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CONTROLLING OF WOOD DESTROYING *ANOBIIDAE* USING PARA-DICHLOROBENZENE IN AEROBIC ATMOSPHERE

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ABSTRACT

The paper presents the results of fumigation of wood-destroying insects using 1,4 dichlorobenzene. Larvae of *Anobium punctatum* De Geer and *Ptilinus pectinicornis* L. were fumigated in the laboratory aerobic atmosphere at the temperature of 20 and 30°C, while settled within wood samples or outside wood. *P. pectinicornis* beetles were fumigated outside at 20°C only. The final concentration of p-dichlorobenzene in the air and insect mortality were recorded. Experiments have been undertaken due to selection necessity of substances supporting the effects of low-oxygen atmosphere, which is used to control wood-destroying insects in the collections of museums. The paper presents experiments of the first phase, in aerobic conditions. The results obtained are encouraging.

KEYWORDS: Fumigation of wood, p-dichlorobenzene, wood-destroying insects, *Anobiidae*.

INTRODUCTION

The list of toxic gases for wood boring insects control has been reduced in recent years, especially after the withdrawal of methyl bromide in Germany in 2002 and in the EU in 2005 (Krehan 2002). Low-oxygen atmosphere of nitrogen or argon is recently of increasing interest for wood-destroying insects controlling in museum collections (Reichmuth et al. 1991, Considine et al. 1993, Koestler 1995, Brokerhof 1999, Binker et al. 2001, Selwitz and Maekawa

1998, Unger and Unger 1992, Hasegawa 2001, Kigawa et al. 2003). Due to very slow action of such atmosphere, attempts are made to toxic substances applying for accelerating the effect of controlling, such as p-dichlorobenzene (Kigawa and Yamano 1996).

Wooden collections are often infested by insects of family *Anobiidae*. The effectiveness of p-dichlorobenzene for controlling wood-destroying insects is not known. It has been rarely used so far, mainly to release fabric from insects of families *Tineidae* (*Microlepidoptera*) (Regan 1982) and *Dermestidae* (Coleoptera) (Karnkowski 1992). This compound is listed as an additive in the composition of such insecticide preparations as: Paracide, Paradow and Fumigant 1 (Unger 1988). Studies on the possible use of p-dichlorobenzene as an additive to low-oxygen atmosphere of nitrogen or argon, which accelerates the controlling of insects of family *Anobiidae*, were conducted as a stage of research on new fumigants, which cab be applied for ridding wood collections of insects. Investigations of p-dichlorobenzene as a fumigant were divided into two stages. At first, the study was carried out under aerobic conditions in the years 2005-2009, at the laboratory of the Division of Wood Protection, Faculty of Wood Technology, WULS. The paper presents the results of these studies, which were then continued in a low-oxygen atmosphere.

MATERIAL AND METHODS

1,4-dichlorobenzene (p-$C_6H_4Cl_2$) from Fluka Company has been used in the experiments. It was placed on Petri dishes with a diameter of 55 mm and then in glass desiccator covered with glass lid, sealed with silicone. The samples of p-dichlorobenzene were weighed before and after fumigation on a laboratory scale Radwag-WSP with the accuracy of 0.01 g, in order to calculate the amount of the substance sublimed. The tests were conducted in Selecta incubators, INCUDIGIT S–2001248 model, at controlled temperature of 20 and 30°C. Humidity of air with p-dichlorobenzene vapour in the desiccator glass was measured using TFA hair hygrometers, TA 100 model.

The first stage of the experiment was carried out using grown larvae of *Anobium punctatum* De Geer and *Ptilinus pectinicornis* L. (*Anobiidae*, Coleoptera) outside wood at both temperatures. The mass of the larvae ranged from 0.02 to 0.04 g each, with the average of 0.03 g. The larvae of *P. pectinicornis* were exposed to the p-dichlorobenzene vapour at the temperature of 20°C in seven time variants of: 0, 6, 12, 18, 24 36 and 48 h, while the larvae of *A. punctatum* in six time variants of: 0, 6, 12, 36 and 48 h. At temperature of 30°C, five time variants of: 0, 3, 12, 18 and 24 h were used in the case *P. pectinicornis* larvae, while six time variants of: 0, 3, 6, 12, 18 and 24 h in the case of *A. punctatum*. The number of individuals used in each variant of the experiment (namely time and temperature) was 20, except the variants concerning *A. punctatum* at the temperature of 20°C for 24 and 48 h, where 26 larvae were used in each.

At this stage, the effects of fumigation with p-dichlorobenzene was investigated for *P. pectinicornis* beetles outside the wood, at the temperature of 20°C, in five time variants of: 0, 1, 3, 4 and 5 h. 20 beetles were used in each variant of the experiment.

Reference experiments, without exposure to p-dichlorobenzene vapours, were conducted as well, using 20 individuals for each temperature, species, and growth stage variant. The total number of individuals used outside wood was 240 larvae of *P. pectinicornis*, 252 larvae of *A. punctatum,* and 100 beetles of *P. pectinicornis*.

After fumigation with p-dichlorobenzene outside wood for the time designed, the insects were removed from the glass and placed on filter paper discs in Petri dishes. The number of live individuals was observed along with the rate of moving out of 20 mm diameter circle on filter

paper discs. Movement was regarded as an attribute of life. The absence of necrosis, chromatosis, and shell tension decrease was assumed as the sign of good condition, as well as the ability to moving beyond the marked circle.

At the second stage of the investigation, the experiments were conducted on grown larvae of both species, while the larvae were settled in wood of black alder (*Alnus glutinosa* Gaertn.) and Scots pine (*Pinus sylvestris* L.), in blocks with dimensions of 30×50 mm (cross-section) and of 100 mm parallel to the grain. The number of blocks and wood properties are shown in the Tab. 1.

Tab. 1: Properties of wood and number of blocks used in each variant in second phase of experiments.

Wood species	**Number of wood samples / larvae per variant of the experiment (time / temperature)**	**Density (kg.m^{-3}) air-dry wood (moisture content 13 %)**	**Annual growth rings**	**Defects of wood**
Sapwood of Scots pine (*Pinus sylvestris* L.)	4 / 20	570	Broad	Few resin pockets
Sapwood of black alder (*Alnus glutinosa* Gaertn.)	4 / 20	450	Broad	No

The larvae of *A. punctatum* were all settled in 20 blocks of *P. sylvestris* sapwood, 4 blocks per each variant of the experiment. The larvae of *P. pectinicornis* were all settled in 40 blocks of *A. glutinosa* sapwood and 40 blocks of *P. sylvestris* sapwood, 4 blocks of both species per variant of experiment. Each wood sample was infested with 5 larvae of *A. punctatum* or *P. pectinicornis*, which were placed in the holes of diameter corresponding to their size. Settled larvae were left for 4 weeks to bore deep in the wood.

The larvae of *P. pectinicornis* in wood were exposed to p-dichlorobenzene vapour at the temperature of 20°C in five time variants of: 0, 4, 7, 14 and 28 days and at temperature of 30°C in five time variants of: 0, 1, 2, 4 and 8 days. The larvae of *A. punctatum* settled in *P. sylvestris* sapwood were fumigated at the temperature of 20°C only, in five time variants of: 0, 4, 8, 16 and 28 days

Total number of 400 larvae of *P. pectinicornis* and 100 larvae of *A. punctatum* were used in the second stage of the investigation.

After designed time of fumigation of the larvae settled in wood, the blocks were removed from the glass. The wood samples were split and the larvae were removed from the blocks. Preliminary qualification of the larvae was conducted and then the larvae were settled back in the blocks of *A. glutinosa* wood, of 15×25×50 mm dimensions. Individuals were placed in holes of 5–7 mm depth and diameters corresponding to the size of the larvae and left for 15 days. The final classification of larvae as alive was based on insects boring into the deeper layers of wood sample, which was recognised by the presence of frass in the hole. Alive and dead individuals were counted to determine mortality in the test group.

The results were verified using Fisher's exact test, because of relatively small sample size. This test is based on the distribution of two independent random variables χ^2, where each of them is divided by the corresponding number of degrees of freedom.

RESULTS

The final concentration of p-dichlorobenzene in the desiccator atmosphere, for different variants and stages of investigation, are shown in Tab. 2, as calculated from mass loss.

Tab. 2: The p-dichlorobenzene concentration g.m^{-3} in aerobic atmosphere in desiccator at the temperatures of 20 and 30°C, at the end of experiment.

	***P.pectinicornis* beetles outside the wood**	***P.pectinicornis* larvae outside the wood**		***A. punctatum* larvae outside the wood**			***P.pectinicornis* larvae in the wood**		***A. punctatum* larvae in the wood**
	Temperature						**Temperature**		
Fumigation time (h)	**20°C**	**20°C**	**30°C**	**20°C**	**30°C**	**Fumigation time (days)**	**20°C**	**30°C**	**20°C**
0	–	–	–	–	–	0	–	–	–
1	16					1		152	
3	23		35		34	2		239	
4	33					4	123	1057	180
5	56					7	472		
6		30		30	36	8		1194	350
12		40	78	30	89	14	1008		
18		42	80		100	16			446
24		60	104	60	190	28	1174		743
36		80		100					
48		104		120					

Obtained results of p dichlorobenzene vapours action on beetles and larvae of *P. pectinicornis* and larvae of *A. punctatum* outside the wood at the temperatures of 20 and 30°C in aerobic atmosphere are shown in the Fig. 1.

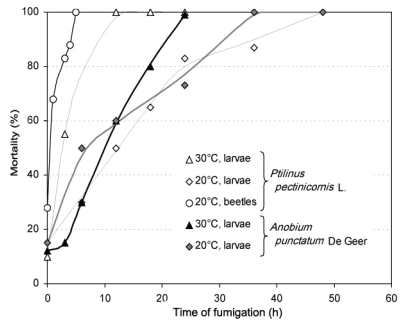

Fig. 1: Dependence of tested insects mortality on the time of fumigating with p-dichlorobenzene, comparison of larvae and beetles, outside the wood.

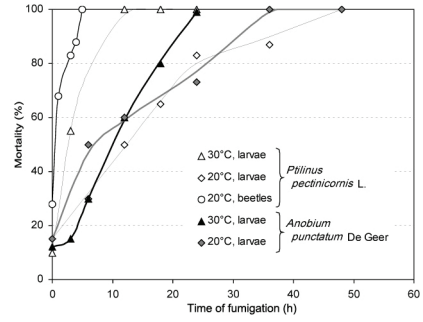

Fig. 2: Dependence of tested insects mortality on the time of fumigating with p-dichlorobenzene, comparison of larvae outside and inside the wood.

The results of p-dichlorobenzene vapours action on larvae of both species examined inside the wood at the temperatures of 20 and 30°C in aerobic atmosphere are shown in the Fig. 2.

The results of the statistical verification of relationship obtained in individual experiments are shown in the Tab. 3.

Tab. 3. Statistical verification of the results obtained.

***Ptilinus pectinicornis* beetles outside the wood**	***Ptilinus pectinicornis* larvae outside the wood**		***Anobium punctatum* larvae outside the wood**		***Ptilinus pectinicornis* larvae in the wood**		***Anobium punctatum* larvae in the wood**
Temperature							
20°C	**20°C**	**30°C**	**20°C**	**30°C**	**20°C**	**30°C**	**20°C**
Results of Fisher's exact test							
F0.05=2.64; F0.95=0.25 F= 4 significant dependence	F0.9=4.19, F0.1=0.2 F=6 significant dependence	F0.05=6.59; F0.95=0.11 F= 9 significant dependence	F0.05=4.53; F0.95=0.2 F= 5 significant dependence	F0.05=2.29, F0.95=0.57 F=4 significant dependence	F0.05=4.207; F0.95=0.16 F=6 significant dependence	F0.05=3.67; F0.95=0.2 F=5 significant dependence	F0.9=5.46; F0.1=0.14 F=7 significant dependence

DISCUSSION

Studies on the toxicity of fumigants towards wood-boring insects are carried in few research centres. Therefore, information on the lethal dose of p-dichlorobenzene for various species is not easy available. Toxicity of p-dichlorobenzene towards insects, such as *Tineidae* and *Dermestidae*, is widely known (Ebeling 1975, Regan 1982). The lethal dose for *Tineidae* is determined to 750 $g.m^{-3}$, while elevated temperature increases insects mortality (Ebeling 1975). The effective time of exposure of the *Tineidae* to p-dichlorobenzene is estimated to 48 h at room temperature (Ebeling 1975). Publications on such toxicity towards wood-boring insects were not found, however.

Discussion of the results obtained is clearer in the case of insects not protected by wood layers. *P. pectinicornis* beetles at the temperature of 20°C were noticeably more sensitive to p dichlorobenzene vapours than the larvae of the same species. The final lethal dose of p dichlorobenzene was almost two times lower (56 $g.m^{-3}$) in the case of beetles tested. The larvae of both examined species showed similar sensitivity to p-dichlorobenzene at the temperature of 20°C. Final lethal dose of p-dichlorobenzene for larvae of both species in these conditions was about 100 $g.m^{-3}$, although it was reached after different times of exposure. The applied temperature of 20°C is very close to the optimum for growth of *A. punctaum* larvae, which is stated to be from 22 to 23°C (Cymorek 1975, Dominik and Starzyk 2004). Optimum temperature for *P. pectinicornis* larvae is unknown – there is no information in the literature cited.

It should be mentioned that Monroe (1969) and Bond (1989), basing on *Sitophilus granarius* L. sensitivity to hydrogen cyanide and methyl bromide, found the decrease of sensitivity to fumigants with the growth of insects. On the other hand, Reichmuth (1988, 1990), in his research on impact of inert gases, like nitrogen and carbon dioxide, observed higher lethality of *S. granarius* L. and *Sitophilus oryzae* L. beetles than the larvae of these species in the same conditions. The total mortality of the tested groups of *S. granarius* L. beetles was achieved just after a week of fumigation, while it took 2 weeks in the case of larvae (Reitchmuth 1988). *S. oryzae* L.

beetles extinct in 95 % after only 32 h exposure to anaerobic atmosphere (Reitchmuth 1990).

Raising the temperature by 10°C should reduce the time of effective controlling at the same dose of fumigant (Monroe 1969, Bond 1989). At this temperature the rate of live processes increases in many species of insects, and sublimation rate of p-dichlorobenzene increases as well. Theoretically, larvae of both species should be killed, even twice as fast as at the temperature of 20°C.

In those cases, at the elevated temperature of 30°C, the larvae of both species showed different sensitivity to p-dichlorobenzene in aerobic atmosphere. Final lethal dose was 78 $g.m^{-3}$ for the larvae of *P. pectinicornis* and 190 $g.m^{-3}$ for the larvae of *A. punctatum*. An opinion may be met in the literature, that the *P. pectinicornis* larvae are more sensitive to liquid wood preservatives at the temperature of approximately 20°C than the *A. punctatum* larvae (Cymorek 1970). Why then, at the temperature of 30°C the larvae of *A. punctatum* are less sensitive to p dichlorobenzene, and require a higher dose of respiratory toxicant? The reason is probably simple. *A. punctatum* is a species of very low thermal tolerance. At the temperature higher than 28°C, the larvae are passing into a thermal coma (Cymorek 1975) and their oxygen demand strongly decreases. Breathing is very slow and thus, *A. punctatum* larvae take poison by the respiratory system slower than *P. pectinicornis* larvae, thus needing more time and higher dose to get the lethal effect. The *P. pectinicornis* beetles massive flights appear from June, when the temperature is relatively high (Dominik and Starzyk 2004). Probably, the optimum temperature of *P. pectinicornis* larvae is also higher and therefore the species larvae do not fall into a thermal coma. If this conclusion is correct, there is no slowing down of the lethal effects manifestation. Thus shorter time or lower dose is needed to control the larvae of *P. pectinicornis*.

In the case of wood fumigation, the temperature increase from 20 to 30°C reduced the time needed to kill *P. pectinicornis* larvae. It would be expected, taking into consideration the information in the literature (Monroe 1969, Bond 1989).

Vapours of p-dichlorobenzene spreads in wood pores more slowly than in the air, which slows down subsequently lethal effect of the larvae of wood-boring insects. Objects subjected to fumigation require long weathering, due to the long-lasting emission of p-dichlorobenzene, which slowly evaporates from the wood to the external environment. Prolonged exposure of the larvae to the gas trapped in the wood internal structure, after the removal of p-dichlorobenzene from a laboratory glass atmosphere, caused smaller effect than the "protection" of the larvae by wood layers. Hiding of the larvae in wood pieces even as small as 30×50×100 mm blocks resulted in a multiplication of fumigation time required to control insects and large increase in p-dichlorobenzene doses in the air.

Undoubtedly, the time needed to kill wood-boring insects depends on the dimensions of fumigated objects and their structure. The p-dichlorobenzene dose of 743 $g.m^{-3}$ was lethal to *A. punctatum* larvae in the pinewood (*P. sylvestris*) samples of 30×50×100 mm, as in the experiment for *Tineidae* (Ebeling 1975). However, wood has much lower permeability for gases than crude wool, which clearly affects the lethal effect towards *Anobiidae*. The complete mortality of *P. pectinicornis* larvae fumigated with p-dichlorobenzene in the pinewood (*P. sylvestris* L.) samples and alder wood (*A. glutinosa*) at 20°C was achieved only after 28 days of exposure, at the dose within the range of 743 $g.m^{-3}$ In the case of *A. punctatum* larvae in pinewood (*P. sylvestris* L.) samples, full mortality was achieved after 16 days by final dose of 446 $g.m^{-3}$.

Applying the wood of Scots pine (*P. sylvestris* L.) allowed increasing the diversity of wood in the experiments. *P. pectinicornis* obviously does not occur naturally in the wood of *P. sylvestris*. However, grown larvae of this species thrive after artificial transfer to the wood of coniferous species, according to investigations of Cymorek (1975) and preliminary experiments performed by the authors. The experiments could therefore be carried out with this species of wood.

CONCLUSIONS

1. 1,4-dichlorobenzene is a toxic substance effectively acting under aerobic conditions for larvae of both examined individuals of *Anobiidae* and beetles of *P. pectinicornis*. Elevated temperature allows shortening the time of exposure needed for wood-boring insects.
2. The larvae of *A. punctatum* and *P. pectinicornis* show similar sensitivity to the p-dichlorobenzene at the temperature of 20°C. The dose of 100 $g.m^{-3}$, obtained at the end of the fumigation, is lethal unless they are protected by layers of wood. At elevated temperature of 30°C *P. pectinicornis* larvae exhibit greater sensitivity to 1,4- paradichlorobenzene in comparison to *A. punctatum* larvae. The first are dead within no more than two days.
3. The beetles of *P. pectinicornis* are more sensitive to p-dichlorobenzene exposure in aerobic atmosphere and die faster than the larvae. The time required to kill the beetles outside wood does not exceed several hours.
4. When fumigated, wood provides a shield for the larvae. The exposure time, needed for full mortality of the larvae, increases to several days.

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