °C and 180 rpm. 1 mL of this sub-culture was then inoculated in 100 mL of PCA for 24 h at 37 °C and 180 rpm. The concentration of this culture was measured by spectrophotometry (Spectronic® 20 Genesys™, Spectronic Instruments, United Kingdom) at 620 nm to standardize bacterial inoculum and was expected to be at 1×10^8 CFU/mL. This solution was used to inoculate wood specimens and to prepare dilutions in sterile water with 0.9% NaCl. A concentration of the inoculum of 8×10^7 cells/mL was chosen, according to Ismail et al. (2014).

P. expansum strain (ATCC-7861) was allowed to grow on malt/agar medium (malt 40 g/L; agar 20 g/L). A spore suspension was generated by growing *P. expansum* on 4 malt/agar plates for 2 weeks at 22 °C. 10 mL of water with 0.9% NaCl and 0.05% Tween® was added to each plate and spores were then collected in an Erlenmeyer flask containing beads. After filtration through sterile gauze, the spore suspension was rinsed three times in 10 mL of sterile water by centrifuging for 20 min at 2000g. Spores were then counted on a Malassez cell to prepare a suspension of 8×10^7 spores/mL. This was used to inoculate wood specimens after dilutions in sterile water with 0.9% NaCl. A concentration of the inoculum of 8×10^7 conidia/mL was chosen, according to Ismail et al. (2014).

2.4. Moisture content of wood specimens

A moisture content of 37% mass/mass was tested as this represents the usual “wet packaging” storage conditions. To obtain this moisture content, wood specimens were soaked in sterilized water for 1 min and weighed. They were then put in an oven at 103 °C for 48 h to determine their dry mass. The moisture content was then measured as described in Ismail et al. (2014) to ensure that wood specimens had a 37% moisture content.

An additional series of wood specimens was prepared with a moisture content of 18% m/m. This represents the usual “dry packaging” conditions of poplar crates. To obtain this moisture content, wood specimens were soaked in sterilized water for 30 s and weighed. They were then put in an oven at 103 °C for 48 h to determine their dry mass. The moisture content was then measured as described in Ismail et al. (2014) to ensure that wood specimens had 18% moisture content.

2.5. Viability control of inocula

A viability control was carried out to determine the concentration of viable microorganisms in tested solutions.

For *P. expansum*, inoculum viability was assessed by spreading 100 µL of serial dilutions on malt/agar plates (malt 40 g/L; agar 20 g/L). After incubation for 48 h at 22 °C, colonies were enumerated to determine the inoculum viability.

For *E. coli*, inoculum viability was assessed by putting 1 mL of serial dilutions on PCA plates. After incubation for 24 h at 37 °C, colonies were enumerated to determine the inoculum viability.

2.6. Inoculation of wood specimens

Sterile wood specimens of 60 × 40 mm, corresponding to a surface area of 24 cm², were used. Each poplar specimen of 18% or 37% moisture content was inoculated in sterile conditions with 300 µL of inocula. After 15 min of inoculum static impregnation, specimens were incubated alone or in contact with an apple for 1 h at room temperature, or 1 week at 10 °C and 85% RH. These conditions correspond to optimum storage conditions in a cold room (http://www.fruits-et-legumes.net/revue_en_ligne/point_sur/fich_pdf/ECO/StockageFLEco.pdf). The wood specimens were

grinded, as already described (Ismail et al., 2014) at different steps: immediately after 15 min of inoculum impregnation (T0), after 1 h of contact with an apple, after 1 week of contact with an apple.

2.7. Inoculation of plates

To ensure that apples did not affect the transfer of microorganisms, PCA plates and malt agar plates of 24 cm² were inoculated in sterile conditions with 300 µL of *E. coli* inoculum and 300 µL of *P. expansum* inoculum, respectively. After 15 min of stabilization, each plate was incubated in contact with an apple. For *P. expansum*, incubation of 1 week at 10 °C and 85% RH was carried out as it is representative of storage conditions in a cold room. For *E. coli*, incubation of 15 min at ambient temperature was carried out to avoid the development of *E. coli* on plates.

2.8. Microbial analysis

After incubation, microorganisms from wood specimens were recovered by grinding as already described in Ismail et al. (2014). The recovery solutions were inoculated by sowing 1 mL of serial dilutions on PCA (for *E. coli*) or 100 µL of serial dilutions on malt/agar (for *P. expansum*). After incubation at 22 °C for *P. expansum* or 37 °C for *E. coli*, colonies were enumerated.

After incubation, apples were peeled using a sterile stainless steel potato peeler to extract microorganisms. 5 cm² of peel, corresponding to the contact surface area with wood specimens, was blended for 2 min using a Stomacher® 80 (Seward, United Kingdom) in 6 mL of sterile water with 0.9% NaCl. Then suspensions were inoculated by sowing 1 mL of serial dilutions on PCA (for *E. coli*) or 100 µL of serial dilutions on malt/agar (for *P. expansum*). After incubation at 22 °C for *P. expansum* or 37 °C for *E. coli*, colonies were enumerated.

2.9. Statistical analysis

The experiments described above were performed in triplicate. Results, expressed in CFU/cm² and transformed into log₁₀ scale, correspond to the mean value ± standard deviation. They were analyzed using the Student t-test ($p < 0.05$) with Minitab® statistical software 16 (Minitab® Inc.). The transfer level (%) was expressed as follows: (CFU/cm² recovered on apples after incubation) × 100/(CFU/cm² inoculated on wood specimens at T0).

3. Results

3.1. Microorganism recovery on poplar specimens

To assess the viability of *E. coli* and *P. expansum* on wood, suspensions of 4.14 log CFU/cm² and 5.57 log CFU/cm², respectively, were inoculated on wood specimens at 37% moisture content. Microbial analyses were performed on wood specimens immediately after inoculation (T0), after 1 h of incubation at room temperature, or 1 week at 10 °C and 85% of relative humidity. The results are presented in Fig. 1. At T0, after the microbiological extraction from wood specimens, 3.95 log₁₀ CFU of *E. coli*/cm² and 5.36 log₁₀ CFU of *P. expansum*/cm² were recovered. In comparison with the amount of viable *E. coli* and *P. expansum* inocula, these results confirm the reliable recovery rates obtained with the chosen extraction method described by Ismail et al. (2014) with both gram-negative bacteria and fungal spores.

3.2. Microorganism survival on poplar

The results in Fig. 1 suggest that *P. expansum* conidia could

Fig. 1. Viability of *E. coli* or *P. expansum* on wood specimens after 1 h and 1 week of incubation. Enumeration corresponds to the mean value of 3 different apple surface enumerations ($n = 3$). * means significantly different from result obtained at T0 ($p < 0.05$, Student t-test).

survive on wood specimens even after 1 week (at 10 °C and 85% of relative humidity), as between 4.94 and 5 log₁₀ CFU/cm² were recovered whatever the incubation time. However, conidia of *Penicillium expansum* did not grow. For *E. coli*, the results after 1 h and 1 week were significantly different from those obtained at T0. They suggest that *E. coli* could not survive on poplar under these conditions.

3.3. Determination of the contact surface area between wooden crates and apples

ImageJ© software was used to quantify the total internal surface area of a poplar wooden crate used for apple storage: 3686.232 cm². Fig. 2 represents the picture of the bottom of a poplar crate after contact with colored apples. All the “black surfaces” were analyzed by ImageJ© to calculate the real contact surface between the bottom of a poplar crate and apples. The average contact area (3 replicates) between a poplar wooden crate and 8 kg of Golden apples was 411.36 cm² ± 9.17. Clearly, the repeatability of this test was not maximal (SD = 9.17), due to the random disposition of apples in the wooden crates.

ImageJ© software was used to quantify the area of a single point contact of Golden apples on poplar: 2.76 cm². This represents only

Fig. 2. Picture of the bottom of the poplar crate after contact with colored apples. Black surfaces represented the real contact surface between the bottom of the poplar crate and the apples in direct contact. This surface area was analyzed by ImageJ©.

Fig. 3. Enumeration of *E. coli* or *P. expansum* on poplar specimens and apples after 1 h and 1 week. Enumeration corresponds to the mean value of 3 different wood specimen enumerations or of 3 different apple surface enumerations. * means significantly different from result obtained at T0 ($p < 0.05$, Student t-test).

1.51% of the total surface area of one apple.

So, in this study, we chose to consider that one contact point between an apple and wood had a surface area of 3 cm² to facilitate the tests, and we chose to analyze 5 cm² of apple peel to cover this contact area completely and to obtain robust results.

3.4. Transfer of microorganisms from poplar specimens to apples

To assess the transfer of *E. coli* and *P. expansum* from wood to apples, suspensions of 4.14 log₁₀ CFU/cm² and 5.57 log₁₀ CFU/cm², respectively, were inoculated in sterile conditions on wood specimens at 37% of moisture content. Apples were then put in contact with wood specimens. After incubation for 1 h at room temperature or 1 week at 10 °C and 85% RH, microbial analyses were performed on wood specimens and on apples to assess the proportion of transfer. The results are presented in Fig. 3. Firstly, analyses of microorganisms on wood specimens in contact with apples were performed. For *E. coli*, the results after 1 week were significantly different from those obtained at T0. For *P. expansum*, from 4.76 to 5.36 log₁₀ CFU/cm² were recovered on wood specimens, whatever the incubation time. These results, coupled with the data of Fig. 1, suggest that the recovery of these two microorganisms from wood specimens was not affected by contact with apples.

Then, analyses were performed on apples (Fig. 3). For *E. coli*, the recoveries were lower than the detection level of the method, suggesting that *E. coli* was not transferred from wood to apples. The corresponding transfer rates (see Table 3) after 1 h or 1 week, which were very low (0.008%), confirmed this hypothesis. For *P. expansum*, 3.21 log₁₀ CFU/cm² were recovered from apples after 1 h and 2.49 log₁₀ CFU/cm² after 1 week. More than 2 log₁₀ CFU/cm² were lost between the recovery on wood at T0 and the recovery on apples after 1 h or 1 week. Corresponding transfer rates (Table 3) after 1 h or 1 week were between 0.08% and 0.25%. These results demonstrate that *E. coli* did not seem to be transferred from wood to

Table 1

Control of transfer – Enumeration of *E. coli* or *P. expansum* on apples after incubation on inoculated agar plates. Enumeration corresponds to the mean value of 3 different apple surface enumerations.

Microorganism	<i>E. coli</i>	<i>P. expansum</i>
Inoculum on agar plates log ₁₀ (CFU/cm ²)	4.11	5.56
Enumeration on apples log ₁₀ (CFU/cm ²)	4.16 ± 0.40	5.30 ± 0.62

Table 2

Enumeration (mean value $\pm$ standard deviation) of *P. expansum* on wood specimens and on apples after 1 week of incubation according to the moisture content of wood. Enumeration corresponds to the mean value of 3 different apple surface enumerations or of 3 different wood specimen enumerations.

Moisture content of wood	18% mass/mass	37% mass/mass
Enumeration on wood specimens without contact Log ₁₀ (CFU/cm ²)	5.49 $\pm$ 0.04	4.94 $\pm$ 0.63
Enumeration on wood specimens after contact with an apple Log ₁₀ (CFU/cm ²)	5.44 $\pm$ 0.15	5.07 $\pm$ 0.31
Enumeration on apples Log ₁₀ (CFU/cm ²)	3.07 $\pm$ 0.65	2.49 $\pm$ 0.08

apples while the transfer of *P. expansum* from poplar to apples was very low.

The results with contact plates applied to the tested Golden apples were negative, demonstrating that disinfection had been fully carried out (data not shown).

To ensure that apple peel does not affect the growth of *E. coli* or *P. expansum* and thus their transfer, control samples were carried out. Plates were inoculated with suspensions of microorganisms and put in contact with apples. After incubation, microbial analyses of apples were carried out (Table 1). For *E. coli*, 4.11 log₁₀ CFU/cm² were inoculated and 4.16 log₁₀ CFU/cm² were recovered on apples. For *P. expansum*, 5.56 log₁₀ CFU/cm² were inoculated and 5.30 log₁₀ CFU/cm² were recovered on apples. These results demonstrate that when microorganisms were inoculated on PCA plates, the transfer to apples was not affected by the peel for both microorganism. Apples did not affect the observed transfer rates.

3.5. Impact of contact duration between inoculated wood and apples on the transfer level

Whatever the contact time considered (1 h or 1 week) between wood inoculated with *E. coli* and apples, the transfer rate was lower than 0.008% (Table 3).

The transfer of *P. expansum* from wood exposed to apples for 1 h was 0.25%, whereas it was 0.08% from wood exposed to apples for 1 week.

3.6. Impact of wood moisture content on the transfer rate

Considering that only the transfer of *P. expansum* from wood specimens to apples was over the detection level, the influence of wood moisture content on transfer rate was assessed for this

Table 3

Transfer rates obtained from wood to apples, in the three separate experiments managed during this study.

Experiment	Condition (wood moisture content – contact time)	Microorganism	Transfer rate (%)
Impact of microorganisms and impact of contact time between wood and apple specimens	37%	<i>E. coli</i>	<0.008
	1 h		<0.008
	37%	<i>P. expansum</i>	0.25
	1 h		0.08
Impact of wood moisture content	37%	<i>P. expansum</i>	0.06
	1 h		0.05
	37%	<i>P. expansum</i>	0.07
	1 h		0.05
Impact of apple batch	37% – 1 h	<i>P. expansum</i>	0.10
	Batch 1		0.06
	37% – 1 week	<i>P. expansum</i>	0.06
	Batch 2		0.06

microorganism.

Two wood moisture contents were tested: 18% m/m, corresponding to dry packaging storage conditions and 37% m/m, corresponding to wet packaging storage conditions. 6.64 log₁₀ CFU of *P. expansum*/cm² were inoculated on wood specimens and apples were put in contact with them. After incubation for 1 week at 10 °C and 85% RH, microbial analyses were performed on wood specimens and on apples (Table 2). Our results demonstrate that there was no significant difference ($p < 0.05$) between recovery rates obtained on wood at two different moisture contents (from 4.94 to 5.49 log₁₀ CFU/cm²) nor on apples in contact with wood (from 2.49 to 3.07 log₁₀ CFU/cm²). Corresponding transfer rates from wood to apple (Table 3) were 0.06% and 0.05% for a wood moisture content of 18% and 37%, respectively. This result demonstrates that wood moisture content did not influence the proportion of microorganism transferred from wood to apples.

3.7. Impact of apple batch variability on the transfer rate

To assess whether the variability of apples coming from different suppliers could impact the transfer of *P. expansum* from wood specimens to apples, two batches were tested. A suspension of 6.64 log CFU/cm² was inoculated on wood specimens at 37% of moisture content. Apples were then put in contact with them. After incubation for 1 h at room temperature or 1 week at 10 °C and 85% RH, microbial analyses were performed on wood specimens and on apples to assess the transfer rate (Fig. 4). The results obtained on wood were identical for both batches. On apples, between 3.25 and 3.46 log₁₀ CFU/cm² were recovered for both batches. Corresponding transfer rates (Table 3) ranged from 0.05% to 0.10% after 1 h or 1 week of contact and demonstrated that apple batches did not influence significantly ($p < 0.05$) the transfer of *P. expansum* from wood to apple peel.

Results with contact plates applied on the tested apples were negative, demonstrating that disinfection had been fully carried out

Fig. 4. Enumeration of *P. expansum* on wood specimens and two apple batches after 1 h and 1 week of incubation. Enumeration corresponds to the mean value of 6 different wood specimen enumerations from 2 experiments.

(data not shown).

Taken together, the results from three separate series of 3 replicates (see Table 3) clearly demonstrate that the transfer of *P. expansum* from poplar specimens to apples did not exceed 0.25%, regardless of the contact duration between wood and apples, the wood moisture content and the apple batch considered.

4. Discussion

In this study, *E. coli* and *P. expansum* behaved differently in terms of survival on poplar in the test conditions (Fig. 1). Once inoculated on poplar specimens and incubated at ambient temperature, viable *E. coli* cells decreased drastically after 1 h of contact. For *P. expansum*, conidia remained at $5 \log_{10}$ CFU/cm² after 1 h and 1 week of contact. The hypothesis of bacteria entrapped in the wood matrix explaining the low recovery of *E. coli* is not valid. Indeed, the recovery methodology to extract microorganisms from wood led to $3.95 \log_{10}$ CFU/cm² versus $4.14 \log_{10}$ CFU/cm² inoculated.

However, the mortality of *E. coli* could be explained by the physiological differences between *E. coli* cells and *P. expansum* conidia. *E. coli* is a gram-negative bacterium whereas *P. expansum* conidia are resistant fungal cells. *E. coli* requires a temperature range of 7 °C–37 °C, a minimum water activity (aw) of 0.95 and an optimum pH of 6–7 (Foods, 1996; Presser, Ratkowski, & Ross, 1997). *P. expansum* is more tolerant to drastic conditions than *E. coli* as its conidia require a temperature range of 0 °C–35 °C, a minimum aw of 0.82 and a pH of 2–10 (ANSES, 2011; Pitt, Hocking, & Diane, 2009). One hypothesis could be that poplar exhibits unfavorable conditions such as a low water activity and a pH of 5.8–6.4 (Balatincez et al., 2014). It may be possible that, despite 37% of moisture content, the local moisture on the wood surface was lower as wood is a material that dries easily. This could lead to a water activity lower than the one required for *E. coli*. Revol-Junelles, Miguindou-Mabiala, Roger-Maigné, and Millière (2005) found that the decrease in *E. coli* survival was probably linked to the desiccation of bacterial cells on poplar surfaces, because of its hygroscopic properties and natural wood extractives. Thus, poplar reduced *E. coli* survival when stored at ambient temperature or at 10 °C and 85% RH (Revol-Junelles et al., 2005).

P. expansum conidia survival on poplar was constant whatever the incubation time. These results suggest that the conditions on poplar, such as surface moisture content and pH, allowed *P. expansum* conidia to survive but not to grow (ANSES, 2011; Pitt et al., 2009).

Wood is a rough and porous material. Poplar, in particular, has numerous small pores, almost uniform and evenly distributed throughout a growth ring. Vessels have a diameter from 50 to 100 µm (Jacquot, Trenard, & Dirol, 1973). Transfer and viability of *E. coli* from poplar to apple were lower than for *P. expansum*. This could be explained by the size of each microorganism. *E. coli* is a bacterium, measuring around 2 µm (Zabel & Morrell, 1992), whereas the conidia of *P. expansum* measure 3×5 µm and frequently aggregate to form chains (Larone, 2002). In this way, *E. coli* could penetrate more easily the first 2–3 mm of the wood surface and thus could be less easily transferred to apple. The penetration of bacteria was demonstrated by Mariani et al. in the case of *L. monocytogenes* within spruce boards (Mariani et al., 2011). Although spruce wood is different, the phenomenon exists.

Poplar also contains extractives such as tannins and quinones. These compounds can be found in wood, bark and leaves. They have antimicrobial action, related to their ability to inactivate microbial adhesins or enzymes, to transport proteins and to complex with polysaccharides and thus the microbial cell wall (Cowan, 1999). *E. coli* is a gram-negative bacterium, containing a thin peptidoglycan layer in its cell wall outside the cell membrane and also

transport proteins called porins. Conidia of *P. expansum* possess a thick and tough cell wall. One hypothesis could be that these compounds complex with the cell wall of *E. coli*, leading to a lower viability for this bacterium than for conidia of *P. expansum*.

In this study, transfer rates obtained for *E. coli* and *P. expansum* from poplar to apples were very low, from 0.01% to 0.25% (from 2 to 4 log of loss between wood and apple), after 1 h of incubation time. This result is fully in accordance with previous studies of transfer of microorganisms from wood to food. Transfer of *L. monocytogenes* from wooden cutting boards to chicken meat was strongly decreased, moving from $4.8 \log_{10}$ CFU/mL to $0.4 \log_{10}$ CFU/mL after 1 h of contact time (Goh et al., 2014). A study with several food-borne pathogens was performed to evaluate the transfer rate between stainless steel surfaces and foods. When the contamination level was moderate ($3 \log_{10}$ CFU/cm²), the recovery of *Salmonella enteritidis* decreased below the detection limit ($4 \log_{10}$ CFU/cm²) within 24 h (Kusumaningrum et al., 2003).

In this study, the microorganism transfer from poplar to apple occurred after one hour of contact (between inoculated wood and apple) and remained similar after one week of contact. This suggests a fast and passive transfer for both microorganisms tested in this study.

Differences in transfer rates could occur according to the quality of the foodstuffs considered, even if transfer rates are very low. Transfer rates of *E. coli* O157:H7 strain to common kitchen surfaces were analyzed using celery, carrot, watermelon and lettuce. After 1 h of drying of the inoculated contact surfaces, freshly-cut celery, lettuce and carrots transferred more bacteria than watermelon (Jensen, Friedrich, Harris, Danyluk, & Schaffner, 2013). The growth of this strain is dependent on the type of vegetable considered (Abadias, Alegre, Oliveira, Altisent, & Viñas, 2012). In another study, the transfer of *E. coli* O157:H7 strain from stainless steel to several food products was, on the whole, higher for meat than for vegetables (Silagyi, Kim, Lo, & Wei, 2009). The transmission of *L. monocytogenes* from a wooden board to chicken after 30 min was higher for cooled chicken meat ($3.2 \log$ CFU/mL) than for hot chicken meat ($0.8 \log$ CFU/mL) (Goh et al., 2014).

Transfer rates of microorganisms could also be influenced by the properties of the “donor material” (poplar in our study) and the “receiver material” (apples in our study). Properties of materials such as surface characteristics and hydrophobicity could influence transfer rates. Wood is a rough and porous material and studies indicate that the greater the roughness is, the lower the transfer is (Midelet & Carpentier, 2002; Pérez-Rodríguez, Valero, Carrasco, García, & Zurera, 2008). The transfer of *L. monocytogenes* to beef was higher for biofilms formed on the polymers PU and PVC than for those formed on stainless steel (Midelet & Carpentier, 2002). The recovery of *Salmonella typhimurium* after an absorption time of 10 min was lower on wood than on plastic (Gough & Dodd, 1998). In a study dealing with adhesion and germination of *P. expansum* conidia on several surfaces, a link was established between wettability and attachment: the more the wettability increased, the more conidia adhesion decreased. Thus, attachment was higher on Teflon and polystyrene than on glass (Amiri et al., 2005). Topography and hydrophobic properties of surfaces seem to contribute greatly to transfer levels (Dawson, Han, Cox, Black, & Simmons, 2007; Knobben, van der Mei, van Horn, & Busscher, 2007).

One perspective could be to assess transfer rates of *E. coli* and *P. expansum* from wood to several foodstuffs and to compare transfer rates from several packaging materials to apples. In such a way, a link could be made between the transfer rates obtained and the surface properties of tested materials. Measurements of surface moisture content on wood could lead to a better understanding of *E. coli* mortality. In the meantime, the use of scanning electron microscopy could give added value by enabling the observation of

bacteria cells and fungal conidia on wood specimens and other packaging materials.

5. Conclusion

This study proved that poplar used for crates leads to *E. coli* mortality within less than one week whereas it maintains the survival of *P. expansum* conidia without allowing its growth.

It also gives evidence that microorganisms, such as a gram-negative bacteria, a contaminant during food processing, and fungal spores, responsible for one of the major postharvest decay, do not transfer easily from wood to foodstuffs.

For both *E. coli* and *P. expansum*, and despite a high artificial contamination of poplar, the transfer rate from poplar to apples did not exceed 0.25%, regardless of the wood moisture content, the contact time between inoculated wood and apples, and the apple supply origin.

This low transfer rate is linked to the poplar material and reinforces the good suitability of wood for contact with foodstuffs.

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