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Fungi Causing Storage Rot of Apple Fruit in Integrated Pest Management System and their Sensitivity to Fungicides

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Abstract. Apple fruit rot can be caused by several fungi. In Northern Europe, the most common storage rot, Bull's eye rot, is caused by *Neofabraea* spp., bitter rot by *Colletotrichum* spp., brown rot by *Monilinia fructigena*, grey mould is caused by *Botrytis cinerea* and *Fusarium* rot by several *Fusarium* species. Blue mold decay caused by *Penicillium expansum* is an important disease in several European countries. Incidence of different causal agents may vary depending on cultivar, climate during growing season and agricultural practices. The main objective of the study was to obtain baseline information about apple rot-causing fungi, their incidence during fruit storage and to evaluate the fungicide sensitivity of most of isolated fungal species. The study was performed during the storage period of apples after the growth season of 2013. Rotten apples were sorted in the storage and part of them was brought to the laboratory in order to obtain fungal isolates. Fungi were identified according to the morphological characteristics and sequencing of the ITS1-5.8S-ITS2 region. During storage in February and March the total percentage of rotten apples in various cultivars varied from 3.6 to 58.9%. All post-harvest diseases described in Northern Europe were detected. In part of the storehouses apple rot caused by *Cadophora luteo-olivacea* was observed. *Alternaria* spp. and *Cladosporium* spp. were detected on few apples as secondary infection agents. Using the most often isolated fungal species, sensitivity tests were performed against five commonly used fungicides. In general, the sensitivity of tested fungi to the fungicides was high with exception of several *Neofabraea* and *Alternaria* isolates.
Key words: *Colletotrichum* spp., *Monilinia fructigena*, *Neofabraea alba*, *Neofabraea malicorticis, Penicillium* spp.

Introduction

Apple rot is an economically significant disease on apple (*Malus´ domestica* Borkh) and can be caused by several filamentous fungi with worldwide distribution like *Penicillium expansum* Link. and *Botrytis cinerea* Pers. that are common and causing significant economic losses in the USA and Europe (Sutton *et al*., 2014). It has been shown that in Northern Europe, particularly in Norway, the most important storage diseases in organically grown apples were caused by *Colletotrichum acutatum* J. H. Simmonds (bitter rot) and *Neofabraea* spp. (Bull's eye rot) up to 64 and 30% respectively from all rotten apples (Borve, Roen, & Stensvand, 2013). Grey mould caused by *Botrytis cinerea*, *Fusarium* rot caused by several *Fusarium* species, brown rot caused by *Monilinia fructigena* (Aderh & Ruhland) Honey and blue mold decay caused by *P. expansum* were found more rarely (Borve, Roen, & Stensvand, 2013). Apple rot studies in Denmark and Germany about organic orchards or orchards not treated with fungicides after petal fall have shown that *Neofabraea alba* (E.J. Guthrie) Verkley and *Neofabraea perennans* Kienholz were the most common storage-rot fungi (up to 62%). Other fungi, such as *Neonectria galligena* (Bres.) Rossman & Samuels, *M. fructigena*, *Cladosporium* spp., *P. expansum*, *Phacidiopycnis washingtonensis* Xiao & J.D.Rogers, *C. acutatum*, *Gibberella avenacea* R.J. Cook, *B. cinerea* were present up to 5% (Maxin *et al*., 2012a). The *Fusarium* rot has been detected on 9 to 30% of apples depending on cultivar stored in Ultra Low Oxygen (ULO) conditions in Croatia (Sever *et al*., 2012). Blue mold decay caused by *P. expansum* is damaging 30 to 60% of cold-stored apples in France, and it is important disease not only in other European countries but also in the USA (Morales *et al*., 2010). It is reported that *Alternaria alternata* causes core rot (Niem *et al.*, 2007) and rot around injuries or at calyx (Sutton *et al*., 2014). The rubbery rot caused by *P. washingtonensis* is considered as a new emerging disease in Northern Europe, and it is reported to cause rot in up to 10% of apples stored in ULO conditions (Weber, 2011).

Apple rot incidence may vary depending on cultivar (Sever *et al*., 2012); Weber, 2011) and harvest time (Borve, Roen, & Stensvand, 2013). Climate conditions during growing season can also have a significant impact, for example, on spore dispersal and following brown rot incidence as it is proved in the conidial dispersal rates in the case of *M. fructigena* in organic and integrated apple orchards in Hungary (Holb, 2008).

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Apples from integrated orchards have a lower disease incidence in comparison to organic orchards in the case of brown rot (Holb, 2008). Similarly, apples from conventional orchards have been reported to have lower levels of storage rots (blue mold, brown mold and Bull's eye rot) in comparison with organic orchards (DeEll & Prange, 1993).

Two mechanisms of infection can be determined, primary and secondary infection. The primary infection usually happens in orchards or during sorting before storage. The secondary infection happens when fungal mycelium and spores spread from infected apples to uninfected apples (Dutot, Nelson, & Tyson, 2013).

The sensitivity of above mentioned apple rot-causing fungi to the fungicides is various. It is reported that *N. alba* shows low sensitivity to fungicides. For instance, in the investigation in the USA it was shown that in *in vivo* conditions from three tested fungicides (copper sulfate, trifloxystrobin, ziram) only the copper sulfate reduced the conidial production by *N. alba* but none of the fungicides used reduced the germination of conidia (Henriquez, Sugar, & Spotts, 2006). Boscalid with pyraclostrobin had shown high efficacy in preharvest application against *P. expansum* and *B. cinerea* (Xiao & Boal, 2009). High resistance of several *P. expansum* isolates has been recorded against benomyl, thiabendazole and diphenylamine (Sholberg *et al.*, 2005). Azoxystrobin, difenoconazole, Polyoxin B and trifloxystrobin have been effective against *A. alternata* causing core rots (Reuveni & Sheglov, 2002).

In the present investigation apples from integrated orchards were examined at the end of the storage period in February and March. The aim of the study was to determine the main causal agents of apple rot during storage and to evaluate the sensitivity of most isolated fungal species to commonly used fungicides.

Materials and Methods

Inspected Apple Storehouses

Apples grown in seven orchards (Table 1) were stored in four storehouses: (1) a farm in Beverina region (northern part of the country) with average

Table 1

Applications of fungicides and other preparations in apple orchards involved in the investigation

Orchard and location	Treatments with fungicides and calcium preparations, dose (kg or L ha^{-1}) and application time in 2013	Total treatments with fungicides
I Beverina region	Funguran OH 1.5 L (06.-07.05.), Score 250 EC 0.2 L (22.05.), Dithane NT 2 kg (28.05.), Chorus 50 WG 0.46 kg (08.-10.06.), Score 250 EC 0.185 L (20.-21.07.), Chorus 50 WG 0.45 kg (12.08.), five treatments with $CaCl_2$, in total 19 kg (18.07.-28.08.)	6
II Bauska region	Funguran OH 1.5 L (03.05.), Dithane NT 3 kg (08.05), Effector 0.3 kg / Chorus 50 WG 0.25 kg (20.05.), Effector 0.3 kg /Score 250 EC 0.25 kg (26.05.), Effector 0.3 kg /Score 250 EC 0.2 L (02.06.)	5
III Pure region A	Champion 4 kg (03.05.), Effector 0.25 kg / Score 250 EC 0.15 L (14.05.), Chorus 50 WG 0.4 kg (20.05.), Score 250 EC 0.15 L /Dithane NT 2 kg (29.05.), Chorus 50 WG 0.4 kg (06.06.), Dithane NT 2 kg (28.06.), Score 250 EC 0.15 L /Dithane NT 2 kg (17.07.)	7
IV Pure region B	Funguran OH 1.5 L (04.05.), Difo 250 EC 0.2 L /Dithane NT 2 kg (11.05.), Chorus 50 WG 0.4 kg (19.05.), Difo 0.2 L /Dithane 2 kg (27.05.), Dithane NT 2 kg (04.06.)	5
V Talsu region	Funguran OH 1.25 L (05.05.), Chorus 50 WG 0.2 kg (13.05.), Chorus 50 WG 0.2 kg/ Effector 0.25 kg h^{-1} (30.05.), Score 250 EC 0.15 L / Effector 0.25 kg (18.06.), Chorus 50 WG 0.2 kg/ Effector 0.25 kg (15.08.)	5
VI Vandzene region	Champion 4 kg (03.05.), Dithane NT 2 kg / Score 250 EC 0.2 L (20.05.), Dithane NT 2 kg / Score 250 EC 0.2 kg (28.05.), Difo 250 EC 0.2 L (14.06.) or Dithane NT 2 kg/ Score 0.2 L (14.06.)	4
VII Jelgava region	Champion 1% (01.05.), Effector 0.3 kg (11.05.), Chorus 50 WG 0.33 kg (30.05.), Dithane NT 2 kg (15.06.), Chorus 50 WG 0.33 kg (27.06.), Effector 0.3 kg/ $CaCl_2$ 1 % (07.07.), Chorus 50 WG 0.33 kg/ $CaCl_2$ 1 % (28.07.)	7

air temperature in the storage 1–3 °C and average relative air humidity 80–90% (orchard I), (2) a farm in Bauska region in the central part of Latvia (2–3 °C, 90%; orchard II); (3) a joint storage for several farmers in Pure region in Western part of Latvia (4–4.5 °C, 80–90%); (4) a farm in Vandzene region in Western part of Latvia (4 °C, 85–90%; orchard VI). In the joint storage in Pure region, the inspected apple cultivars had been grown in various orchards: 'Sinap Orlovskij' in the orchard III, 'Belorusskoje Malinovoje' on the farm in Pure region (orchard IV) and 'Antej' on the farm in Talsi region (orchard V). From apples originating from the orchard VII few fungal isolates were obtained for comparison in the fungicide sensitivity tests, the storage site itself was not inspected.

The apple storehouses were inspected in February and March, 2014. In the storage, healthy and rotten apples in the containers were counted, one container for each cultivar containing from 600 to 27000 apples. Diseases were identified according to their symptoms on the location or later in the laboratory. The following apple cultivars were monitored: 'Ligol', 'Tellissaare', 'Antej', 'Sinap Orlovskij', 'Belorusskoje Malinovoje', 'Auksis', 'Lobo' and 'Forele'.

The applications of fungicides and calcium preparations in the mentioned apple orchards in 2013 are listed in Table 1. It has to be admitted that farmers in Latvia only occasionally use fungicides targeted specially against apple fruit rot. Only treatments against apple scab are usually carried out.

Isolation and Identification of Fungi

The fungal material (mycelium and spores) from rotten apples was directly subjected to light microscopy or after a few days long incubation of rotten apples in the incubator (20 ± 2 °C). From the rotten apples pure cultures of fungi were isolated on the potato dextrose agar (PDA) (Biolife, Italy). Fungal cultures were identified using morphological characteristics (Crous *et al*., 2019; Sutton *et al*., 2014); Watanabe, 2002) or with molecular biology methods sequencing part of the ITS1-5.8S-ITS2 region. Genomic DNA from approximately 0.25 g of mycelia was extracted using the E.Z.N.A. Fungal DNA Mini kit (Omega Bio-Tek, USA). The following primers were used: ITS4, universal for eukaryotes (White *et al*., 1990); ITS1F, specific for fungi (Gardes & Bruns, 1993). The PCR reactions in SensoQuest Labcycler 48 (SensoQuest, Germany) were carried out in 25 µl volume. The mixture contained 12.5 µl Hot Start Master Mix 2X, 0.375 µl Bovine Serum Albumin 20 mg ml^{-1} (all reagents from Thermo Scientific Fermentas Molecular Biology Solutions, Lithuania), 0.5 µl of each 10 µM primer (Integrated DNA Technologies, USA), 10.125 µl sterile distilled water and 1 µl of DNA template. The PCR conditions were as follows: the initial denaturation step of 4 min at 95 °C, 40 s of denaturation at 95 °C, 40 s of annealing at 52 °C, 1 min of primer extension at 72 °C (30 cycles) and final extension 10 min at 72 °C.

Amplified DNA fragments were treated with FastAP™ Thermosensitive Alkaline Phosphatase and Exonuclease I (Thermo Scientific Fermentas Molecular Biology Solutions, Lithuania) and sequenced by Macrogen Europe (Amsterdam, the Netherlands). Double stranded sequences of PCR amplicons were assembled using Staden Package 1.6.0. (Bonfield, Smith, & Staden, 1995). The resulting consensus sequences were used in BLASTN homology search against the National Centre for Biotechnology Information nucleotide database (http://www.ncbi.nlm.nih.gov).

Identification of such fungi as *Cadophora luteo-olivacea* (J.F.H. Beyma) T.C. Harr. & McNew and *Alternaria alternata* (Fr.) Keissl. apple pathotype (*A. mali* Roberts) was further proved by specific PCR using the following primers: Clm-REV and Clu-FOR or Cma-FOR specific to ITS1-5.8S-ITS2 region of *C. luteo-olivacea* or *C. malorum*, respectively (Spadaro *et al*., 2011) and LinF1 and LinR specific to *AMT* gene of *A. alternata* apple pathotype (*A. mali*) (Johnson *et al.*, 2000).

Sequences of the ITS1-5.8S-ITS2 region of 59 fungal isolates were submitted to the GenBank (accession numbers KP903568-KP903626, KT923785-KT923789). All isolates were preserved in –86 ºC freezer in the collection of Latvian Plant Protection Centre.

Application of Koch's Postulates

With most often isolated fungal species from this and previous investigation of Latvian Plant Protection Centre (Volkova & Juhnevica-Radenkova, 2015) the Koch's postulates were applied using the basic principles of the methodology described in other investigations (Mari *et.al*., 2012; Xiao & Rogers, 2004). Inoculation was performed on apples sterilized for 2 min in 2% hypochlorite solution and rinsed with deionized water. In apples, the inoculation, a manual cut with a sterile scalpel up to 3 mm deep, with a small piece of fungal mycelia and/or spores from the cultures grown 14 days on PDA was done. After inoculation the apples were placed in sterile plastic boxes on the wet paper towel and incubated at 20 ± 2 °C up to 39 days. After incubation re-isolation of the pathogenic fungi was performed and these were identified according to the morphological characteristics.

Fungicide Sensitivity Tests

With most often isolated fungal species (*N. alba*, 11 isolates; *N. malicorticis*, five isolates; *C. luteo-olivacea*, three isolates; *A. alternata*, six isolates) representing various orchards fungicide sensitivity tests were carried out against five fungicides (Table 2)

Table 2

Fungicides and their concentrations used in the fungicide sensitivity tests

Fungicide, active substance, concentration	Application target	Recommended dose	Concentration used in the experiment (%)				
			1	2	3	4	5
Effector, dithianon, 700 g kg^{-1}	Apple fruit rot, apple scab	0.05-0.1%	0.025	0.05	0.075	0.1	0.2
Score 250 EC, difenoconazole 250 g l^{-1}	Apple scab, powdery mildew	0.01-0.05%	0.005	0.01	0.03	0.05	0.1
Chorus 50 WG, cyprodinil 500 g kg^{-1}	Apple scab, fruit rot of plums	0.3-0.45 kg ha^{-1}	0.03	0.06	0.075	0.09	0.18
Dithane NT, mancozeb 750 g kg^{-1}	Apple scab	2.0 kg ha^{-1}	0.1	0.2	0.3	0.4	0.6
Signum, boscalid 26.7 %, pyraclostrobin 6.7%	Fruit rot of plum and cherry	0.75-1.0 kg ha^{-1}	0.075	0.15	0.17	0.2	0.4

Table 3

List of isolates used in the fungicide sensitivity tests

Species	Orchard, cultivar	Accession number in Genbank	Species	Orchard, cultivar	Accession number in Genbank
N. alba	I, 'Beloruskoje Malinovoje'*	Not submitted	*N. malicorticis*	I, 'Beloruskoje Malinovoje'	KP903612
N. alba	I, 'Beloruskoje Malinovoje'	KT923785	*N. alba*	V, 'Antej'	KP903587
N. malicorticis	I, 'Sinap Orlovskij'	KP903615	*N. malicorticis*	VI, 'Forele'	KP903625
N. alba	I, 'Tellissaare'*	Not submitted	*C. luteo-olivacea*	III, 'Sinap Orlovskij'	KP903599
N. alba	II, 'Ligol'	KT923786	*C. luteo-olivacea*	IV, 'Beloruskoje Malinovoje'	KP903596
N. alba	II, 'Lobo'	KP903582	*C. luteo-olivacea*	VI, 'Forele'	KP903623
N. alba	II, 'Tellissaare'	KT923787	*A. alternata*	II, 'Auksis'	KP903568
N. alba	III, 'Sinap Orlovskij'	KT923788	*A. alternata*	II, 'Ligol'	KP903579
N. alba	IV, 'Beloruskoje Malinovoje'	KT923789	*A. alternata*	II, 'Tellissaare'	KP903586
N. alba	VII, 'Auksis'	KP903614	*A. alternata*	II, 'Auksis'	KP903571
N. alba	VII, 'Auksis'	Not submitted	*A. alternata*	VI, 'Beloruskoje Malinovoje'	KP903621
N. malicorticis	I, 'Sinap Orlovskij'	KP903618	*A. alternata*	III, 'Sinap Orlovskij '	KP903601
N. malicorticis	I, 'Sinap Orlovskij'	KP903591	-	-	-

*- isolate obtained from the previous investigation (Volkova & Juhnevica-Radenkova, 2015).

using *in vitro* agar plate test (Russell, 2002). Two additional *N. alba* isolates originated from the orchard VII (Table 1). A complete list of isolates is given in Table 3. Each fungal isolate was grown in two replicates on PDA supplemented with particular fungicide in each concentration after autoclaving just before the test. Concentrations 1 and 5 were twice lower or twice higher than minimal and maximal doses recommended by a producer. Other concentrations were within the recommended range. The recommended dose for Dithane NT corresponded to the concentration 4. The theoretical spraying volume used for calculations was 500 l ha^{-1}. Fungi were incubated at 25 °C for three weeks; after that the diameter of fungal colony was measured.

Statistical Analysis

The statistical analysis was performed with data from the fungicide sensitivity tests comparing the average colony diameters of isolates at presence of various fungicides and in control plates, and within isolates from the same species and among various species. The F-test, t-test ($\alpha = 0.05$) and correlation analysis were done with *Excel* (Microsoft, USA). Both Pearson correlation coefficients (*r*) and determination coefficients (R^2) were calculated to determine the correlation strength among fungicide doses and average growth results of every species.

Results

In February and March, the total percentage of rotten apples of various cultivars and in different storage sites ranged from 3.55 to 58.88% (Table 4). Both types of infections - primary and secondary, were observed,. From infected apples *Neofabraea* spp. ranged from 0.33 to 50.88% depending on the cultivar. The most severely affected cultivar was 'Forele'. *Fusarium* spp. caused rot ranged from 0 to 2.22%, *Penicillium* spp. from 0.04 to 5.34%, *Colletotrichum* spp. from 0.12 to 3.13%, *Botrytis* spp. from 0 to 3.37%, *Monilinia* spp. from 0 to 21.08% (the most affected cultivar was 'Lobo'). In

Table 4

Total percentage of rotten apples of various cultivars and in different storage sites and percentage of particular rot type

Cultivar	*Neofabraea spp.*[1]	*Fusarium spp.*[2]	*Penicillium spp.*[3]	*Colletotrichum spp.*[4]	*Botrytis cinerea*	*Monilinia fructigena*	*Alternaria alternata*	*Cladosporium* spp.	*C. luteo-olivacea*	Other[5]	Total number of apples	Rotted apples (%)
Storage in Bauska region												
'Tellissaare'	0.33	0.15	0.37	0.33	3.37	0.18	0.04	0.18	0.00	0.00	2728	4.95
'Auksis'	3.29	2.22	5.34	1.51	0.27	0.00	0.31	0.00	0.00	0.00	2249	12.89
'Lobo'	0.90	0.30	0.60	0.15	0.00	21.08	0.00	0.30	0.00	0.00	664	23.34
'Ligol'	0.95	0.24	2.25	0.12	0.06	0.12	0.06	0.00	0.00	0.00	1690	3.55
Joint storage for several farmers in Pure region												
'Antej'	3.80	0.00	0.29	1.58	0.06	0.29	0.06	0.00	0.06	0.00	1709	6.14
'Beloruskoje Malinovoje'	6.38	0.00	0.51	2.42	0.05	0.10	0.00	0.00	0.00	0.41	1945	9.87
'Sinap Orlovskij'	4.24	0.19	0.64	2.44	0.51	0.51	0.06	0.26	0.06	0.32	1557	9.31
Storage in Vandzene region												
'Beloruskoje Malinovoje'	9.26	0.04	0.53	1.24	0.89	0.09	0.04	0.00	0.00	0.75	2258	12.84
'Forele'	50.88	0.25	1.00	3.13	2.88	0.50	0.00	0.13	0.13	0.00	800	58.88
Storage in Beverina region												
'Sinap Orlovskij'	1.59	0.04	0.04	0.09	0.04	0.13	0.00	0.00	0.00	2.04	1562	5.76
'Beloruskoje Malinovoje'	8.00	0.13	0.13	0.00	1.00	1.13	0.38	0.00	0.00	4.38	1215	9.96

1 – *N. alba*, *N. malicorticis*; 2 – *F. avenaceum* (*G. avenacea*), *Fusarium* spp.; 3 – *P. brevicompactum* Dierckx, *P. echinulatum* Raper & Thom ex Fassat., *P. expansum, P. solitum* Westling; 4 – *C. acutatum*, *C. gloeosporioides* (Penz.) Penz. & Sacc.; 5 - *Aureobasidium pullulans* (de Bary) G. Arnaud, *Diaporthe eres Nitschke* (*Phomopsis velata* (Nitzschke ex Sacc.) Traverso), *N. galligena*.

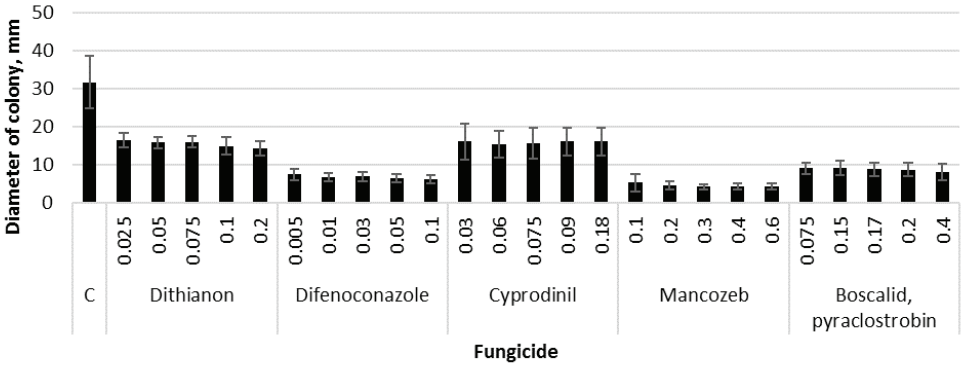

Figure 1. Sensitivity of *N. alba* isolates to five fungicides (C – control without fungicides). Error bars indicate standard deviations (±SD), $n = 11$.

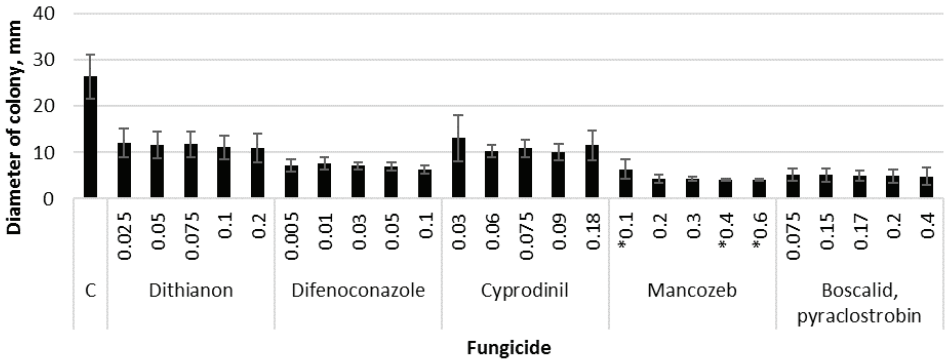

Figure 2. Sensitivity of *N. malicorticis* isolates to five fungicides (C – control without fungicides). Statistically significant differences in dose response are indicated with an asterisk. Error bars indicate standard deviations (±SD), $n = 5$.

two storehouses apple rot caused by *C. luteo-olivacea* was detected, and its identity was successfully proved by PCR with primers specific to this species (data not shown). *A. alternata* and *Cladosporium* spp. were detected on a few apples as secondary infection agents. None of the *A. alternata* isolates gave positive amplification products with primers specific to *AMT* gene.

Application of Koch's postulates was successfully carried out with *C. gloeosporioides*, *N. alba*, *F. avenaceum* and *Penicillium expansum* on six apple varrieties: 'Auksis', 'Belorusskoje Malinovoje', 'Iedzēnu', 'Sinap Orlovskij', 'Tellissaare' and 'Saltanat', and with *C. luteo-olivacea* on 'Auksis' and 'Zarja Alatau'.

Results of fungicide sensitivity tests are shown in Figure 1 to 4. Isolates of *N. alba* and *N. malicorticis* were sensitive to difenoconazole, mancozeb and boscalid with pyraclostrobin which reduced the growth of these fungi by 70–80%. Dithianon and cyprodinil reduced the fungal growth only by 50% (Figures 1 and 2). In general, isolates of *N. malicorticis* were significantly slowly growing ($p < 0.001$), but the average response to fungicides was not significantly different.

Three isolates of *N. alba* from the orchards IV, V and VII were significantly less sensitive to the tested fungicides in comparison to the rest of isolates ($p < 0.05$), especially to the dithianon and cyprodinil. The growth of these isolates in the presence of the dithianon was reduced only by 41, 45 and 43%, respectively, at the highest fungicide concentration. In the presence of the cyprodinil the growth was reduced only by 41, 36 and 35%, respectively, at the presence of the highest fungicide concentration.

From five isolates of *N. malicorticis* one isolate originating from the orchard I (KP903612) showed less sensitivity to dithianon in comparison to the rest of isolates. The growth of this isolate was reduced only by 51 *vs*. 64% in the presence of the highest fungicide concentration.

Differences of dose response of *N. alba* isolates to tested fungicides were not statistically significant. In the case of *N. malicorticis* statistically significant

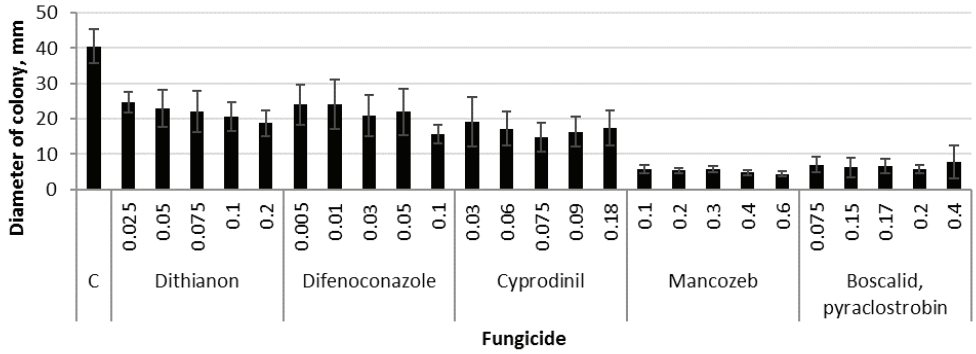

Figure 3. Sensitivity of C. *luteo-olivacea* isolates to five fungicides (C – control without fungicides). Error bars indicate standard deviations (±SD), $n = 4$.

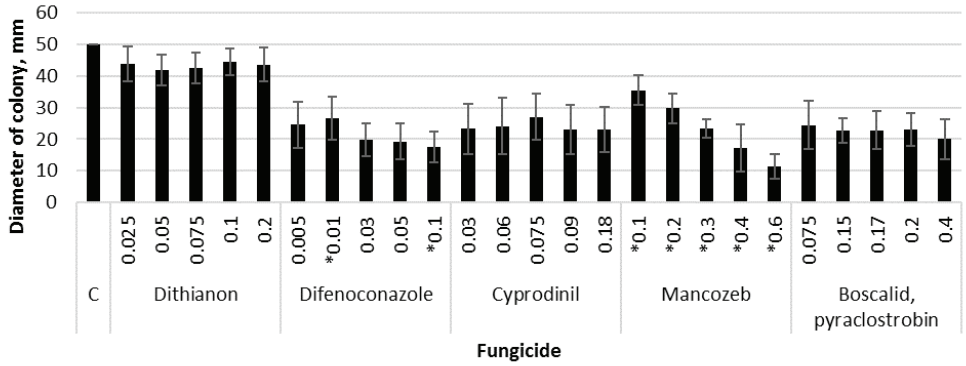

Figure 4. Sensitivity of *A. alternata* isolates to five fungicides (C – control without fungicides). Statistically significant differences in dose response are indicated with an asterisk. Error bars indicate standard deviations (±SD), $n = 7$.

differences ($p < 0.05$) in the dose response were detected only in the case of mancozeb ($r = -0.70$, $R^2 = 0.49$).

Isolates of C. *luteo-olivacea* were sensitive only to mancozeb and boscalid with pyraclostrobin, which reduced the growth of these fungi on average by 85%. Other fungicides were less effective, reducing the growth of these fungi only by 50–60% (Figure 3). Differences of dose response of C. *luteo-olivacea* isolates against tested fungicides were not statistically significant although the correlation between dithianon and difenoconazole doses and average colony diameters was strong ($r = 0.95$, $R^2 = 0.92$).

Isolates of *A. alternata* were medium sensitive to difenoconazole, cyprodinil, mancozeb and boscalid and pyraclostrobin, on average reducing the fungal growth by 50%, the dithianon was the least effective reducing the growth only by 13% (Figure 4). One isolate from the orchard II was 100% resistant to all concentrations of the dithianon. On average, *A. alternata* isolates showed statistically significant differences in dose response against mancozeb ($r = -0.98$, $R^2 = 0.97$) and particularly against difenoconazole ($r = -0.85$, $R^2 = 0.72$).

Discussion

The species spectrum of apple rot-associated fungi identified in the present study was mainly identical to the causing agent spectrum described in the literature. The exception was the rubbery rot caused by *P. washingtonensis* that is considered as a new emerging disease in Northern Europe, and it is reported to cause rot in up to 10% of apples stored in ULO conditions (Weber, 2011), but it was not detected in the present investigation. The second exception was side rot caused by *C. luteo-olivacea* that is not so common in other studies, but it was detected in two storage sites in Latvia. This pathogen is reported as important kiwi pathogen especially during long storage periods, but it has also been found on apples (Spadaro *et al*., 2011).

From the apples in three storage sites several isolates of *Phomopsis velata* (imperfect stage of *Diaporthe eres*) were isolated. This fungus has been previously described as common pathogen of peach

(*Prunus persica* (L.) Stokes) causing shoot blight and canker disease of peach in Greece (Thomidis & Michailides, 2009) and minor disease of apple and pear in North America, Europe and Japan causing cankers and fruit decay (Sutton *et al*., 2014).

Several *Penicillium* species (*P. brevicompactum*, *P. echinulatum*, *P. expansum, P. solitum*. *P. expansum* and *P. solitum*) were isolated, which are common apple rot-causing pathogens (Sholberg *et al.*, 2005). Isolates of *P. brevicompactum* have been isolated from rotten apples in Canada (Sholberg & Haag, 1996). *P. echinulatum* have been found on lipid-rich substrates and wood residues (Frisvad & Samson, 2004).

Aureobasidium pullulans was isolated from the cultivar 'Beloruskoje Malinovoje' (orchard I) only once and has been characterized as causing agent of miscellaneous postharvest diseases of apples (Sutton *et al*., 2014).

N. galligena was isolated from the cultivar 'Lobo' (orchard II) only once and is reported as rot -causing agent of apples (Maxin *et al*., 2012a, 2012b).

The proportion of rotten apples varied from 3.55 to 58.88% and was more cultivar-specific, not related to the number of fungicide treatments or storage conditions. For example, in the storage of orchard II four cultivars were examined having the same fungicide treatments and storage conditions, but the respective proportion of rotten apples varied from 3.55 ('Ligol') to 23.34% ('Lobo'). Differences of cultivars in infection level have been described in other investigations, for instance, in the case of infections with *Fusarium* spp. (Sever *et al*., 2012).

It is reported that *N. alba* shows low sensitivity to fungicides. For instance, in the study in Southern Oregon it was detected that in *in vivo* conditions only copper sulfate reduced the conidial production by *N. alba* but none of the fungicides used (copper sulfate, trifloxystrobin, ziram) reduced the germination of conidia (Henriquez, Sugar, & Spotts, 2006). In the present investigation isolates of *N. alba* as well *N. malicorticis* showed high sensitivity to the tested fungicides: difenoconazole, mancozeb and boscalid with pyraclostrobin reduced the growth of these fungi by 70–80%, dithianon and cyprodinil reduced the fungal growth by 50%.

Three isolates of *N. alba* from the orchards IV, V and VII were less sensitive to tested fungicides, especially to dithianon and cyprodinil. The orchard VII had one of the highest numbers of fungicide treatments in 2013 (seven), but the orchards IV and V had an average number of treatments (Table 1). On the orchard VII the dithianon-containing fungicides were used twice and cyprodinil-containing fungicides were used three times in the growing season of 2013.

From five *N. malicorticis* isolates one isolate originating from the orchard I showed less sensitivity to dithianon and cyprodinil in comparison with the rest of the isolates. Orchard I had one of the highest numbers of fungicide treatments in 2013 (six). Orchard VI from which one isolate of *N. malicorticis* originated had smaller numbers of treatments – four. In the orchard I the dithianon was not used but cyprodinil-containing fungicides were used twice. In orchard VI fungicides containing dithianon or cyprodinil were not used in 2013.

Sensitivity of *A. alternata* isolates to tested fungicides was medium high by comparison to other fungi and also to other investigations. For example, in the study in Lithuania, it was detected that the growth of *A. alternata* isolated from lovage and cabbage was reduced by 80% in the case of boscalid with pyraclostrobin (Signum) in comparison with 55% in the present study (Surviliene & Dambrauskiene, 2006). One isolate from the orchard II was 100% resistant to all concentrations of the dithianon. The dithianon in this orchard was applied three times during 2013 and up to five times in the previous two years.

In fungicide efficacy trials in Czech Republic, it was stated that strobilurines were the most effective fungicides against the storage rot of cultivar 'Golden Delicious' caused by *N. alba*, *N. malicorticis* and *Penicillium* spp. (Minář, 2006). Slightly lower efficacy was shown by tolylfluanid, dodine and captan. Dithianon-containing fungicide was the least effective as it was observed in the present investigation. Since 2015 dodine is registered in Latvia against apple scab. Captan is registered against apple scab and fruit rot, but the strobilurin-containing fungicide Signum which is registered against fruit rot of plum and cherry can be used with special individual permissions on apple.

In the present investigation, the cyprodinil-containing fungicide showed the lowest inhibition effect on tested fungal species. However, it has shown efficacy against postharvest apple rot caused by *B. cinerea* in Canada (Sholberg, Bedford, & Stokes, 2003).

A few isolates with reduced sensitivity to tested fungicides found in the present study illustrate the tendency that larger numbers of fungicide applications are causing lesser sensitivity of pathogenic fungi against fungicides. Development of fungicide resistance of pathogens is one of the problems demanding for new control strategies (De Capdeville *et al*., 2002).

Conclusions

1. The most common apple fruit rot causing agents were *Neofabraea alba*, *Neofabraea malicorticis*,

Fusarium spp., *Penicillium* spp., *Colletotrichum* spp., *Botrytis cinerea*, *Monilinia fructigena*. The following species *Cadophora luteo-olivacea*, *Phomopsis velata* and *Alternaria alternata* were considered of minor importance.

2. The most severely affected cultivar by *Neofabraea* spp. was 'Forele' but the most affected cultivar by *Monilinia* spp. was 'Lobo'. The least infected cultivars in general were 'Tellissaare' and 'Ligol'.
3. In general, the sensitivity of tested fungi to the fungicides was high with exception of particular *Neofabraea* and *Alternaria* isolates. The most effective fungicides were difenoconazole, mancozeb and pyraclostrobin-containing fungicides. Recent changes in the list of fungicides that are registered in Latvia on apples can help growers to diversify the fungicide spectrum including in the treatments dodine- and captan-containing fungicides which have shown good efficacy against storage diseases of apples in other countries.
4. The investigation should be continued increasing the number of orchards, storehouses and cultivars.

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Disparity in Discolouration of Thermally Modified Wood Exposed to Solar and Artificial Ultraviolet Irradiation

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Abstract. Artificial weathering is a widely used method for predicting wood behaviour during its service life. A study was carried out to compare the colour change of thermally modified aspen (*Populus tremula* L.*)* and grey alder (*Alnus incana* (L.) Moench) wood during natural solar and artificial ultra violet (UV) irradiation. Thermally modified wood specimens were exposed for 30 h to artificial UV irradiation at two different intensities, i.e. 1.36 W m^{-2} at 340 nm and 0.68 W m^{-2} at 340 nm, as well as to solar irradiation outdoors and indoors. After 2.5, 5 and 10 h exposure, colour parameters and reflectance spectra (360–740 nm) were determined. Colour was expressed in accordance with the CIELAB colour model as colour parameters L*, a*, b*. The total colour change ΔEab was calculated from colour parameter differences ΔL*, Δa*, Δb*. The colour changes caused by solar and artificial UV irradiation had a similar pattern for both thermally modified hardwood species under study. Changes in the individual colour parameters in the course of the experiment altered their direction, which implies that discolouration of thermally modified wood is a complicated and dynamic process with various and different chemical transformations in wood chromophores. Colour and reflectance changes had similar trends for different intensities of the same type of irradiation, but they differed for various irradiation types – natural solar or artificial UV irradiation. Greater discolouration was detected for the specimens exposed to both solar irradiations - outdoors and indoors. The results suggest that the fluorescent lamps of the UVA-340 type, which only imitate well the sunlight UV spectrum from 290 nm to 365 nm, do not fully simulate the changes in thermally modified wood induced by solar radiation.
Keywords: Thermally modified wood, Discolouration, Solar irradiation, Artificial UV irradiation.

Introduction

Thermal modification is known as an effective method to improve some properties of wood such as dimensional stability, resistance against biodegradation and decrease of hygroscopicity (Esteves & Pereira, 2009). Unfortunately, wood mechanical strength decreases due to thermal treatment (Boonstra *et al*., 2007). Therefore, major application fields of thermally modified wood are, for example, garden furniture, decking, spa areas, swimming pools and floorings (Bächle *et al*., 2010). When wood is exposed outdoors, a complex combination of chemical and mechanical factors contributes to what is described as weathering, i.e. a complex set of reactions induced by solar radiation, water (rain, dew, snow and humidity), temperature and atmospheric pollution (sulphur dioxide, nitrogen dioxide, ozone, dust, etc.) (Williams, 2005). UV light is known to be the main reason for degradation and discolouration of wood surface during weathering (Ayadi *et al*., 2003). Artificial weathering is extensively used to predict the behaviour of wood outdoors as the process is controllable and reproducible. Another advantage of artificial weathering is that results can be obtained in a relatively short time. A lot of information about the artificial weathering of unmodified (Derbyshire & Miller, 1981; Oltean, Teischinger, & Hansmann, 2008; Pandey & Vuorinen, 2008; Tolvaj & Varga, 2012) as well as thermally modified wood (Ayadi *et al*., 2003; Deka *et al*., 2008; Miklečić *et al*., 2011; Huang *et al*., 2012) is available. Different types of light sources are applied to simulate solar radiation. Mostly xenon arc (Tolvaj & Mitsui, 2005; Srinivas & Pandey, 2012) and fluorescent (Chang *et al*., 2010; Miklečić *et al*., 2011) lamps are used.

Light provokes chemical reactions on wood surface. As wood discolours as a result of these reactions, it is easy to monitor the weathering process through determining wood colour changes (Ayadi *et al.* 2003; Kishino & Nakano 2004; Pastore *et al.* 2004; Temiz *et al.* 2005; Mitsui 2006; Sharratt *et al.* 2009). The UVA type fluorescent lamps are widely used in the case of the artificial weathering of thermally modified wood. There are some studies about unmodified wood in which the changes in wood during exposure to artificial UV irradiation and solar irradiation outdoors are compared (Derbyshire & Miller 1981; Sudiyani *et al.* 1999; Tolvaj & Mitsui, 2005; Deka & Petric 2008). Unfortunately, there is hardly any knowledge about peculiarities of thermally

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modified wood discolouration caused by artificial and natural solar irradiation. However, the information on how well the artificial weathering is able to imitate natural processes is an important issue concerning investigation and prediction of material behaviour during its application.

The objective of the present study was to evaluate how well artificial weathering by using fluorescent lamps of the UVA-340 type is able to imitate the changes in thermally modified wood colour during exposure to solar radiation.

Materials and Methods

Wood specimens

Two common Latvian hardwood species, i.e. aspen and grey alder were used for the study. Aspen and grey alder boards measuring 1000 x 100 x 40 mm were hydrothermally modified in an experimental wood modification device in a water vapour medium for 1 h at 170 °C under elevated pressure (0.8 MPa). Before preparing specimens for weathering experiments, the boards were conditioned at 20 °C and 65% RH until the equilibrium moisture content was reached. Specimens measuring 150 x 70 x 15 mm were cut from the conditioned boards to be further used for the artificial UV irradiation at two intensities and for exposure to sunlight outdoors and indoors. For each experiment, three specimens from each species were prepared.

Solar irradiation tests

One set of specimens was exposed to solar irradiation on a horizontal surface outdoors. This test was labelled as "Outdoor". Another set was placed on a horizontal surface indoors in a place where specimens were subjected to sunlight filtered through the window glass. This test was labelled as "Indoor". The experiment site was located in Riga – 56°58' N, 24°11' E. During the experiment, the air temperature at the surface of the specimens varied from 20 to 28 °C and relative humidity from 20 to 43%. The total exposure time was 30 h. After 2.5, 5 and 10 h exposure, colour parameters and reflectance spectra of the specimens were recorded. Every hour outdoors and indoors, the total ultraviolet (UV) radiation flux density in a range of 290–390 nm was measured using an UV light meter. The irradiation doses received by specimens were calculated from these data. The experiment was carried out from June to August and the specimens were only exposed to irradiation during the hours when the UV radiation flux density outdoors was not less than 10 W m^{-2}. Wood was kept in the dark in the laboratory while it was not exposed to irradiation.

Artificial UV irradiation tests

Artificial UV irradiation was performed in an accelerated weathering tester QUV equipped with fluorescent lamps of UVA-340 type, which are commonly used in artificial weathering and provide good simulation of sunlight in the short wavelength region from 365 nm to the solar cut-off of 295 nm with a peak emission at 340 nm.

The artificial UV irradiation was carried out in two modes, which differed in irradiation intensities. In a test labelled "Intense", the UV radiation flux density at 340 nm was 1.36 W m^{-2} and in a test labelled "Mild", it was 0.68 W m^{-2}. The total UV radiation flux densities in a range of 290 to 390 nm were 30.5 W m^{-2} in the Intense test and 17.5 W m^{-2} in the Mild test. During both artificial weathering tests the temperature in the weathering chamber was 60°C. Total exposure time was 30 h. Tests were suspended after 2.5, 5 and 10 h to perform spectrophotometric measurements.

Spectrophotometric measurements

Reflectance spectra and wood colour was measured with a spectrophotometer CM-2500d (standard illuminant D65, d/8° measuring geometry, 10° standard observer, measuring area Ø 8 mm) before and after irradiation as well as after 2.5, 5 and 10 h exposure. Colour was expressed in accordance with three dimensional colour space CIELAB where parameter L* indicates the lightness in a range from black (0) to white (100), while parameters a* and b* define the position in green-red and blue-yellow axis respectively. The total colour change ΔEab is a distance between two points in the colour space and was calculated from the colour parameter differences between the initial and resulting values ΔL*, Δa*, Δb* (e.g. $\Delta L^* = L^*_{resulting} - L^*initial$) according to the formula:

$$\Delta E ab = \left(\left(\Delta L^* \right)^2 + \left(\Delta a^* \right)^2 + \left(\Delta b^* \right)^2 \right)^{\frac{1}{2}}$$

Reflectance spectra were recorded in a range of 360–740 nm, measuring the reflectance at 10 nm intervals. Colour parameters and reflectance spectra were measured at five, always the same positions on each specimen and average values were calculated.

Results

Variation of the UV radiation flux density in a range of 290–390 nm for all tests of this study is shown in Figure 1. The UV radiation in both artificial weathering tests was constant during the whole experiment. UV radiation intensity in both solar radiation tests varied from day to day as well as it varied from hour to hour depending on the time of day.

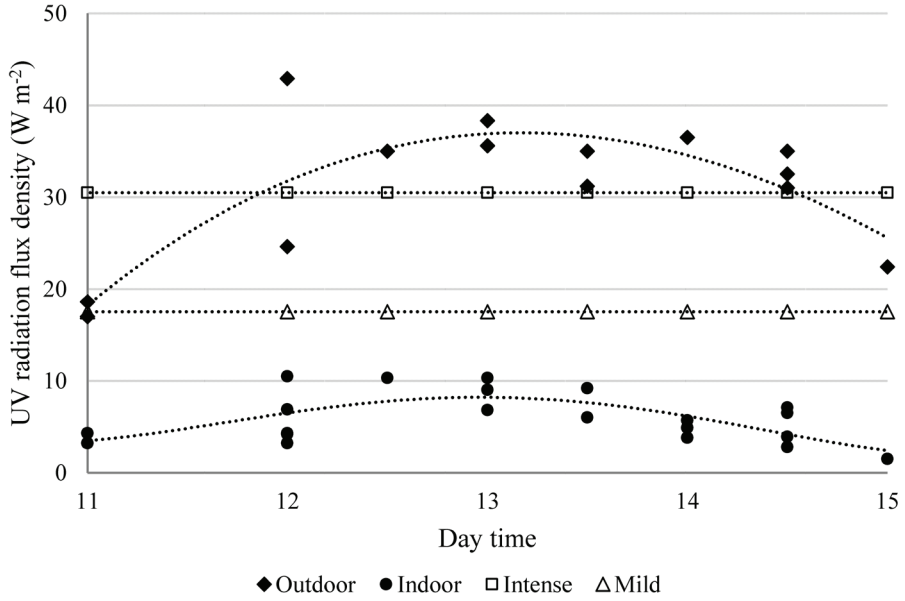

Figure 1. Variation of the UV radiation flux density in a range of 290 – 390 nm during Outdoor, Indoor, Intense, and Mild tests.

During the experiment, the highest radiation in the UV range in both solar tests was detected between 12 pm to 14 pm. As the UV intensity varied during solar irradiation tests, the UV irradiation doses received by the specimens during exposure were calculated (Table 1).

The highest total UV irradiation dose in a wavelength range of 290–390 nm, received by the specimens during 30 h of exposure was for the specimens artificially irradiated in the Intense test – 3300 kJ m^{-2}. Much lower and almost equal UV irradiation doses during 30 h exposure received specimens in the Outdoor and Mild tests – 2000 kJ m^{-2} and 1900 kJ m^{-2}, respectively, whereas the specimens exposed to solar irradiation through the window glass in the Indoor test were only reached by 24% of that of the full solar UV radiation in the Outdoor test.

At the end of the experiment, considerable colour changes ΔEab were detected for all studied irradiation conditions for both wood species (Figure 2). However, less discolouration was observed for specimens subjected to exclusively UV irradiation in both artificial weathering tests despite the substantial UV radiation dosages received by the specimens in these tests.

Thermally modified wood exposed to solar and artificial UV irradiation differed not only by the magnitude of total discolouration, but also by changes in individual colour parameters (Table 2). However, the pattern of colour change was similar for both thermally modified aspen and grey alder wood.

In both solar irradiation tests from the very beginning the lightness L* of both species increased ($\Delta L^* > 0$), which means that wood became lighter. Unlike, in both artificial UV irradiation tests at the initial stage of the exposure darkening of wood was

Table 1

UV irradiation (290 – 390 nm) doses received by specimens during solar and artificial UV exposure for different periods of time

Exposure time, h	Irradiation doses, kJ m^{-2}			
	Outdoor	Indoor	Intense	Mild
2.5	250	60	270	160
5	450	110	550	320
10	960	230	1100	630
30	2000	470	3300	1900

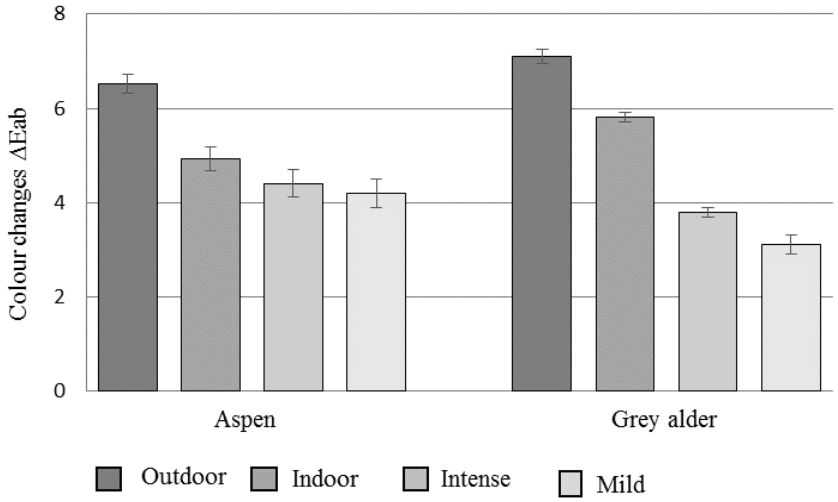

Figure 2. Colour change ΔEab of thermally modified aspen and grey alder wood during exposure to different irradiation conditions for 30 h.

detected (ΔL* < 0), which was followed by increase in lightness during the further exposure. For the specimens exposed in the Intense test, positive lightness change values ΔL* were fixed after 5 h irradiation while for those in the Mild test after 10 h of exposure.

The redness parameter a* declined (Δa* < 0) during the whole experiment for specimens exposed to all weathering conditions. Slightly greater changes in redness at the end of the experiment were found for specimens subjected to the artificial UV irradiation. However, the most distinctive behaviour in solar

Table 2

Changes in the colour parameters ΔL*, Δa* and Δb* of thermally modified aspen and grey alder wood due to solar and artificial UV irradiation for different periods of time

Exposure time, h	Aspen			Grey alder		
	ΔL*	Δa*	Δb*	ΔL*	Δa*	Δb*
	Outdoor test					
2,5	1.5±0.3	-0.7±0.0	0.1±0.1	1.7±0.1	-0.6±0.1	0.0±0.2
5	2.4±0.2	-1.0±0.1	0.2±0.1	2.5±0.2	-0.7±0.1	0.5±0.3
10	3.8±0.5	-1.4±0.1	0.8±0.3	3.9±0.3	-0.9±0.1	1.4±0.4
30	6.0±0.7	-1.0±0.1	2.5±0.8	6.3±0.3	-0.3±0.1	3.5±0.3
	Indoor test					
2,5	0.5±0.2	-0.6±0.3	-0.6±0.3	0.8±0.3	-0.8±0.1	-0.9±0.4
5	1.8±0.2	-0.7±0.3	-0.2±0.3	1.4±0.3	-0.7±0.1	0.0±0.4
10	2.0±0.2	-0.8±0.2	0.0±0.1	2.3±0.4	-0.9±0.1	0.3±0.1
30	4.4±0.4	-0.5±0.3	2.2±0.3	5.2±0.3	-0.7±0.2	2.8±0.6
	Intense test					
2,5	-0.6±0.2	-0.7±0.1	-2.1±0.5	-0.4±0.1	-1.0±0.0	-1.7±0.1
5	0.4±0.1	-0.9±0.1	-2.3±0.2	0.5±0.1	-1.2±0.1	-1.4±0.1
10	0.7±0.3	-1.4±0.1	-2.4±0.1	0.6±0.3	-1.3±0.1	-1.2±0.1
30	4.2±0.5	-1.8±0.3	-1.6±0.4	3.2±0.5	-1.6±0.2	-0.2±0.4
	Mild test					
2,5	-2.2±0.2	-0.7±0.1	-3.1±0.3	-1.2±0.2	-0.6±0.1	-2.1±0.2
5	-1.5±0.4	-0.9±0.2	-3.0±0.5	-1.2±0.2	-0.8±0.3	-1.9±0.4
10	0.3±0.2	-1.5±0.2	-2.7±0.2	0.3±0.1	-1.3±0.2	-1.8±0.2
30	2.4±0.3	-1.6±0.2	-2.4±0.5	2.3±0.6	-1.7±0.4	-0.8±0.2

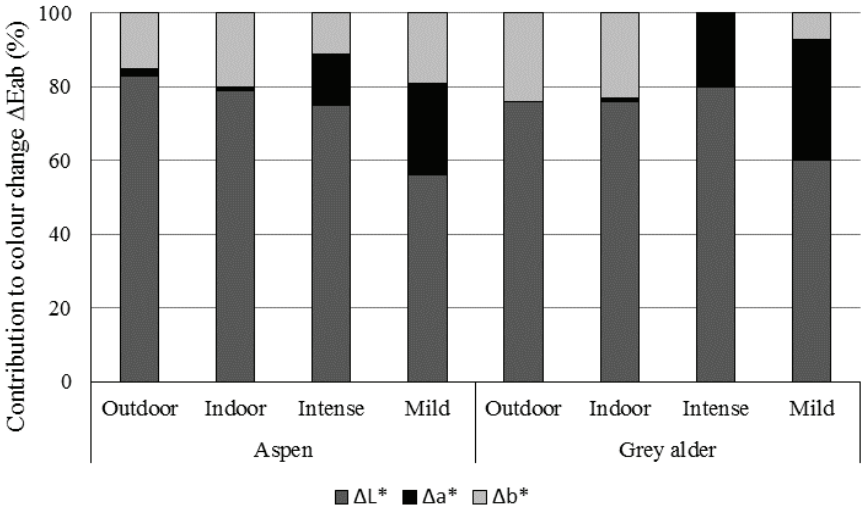

Figure 3.Contribution of the colour parameter changes (ΔL*, Δa, Δb*) to the total colour change ΔEab of thermally modified aspen and grey alder wood during exposure to different irradiation conditions for 30 h.

and artificial weathering tests was observed for the yellowness parameter b*. In the Outdoor test, parameter b* values increased (Δb* > 0) during the whole experiment. In the Indoor test, small decrease of the parameter b* was observed during initial exposure after which the parameter b* increased, and its values exceeded the original values (Δb* > 0) after a 10 h exposure for aspen wood and after a 5 h exposure for grey alder wood. In both artificial UV irradiation tests, the initial decrease of the parameter b*, which was significantly greater than that in the Indoor test, was observed. During the subsequent exposure, the parameter b* increased in both artificial irradiation tests but at the end of the experiment, the parameter b* values were still lower than their original ones (Δb* < 0).

Figure 3 represents the effect of solar and artificial UV irradiation on the contribution of the individual colour parameters to the total colour change ΔEab.

During all tests, changes in lightness parameter L* were the major contributor to discolouration. The redness parameter a* contributed less than 2% to the total discolouration during Outdoor and Indoor tests, while significant contribution of redness parameter a* was detected for both artificial weathering tests with Δa* value even contributing 33% in the total discolouration for grey alder wood exposed in the Mild test. Concerning contribution of chromaticity parameters a* and b* in the total discolouration, a trend was observed that changes in yellowness parameter b* were greater for specimens in the solar tests while changes in the redness parameter a* prevailed in the artificial weathering tests.

Changes in reflectance spectra at the end of the experiment, i.e. after a 30 h exposure, are shown in Fig. 4 in a form of difference reflectance spectra, which were calculated by subtracting the reflectance spectrum of wood after and before exposure.

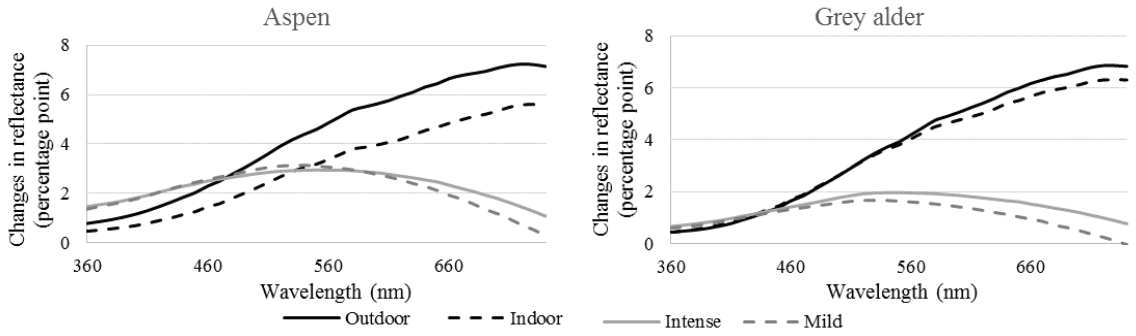

Figure 4. Difference reflectance spectra of thermally modified aspen and grey alder wood exposed to different irradiation conditions for 30 h.

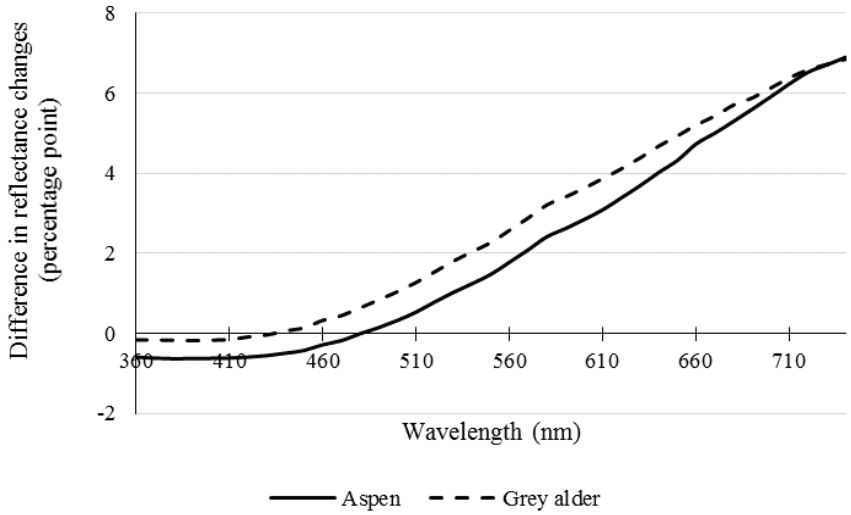

Figure 5. Difference of reflectance changes between specimens exposed to equivalent UV radiation dosages in the solar (Outdoor) and artificial UV (Mild) tests for thermally modified aspen and grey alder wood.

It can be seen that the changes in reflectance had similar trends for thermally modified wood of both species under study. For all studied irradiation conditions, reflectance increased over the whole inspected wavelength range. Only a slight increase in reflectance was detected in the shorter wavelength range for both solar and artificial irradiation. Reflectance of specimens exposed to the artificial UV irradiation changed less and quite evenly over the whole inspected wavelength range with a slight increase around 500 nm and hardly any changes in the wavelength range above 700 nm. However, substantial increase of reflectance in the wavelength region above 500 nm was observed for specimens exposed to the solar irradiation outdoors and indoors.

To compare the effect of solar and UV radiation on changes in reflectance of thermally modified wood, the difference between changes in reflectance of Outdoor and Mild tests, during which the specimens received almost equivalent dosages of the UV radiation (Table 1), were calculated by subtracting the respective values (Figure 5).

It can be seen that difference between reflectance changes due to solar and artificial UV irradiation was similar for thermally modified aspen and grey alder wood. Hardly any difference was detected in the wavelength range up to 500 nm, while substantially greater increase in reflectance was found at the longer wavelength range for specimens in the Outdoor test.

Discussion

Nevertheless, the recorded UV radiation flux density of UV radiation in the solar spectrum at midday during the Outdoor test was even higher than that used in the Intense UV test, when wood was exposed to the artificial UV irradiation of the highest intensity (Figure 1), the total UV radiation dose received by specimens in the Outdoor test was only about 60% from that received by the specimens in the Intense test. During the whole experiment, UV radiation flux density of the sunlight filtered through the window glass to which wood was exposed in the Indoor test was lower than that used in both artificial weathering tests. Moreover, the magnitude of the UV radiation dose received by the Indoor test specimens at the end of the experiment was less than that received by the specimens in the Intense UV test during the first 5 h of exposure. It should be mentioned that UV radiation represents only 5% from the solar radiation reaching the Earth surface (Deka *et al*., 2008).

As the specimens were only exposed to the irradiation for 30 h, the substantial colour change indicates that the process of thermally modified wood discolouration is quite fast. Similar observation about very rapid discolouration in the initial period of both unmodified and thermally modified wood exposure to different light sources has been reported by several researchers (Ayadi *et al.* 2003; Deka *et al*., 2008; Oltean, Teischinger, & Hansmann, 2008; Tolvaj & Varga, 2012; Hauptmann *et al*., 2014).

It has been well documented that UV radiation causes greater or lesser discolouration in thermally modified wood. Our results suggest that the part of solar radiation (radiation above 365 nm) that is not represented in the spectrum of the UVA-340 fluorescent lamps, which are widely used in the artificial weathering tests, significantly influenced the discolouration process of thermally modified wood (Figure 2). Greater discolouration was detected even

for the specimens in the Indoor test when the total received UV radiation (290 – 390 nm) dose was 14% and 25% from that received by the specimens in the Intense and Mild tests, respectively. Besides, the discolouration of the Indoor test specimens indicate that, although the window glass absorbs part of solar radiation, the transmitted radiation can provoke substantial colour changes in thermally modified wood. These results suggest that UVA type lamps, which only imitate well the sunlight UV spectrum from 290 nm to 365 nm, do not fully simulate changes induced by solar radiation in thermally modified wood. The longer wavelength range of UV radiation and the short-wavelength range of visible light are an important initiator of discolouration for thermally modified wood.

Total colour changes depend on changes in individual colour parameters. Different trends of individual colour parameter changes were observed for solar and artificial weathering tests (Table 2). However, during the time window of the experiment, the pattern of colour parameter changes was similar for both thermally modified aspen and grey alder wood. As it can be seen, changes in the individual colour parameters in the course of the experiment altered their direction. It implies that discolouration of thermally modified wood is a complicated and dynamic process with various and different chemical transformations in wood chromophores. Several researchers have reported results of thermally modified wood discolouration due to artificial weathering (Deka & Petric 2008; Miklečić *et al*., 2011; Huang *et al*., 2012). Common trend of an increase in the lightness parameter L* due to weathering was observed in all reported studies. However, concerning chromaticity parameters a* and b*, different patterns have been observed. Deka & Petric (2008) detected decrease in redness parameter a* and increase in yellowness parameter b*, while Huang, Kocaefe D., Kocaefe Y., Boluk & Pichette (2012) found decrease in both parameters a* and b* during artificial weathering which agrees with the results established in the present study. In its turn, Miklečić, Jirouš-Rajković, Antonović & Španić (2011) observed different trends of colour parameter changes for thermally modified ash wood depending on the thermal treatment temperature. They investigated artificial weathering of ash wood modified at 190 °C and 212 °C and observed increase in a* and b* for ash treated at 190 °C and increase in a* and decrease in b* for ash treated at 212 °C. The differences in discolouration patterns may be attributed to different wood species as well as different thermal treatment conditions used in the studies.

The dissimilarity between effect of solar and artificial irradiation on thermally modified wood is well illustrated by the contribution of the individual colour parameters to the total colour change ΔEab (Figure 3). It can be seen that for all irradiation systems changes in lightness parameter L* contributed more than 50% to the discolouration. However, in the cases of artificial weathering, redness parameter a* contributed significantly to the total discolouration, while hardly any impact of changes in this parameter was detected for specimens exposed to solar irradiation.

The fact that there are differences in colour change between the specimens exposed to solar and artificial UV irradiation is also confirmed by changes in reflectance spectra in the visible light range of 360–740 nm (Figures 4 and 5). Reflectance spectra characterise wood chromophores and changes in their concentration (Pandey & Vuorinen, 2008). The changes in reflectance clearly indicate that there are dissimilarities for various types of irradiation, while similar tendencies were observed for the specimens exposed to the irradiation of different intensities of the same type, i.e. solar or artificial UV irradiation.

The most similar UV radiation doses that specimens received during the experiment were for the Outdoor and Mild test specimens (Table 1). In the Indoor test, the UV dosage received by the specimens was much lower. Nevertheless, changes in reflectance after a 30 h exposure (Figure 4) are more similar for both the solar irradiation – Outdoor and Indoor tests. It implies that the UV radiation is not the dominant factor influencing thermally modified wood during exposure to solar irradiation.

Comparing the effect of radiation comprising similar UV doses but different spectral composition on changes in thermally modified wood reflectance (Figure 5), it is clearly seen that additional visible light irradiation in the Outdoor test resulted in significantly greater changes in reflectance in the wavelength range above 500 nm. Changes in reflectance in the shorter wavelength region are quite similar for both the solar and artificial UV irradiation processes. Moreover, negative values in the short-wavelength range indicate on even slightly more changes in reflectance in this wavelength range for specimens in the Mild test than that of the Outdoor test. It indicates that the chromophores, with characteristic reflectance in the range up to 500 nm, are relatively stable to visible light and can be altered mostly by the UV radiation. On the other hand, the markedly higher changes in the wavelength range above 500 nm of the specimens exposed to solar radiation suggest that thermally modified wood contains significant amount

of chromophores which can be transformed also by the light of relatively less photons energy.

Conclusions

The colour change caused by solar and artificial UV irradiation has a similar pattern for both thermally modified hardwood species under study, namely, aspen and grey alder wood. Colour change was similar for different intensities of the same type of irradiation but differed for various irradiation types, i.e. solar or artificial UV irradiation. Noticeably greater colour changes were detected for specimens exposed to solar irradiation outdoors and indoors compared to specimens exposed to artificial UV radiation regardless the higher UV radiation doses received by the specimens during the artificial weathering. It implies that thermally modified wood contains significant amount of chromophores which can be transformed by the light of relatively less photons energy. Analyses of changes in colour parameters indicate that discolouration of thermally modified wood is a complicated and dynamic process with various and different chemical transformations in wood chromophores.

Results of the present study clearly show that the region of the solar spectrum that is not included in the spectrum of UVA-341 lamps but accounts for a substantial part of the solar radiation, namely, UV radiation of the longer wavelength range above 365 nm and possibly also some range of visible light, is an important initiator of chemical reactions in thermally modified wood. This suggests that UVA-340 type lamps, which only imitate well the sunlight UV spectrum from 290 nm to 365 nm, do not fully imitate the changes induced by solar radiation in thermally modified wood.

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Prevalent Parasitosis in Beef and Dairy Cattle Farms in Vidzeme Region

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Abstract. The aim of the study was to investigate the beef and dairy cow parasitosis epizootic situation in Vidzeme region. Research was done throughout Vidzeme territory during the period of the years 2013-2014. The total number of animals examined was: 273 dairy and 90 young beef cattle aged from 6 months to two years and 248 dairy and 29 beef cows older than two years. For the diagnosis of helminthes standardized ovoscopic and larvoscopic methods were used. For the diagnosis of protozoa flotation and modified Ziehl-Neelsen methods were used. The main species in the samples were *Cryptosporidium* spp., *Eimeria* spp. and *Strongylus* spp. In the young dairy and beef cattle aged from 6 months to two years and cattle older than two years *Cryptosporidium* spp. invasion accordingly was 32.6% and 19% (dairy cattle) and 62.2% and 65.5% (beef cattle); the invasion of *Eimeria* spp. 30% and 7.3% (dairy cattle) and 55.6% and 10.3% (beef cattle); and the invasion of *Strongylus* spp. was 17.6% and 13.7% (dairy cattle) and 43.3% and 27.6% (beef cattle). Both dairy and beef cattle were infected with *Moniezia* spp., *Paramphistomum* spp., *Strongyloides* spp. Dairy cows aged from 6 months to two years had *Trichuris* spp., *Dictyocaulus* spp. and *Neoscaris* spp. invasion.
Key words: cryptosporidium, eimeria, strongylus, parasitosis, cattle, Latvia.

Introduction

In Latvia, there have been no requirements for farmers to carry out any coprological examination of cattle in the last 20 years. Only few researches have been carried out in the last 5-10 years (Keidāne, Krūklīte, & Medne, 2012; Lassen, 2011). Thereby, the situation regarding the most common cattle parasitosis in this country has still not been fully described.

In addition to climate change and the import of cattle, there is a great opportunity to introduce the parasite species which are not registered in the republic (Demiaszkiewicz, 2014).

Overall, worldwide cattle show a very high prevalence (95.5%) of parasite infections. Out of this percentage, 75.1% had multiple parasites while 20.4% had a single parasite infection. Prevalence of *Strongyles* spp. (63.1%) was the highest, followed by *Fasciola* spp. (51.1%), *Eimeria* spp. (29.4%), *Paramphistomum* spp. (25.9%), *Schistosoma* spp. (21.7%), *Ascaris* spp. (6.1%) and then *Moniezia* spp. (2.3%) (Squire, Amafu-Dey & Beyuo,2013; Уркхарт , 2000). The prevention of parasitic diseases is based on the study of the epizootic situation and planning of preventive measures (Jasmer, Lahmers, & Brown,2007; Heinrichs *et al.*, 2003; Forbes *et al*., 2000; Уркхарт *et.al*., 2000).

The aim of this study was to find out prevalent parasitosis in dairy and beef cattle herds in Vidzeme region.

Materials and Methods

The research was done in Vidzeme region in the period from 2013-2014. The dairy and beef cattle were investigated. Animals were divided into the following four groups: dairy cattle aged from six months to two years (n = 273) and older than two years (n = 248); beef cattle aged from six months to two years (n = 90) and older than 2 years (n = 29). Samples were obtained from 62 cattle farms: 50 dairy cattle farms and 12 beef cattle farms. Laboratory tests were made in the laboratory of the department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Agriculture.

Rectal fecal samples collected into plastic bags were kept in a refrigerator at 4˚C prior to examination. For the diagnosis of helminthes standardized ovoscopic and larvoscopic methods were used (Roepstorff & Nansen, 1998). For the diagnosis of protozoa flotation and modified Ziehl-Neelsen methods (Henriksen & Pohlenz, 1981) were used.

Parasitosis invasion extensive margin or prevalence (IE) was computed by dividing the number of infected animals by the total number of animals in a group. The correlation between the age of the animals and the validity of invasion was calculated using the t-test function in Microsoft Excel 2013 programme.

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Results and Discussion

The present study showed that in Vidzeme parasitosis were found in both age groups of dairy (Figure 1) and beef cattle (Figure 2).

In both age groups of dairy cattle, the highest infestation extensive margin (IE)was showed by *Cryptosporidium* spp. IE 32.6% and 19% ($p < 0.05$), *Eimeria* spp. IE 30% and 7.3% ($p < 0.01$) and *Strongylus* spp. IE 17.6% and 13.7% ($p < 0.05$) correspondingly. The prevalence of *Eimeria* spp. and *Strongylus* spp. in both groups is quite similar to studies in other countries (Kounty *et al.*, 2012; Knubben-Schweizer *et al*., 2010; Lassen & Talvik, 2009; Forbes *et al*., 2000).

In group of young cattle from six months to two years in addition to the above mentioned invasions, we diagnosed *Moniezia* spp. IE 6.2%, *Neoscaris* spp. (ascarids) IE 2.6%, *Trichuris* spp. IE 1.5%, *Fasciola* spp. IE 1.7%, *Paramphistomum* spp. IE 1.7%, *Strongyloides* spp. IE 1.1% and *Dictyocaulus* spp. IE 1.7%.

In cattle older than two years, we diagnosed *Moniezia* spp. IE 3%, *Trichuris* spp. IE 0.4%, *Fasciola* spp. IE 1.2%, *Paramphistomum* spp. IE 0.8% and *Dictyocaulus* spp. IE 0.4%.

Neoscaris vitulorum or calf ascarids was diagnosed in young cattle aged six months to two years IE 2.6%. As we know, adult cattle do not suffer from ascarids (Davila, Irsic, & Greiner, 2010; Zajac & Conboy, 2006).

Dictyocaulus spp. invasion in cattle from six months to two years was IE 1.7%, in cows older than two years IE 0.4% ($p > 0.05$). It is noted that the invasion of *Dictyocaulus*is spp. is more often diagnosed in young animals. (Elsheikla, 2011; Holzhauer *et al*., 2011; Hoglund, Ganheim, & Alenius, 2003). The diagnosed invasion of *Dictyocaulus* spp. in the cattle older than two years could be explained by the import of cattle from different regions of the world.

Describing the invasion of *Fasciola* spp. IE 1.7% and 1.2%, *Paramphistomum* spp. IE 1.7% and 0.8%, it can be concluded that the situation in Vidzeme region is similar to that of the previous studies about Latvia (Keidāne, Krūklīte, & Medne, 2012), but comparing with the research conducted in other countries, the prevalence of parasites in Latvia is lower. Similarly, a study in Austria found that cattle *Fasciola* spp. IE was 16% (Knubben-Schweizer *et al*., 2010). Regarding *Paramphistomum* spp., in Turkey the average IE throughout the year was 10.4% (Ozdal *et al*., 2010).

In beef cattle, like in dairy cattle, the highest IE both for young cattle from six months to two years and cattle older than two years has been observed in *Cryptosporidium* spp. IE 62.2% and 65.5% ($p < 0.01$), *Eimeria* spp. 55.6% and 10.3% ($p < 0.01$) and *Strongylus* spp. IE 43.3% and 27.6% ($p < 0.01$) invasions (Figure 2). Completely different results were obtained from researchers in Alberta, Canada – the prevalence of *Cryptosporidium* spp. in beef cattle calves is 5% (Ralston, McAllister, & Olson, 2003; Gow & Waldner, 2006). Compared with other countries, *Eimeria* spp. IE in Latvia is slightly higher: studies in Turkey found *Eimeria* spp. IE for calves in beef cattle herds 27.2% (Cieek *et al*., 2007), 33.3% in Hungary (Farkas, Szeidemann & Majors,2007), 22.6% in Brazil (Almeda *et al*., 2011). *Strongylus* spp. IE in Latvia is lower than in other countries, for example, in Thailand IE is 100% (Lwin, 2011).

Along with the invasions mentioned above, young cattle were infected with *Moniezia* spp. IE 8.9% and *Strongyloides* spp. IE 1.1%, whereas in cattle older than two years *Paramphistomum* spp. IE 3.4% was diagnosed. In comparison with other countries, the invasion of these parasitosis in our country is

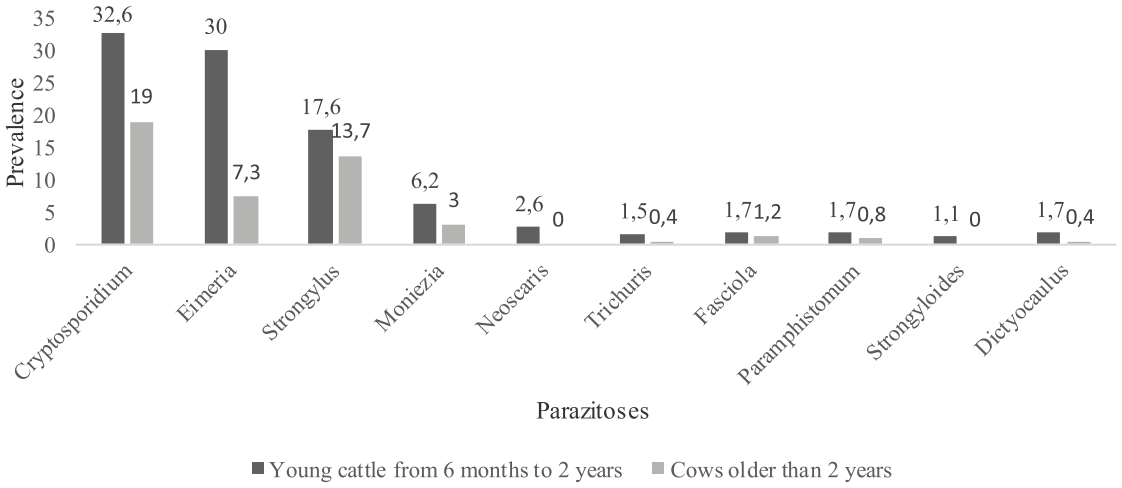

Figure 1. Prevalence (%) of parasite invasion in dairy cattle.

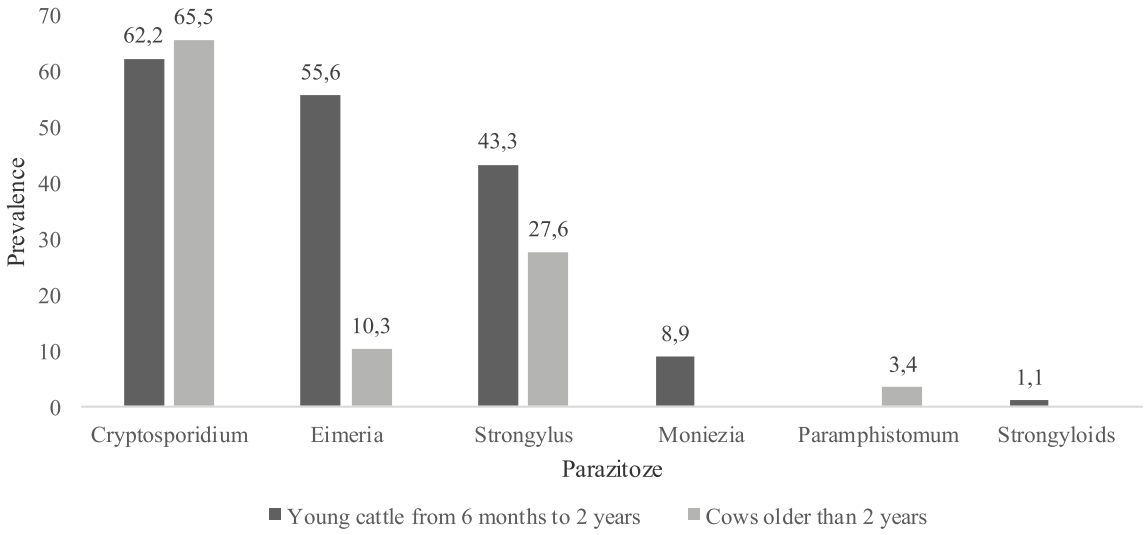

Figure 2. Prevalence (%) of parasite invasion in beef cattle.

much lower. The research in Spain reported about *Paramphistomum* spp. IE was 29% (Gonzales-Warleta *et al*., 2013), in Thailand IE was 6.4% in beef cattle under 1 year old, and IE was 23.3% of cattle from one to two years old (Lwin, 2011). Similar IE has been mentioned regarding *Moniezia* spp.: in Thailand *Moniezia* spp. IE in cattle under one year was 12.9%, from one to two years old it was 23.3% (Lwin, 2011). Relatively higher *Strongyloides* spp. IE was found in the Czech Republic - 4.3% (Kvač & Vitovec, 2007).

In general, younger animals are most likely to show signs of parasitism, while mature cows acquire a degree of immunity to parasites that reside in the gastrointestinal tract. Dairy cattle in a dry lot are less likely to have heavy worm infection than beef cattle on pastures (Gadberry & Powell, 2011).

The results show that we should pay more attention to studies of cryptosporidiosis in Latvia because this disease has not been thoroughly investigated. Our research showed a high *Cryptosporidium* spp. invasion both in dairy and beef cattle herds. According to the sources of scientific literature, cryptosporidiosis is a serious invasion that is registered not only in the tropical zone countries (Kounty *et al*., 2012; Уркхарт *et al*., 2000), but also in Europe and it is shown by the recent research conducted in Latvia (Keidāne, Krūklīte, & Medne, 2012; Lassen, 2011). We didn't include calves aged from the first to the tenth day of life in this study, which are in a high-risk group. Therefore, we will continue studies of the *Cryptosporidium* spp. invasion, treatment and prevention.

Conclusions

- In dairy cattle herds in the age group of six months to two years were diagnosed with low invasion of ascarides, *Dictyocaulus* spp. and *Trichuris* spp.
- Dairy cattle aged from six months to two years and cattle older than two years were more frequently infected with *Cryptosporidium* spp. IE 32.6% and 19%, *Eimeria* spp. IE 30% and 7.3% and strongylatoses of digestive system IE 17.6% and 3.7%.
- Beef cattle aged from six months to two years and cattle older than two years, were more frequently infected with *Cryptosporidium* spp. IE 62.2% and 65.5%, *Eimeria* spp. IE 55.6% and 10.3% and *Strongylus* spp. 43.3% and 27.6%.
- Both dairy and beef cattle herds were infected with *Moniezia* spp., *Paramphistomum* spp. and *Strongyloides* spp.

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Leachates of Thermally Modified Pine (*Pinus sylvestris* L.) Wood

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Abstract. During the last decades, thermally modified wood has become an object of interest among the wood scientists as an environmentally friendly material, because nowadays environmental aspects of materials have become more and more significant. Leaching is one of the processes that occurs in outdoor use. The aim of this study was to evaluate concentration of potentially hazardous substances in leachates of thermally modified pine wood. Scots pine (*Pinus sylvestris* L.) wood was thermally modified using Wood Treatment Technology (WTT) company device at 170 °C for 1 hour (TMP170/1) and at 160 °C for 3 hours (TMP160/3) and the mass loss was calculated. Material preparation and leaching procedure was made according to standard LVS EN 84:2000. In obtained leachates, the content of sugars, acetic acid, furfural and tannic acid were determined. Results showed that the total wood mass loss was 7.1 ± 1.4% (n=20) for TMP170/1 and 4.0 ± 1.6% (n=20) for TMP160/3. The initial leaching velocity differs between both modes and is higher for TMP160/3. The velocity decreases exponentially with immersion time and reaches plateau after 7th (5 days) immersion, but leaching still continues after the 9th immersion (14 days). The main components in leachates were tannic acid and pentoses. Among all studied compounds furfural is the hardest leachable one. Thermally modified wood treated at TMP170/1 is more environmentally friendly due to less water leachable substances. It is worth looking forward by investigating volatile organic compounds emissions in the air as it also could give high impact on human health.
Key words: Thermally modified wood, leachates, furfural, softwood.

Introduction

Thermal modification of wood has been investigated during the last decades as an alternative approach to obtain bio-durable wood (International ThermoWood Association, 2003). The properties of material both mechanical (Esteves & Pereira, 2008; Korkut, Akgül, & Dündar, 2008; Srinivas & Pandey, 2012) and decay resistance (Boonstra *et al.*, 2006) have been widely investigated. Relatively fewer researches are devoted to the environmental impact of this material (Kamdem, Pizzi, & Triboulot, 2000; Peters, Fischer, & Fischer, 2008; Vetter *et al.*, 2008). As the only chemical agent used in this treatment is water, it is considered to be an environmentally friendly method (Finnish ThermoWood Association, 2008). Nowadays evaluation of material impact on environment life cycle assessment has become more and more significant (European Commission, 2014). This system approach requires a lot of input data, and it is considered to be improved whenever new data appears (LVS EN ISO 14044: 2006). Life cycle assessment of thermally treated and untreated maritime pine boards (Ferreira *et al.*, 2014) shows that thermally treated wood has a high weight of impact on human health (100%); therefore, it is essential to provide additional data about its service life.

One of the processes in material usage outdoors is leaching. It is also a standardized procedure for accelerated ageing (LVS EN 84:2000) and is commonly used for impregnated wood testing. Leachates of thermally modified wood consist mainly of wood constituents degradation products. Total mass loss is the one of the main characteristics of degradation degree in thermal treatment; it depends on the wood species, heating medium, temperature and treatment time, among which the temperature has the most influence. Hemicelluloses are the most easily degradable wood components and form hexoses and pentoses (sugars) when exposed to high temperatures, which in turn degrade further forming furfural from pentoses (Esteves & Pereira, 2008). The acetic acid is formed during the thermolysis of the acetyl radicals linked to xylose in xylans. Leachates contain not only the mentioned wood thermal degradation products, but also extractives. Tannins belong to water-soluble group of extractives - large polyphenol compounds containing hydroxyl groups that bound to wood macromolecules.

The aim of this study was to compare pine wood, modified in two different regimes in relevance of leaching by determining the total mass loss during the thermal treatment, as well as detecting concentration of hazardous substances in leachates of thermally modified pine wood.

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Materials and Methods

The experiments were carried out in the Laboratory of Wood Biodegradation and Protection of the Latvia State Institute of Wood Chemistry.

Thermal modification. Scots pine (*Pinus sylvestris* L.) was chosen for this study as it is the most common wood species in Latvia. Pine wood was thermally modified using Wood Treatment Technology (WTT) device. The dimensions of the wood boards before the thermal treatment were 1000×100×25 mm. The treatment was conducted in a water vapour medium under elevated pressure (0.6 MPa) at 170 ºC temperature for 1 h (TMP170/1) and other set of samples at 160 ºC temperature for 3 h (TMP160/3). Afterwards, modified boards were conditioned at 20 ºC and 65% relative humidity, measured and weighted in order to calculate mass loss.

Leaching. Material preparation and leaching procedure was made according to standard LVS EN 84:2000. The leaching procedure consisted of an initial impregnation with distilled water under 4 kPa vacuum for 20 min. The water was subsequently replaced 2 h after the impregnation, continuing at 24 h and 48 h, and another seven times in the next 12 days at intervals of not less than 1 day and not more than 3 days. The leachates were collected, their volume measured, and part of them stored in 5 °C.

Sugar analysis. For sugar analysis 0.1 mL of 10% sulphuric acid and 1 mL 0.2 *M* sodium periodate solution were added to approximately 10 g of leachate after that sample was kept in 40 °C for 5 h, and then 5 mL of fresh 10% ammonium molibdate solution was added. Fifteen minutes later 1 mL of glacial acetic acid and 1 mL of 10% potassium iodate solution were added. After 15 minutes samples were titrated with 0.1 *M* standardized sodium thiosulphate (against potassium dihromate) solution. The results were recalculated to the relative mass of hexoses or pentoses (w%). In the described method, the following reactions (Eq. 1, Eq. 2) take place:

$$C_6H_{12}O_6 + 5NaIO_4 \rightarrow \rightarrow 5HCOOH + H_2CO + 5NaIO_3 \quad (1)$$

$$C_5H_{10}O_5 + 4NaIO_4 \rightarrow \rightarrow 4HCOOH + H_2CO + 4NaIO_3 \quad (2)$$

Tannin analysis. Content of tannins in leachates was obtained by measuring absorption of samples at 280 nm and plotting the results against calibration curve of tannic acid. Measurements were made with UV-VIS Spectrometer Genesys™ 10.

Acid analysis. Content of short chain carboxylic acids was determined by titrating the acetic acid within the leachates with standardized 0.1 *M* potassium hydroxide solution (standard – potassium hydrogenpfhtalate).

Aldehyde analysis. In order to find out approximate content of aldehydes, 5 mL of 0.5 *M* hydroksylamine hydrochloride solution were added to 10 g of sample. After 2.5 h 20 mL propanol-2 were added and three parallel samples were titrated with standardized 0.1 *M* potassium hydroxide solution (standard – potassium hydrogenphtalate). The following reaction (Eq. 3) occurs:

$$RCHO + H_2NOH \cdot HCl \rightarrow \rightarrow RCHNOH + HCl + H_2O \quad (3)$$

The results were expressed as relative mass of furfural in sample.

The results were processed by mathematical and statistical methods with software Microsoft EXCEL 2010. Mean values and standard deviations of the samples were calculated.

Results and Discussion

This study also reveals that temperature is the most significant factor in thermal modification - although treatment time at 160 °C was three times longer (3 h) than in 170 °C (1 h) mass loss at lower temperature (4.0 ± 1.6%, n=20) was almost two times smaller than in higher temperature (7.1 ± 1.4%, n=20). The standard deviations are relatively high and only slightly differ between both treatments, more likely due to the variety of the pine wood itself. Metsä-Kortelainen (2011) in ThermoWood® process has obtained that at 170 °C 3 h modified pine sapwood has a mass loss of about 2%, which is 3 times less than results obtained in this study. It is more likely due to the evaluate pressure in thermal modification used in this study.

Results show that after 14 days the total amount of leached substances was 21% higher from pine wood thermally modified at 160 °C (Table 1) than from pine wood thermally modified at 170 °C (Table 2). Sugars were the main components (38%) leached from TMP160/3 while for TMP170/1 sugars were only 26% due to further degradation of sugars forming carboxylic acids and aldehydes. The main component leached from TMP170/1 was the tannic acid (43%). Also, in the leachates of TMP160/3 (35%) the content of tannic acid was high.

Results of Graf, Wagner, Begander, Trinkaus, & Boechzelt (2005) showed that the main components of leachates include acetic acid, furfural and furfural derivatives (80% from almost 100 compounds). In this study acetic acid constitutes 15% (TMP160/3) and 20% (TMP170/1) of total leached and identified

Table 1

Content of organic substances in leachates of pine wood, thermally modified at 160 °C for 3 h, mg of leached substance per g absolute dry sample

No. of immersion	Time, h	Acetic acid	Stdev.	Furfural	Stdev.	Tannic acid	Stdev.	Sugars	Stdev.
1	4	0.40	0.006	0.24	0.097	1.12	0.0002	0.92	0.01
2	24	2.08	0.009	1.39	0.098	4.35	0.0041	4.38	0.09
3	27	1.46	0.000	1.18	0.136	2.99	0.0045	3.37	0.16
4	48	1.74	0.003	1.55	0.045	3.91	0.0018	4.66	0.10
5	28	0.67	0.010	0.73	0.064	1.87	0.0109	1.79	0.05
6	43	0.55	0.002	0.73	0.036	1.70	0.0018	1.85	0.06
7	52	0.31	0.012	0.27	0.182	0.86	0.0055	1.08	0.01
8	48	0.32	0.090	0.27	0.180	0.80	0.0018	1.05	0.03
9	62	0.25	0.008	0.36	0.004	0.89	0.0008	1.09	0.01
	Total	7.8		6.7		18.5		20.2	

Table 2

Content of organic substances in leachates of pine wood, thermally modified at 170 °C for 1 h, mg of leached substance per g absolute dry sample

No. of immersion	Time, h	Acetic acid	Stdev.	Furfural	Stdev.	Tannic acid	Stdev.	Sugars	Stdev.
1	4	0.53	0.005	0.16	0.005	1.28	0.0004	0.60	0.01
2	24	1.96	0.031	0.46	0.231	3.63	0.0015	2.14	0.08
3	27	1.63	0.002	0.95	0.130	2.97	0.0104	1.75	0.03
4	48	1.96	0.061	1.12	0.173	3.71	0.0006	2.48	0.06
5	28	0.74	0.009	0.61	0.035	1.74	0.0004	0.96	0.04
6	43	0.66	0.007	0.61	0.259	1.79	0.0017	1.08	0.02
7	52	0.30	0.006	0.43	0.112	0.80	0.0012	0.53	0.01
8	48	0.35	0.017	0.26	0.095	1.12	0.0005	0.73	0.02
9	62	0.27	0.004	0.34	0.026	1.00	0.0017	0.62	0.00
	Total	8.4		4.9		18.0		10.9	

amount of substances but furfural 13% and 12% respectively. This discrepancy can be explained by the sensitivity of used methods. Graf, Karlsson and colleagues had used gas chromatography-mass spectrometry and identified single compounds while in this study, results represent a group of substances with similar composition - pentoses represent sugar content; acetic acid represents content of short chain carboxylic acids; furfural represents the content of aldehydes and tannic acid represents the content of aromatic phenolic compounds. Karlsson, Torniainen, Dagbro, Granlund, & Moren (2012) state that under saturated steam at 170 °C thermally modified pine wood leaches 0.586% of dry mass 5-(hydroxymethyl) furfural and 0.029% furfural, which is in total 0.615% furan compounds of dry mass. Recalculating results in this research, obtained values are 0.672% for TMP160/3 and 0.494% for TMP170/1. This could be explained due to the furfural leaching during thermal modification in WTT process or due to furfural polymerization with lignin destruction products, forming resins.

It could be also relevant to prolong the experiment because after the 9th immersion (14 days) curves of total furfural content have not reached plateau (Figure 1). The same pattern can be observed for curves of tannic acid, acetic acid and sugars.

The sugar leaching velocity decreases exponentially depending on water immersion time (Figue 2). The velocity of leaching of the sugars from pine wood, thermally modified at TMP160/3 is the highest at the beginning (0.32 mg h^{-1} g^{-1}), and

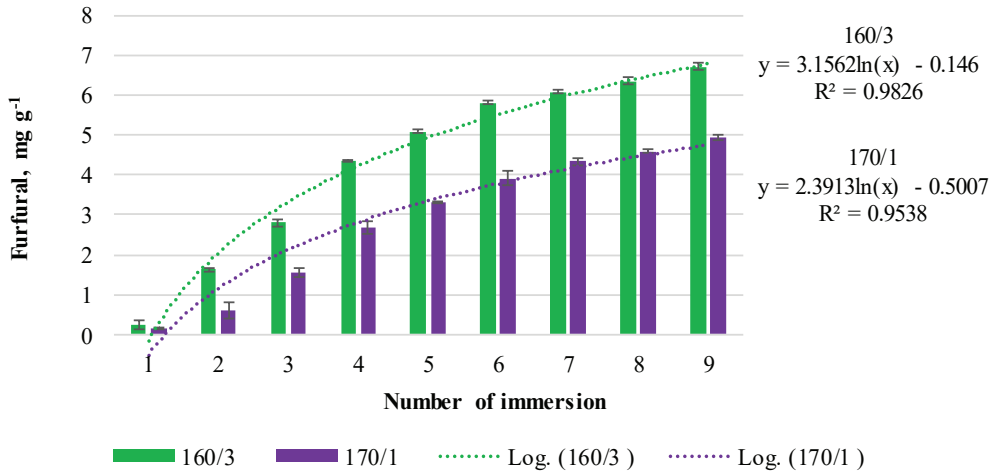

Figure 1. Total content of furfural in leachates of thermally modified pine wood treated in two different modes depending on the number of immersions.

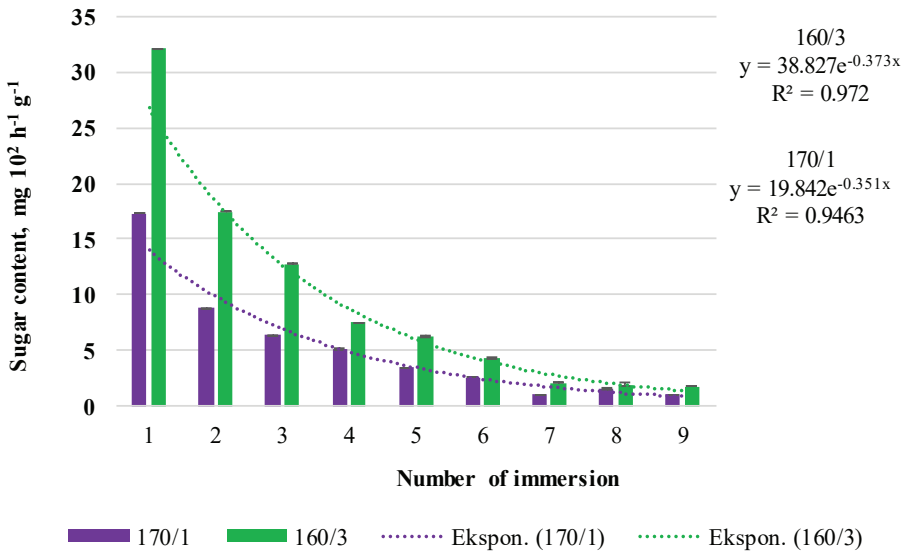

Figure 2. Sugar leaching velocity of thermally modified pine wood treated in two different modes depending on the number of immersions.

decreases with repeated number of water replacement and after the 7th immersion reaches plateau at 0.02 mg h^{-1} g^{-1}. For thermally modified wood treated at TMP170/1, initial sugar leaching velocity is almost twice as slow (0.17 mg h^{-1} g^{-1}) and likewise it reaches plateau after the 7th immersion at 0.02 mg h^{-1} g^{-1}.

The same pattern can be observed with furfural leaching – initial velocity rate for TMP160/3 treated wood is 0.08 mg h^{-1} g^{-1} while for TMP170/1 only 0.04 mg h^{-1} g^{-1}, reaching plateau after the 7th immersion at 0.01 mg h^{-1} g^{-1}. Tannic acid and acetic acid substances have similar leaching velocity curves to those of the previously described - sugars and furfural although there is no significant difference between initial velocities for wood modified at TMP170/1 and TMP160/3. They are 0.37 and 0.38 mg h^{-1} g^{-1} for tannic acid, 0.15 and 0.14 mg h^{-1} g^{-1} for acetic acid with plateau at 0.015 and 0.006 mg h^{-1} g^{-1} respectively.

Unfortunately, by the methods used it was not possible to distinquish hexoses and pentoses. Results of Karlsson, Torniainen, Dagbro, Granlund, & Moren (2012) showed that leachates of pine wood thermally modified for 3 hours at 170 °C contain 37% more hexoses than pentoses. As this result is obtained with gas chromatography-flame ionization detector, it is more likely that difference in sugar amounts of leachate exist, but are above sensitivity of approach used in this study.

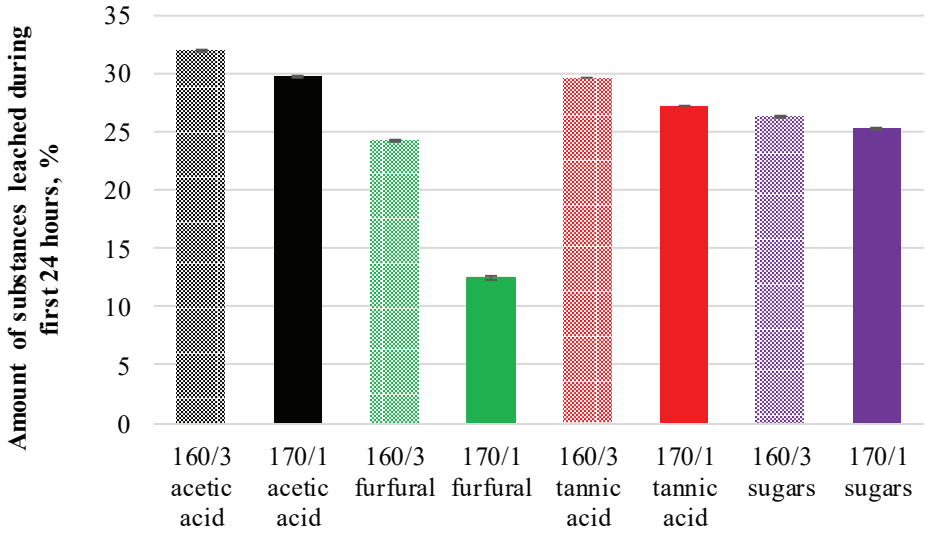

Figure 3. Amount of substances leached during the first 24 hours as percentage of total leached amount.

Comparing percentage of the total amount of all studied constituents of leachates after the first 24 hours of experiment, reveal that the acetic acid leaches most easily, followed by tannic acid and sugars (Figure 3). Furfural is more difficult to leach.

All studied compounds leach more easily from pine wood treated at TMP160/3 and in greater total amount than from pine wood modified at TMP170/1. It is more likely due to wood cell wall shrinkage after thermal treatment that increases with temperature. In addition, due to the thermal degradation of wood components, the content of hydroxyl groups decreases leading to increased hydrophobity and more difficult wettability. According to obtained results of wood mass loss during the thermal treatment, degradation process in TMP170/1 occurs at greater degree than in TMP160/3 modified wood, thereby reducing the amount of substances accessible to leach. Mass loss in TMP170/1 is 7.5±1.4, which is comparable with results obtained in other studies – 7.3% (Esteves *et al.*, 2007); 6.9% (Brito *et al.*, 2008), whereas mass loss in TMP160/3 is 4.0±1.6.

Conclusions

As expected, the leaching velocity of investigated substances – acetic acid, furfural, tannic acid and sugars - decreases exponentially with repeated immersions. Although the velocity plateau is reached after the 7th immersion (9 days), it is worth to prolong the experiment because after the 9th immersion (14 days) the investigated substances still continue to leach. Pine wood thermally modified for 1 hour at 170 °C in WTT is more environmentally friendly due to less in water leachable substances. Hereafter, it is worth looking forward by investigating volatile organic compounds emissions in the air as it also could give high impact on human health.

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The Composition and Use Value of Tree Biomass Ash

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Abstract. Wood-based ash landfilling is increasing issue not only in Latvia but in the whole world as more biomass is used for energy production. Utilization of wood burning waste as fertilizer is already used worldwide, but there is lack of information about chemical composition of wood ash obtained from Latvia plants, so the aim of this study was to determine chemical composition and analyse possible utilization options of wood-based ash from Latvia plants. Therefore wood ash samples from 53 companies were collected, sieved and chemical composition of samples was determined. It was concluded that within higher capacity of furnace more coarse fraction of wood ash was observed which is less valuable as fertilizer. Wood ash is good liming material consisting alkali compounds and other biogenic elements but also heavy metals, which are pollutants and could cause environmental problems.
Key words: wood ash, chemical composition, forest fertilization.

Introduction

A demand of alternative energy is increasing, as fossil fuel resources are decreasing. High forest coverage and soil fertility are factors which make biomass energy profitable for Latvia conditions. In recent years, forest stands are managed more intensively. It has been forecasted that consumption of energy wood in 2015 will increase for about 1.53 milj. m^3 comparing to 2011 (Būmanis *et al.*, 2012). To provide energy, either more energy plantations of plants with wood-based biomass should be made or more wood residues should be delivered from forest harvesting (branches, tree tops, stumps). Both actions in long term could lead to soil degradation. Increasing consumption of woody biomass for energy production will lead to waste (wood ash), which usually is landfilled, overproduction. Yet, there are other alternative utilization ways (Pitman, 2006; James *et al.*, 2012; Siddique, 2012). Wood ash use for soil amendment is the way how to return nutrients removed by harvesting and solve ash utilization problem.

Wood-based ash contains almost all biogen elements and minerals. Ash alkalies compounds reduce soil acidity (Saarsalmi, Mälkönen, & Piirainen, 2001; Ozolinčius *et al.*, 2005), which provides favorable conditions for microbial growth that improve decomposition of organic matter and nutrient release (Saarsalmi *et al.*, 2014), yet many studies have shown different results of tree growth in response of wood ash spreading in forest. Wood ash treated forest stand annual increments vary from 1 m^3 ha^{-1} (Saarsalmi *et al.*, 2014) to 4 m^3 ha^{-1} (Emilsson, 2006).

Wood ash chemical properties depend on many factors (subsidiary fuel type, combustion system and season). But even within one tree specie element composition can differ significantly (Reimanna *et al*., 2008). Qualitative composition of wood ash differs significantly within technology used for biomass combustion (Pitman, 2006). In Latvia, plants are mostly used in relatively small reciprocating grate furnaces with 1 MW capacity. In recent years many plants have been built with high capacity furnaces (20 MW), which produce significantly higher rates of ash.

It is impossible to provide energy plants with large volumes of uniform wood-based fuel so it is pointless to analyse separate tree species ash chemical content. For large scale ash utilization other factors should be analysed, for example, type of furnaces or capacity. The aim of this study is to determine composition and use value of tree biomass ash from Latvia plants.

Materials and Methods

Samples of wood-based ashes from reciprocating grate furnaces with or without ash stabilizing water beds of 53 different companies were received. Ash samples were sieved and divided into three fractions – fine (particle size < 3.15 mm), average (3.15 – 16 mm) and coarse (> 16 mm). For each fraction composition of chemical elements, pH, moisture and density were determined. To determine total carbon (C_{tot}) elementanalizator LECO CR-12 was used (LVS ISO 10694). Content of carbonates (C_{carb}) was determined using Eijkelkamp calcimeter adding 20% hydrochloric acid (HCl) (according to LVS ISO 10693: 1995 standard). Organic carbon was calculated as residual between C_{tot} and C_{carb}.

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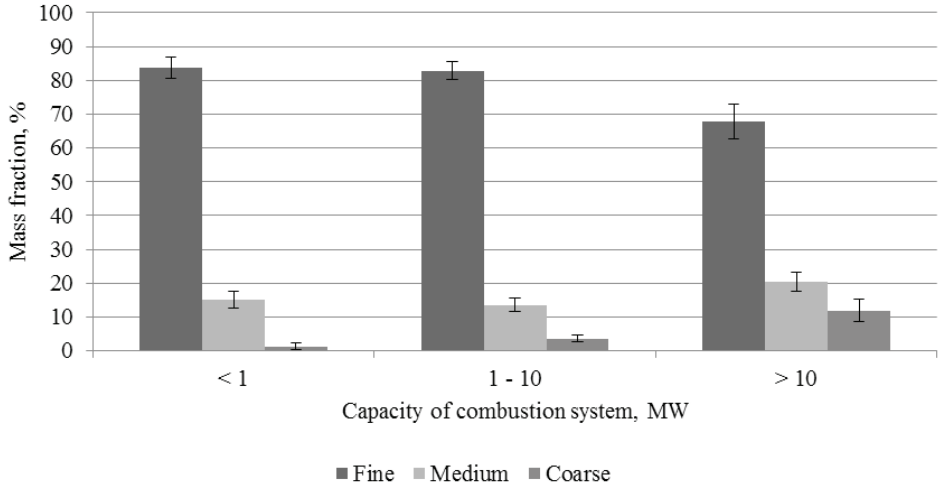

Figure 1. Mechanical composition of wood ash depending on combustion system capacity.

Total sulfur (S) was determined using ELTRA CS-530 method, oxidizing S to SO_2 at 1340 °C (ELTRA CS 530 methodology). To determine nitrogen (N), the modified Kjeldal method was used (LVS ISO 11261). Phosphates were determined calorimetric in aqua regia (LVS 298 (2002), LVS ISO 11466: 1995, LVS EN 14672 (2006)). Total potassium (K), calcium (Ca), magnesium (Mg) and manganese (Mn) were determined using an atom absorption spektrofotometer (ISO 11466:1005). Heavy metals cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) were determined according to LVS ISO 11047 standard using flame emission method. Furnaces were divided into three groups according to their capacity – furnaces with up to one MW, 1 to 10 MW and 10 to 20 MW. Statistical analysis was made of received results within capacity groups. Considering that Ca and Mg could be found as carbonates or hydroxides and oxides proportion of these elements attached to carbonates was calculated. T-test (α=0.05) was used for significance analysis and correlation analysis was used for data evaluation.

Results and Discussion

Combustion system capacity was used to compare wood ash properties from different furnaces, assuming that temperature is higher and temperature regime is more stable in high capacity combustion systems. Results showed that by increasing boiler capacity, proportion of coarse ash fraction (Figure 1), which is explained by fusion of small particles at higher temperature, increases. Fusion of particles starts at 700 °C and fusion intensity increases within temperature (Vassilev, Baxter, & Vassileva, 2013).

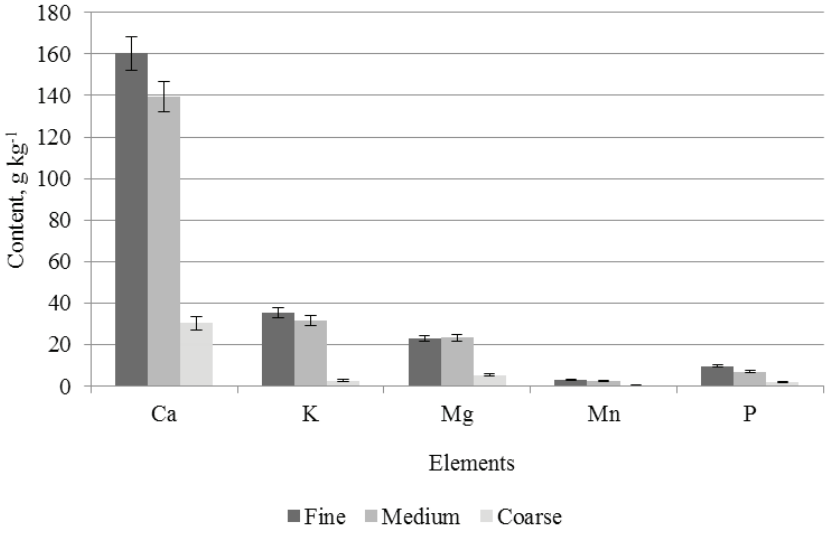

Figure 2. Mineral content in different ash fractions.

However, fine ash dominates in every furnace and form more than 69% of total ash.

Coarse ash fraction significantly (α=0.05) differs from fine and average fractions containing less biogenic elements (Figure 2). Due to low element content, coarse ash fraction should be sieved off before application in forest. According to preliminary results of ash utilization in Finland, it could be used for road construction (Vanhanen, Dahl, & Joensuu, 2014). However, chemical composition between fine and medium fractions does not differ significantly.

During research it was found that wood ash is favourable liming material because of relatively high consistance of alkali metals. By using 1 tonne of sieved wood ash, it is possible to bring in soil 170 kg of liming material (150 kg Ca and 20 kg Mg) (Figure 2).

Liming properties are defined by alkali compounds which could be carbonates or oxides and hydroxides. It was found that between ash moisture and proportion of Ca and Mg expressed as carbonates there was a moderate and positive correlation (r_{act} = |0.785| > $r_{(0.05;30)}$ = 0.361) (Figure 3). The dryer is the ash, the higher proportion of Ca and Mg is expressed as hydroxides, which can cause plant damage during fresh ash spreading. To prevent negative effect on vegetation, stabilisation (moisturising or hardening) of dry ash before spreading is required. Results show that wood ash taken from furnaces with water beds are more suitable as forest fertilizer.

Since coarse ash is not favourable as fertilizer, further only fine and medium ash fractions are analysed. Higher K content was found in ash produced by small and medium (less than 10 MW) furnaces (Table 2), which could be explained by higher temperature regime in large scale furnaces. Boiling point of potassium is at 760 °C. Above this temperature evaporation of K compounds increases (Misra, Ragland, & Baker, 1993; Vassilev, Baxter, & Vassileva, 2013). Content of organic carbon also differs significantly between combustion systems and level of this element is lower in high capacity furnaces because of higher combustion rates. When temperature decreases volatile elements adsorb on finest ash particles (Saqib & Bäckström, 2014) which explains higher content of K in fine ash fraction.

Phosphorus is a non-volatile element (Vassilev, Baxter, & Vassileva, 2013) that is why the content of P in wood ash from different furnaces is not significant and depends more on other factors like biomass type. Determined elements form about 30 % of total ash. While ash also consists of chemically bounded oxygen, composition of ash from biomass is dominated by Si (silicon) (Nunes, Matias, & Catalão, 2016). High silicon content in wood biomass ash is a reason of coarse fraction formation.

Like K also cadmium (Cd) is a volatile element (boiling point 767 °C) and its content in wood ash highly depends on combustion temperature and is one of the elements whose concentration is not multivariate as high as other heavy metals (Table 2). Other heavy metal content is less stable, which is a problem if ash is considered as a fertilizer. Concern of environmental pollution is the main obstacle of wood biomass ash use for soil amendment.

In Latvia, no restrictions have been set about wood-based ash use for forest soil amendment, however, in other countries maximum content of heavy metals is limiting biomass ash use as fertilizer (Eijk, Obernberger, & Supancic, 2012) (Table 3). During some studies no significant impact or trace element accumulation by wood-based ash application in living organisms has been found yet (Perkiomaki *et al.*, 2003; Perkiömäki & Fritze, 2005; Omil, Piñeiro,

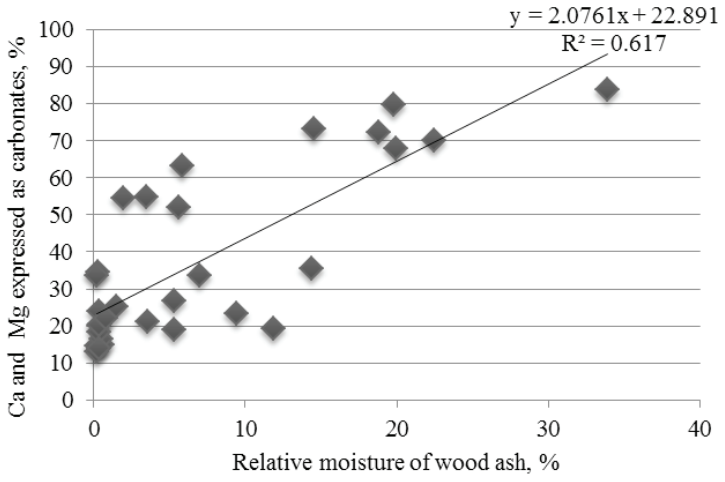

Figure 3. Relation between relative moisture of ash and alkali metals expressed as carbonates.

Table 2

Wood ash chemical composition according to furnace power

Element	Capacity of combustion system, MW and wood ash fraction					
	< 1		1 - 10		> 10	
	Fine	Medium	Fine	Medium	Fine	Medium
$C_{carb.}$, g kg^{-1}	31.09± 8.35	20.71± 6.87	26.00± 3.41	17.14± 3.60	15.00± 3.96	10.88± 3.23
$C_{org.}$, g kg^{-1}	148.73± 127.55	213.5± 122.35	57.52± 29.61	122.41± 45.82	20.84± 8.61	52.46± 34.00
$S_{tot.}$, mg kg^{-1}	27.00± 9.28	17.49± 7.11	13.80± 4.96	31.76± 10.15	11.37± 4.25	20.90± 6.28
$N_{tot.}$, g kg^{-1}	-	1.72± 0.58	0.34± 0.06	0.60± 0.15	0.26± 0.09	0.25± 0.08
P, g kg^{-1}	10.75± 2.67	6.64± 1.58	11.12± 0.76	8.00± 0.60	7.82± 0.91	5.73± 0.62
K, g kg^{-1}	46.34± 10.88	42.50± 11.96	43.01± 3.39	38.96± 3.50	24.05± 2.86	20.64± 3.07
Ca, g kg^{-1}	155.95± 25.67	138.78± 24.12	186.98± 10.00	159.81± 9.75	128.95± 12.54	114.88± 11.33
Mg, g kg^{-1}	28.11± 8.16	27.63± 11.34	25.76± 1.53	26.05± 2.00	18.41± 1.75	19.05± 2.12
Mn, g kg^{-1}	3.81± 0.93	3.04± 0.86	3.72± 0.31	2.98± 0.33	2.10± 0.26	1.76± 0.25
Fe, g kg^{-1}	11.58± 4.12	13.58± 4.74	6.60± 1.91	11.82± 3.82	4.72± 0.25	4.52± 0.52
Cd, mg kg^{-1}	15.06± 4.53	12.83± 2.71	15.6± 2.14	14.95± 2.43	9.20± 1.54	8.79± 1.20
Pb, mg kg^{-1}	70.09± 24.86	73.7± 15.35	60.76± 7.76	62.67± 7.86	45.54± 8.25	88.75± 11.42
Cr, mg kg^{-1}	143.36± 24.46	215.9± 59.06	103.26± 11.58	208.77± 28.89	229.99± 35.70	298.01± 59.13
Ni, mg kg^{-1}	28.08± 6.40	29.85± 7.19	38.00± 7.28	49.80± 11.39	23.84± 2.29	36.46± 4.70
Zn, mg kg^{-1}	-	-	286.29± 56.65	192.44± 53.1	490.40± 159.79	409.42± 165.26
Cu, mg kg^{-1}	203.97± 39.35	219.76± 71.22	218.09± 31.49	232.38± 47.76	99.86± 17.03	72.30± 10.88

Table 3

Existing limiting values of heavy metals in biomass ash for the application on forest lands, mg kg^{-1}

Trace element	Germany	Denmark	Sweden	Finland
Cd	1.5	20	30	25
Cr	2.0	100	100	300
Cu	-	-	400	700
Ni	80	60	70	150
Pb	150	250	300	150
Zn	-	-	7000	4500

& Merino, 2007). High differences of limiting heavy metal contents between countries, evidence for hazardous effect of heavy metal contamination has not been studied sufficiently yet. Some of trace elements are favourable for plant growth as microelements, for example, Cu and Zn.

Conclusions

Coarse ash fraction is more often found in higher capacity furnaces and is less valuable as fertilizer, so particles larger than 16 mm should be sieved off before transportation to treatment sites.

Between ash moisture and proportion of Ca and Mg attached to carbonates is moderate and positive correlation (r = 0.79) which suggests that fresh ash are not suitable for spreading in forest at vegetation period.

Qualitative composition of wood ash depends on volatilization of elements in high temperatures, higher concentrations of potassium have been found in ash from lower capacity furnaces, which makes wood ash more valuable as fertilizer, but also cadmium concentration is higher, which might cause higher contamination risk.

This is more likely preliminary overview of wood-based ash chemical composition from Latvia plants, no precise ash determining values could be set while wood based ash content depends on many factors. Capacity of furnaces could not be used for ash chemical content prediction and classification, because of high variation of element content, further investigations should be made.

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