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ORIGINAL ARTICLE

Effectiveness of new molecules against widespread moulds for food-safe hardwood and softwood packaging

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Abstract

Wood packaging materials (WPMs) are widely used for collecting, storing and trading a wide range of products, including fresh fruit, vegetables and grains. The occurrence of moulds on WPMs used in the food industry must be avoided at every stage of the supply chain. This study aimed at (1) characterising fungal mould populations developing on fresh boards of hardwoods (European beech and poplar) and softwoods (Norway spruce and eastern white pine) commonly used by the packaging industry, and (2) assessing the effectiveness of two new molecules approved to come in contact with food, potassium sorbate and copper-8-quinolinolate, against mould growth and sporulation. A total of 322 fungal isolates belonging to 182 putatively different morphotypes were obtained. Spruce and beech boards were found to harbour a higher number of putatively different morphotypes compared to poplar and pine. The spectrum of fungi mostly included *Ascomycota* and the most abundant taxa were *Trichoderma* spp. and *Penicillium* spp. The effectiveness of the two new molecules (potassium sorbate approved for the use in both Europe and USA, and copper-8-quinolinolate approved for the use in USA only) was assessed on treated test pieces by inoculating conidial suspensions combining the three most common fungal species for each wooden material. Both preservatives showed comparable effectiveness and significantly reduced ($P < 0.05$) mould mycelial growth and sporulation on all the tested wooden materials compared to untreated controls, representing a suitable option for the control of moulds on WPMs.

1 Introduction

Inefficient post-harvest procedures, including inadequate packaging for the wholesale market of fresh fruit, vegetables and grains, can cause severe economic losses to horticultural markets worldwide (Camargo and Perdas 2002; Cortez et al. 2002; Sharma et al. 2009). A range of materials can be used for fruit and vegetable packaging, including wooden products, corrugated cardboard and plastic. The most suitable packaging depends on the crop, the region, the length and nature of the market, the environmental conditions, the availability and costs of materials and the post-harvest procedures (Vigneault et al. 2006).

Crates, pallets and boxes are the major wood packaging materials (WPMs) for collecting, storing and trading a wide range of products, including fresh fruit, vegetables and grains. Eighty-five percent of goods are transported worldwide either on or within WPMs (FEFPEB Position Statement 2019). WPMs are manufactured of solid wood (e.g., sawn boards, blocks, etc.) or of engineered wood-based products (e.g., plywood, oriented strand board—OSB, medium-density fibreboard—MDF, etc.). Their success worldwide is related to the local availability, price, ease of processing and mechanical performance, considering that the use of wood enables to obtain light and robust structures suited to transport heavy loads (Andreolli et al. 2017). In addition, the environmental impact of WPMs is considerably lower compared to plastic packaging (Bergman et al. 2014; Kočí 2019). For the production of WPMs, a wide range of wood species can be used, including ash (*Fraxinus* spp.), beech (*Fagus* spp.), oak (*Quercus* spp.), and poplar (*Populus*

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spp.) among broadleaves, and pine (*Pinus* spp.) and spruce (*Picea* spp.) among conifers (Aviat et al. 2016).

Moulds are fungi growing on a large variety of substrates (Guynot et al. 2005) and mostly belonging to the genera *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., *Fusarium* spp., *Mucor* spp., *Penicillium* spp., *Rhizopus* spp., and *Trichoderma* spp. (Samson 1985; Grant et al. 1989; Henz and Cardoso 2005; Clausen and Yang 2007; Yang and Clausen 2007). While the establishment of moulds commonly occurs by means of mitospores (i.e., conidia) which are ubiquitous in the air, optimal temperatures, high moisture conditions and nutrient availability may lead to the rapid growth of these fungi on wood and wood product surfaces. A number of papers have been published on this topic, with special emphasis on moulds growing on building materials (e.g., Nielsen 2003; Nielsen et al. 2004; Andersen et al. 2011; Johansson et al. 2012), including their adverse effects on human health (Dales et al. 1998; Koskinen et al. 1999; Purokivi et al. 2001). Recently, Lie et al. (2019) investigated how agar plate screening tests and water uptake tests can predict mould growth on exterior wooden claddings.

Since the 1990s, a number of scientific studies focused on wood in contact with food have been published to investigate the survival and the potential transfer from wood of microorganisms relevant to food hygiene and human health (Aviat et al. 2016 and references therein; Rico-Munoz 2017). Most of them targeted bacteria, while little is known about mould fungi.

Although the wood for hygienic reasons is in general deemed suitable for the use in the food industry, especially if compared with other packaging materials (Worfel et al. 1995; Schönwälder et al. 2000; Fink et al. 2013; Aviat et al. 2016), the occurrence of moulds on WPMs used in the food industry must be avoided at every stage of the supply chain (Sela et al. 2017; Snyder and Worobo 2018). Fungal growth, involving spore germination and hyphal extension eventually forming visible mycelium, makes the wood aesthetically and hygienically unacceptable to carry food products (Filip et al. 2012; Fink et al. 2013; Aviat et al. 2016 and references therein; Rico-Munoz 2017). First of all, moulds can modify organoleptic characteristics of fruit and vegetables and sometimes they are also responsible for post-harvest diseases (Kora et al. 2005). Furthermore, some mould genera such as *Aspergillus*, *Penicillium* and *Fusarium* produce metabolites (e.g., mycotoxins) associated with a range of human diseases (Nielsen 2003; Dao et al. 2008; Egbuta et al. 2017). *Aspergillus niger* Tiegh., *Penicillium chrysogenum* Thom., *P. commune* Thom. and *P. expansum* Link represent only some of the fungal species with potentially relevant implications for human health (Aviat et al. 2016 and references therein; Egbuta et al. 2017). Recently, the occurrence of spores of heat resistant moulds (e.g., *Paecilomyces* spp., *Aspergillus* spp., *Talaromyces* spp.) was demonstrated in food and beverage processing environments; in particular wooden pallets showed the highest spore counts (Rico-Munoz 2017).

Fungal growth is also responsible for the release of mitospores (i.e., conidia), thereby increasing the level of airborne inoculum and the dispersal potential of moulds. Indeed, mitospores of moulds are very tiny and light, which allow them to travel through the air (Carreras 2006; Yang et al. 2007a).

The quantification of microbial contamination of wooden surfaces can be performed by using different methods including agar-contact plate and swabbing (Miller 1996; Lortal et al. 2009) as well as stomacher, ultrasonic sound and brushing methods (Mariani et al. 2007; Le Bayon et al. 2010). The detection threshold of each of these methods depends on their recovery rate considering that wooden surfaces are porous and microorganisms can be trapped within cavities. However, no standard recovery methods are available for wooden surfaces and no scientific evidence shows that trapped microorganisms can be likely transferred to the surface again (Aviat et al. 2016).

In Europe, WPMs like other packaging are subjected to the European Regulation (EC) No (1935/2004) on materials and articles intended to come into contact with food, and to the Commission Regulation (EC) No (2023/2006) on good manufacturing practice for the above materials and articles. Based on these two regulations, contact materials must not transfer their constituents to food and must be manufactured according to the rules on Good Manufacturing Practices (GMPs) in order to preserve human health, to avoid unacceptable changes in the food, and to prevent the deterioration of its organoleptic characteristics. National measures have been adopted to cope with these general principles, providing detailed rules to fit the requirements for the specific uses of the different materials (e.g., DGCCRF 2012).

The International Standard for Phytosanitary Measures ISPM-15 (FAO 2017a) includes measures that are mandatory for importing WPMs into Europe and are intended to eradicate insects and quarantine microorganisms from wooden packaging. The commonly used heat treatment (HT) required by ISPM-15 hinges on exposing wood to a temperature of 56 °C for at least 30 min so that these parameters reach the central core of the material. While this treatment is generally effective in killing insects, it is not expected to prevent the growth of moulds, which rather may be favoured by the treatment conditions. HT process, in fact, draws moisture and sugar (food source) to the surface of the wood, which is also warm and wet: this determines ideal conditions for the growth of mould (Lambertz and Welling 2010; Iline et al. 2014; FAO 2017b). The kiln drying (KD) treatment used for drying wood at a moisture content lower than 15% is generally effective in

Table 1 Aims and outputs of the main steps of the overall experimental design

Step	Aim	Output
1	Characterisation of mould populations on fresh-cut boards for wooden packaging of poplar, European beech, eastern white pine and Norway spruce	Moulds spectrum organised in fungal morphotypes based on macro- and micro-morphological features with the corresponding isolation frequency (IF, see Online Resource 1)
2	Selection and identification of the three major and representative fungal taxa (highest values of IF) for each wood species to be used in the subsequent experiment to test the effectiveness of two new molecules	Nine representative fungal taxa (see Table 2) for subsequent experiment on the effectiveness of two new molecules
3	Inoculation of mixed conidial suspensions of the representative fungal taxa on treated and untreated test pieces of each wood species. The new tested molecules are Celbrite FS2® (potassium sorbate) and PQ-80® (copper-8-quinolinolate)	Estimation of the wood area colonised by the fungal mycelium (% mycelial growth; see Table 3) and quantification of the sporulation ability (conidial concentration conidia/ml; see Table 4)
4	Comparison among treatments through the bootstrap hypothesis testing method carried out on wood area colonized by the fungal mycelium and on conidial concentration	Demonstration of the effectiveness of Celbrite FS2® and PQ-80®. The molecules were comparable in reducing both the mycelial growth and the conidial production of moulds (see statistical results in Tables 3 and 4)

containing fungal growth but it is rather expensive, making it an unsuitable method to avoid fungal contaminations.

An alternative approach to prevent mould growth on wood-based materials relies on treatments with non-toxic and non-volatile fungicides (Clausen and Yang 2007; Yang and Clausen 2007; Reinprecht 2010). An effective fungicide should prevent spore germination and increase the service life of wooden products under conditions of high humidity. Eco-friendly treatments based on the use of biological control agents or of natural extracts (e.g., plant extracts) represent intriguing alternatives to use of chemicals (Yang and Rossignol 1999; Yang et al. 2007a, b). However, when used on wood materials expected to come in contact with food, any treatment regardless of its origin must be sufficiently safe. In the last decades, experiments were performed to assess the effectiveness of different plant-associated substances such as flavonoids, tannins, and essential oils as safe preservatives against moulds, yeasts and bacteria (Rauha et al. 2000; Plumed-Ferrer et al. 2013). Laboratory tests were performed to assess the effectiveness of condensed tannins and potassium sorbate in reducing mycelial growth and sporulation of four fast-growing mould species [*Penicillium chrysogenum*, *Trichoderma longibrachiatum* Rifai, *Cladosporium cladosporioides* (Fresen) G. A. de Vries, and *Aspergillus niger*] developing on high-density fibreboard (HDF) used for the production of crates for fruit and vegetables (Giordano et al. 2011). The patterns of establishment and growth of moulds, in fact, can be considered similar in solid wood and wood-based products such as HDF.

In this study, fungal mould populations developing on boards obtained from the main hardwoods and softwoods used in the packaging industry were characterised. To the best of the authors' knowledge, this is the first extensive investigation on mould associated with unseasoned boards (moisture content > 30%) for wooden packaging.

In addition, the effectiveness of two new molecules approved to come in contact with food was assessed in reducing mycelial growth and sporulation of the most common mould species on wooden packaging. This can be relevant to protect wooden packaging during storage after ISPM-15 heat treatment that, when performed on unseasoned timber, can lead to conditions favourable to the development of moulds. Experiments were performed in more extreme conditions, in terms of temperature and relative humidity, than that occurring in post-harvest to maximise moulds growth and sporulation.

2 Materials and methods

The overall experimental design is summarised in Table 1.

2.1 Wood material, fungal isolations and identification

The characterization of mould populations was conducted on fresh boards of $0.39 \times 0.08 \times 0.025$ m (length, width, thickness) in size of four wood species commonly used by the packaging industry: two hardwoods, i.e. poplar (*Populus* spp.) and European beech (*Fagus sylvatica* L.), and two softwoods, i.e. eastern white pine (*Pinus strobus* L.) and Norway spruce [*Picea abies* (L.) Karst.]. Three to ten boards per each of the wood species were randomly sampled from eight wood-packaging manufacturers located in Northern Italy. The boards were directly taken from the

manufacturers' yards where they were stored in open-air for periods ranging from few days to some weeks, so that their moisture content was always > 30%. To avoid cross-contaminations, boards were singly placed in plastic bags, transferred to the laboratory and stored at 5 °C prior to testing.

Subsequently, the boards were individually sealed in a new plastic bag and incubated horizontally in the dark for 2 weeks in a growth chamber set at a temperature of 25 ± 1 °C. Sterilized filter papers dampened with sterile water were included in each bag to ensure high relative humidity throughout the incubation period. It should be emphasised that the above-mentioned experimental conditions were particularly favourable to mould growth and sporulation, and should be considered extremes with respect to ordinary conditions occurring during manufacture, storage and use of WPMs intended for food contact.

Fungal isolations were performed from boards by transferring small wood samples (0.005 × 0.005 × 0.002 m) onto 9 cm diameter Petri plates filled with 2% malt extract agar (MEA; 20 g malt extract, 20 g agar, 1000 ml distilled water). For each board, at least 20 wood samples were plated by taking them from areas where moulds were visible. To reduce fast-growing fungi without removing all superficial fungal inoculum, wood samples were previously surface disinfected by dipping in 5% sodium hypochlorite (NaClO) for 5 s and rinsed in sterile distilled water for 10 s. Petri plates were incubated in the light at room temperature (22 °C) for up to 2 weeks depending on the growth rates of the fungal colonies. Growing colonies were individually transferred to 2% MEA.

For each wood species, the purified fungal colonies were grouped in different morphotypes based on growth morphology and macroscopic features. Identification at the genus level was achieved by macro- and micro-morphological examination of colonies by using taxonomic guides and standard procedures (Pitt 1979; Domsch et al. 1980; von Arx 1981; Kiffer and Morelet 1997). The relative isolation frequency (IF) was computed as the percentage of colonies for each fungal morphotype on the total number of colonies obtained for each wood species.

Three major and representative fungal morphotypes were selected for each wood species to be used in the subsequent experiment on the effectiveness of treatments by combining the highest values of IF and the most distinctive morphological characters. All selected morphotypes were identified at species level based on macro- and micro-morphological features of colonies. To confirm the morphological identification, the fungal DNA was extracted and the internal transcribed spacer (ITS) region was amplified and sequenced with universal fungal primers ITS1 and ITS4 (White et al. 1990). Sequences were compared with those of known fungi using the National Center for Biotechnology Information's GenBank nucleotide BLAST search.

2.2 Effectiveness of treatments

Test pieces of poplar, European beech, eastern white pine and Norway spruce (10 × 50 × 1 mm) were treated by dipping either in Celbrite FS2® (15% potassium sorbate as active ingredient; Koppers Performance Chemicals, Germany) for 20 min or in PQ-80® (10% solution of copper-8-quinolinolate as active ingredient; ISK Biocides Inc., Memphis, Tennessee) for 10 min. Currently, Celbrite FS2® is approved for the use in both Europe and USA, while PQ-80® in USA only. After dipping, test pieces were air-dried for 48 h. Treated and untreated test pieces (included in the experiment as controls) were sealed in plastic bags and sterilized with γ rays (2.5 Mrad) using Cobalt-60 radioisotopes at Gammatom (Guanzate–CO, Italy).

The effectiveness of treatments against moulds was assessed by inoculating treated and untreated test pieces with a mixed conidial suspension of the three selected fungal species for each wood species. Mixed conidial suspensions were primarily used to mimic "natural" contamination conditions during the service life of WPMs. In addition, mixed conidial suspensions were recommended to determine the spectrum of activity of new molecules or compounds for preservative/antimicrobial aims (Bush et al. 1946; Siegert 2012; Koziróg et al. 2016).

Subcultures of the above fungal species were prepared aseptically by transferring small pieces of mycelium or conidia masses from individual colonies to fresh 2% MEA. After incubation at room temperature for 10 days, conidia were collected in Eppendorf tubes (50 ml) after flooding the surface of the plates with 5 ml of sterile water. The concentration of conidia was assessed by using a counting Bürker chamber and each conidial suspension was standardized at 10^3 conidia/ml. The final suspension was prepared by mixing together the three conidial suspensions of three fungal species for each wood species.

According to Giordano et al. (2011), 10 test pieces treated with each of the two chemical preservatives and 10 untreated test pieces were singly and uniformly inoculated with 1 ml of each of the above conidial suspensions using a sterile Pasteur pipette. Ten untreated and non-inoculated test pieces were included in the experiment as controls to assess the level of natural fungal contamination.

After inoculations, test pieces were singly placed, horizontally, in 15 cm diameter Petri plates containing a piece of sterile filter paper dampened with 4 ml of sterile water, to prevent drying. The samples were incubated at room temperature for two weeks in the light. Samples were examined 7, 10 and 14 days after inoculations by visual estimation of the wood area colonized by the fungal mycelium, with a detection threshold corresponding to the graphics error (distance between two points), a value derived from the cartography field and conventionally equal to 0.2 mm. Given the crucial

role played by airborne inoculum (i.e., conidia) in the post-harvest infection biology of these fungi, the effectiveness of treatments in reducing the sporulation ability of fungi was also assessed at the end of the experiment. The growing mycelium on each test piece was collected by rubbing with a sterile piece of gauze. The gauze was placed in a Falcon tube (50 ml) containing 10 ml of sterile water, and conidia were suspended by vortexing tubes for 1 min. Thanks to the distinctive morphological characteristics of three mixed fungal species, the concentration of conidia (conidia/ml) of each fungal species was determined by using a Bürker chamber as previously described; in this case, the detection threshold corresponds to the Bürker chamber detection limit (10^3 conidia/ml).

2.3 Data interpretation and analysis

The 95% bias corrected and accelerated confidence interval was calculated from 10^4 bootstrap samplings (DiCiccio and Efron 1996) to analyse the wood area colonized by the fungal mycelium and concentration of conidia, with the exception of those averages resulting from constant values (see results). Based on the above confidence intervals, treatment comparison was performed with the bootstrap hypothesis testing method described in Crawley (2013). Statistical analyses were conducted with R version 3.6.0 (R Core Team 2019).

3 Results and discussion

A total of 322 fungal isolates belonging to 182 putatively different morphotypes was obtained in this study (Online Resource 1). Ninety-nine additional isolates were contaminated, and it was not possible to obtain purified colonies for the identification. These isolates were excluded from all analyses.

Norway spruce and European beech boards were found to harbour a higher number of putatively different morphotypes, 74 and 68 corresponding to 155 and 92 fungal isolates, respectively, compared to boards of the other two wood species. An almost equal number of isolates (36) was obtained from poplar and eastern white pine, corresponding to 23 and 26 putatively different morphotypes, respectively (Online Resource 1).

One hundred fifty-six bacterial colonies were also counted of which 75 from Norway spruce, 50 from poplar, 16 from eastern white pine, and 15 from European beech. However, the above bacterial colonies were not identified since this study was not designed to investigate bacterial populations developing on boards.

The spectrum of fungi isolated from boards mostly included taxa belonging to *Ascomycota*; the only *Zygomycota* was *Mucor* sp., absent in eastern white pine boards but with IF > 8.0 in Norway spruce and poplar boards and IF > 1.0 in European beech boards. The most abundant taxon was *Trichoderma* spp. with 110 putatively different morphotypes followed by *Penicillium* spp. with 31 putatively different morphotypes. *Trichoderma* spp. and *Penicillium* spp., detected in both hardwoods and softwoods (Online Resource 1), included well-known fast-growing species previously reported to readily, and often preferentially, colonize solid wood materials under moisture conditions and in a temperature range conducive to spore germination (Flannigan and Miller 1993; Kang and Morrell 2000; Pasanen et al. 2000; Seifert and Frisvad 2000; Nielsen et al. 2004; Clausen and Yang 2007; Yang and Clausen 2007). It should be emphasised that two isolated fungal species, *Penicillium commune* and *Aspergillus niger*, are listed among those responsible for potentially relevant implications for human health (Aviat et al. 2016 and references therein; Egbuta et al. 2017).

This is the first extensive investigation on fungal mould populations associated with fresh-cut boards for wooden packaging. Previously, Henz and Cardoso (2005) assessed, under different RH conditions, the growth of fungi on the surface of solid pine wood for assembling the "K" box, a standard crate for packing, transporting and trading vegetables in Brazil. In their study, the predominant fungi growing on the wood surface were *Trichoderma harzianum* Rifai and *Rhizopus stolonifer* (Ehrenb.) Vuill. with small colonies of *Aspergillus* spp. and *Penicillium* spp.

All fungi showing high IF and selected to be used in the experiments to assess the effectiveness of treatments were identified at species level by combining morphological and molecular analyses (Table 2) and these were: *Trichoderma longibrachiatum* (IF: 14.0%), *Mucor* sp. (IF: 8.3%) and *Trichothecium roseum* (Pers.) Link (IF: 2.8%) for poplar; *Trichoderma citrinoviride* Bissett (IF: 5.4%), *Aspergillus niger* (IF: 7.6%) and *Penicillium commune* (IF: 3.3%) for European beech; *Trichoderma harzianum* (IF: 5.6%), *Chaetomium globosum* Kunze (IF: 16.7%) and *Penicillium commune* (IF: 2.8%) for eastern white pine; and *Trichoderma atroviride* P. Karst. (IF: 12.3%), *Mucor* sp. (IF: 8.4%) and *Chaetomium globosum* (IF: 2.6%) for Norway spruce. Previously, fungal genera such as *Aspergillus*, *Penicillium* and *Trichoderma* were used in other studies to test the effectiveness of chemical preservatives for the inhibition of moulds on wood in indoor applications (Price et al. 2002; Clausen and Yang 2003, 2005, 2007; Yang and Clausen 2007; Yang et al. 2007a; Tiitta et al. 2009).

Mycelial growth and abundance of conidia on test pieces 14 days after inoculation are shown in Tables 3 and 4. It should be noted that mould growth remained undetected on untreated and non-inoculated test pieces. Celbrite FS2® and PQ-80® reduced significantly ($P < 0.05$) both the mycelial

Table 2 Results of molecular identification at species level of selected morphotypes through amplification and sequencing of the internal transcribed spacer (ITS) region

Morphotype ID	Species identification	Wood species	Sequence similarity (%)	GenBank accession n.[a]
M6	*Trichoderma atroviride*	Norway spruce	100	MG972798.1
M46	*T. longibrachiatum*	Poplar	100	MG650610.1
M64	*T. harzianum*	Eastern white pine	100	KY2858891.1
M79	*T. citrinoviride*	European beech	100	MG972803.1
M111	*Penicillium commune*	European beech/eastern white pine	99	GQ458026.1
M142	*Aspergillus niger*	European beech	100	MK461093.1
M143	*Mucor* sp.	Norway spruce/Poplar	92	AY141178.1
M146	*Chaetomium globosum*	eastern white pine/Norway spruce	100	MF476082.1
M161	*Trichothecium roseum*	Poplar	100	EU552162.1

For morphotype ID, see Online Resource 1

[a]Accession number refers to closest match in BLAST

growth and the concentration of conidia of all the inoculated fungi compared to controls. The only exception was *C. globosum* whose conidia were not found on untreated test pieces of eastern white pine and Norway spruce. The reason of this exception is unclear since conidia were collected from actively growing colonies and because *C. globosum* was previously effectively used in mixed inoculation experiments with other fungal species, thereby excluding this species may be negatively affected by interspecific competition (Bush et al. 1946; Koziróg et al. 2016).

The present results are in agreement with Giordano et al. (2011) and Clausen and Yang (2003) who reported the effectiveness of potassium sorbate against fungal moulds on HDF samples used for fruit and vegetable crate production and on stakes of unseasoned southern pine, respectively.

Celbrite FS2® and PQ-80® were selected based on their previously reported antimicrobial and antifungal properties. Potassium sorbate, the active ingredient in Celbrite FS2®, is a common processed-food preservative with a broad-spectrum activity against moulds and yeasts (Sofos and Busta 1981; Al-Ashmawy and Ibrahim 2009). It is accepted worldwide for the use in food, such as cheese, wine, yogurt, dried meats, etc. (Marín et al. 2002). One of the main characteristics of this salt is that it doesn't affect the taste, colour or flavour of food. PQ-80® is an effective and water-soluble fungicide to control sapstain and moulds on both hardwoods and softwoods. Although not approved for the use in European countries (Ruddick 2011), copper-8-quinolinolate, the active ingredient in PQ-80®, is the only U.S. Environmental Protection Agency (EPA)-registered preservative allowed by the U.S. Food and Drug Administration (FDA) for treatment of wood used in direct contact with fruit, vegetables and other foodstuffs, including pallets, boxes and bins, mushroom trays, and nursery trays and flats (Lebow 2010; CFR TITLE 21, § 178.3800 2019; EPA REG. NO. 1022-489-71581 2015).

Table 3 Average mycelial growth on test pieces of poplar, European beech, eastern white pine and Norway spruce wood 14 days after inoculation with Celbrite FS2® and PQ-80®

Wood species	Treatment	Mycelial growth (%)
Poplar	Celbrite FS2®	0 b
	PQ-80®	0 b
	Untreated	77.5 (55–92.5) a
	Untreated non-inoculated	0 b
European beech	Celbrite FS2®	0 b
	PQ-80®	0 b
	Untreated	94 (78–98) a
	Untreated non-inoculated	0 b
Eastern white pine	Celbrite FS2®	0 b
	PQ-80®	0 b
	Untreated	71 (32–91) a
	Untreated non-inoculated	0 b
Norway spruce	Celbrite FS2®	0 b
	PQ-80®	0 b
	Untreated	92 (60–100) a
	Untreated non-inoculated	0 b

Average values are reported along with their associated 95% bias corrected and accelerated confidence interval (in brackets), with the exception of average values deriving from constant values (i.e. 0 variance within treatment)

Different letters indicate average values significantly different ($P < 0.05$)

Based on the present results, potassium sorbate and copper-8-quinolinolate were comparable in their effectiveness in reducing both the mycelial growth and the conidial production of moulds of WPMs. In both cases, no mould growth and fungal sporulation were observed. Their effectiveness was further supported by the fact that the present experimental conditions, in terms of temperature and relative humidity,

Table 4 Conidial concentration (conidia/ml) on test pieces of poplar, European beech, eastern white pine and Norway spruce wood 14 days after inoculation with Celbrite FS2® and PQ-80®

Wood species	Treatment	Conidia/ml		
		Trichoderma longibrachiatum	*Mucor* sp.	*Trichothecium roseum*
Poplar	Celbrite FS2®	0 b	0 b	0 b
	PQ-80®	0 b	0 b	0 b
	Untreated	$6.9\ 10^6$($3.8\ 10^6$–$1.2\ 10^7$) a	$4.6\ 10^5$($2.3\ 10^5$–$9.0\ 10^5$) a	$2.1\ 10^4$($8.3\ 10^3$–$3.5\ 10^4$) a
	Untreated non-inoculated	0 b	0 b	0 b
		Trichoderma citrinoviride	*Aspergillus niger*	*Penicillium commune*
European beech	Celbrite FS2®	0 b	0 b	0 b
	PQ-80®	0 b	0 b	0 b
	Untreated	$6.9\ 10^6$($3.0\ 10^6$–$2.1\ 10^7$) a	$2.0\ 10^6$($1.1\ 10^6$–$3.7\ 10^6$) a	$2.6\ 10^6$($1.9\ 10^6$–$3.2\ 10^6$) a
	Untreated non-inoculated	0 b	0 b	0 b
		Trichoderma harzianum	*Chaetomium globosum*	*Penicillium commune*
Eastern white pine	Celbrite FS2®	0 b	0 a	0 b
	PQ-80®	0 b	0 a	0 b
	Untreated	$5.3\ 10^6$($1.9\ 10^6$–$1.7\ 10^7$) a	0 a	$1.5\ 10^6$($7.0\ 10^5$–$2.5\ 10^6$) a
	Untreated non-inoculated	0 b	0 a	0 b
		Trichoderma atroviride	*Mucor* sp.	*Chaetomium globosum*
Norway spruce	Celbrite FS2®	0 b	0 b	0 a
	PQ-80®	0 b	0 b	0 a
	Untreated	$1.3\ 10^7$($8.1\ 10^6$–$1.9\ 10^7$) a	$2.6\ 10^6$($1.7\ 10^5$–$1.0\ 10^7$) a	0 a
	Untreated non-inoculated	0 b	0 b	0 a

Average values are reported along with their associated 95% bias corrected and accelerated confidence interval (in brackets), with the exception of average values deriving from constant values (i.e. 0 variance within treatment). For each wood species, different letters (a, b) indicate average values of conidial concentration for a specific fungal species (e.g., for poplar *Trichoderma longibrachiatum*) significantly different ($P < 0.05$) among treatments (Celbrite FS2®, PQ-80®, Untreated and Untreated non-inoculated)

were particularly favourable to mould growth and sporulation, and should be considered extremes with respect to ordinary conditions occurring in the post-harvest.

4 Conclusion

Although wood for hygienic reasons is in general deemed suitable for the use in the food industry, the compliance of WPMs for logistic operations related to food must be assured at each stage of the supply chain. In this context moulds can represent a serious threat not only to the integrity of fresh fruit, vegetables and grains, but also to the human health.

In the first case, moulds can be responsible for organoleptic modifications or post-harvest diseases; in the second one, some moulds (e.g., *Penicillium* spp., *Aspergillus* spp.) can have potentially relevant medical implications (e.g., mycotoxins).

In this study, a first extensive investigation on fungal mould populations associated with fresh boards of hardwoods and softwoods commonly used by the packaging industry was provided. The spectrum of fungi mainly included *Ascomycota* and the most abundant taxa were *Trichoderma* spp. and *Penicillium* spp. previously reported to readily, and often preferentially, colonize wood materials.

In addition, the effectiveness of two new molecules in reducing mycelial growth and sporulation of moulds was demonstrated, representing a suitable option for the control of moulds on WPMs. The tested molecules are potassium sorbate (Celbrite FS2®), available in both the EU and USA, and copper-8-quinolinolate (PQ-80®), currently available only in the USA, which is the producer's reference market. Both products are safe in use and admitted for food contact.

To achieve a more complete picture of fungal mould populations associated with WPMs and to clarify their potential implications to the human health, further investigations are needed on different kinds of WPMs and during all manufacturing steps. Finally, from a practical perspective, the optimization of the application of potassium sorbate and copper-8-quinolinolate on industrial scale and the assessment of their persistence in the wooden packaging are also needed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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