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Radovan Despot

Mechanism of infection of fir wood joinery; Part 2: Sequence and intensity of attack of microorganisms

Mehanizam infekcije jelove građevne stolarije; dio 2: Slijed pojavnosti i jačina napada mikroorganizama

Original scientific paper - Izvorni znanstveni rad Received - primljeno: 17. 07. 1998. • Accepted - prihvaćeno: 24. 09. 1998. UDK 634*812; 634*833.15; 634*829.1

SUMMARY • In Croatia the primary raw material for joinery production is silver fir wood (Abies alba Mill). L-joints made of home-grown fir sapwood and prepared according to EN 330: 1993. were used to establish the colonisation and infection of micro-organisms to compare the performance of untreated and 1% FnBTO treated L-joints by ten-minute immersion. The L-joints surfaces were coated with two types of liquid coat, and exposed on three different climate sites in Croatia. The first type of coat was alkyd paint and the second was a stain, in three different colours: white, brown and black. The untreated L-joints were examined after 1, 2, 3, 4, 6, and 12 months and treated after 12 months of exposure. The influence of the climate, and the type of coat were the most important factors which affected the rate of colonisation. In Zalesine, a mountain site with the highest average air humidity and the greatest amount of precipitation, colonisation was fastest and strongest. The lowest bacterial and fungal colonisation occurred in L-joints exposed in Rovinj, a site on the Adriatic coast, particularly on those L-joints coated with a darker stain. It was due to the well known vaporous diffusivity of the stains and the fact that Rovinj had the largest number of sunny days during the first two months of exposure when the dark stain surfaces absorbed many more of the sun's heat rays which caused accelerated seasoning, lower moisture contents and lower

Author is an assistant professor at the Faculty of Forestry of the Zagreb University.

Autor je docent na Šumarskom fakultetu Sveučilišta u Zagrebu.

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bacterial colonisation. The influence of coat colours later was not significant. The differences between one year exposed untreated and treated L-joints were significant according to the moisture content, permeability and fungal colonistaion. The colonisation and fungal attack of treated L-joints was lower, particularly of those coated with stain. The most frequently isolated fungi were Alternaria alternata (Fr.) Keissler, Gloeophyllum trabeum (Fr.) Pers. and Aurobasidium pullulans (de Bary) Arnaud.

Key words: Fir-wood joinery, L-joints, preservation, bacterial and fungal colonisation, biodeterioration, wood decay.

SAŽETAK • Osnovni je zadatak ovog rada bio na temelju dugotrajnog izlaganja simuliranih elemenata vanjske građevne stolarije, tzv. L-spojeva, izrađenih od domaće drvne sirovine i premazanih domaćim premazima za drvo, ustanoviti pojavu, slijed, mehanizam infekcije, odnosno proces trulenja drva.

Za potrebe ispitivanja, izrađene su dvije skupine L-spojeva. U prvoj su bili L-spojevi prethodno zaštićeni sredstvom za zaštitu drva od mikroorganizama, a u dugoj grupi su bili kemijski nezaštićeni L-spojevi. Zaštićeni i nezaštićeni L-spojevi premazani su potom lazurnim, odnosno alkidnim premazom hrvatskog proizvođača i to u jednom od tri tona, bijelom, smeđem i crnom. Za kemijsku zaštitu L-spojeva prve skupine upotrbljena je 1%-tna otopina TnBTO-a, a zaštita je bila obavljena desetominutnim potapanjem. Za mjesta izlaganja odabrani su Zagreb, mjesto s naznakama tipične kontinentalne klime, Zalesine, s naznakama planinske vlažne klime i Rovinj, s naznakama sredozemne suhe klime. Nezaštićeni L-spojevi sukcesivno su ispitivani na sva tri mjesta izlaganja nakon 1, 2, 3, 4, 6 i 12 mjeseci izlaganja, a zaštićeni L-spojevi koji su bili izloženi samo u Zagrebu, ispitivani su samo nakon 12 mjeseci izlaganja. Rezultati istraživanja pokazali su da je slijed infekcije odnosno naseljavanja drva mikroorganizmima u prirodi istovjetan u jelovine kao i u ostalih vrsta drva, neovisno o mjestu izlaganja, kemijskoj zaštiti, odnosno vrsti i tonu premaza. Klima i vrsta premaza bili su najvažniji čimbenici uvjetovanja veličine i intenziteta napada L-spojeva mikroorganizmima. U Zalesinama, planinskome mjestu s velikom prosječnom vlažnošću zraka i velikim brojem kišnih dana u godini, naseljavanje i infekcija mikroorganizmima bili su najbrži i najjači. Nasuprot tome, naslabije naseljavanje i infekcija zabilježene su u L-spojevima izlaganim u Rovinju. Neovisno o mjestu i duljini izlaganja, pokazalo se da je zbog dobro poznate paropropusnosti lazurnih premaza infekcija mikroorganizmima bila slabija na L-spojevima premazanim lazurom. Tamnije lazurirane površine jače su apsorbirale toplinske zrake, što je pak rezultiralo bržim sušenjem, manjim sadržajima vode i slabijim naseljavanjem bakterija i gljiva u drvo. Neovisno o vrsti premaza, utjecaj tonova premaza pokazao se značajnim za sadržaj vode, osobito tijekom prva dva mjeseca izlaganja, kada je toplinsko djelovanje sunca bilo izrazitije, a zagrijavanje tamnijih premaza jače.

Ovisno pak o vrsti premaza i mjestu izlaganja, najjača mikrobiološka aktivnost zabilježena je na kemijski nezaštićenim L-spojevima premazanim bijelim alkidnim premazom i izlaganim u Zalesinama, a najslabija na tamnim lazurama premazanim L-spojevima izlaganim u Rovinju. Između nezaštićenih i zaštićenih L-spojeva, neovisno o vrsti premaza, zabilježene su značajnije razlike u intenzitetu napada mikroorganizama i infekciji. U odnosu prema nezaštićenim L-spojevima, u zaštićenim je L-spojevima, osobito onima premazanim lazurom, ••••• R. Despot: Mechanism of Infection of Fir wood ...

ustanovljen znatno manji broj gljiva uzročnika meke truleži, odnosno gljiva iz reda basidiomyceta, uzročnika prave truleži.

Od vrsta gljiva, najčešće su izolirane Gloeophyllum trabeum (Fr.) Pers., uzročnik smeđe truleži, odnosno gljive Aurobasidium pullulans (de Bary) Arnaud. i Alternaria alternata (Fr.) Keissler., uzročnici promjene boje i tzv. meke truleži.

Ključne riječi: jelova vanjska stolarija, L-spoj, kemijska zaštita, kolonizacija bakterija i gljiva, biološka razgradnja, trulež drva

1.INTRODUCTION 1. Uvod

As it was mentioned in the previous article (Despot 1998a, Part 1.), the decaying of external joinery is a long process, so the major problem is the long-term nature of all exposure trials (Carey 1980, 1982, Carey and Bravery 1984, 1985, 1987. 1989). L-joints method (EN 330: 1993) which has been used and described in that article produced numerous data about the changes of the moisture content and permeability of untreated and treated fir L-joints exposed at three different sites in Croatia. The aim of this article was to establish the influence of the mentioned parameters on the sequence of colonisation and the mechanism of infection of fir-wood joinery.

2. MATERIALS AND METHODS 2. Materijal i metode

2.1. Materials

2.1. Materijali

The preparation and exposure of fir Ljoints was described in the previous article (Despot 1998). L-joints were prepared from an air dry stock of Croatian-grown silver fir sapwood (Abies alba, Mill.) according to EN 330: 1993.

2.2. Methods 2.2. Metode rada

2.2.1. Preparation and Exposure of Samples

2.2.1. Izrada i izlaganje uzoraka

The paragraph was also described in the previous article - Part 1(Despot 1998).

2.2.2. Sampling

2.2.2. Uzrokovanje

Each L-joint group consisted of three replicas. After each exposure period, the Ljoint groups were observed for any visible signs of deterioration. The end seal overlap-

ping the paint film was removed to facilitate accurate sawing, and the horizontal member was sawn into samples for measurements of moisture content, permeability and the isolation of the micro-organisms.

As the moisture content and permeability determination was described in the previous article-Part 1. (Despot 1998), in this article only the isolation studies and their results are presented.

2.2.2.1. Isolation studies 2.2.2.1. Izolacija

A 6 mm thick strip from the other side of each replica was sampled on its freshly sawn face at 6 set positions. Four samples, each approximately 15 mm long, were cut from each position using 6 mm wide Ushaped gouge, and planted on one of the following media:

- nutrient agar (NA),
- 2% malt agar (2M),
- 5% malt agar containing 10 ppm
 - benomyl (BEN 10),
- starch casein nitrate agar containing 350 ppm rose bengal (SCN).

The first, the most uniform in shape, was streaked across the substrate and then planted on to a plate of nutrient agar, to assess the bacteria present, using the method described in the previous works (Carey 1979, Carey 1982). Fungi growing on the other three media were subcultured, purified using normal mycological techniques, characterised, and eventually identified. The plates were incubated at 22°C. The nutrient agar plates were observed for bacterial growth after 4 days, and then rejected. The remaining plates were incubated for a minimum of six weeks and were frequently observed. In order to determine the significant decay in the replicas the mycelia of all isolated basidiomycets were subcultured into test tubes with a sawdust substrate which consisted of 1 kg Spruce sapwood sawdust, 30 g maize meal and 20 g bone meal, all mixed with distilled water and sterilised.

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Table 1.

The distribution of fungal isolates from the L-joints coated with TEAK STAIN coat. A typical sample where generally a small number of isolates confirmed the lower fungal infection and colonisation. Raspored izolata gljiva izoliranih iz L-spojeva premazanih s SMEĐIM LAZURNIM premazom. Tipičan primjer kod kojeg je općenito manji broj izolata potvrdio manju infekciju i manju kolonizaciju gljivama.

Table 2

The distribution of fungal isolates from the L-joints coated with WHITE ALKYD coat. A typical sample where generally a large number of isolates confirmed the stronger fungal infection and greater colonisation. Raspored izolata gljiva izoliranih iz L-spojeva premazanih s BIJELIM ALKIDNIM premazom. - Tipičan primjer kod kojeg je općenito veći broj izolata potvrdio jaču infekciju i veću kolonizaciju gljivama.



Legend: $\Box = 1$ isolate

Legenda: $\Box = 1$ izolat



Legenda: $\Box = 1$ isolate

3. RESULTS 3. Rezultati

The results of moisture content and permeability investigations were presented in the previous article (Despot 1998) Part 1.

3.1. Colonisation of microorganisms 3.1. Naseljavanje mikroorganizama

The examinations on all the L-joints showed that micro-organisms had invaded the L-joints and appeared to have entered via the joint. As it was established, the bacteria and fungi were isolated, first close to the joint, and then along the length of the members. Depending on the exposure site the intensity of the bacterial colonisation was similar to the intensity of fungal colonisation. During the first months of exposure the bacteria and fungi had mainly colonised the first three positions. Patterns in the sequence of colonisation were investigated and the deterioration by fungi isolated summarised. As shown the intensity of infection and the number of fungal isolates depended mainly on the type of coat of paint and on the site climate charasteristics. The average least fungal colonisation and infection occurred in the Ljoints coated with brown (teak) stain (Table 1). On the other hand the average greatest fungal colonisation and infection occurred in the L-joints coated with white alkyd paint (Table 2).

The fungi isolated were classified according to their effect on the wood. Those classified as mould fungi caused neither a blue stain nor soft rot. Those with pigmented hyphae were classified as blue stain fungi unless they were also capable of causing soft rot. The isolates of Basidiomycotina were determined and classified according to the presence of charasteristic "clamps" connections, thus *Sistotrema brinkmanii* was also included. The number of isolates and frequency of those basidiomycetes which produced true wood decay (without *S. brinkmanii*) is presented in Figures 2a-c and 3a-c. The distribution of isolates from all the untreated and treated L-joints according to the positions relative to the joint was defined maximally with six isolates for each position of the three replicas.

4. DISCUSSION 4. Rasprava

4.1. Permeability & Moisture Content4.1 Permeabilnost i sadržaj vode

As the author confirmed in the previous article (Despot 1998), regardless of the type of coat the greatest average moisture contents and greater permeability, occurred in the L-joints exposed in Zalesina and the least average moisture contents and lower permeability occurred in L-joints exposed in Zagreb and Rovinj. Regardless of the exposure site and exposure period the average moisture contents and permeability which occurred were always higher in the L-joints coated with alkyd coats, particularly in those coated with white alkyd paint and exposed in Zagreb and Zalesina.

Because of the well known stain vapour diffusivity the least average moisture contents and lower permeability occurred in the L-joints coated with stain, particularly in those coated with brown and black stain and exposed in Zagreb and Rovinj.

Regardless of the type of coat the influence of colour was noticeable during the first two months of exposure, particularly



The comparison of bacterial colonisation occurred in two *L*-*joints*, *both exposed* 3 months in Zagreb. Upper "petri dish" belongs to L-joint coated with white stain (2 JLS - 18), and lower "petri dish" belongs to L-joint coated with black alkyd coat (2 JAD -16) a - positions 1, 2 and 3; b - positions 4, 5, and 6 • Usporedba bakterijske kolonizacije zapažene na dva L-spoja oba izlagana 3 mjeseca u Zagrebu. Gornja petrijevka pripada L-spoju premazanom bijelom lazurom (2 JLS - 18), a donja petrijevka pripada L-spo ju premazanom crnim alkidnim premazom (2 JAD -16); a - pozicije 1, 2, i 3; b - pozicije 4, 5 i 6





Figure 2.a

The appearance of true wood decay fungi in L-joints coated with STAIN COATS and exposed in Zagreb • Pojavnost prave truleži u L-spojevima premazanim lazurom i izlaganih u Zagrebu



Figure 2.b

The appearance of true wood decay fungi in L-joints coated with STAIN COATS and exposed in Zalesine • Pojavnost prave truleži u L-spojevima premazanim lazurom i izlaganih u Zalesinama

Figure 2.c

The appearance of true wood decay fungi in L-joints coated with STAIN COATS and exposed in Rovinj • Pojavnost prave truleži u L-spojevima premazanim lazurom i izlaganih u Rovinju with the L-joints coated with brown and black alkyd paint and exposed in Zagreb and Zalesine. The treated L-joints coated with alkyd coats had greater moisture contents and greater permeability than those L-joints coated with stain.

4.2. Bacterial colonisation 4.2. Naseljavanje bakterija

With regard to the site climate characteristics the intensity of bacterial attack always depended on the amount of precipitation. In a longer dry period the bacteria could not develop and did not invade through the tenon. Regardless of the exposure site bacterial colonisation was more intensive and occurred more frequently in the L-joints coated with alkyd paint than in those coated with stain. The greater moisture contents of the L-joints coated with alkyd coat, consequently caused a higher bacterial colonisation. As shown in Figure 1., the typical differences in bacterial colonisation between L-joints coated with alkyd coat and Ljoints coated with stain occurred after 3 months of exposure in Zagreb. In the lower petri dish which belongs to the L-joint coated with black alkyd paint (L-joint No. 2 JAD 16) bacteria colonised all 6 positions, but at the same time in the upper petri dish which belongs to the L-joint coated with white stain (2 JLS -18), the bacteria did not invade. Almost the same lower bacterial colonisation of the L-joints coated with stain occurred in other sites, particularly in Rovinj where during the first three months of exposure there was no bacteria action in the untreated Ljoints coated with darker stains. After 12 months of exposure, all untreated L-joints coated with alkyd coats were colonised by bacteria much stronger than those coated with stain, particularly in Zalesine. At the same time in Zagreb and Rovinj it was the end of a long dry summer period, so the Ljoints coated coated with the coat of stain were significantly lower colonised by bacteria, particularly those exposed in Rovinj. Of all the treated L-joints, the greatest bacterial colonisation occurred in the L-joints coated with a white alkyd coat and the lowest in the L-joints coated with a brown coat of stain.

4.3. Fungal colonisation 4.3 Naseljavanje gljiva

After the first month of exposure, all the untreated L-joints on all the sites became colonised rapidly by fungi. It coincided with a period of heavy rainfall particularly in Zagreb and Zalesine. It was especially unusual for Zagreb since September is known to be a dry month without much rainfall. During October 1995 in Zagreb there was no significant rainfall but the consequences of the previous rainfall were decisive on the further colonisation. With the next rainy period the number of fungal isolates increased till the beginning of winter. The winter was snowy and rainy in Zagreb and there were no significant differences between L-joints coated with alkyd and a coat of stain and exposed 4 and 6 months. Even the L-joints coated with stain were somewhat more infected with blue stain and moulds than the L-joints coated with alkyd coats. However, neither type of organism was able to extend further from the joint during the dry period, particularly in the Ljoints coated with stains and exposed in Rovinj. It was confirmed at last, after 12 months of exposure.

As Carey confirmed before (1982, 1983), the sequence of colonisers observed cannot claim to have established a succession since there is no indication of a decline of numbers following the initial build-up of a particular group but rather the maintenance of a stable population. Thus the groups, if not the species within them, coexist and do not displace one another.

Moulds and blue stain fungi Plijesan i gljive uzričnici modrila

During the first four months of exposure on all the sites the main fungi were blue stain fungi and moulds. The number of isolates of mould fungi increased after longer exposure periods and was higher in Zalesine than in Zagreb and Rovinj. Regardless of the exposure site and period of exposure the number of isolates and mould species was lower in the L-joints coated with stain than in the L-joints coated with alkyd coat. The exception were the L-joints coated with brown stain and exposed for one month in Zagreb and Rovinj.

Regardless of the exposure site the most frequent mould fungi isolated from the L-joints coated with stain were Penicillium spp., Trichoderma spp., and Aspergillus spp. L-joints, and from the L-joints coated with alkyd paint were Penicillium spp., Trichoderma spp., and Mucor spp. Besides the mentioned moulds the most frequent mould from the Zagreb L-joints was Gliocladium sp., from Zalesine's L-joints Torula sp. and from Rovinj's L-joints Aspergillius sp. A decrease of moulds was established after the fourth and sixth month of exposure, particularly with the L-joints coated with stain and exposed in Rovinj. After 12 months of exposure the number of mould records,

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Figure 3.a

The appearance of true wood decay fungi in L-joints coated with ALKYD COATS and exposed in Zagreb • Pojavnost prave truleži u L-spojevima premazanim ALKIDNIM premazom i izlaganih u Zagrebu



Figure 3.b

The appearance of true wood decay fungi in L-joints coated with ALKYD COATS and exposed in Zalesine • Pojavnost prave truleži u L-spojevima premazanim ALKIDNIM premazom i izlaganih u Zalesinama

Figure 3.c

The appearance of true wood decay fungi in L-joints coated with ALKYD COATS and exposed in Rovinj • Pojavnost prave truleži u L-spojevima premazanim ALKIDNIM premazom i izlaganih u Rovinju named "secondary moulds" rapidly decreased. Those mould fungi were initially of low incidence but there was some indication of larger numbers occurring in the untreated material after the colonisation by the Basidiomycotina (Carey, 1983).

After the first month of exposure blue stain fungi were isolated in large numbers. In all the L-joints they developed intensively adjacent to the joint, and had penetrated at least 30 mm from the joint (positions 1, 2 & 3). A much larger number of blue stain fungi was isolated from the L-joints coated with alkyd paint, particularly from those exposed in Zalesine and Zagreb. The most frequent blue stain fungi were Aureobasidium pullulans (de Bary) Arnaud., Alternaria alternata (Fr.) Keissler., Cladosporium sp. and particularly fungi from the class of Coelomycetes and the order of Sphaeropsidales. A. pullulans was most frequent in Zagreb and Rovinj and A., alternata in Zalesine. From the L-joints exposed in Zagreb Bispora sp., Diploccocum sp., and from the L-joints exposed in Zalesine Graphyum sp. and Stemphyllium sp. were also often isolated.

Later many of the before mentioned moulds and these blue stain fungicaused softrot.

Soft rot Meka trulež

The presence of soft rot firstly occurred in the L-joints coated with the alkyd coat and exposed for one month in Zagreb and Zalesine. Those soft rot fungi occurred only close to the joint, suggesting that they were later colonisers than the blue stain fungi. Soft rot appeared more frequently in the L-joints coated with alkyd coat than in the L-joints coated with stain, and more often in Zalesine and Zagreb than in Rovinj. The exception were two L-joints coated with black stain. One was exposed 4 months in Zagreb, and the other was exposed for 6 months in Rovinj. In these L-joints the presence of soft rot fungi was greater than in the other Ljoints coated with stain. It happened due to the high moisture contents in these L-joints. Generally, when the average moisture contents were higher the colonisation of soft rot fungi was greater, and vice versa.

The most frequntly isolated soft rot fungi from the L-joints exposed in Zalesine were Alternaria sp., Fusarium sp:, Humicola sp., Papulospora sp. and Phialophora sp. At the same time Alternaria sp. and Fusarium sp. were often isolated in Zagreb, and in Rovinj particularly Fusarium sp. The Chaetomium globosum (Fr) Kunze., which first caused mould and later soft rot, was isolated only from two L-joints, the one coated with white alkyd coat and exposed in Zalesine, and the other coated with brown alkyd coat exposed in Zagreb. Both L-joints were exposed for three months.

Basidiomycotina, wood decay fungi Basidiomikotine, gljive uzročnici truleži drva

The last group of fungi to appear were the Basidiomycetes, the organisms which cause significant decay of joinery in service. Regardless of the type of coat, the Basidiomycotina were more frequently isolated from the L-joints exposed in Zalesine than from the L-joints exposed in other sites. Regardless of the exposure site, the Basidiomycotina more frequently colonised L-joints coated with alkyd coat than the Ljoints coated with stain coat. The appearance of basidiomycetes on the L-joints exposed in Rovinj was lower, particularly on those coated with stain. But there were two exceptions. One in the L-joint coated with black alkyd coat and 6 months exposure in Rovinj, and the other in the L-joint coated with black stain and 6 months exposure in Zagreb. Those L-joints were completely colonised with basidiomycetes, and therefore completely decayed.

Only two species were determined, Gloeophyllum trabeum (Fr.) Pers. and Schizophyllum commune (Fr.). The identification was possible due to the appearance of their carpofores and fructifications. The presence of other wood decay fungi was established using a saw dust isolation method. If the colour of the sawdust was brown it was attributed to white rot. It happened only in the few L-joints coated with alkyd coat and exposed in Zalesine. In all other cases the colour of sawdust was golden brown and was attributed to brown rot. If there was no change of sawdust colour inside the test tube, it was supposed that Basidiomycotina were not able to produce decay so we attributed it to Sistotrema brinkmanii. The other factor which sometimes helped in that determination was the specific smell of S. brinkmanii

Preservative treatment Tretiranje zaštitnim sredstvom

Preservative treatment had little effect on colonising the bacteria, but the colonisation by the fungi particularly by Basidiomycotina was affected. Except for the two isolates of basidiomycetes which were isolated close to the joint of two L-joints coated with white and black alkyd paint, there were no basidiomycetes in the treated L-joints. With TnBTO treatment the rate of colonisation of the blue stain fungi was similar to that of the untreated material although the numbers of isolates were generally lower. This could be expected based on the known poor performance of TnBTO against the blue stain in service.

With regard to the coat type, the rate of blue stain colonisation fungi in the treated L-joints coated with stain was greater than in the treated L-joints coated with alkyd coat. On the other hand the rate of soft rot fungi was the highest in the treated L-joints coated with white alkyd coat. The presence of soft rot in the treated L-joints coated with stain was again lower.

5. CONCLUSION 5. Zaključak

The processes of the colonisation of untreated fir L-joints, coated with alkyd and coats of stain and exposed on three sites in Croatia, show the known sequence of colonisation of microorganisms in the following order: bacteria, followed by moulds, blue stain fungi, soft rot fungi, and basidiomycetes.

The intensity of the colonisation depended on two main factors. The first factor was the influence of the climate characteristics of each exposure site, and second was the type of coat. The influence of colour was significant during the first two or three months but not later. The highest and strongest colonisation occurred in Zalesine, the site with the highest average air humiditiy and the largest amount of precipitation during all of the exposure, particularly in the L-joints coated with white alkyd coat. The strongest colonisation noticed was followed with significantly greater moisture contents and greater porosity and permeability. In Rovinj, a site with a typical dry Mediterranian climate, the average moisture contents, porosity and bacterial degradation were lower, and fungal colonisation was lower and slower. Regardless of type and colour of coat of paint the intensity of the colonisation occurring in the L-joints exposed in Zagreb was somewhere between the colonisations which occurred in Zalesine and those in Rovinj.

The preservative treatments investigated did not modify the sequence of colonisation the various groups of organisms but they delayed of progress of the sequence and reduced the intensity of the colonisation as indicated by the overall number of isolates; the treatment with TnBTO was useful particularly in the case of Basidiomycotina. Thus, it may be possible to use the differences detected during the early stages of exposure as the basis for a realistic prediction of the likely performance in practice of new preservatives, without having to wait many years for the results of conventional field trials.

The data from the destructive examination of untreated fir L-joints in comparison with the treated fir L-joints can be used to predict fungal colonisation and overall preservative effectiveness after each period of exposure, and on any site in Croatia. Regardless of the site of exposure, during the first year of exposure, the L-joints coated with stains showed a better resistance to microorganisms.

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