**Preliminary research on mortality of wood destroying Anobiidae
(Coleoptera) following the application of p-dichlorobenzene
in combination with low oxygen atmosphere fumigation**

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ABSTRACT. Experiments were undertaken in connection with the need, indicated in the literature, for the proper choice of a substance enhancing the effect of a low oxygen atmosphere used as a means of controlling wood boring insects in museum collections and historical objects. A low oxygen atmosphere itself causes the death of wood boring insects after a relatively long period of application. Thus, attempts were made to use p-dichlorobenzene to accelerate the effects of a low oxygen atmosphere in the control of wood boring insects. The experiments were performed as a continuation of preliminary research on the effect of p-dichlorobenzene on Anobiidae in an aerobic atmosphere. The paper presents the results of gassing *Anobium punctatum* and *Ptilinus pectinicornis* (Coleoptera: Anobiidae) larvae with 1,4-dichlorobenzene in a low oxygen atmosphere of nitrogen and argon. The experiments were performed in laboratory conditions at a temperature of 20°C on larvae extracted from wood. The results do not show a faster elimination of larvae of either species using p-dichlorobenzene in a low oxygen atmosphere as compared to using p-dichlorobenzene in an aerobic atmosphere. Complete eradication of larvae in the test groups was obtained after 48 hours when using p-dichlorobenzene in nitrogen. The results obtained when using p-dichlorobenzene in argon appear to be worse at fumigation times shorter than 48 hours.

KEY WORDS: fumigation of wood, p-dichlorobenzene, anoxia, wood boring insects, *Anobiidae*.

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INTRODUCTION

The list of toxic gases applicable to wood disinfestation has recently been reduced, especially since methyl bromide was withdrawn from use in Germany in 2002 and in the European Union in 2005 (KREHAN 2002). For controlling wood boring insects in museum collections, the popularity of the low oxygen atmosphere method has been growing (VALENTIN 1990, VALENTIN & PREUSSER 1990a, 1990b, REICHMUTH et al. 1991, CONSIDINE et al. 1993, KOESTLER 1993, 1996, RUST & KENNEDY 1993, KOESTLER & MATHEWS 1994, BROKERHOF 1999, KOESTLER et al. 2000, UNGER & UNGER 1995, HASEGAWA 2001, BINKER et al. 2001, KIGAWA et al. 2003). Because of the very slow effect of a low oxygen atmosphere, however, attempts have been made to use toxic substances, such as p-dichlorobenzene, in order to accelerate the process (KIGAWA & YAMANO 1996). The present research examines this idea.

The wood of museum exhibits and historical objects is often attacked by insects from the Anobiidae family. The effectiveness of using p-dichlorobenzene as a means of controlling wood boring insects, however, has been relatively poorly examined (WÓJCIK et al. 2014). P-dichlorobenzene has so far been utilised mostly for the disinfestation of fabrics attacked by insects of the Tineidae (Lepidoptera) and Dermestidae (Coleoptera) families (REGAN 1982). This chemical compound is contained in preparations designed for fabric disinfestation such as Paracide, Paradow and Fumigant 1 (UNGER 1988). Research into the possible utilisation of p-dichlorobenzene in combination with a low oxygen atmosphere as a means of accelerating Anobiidae control was performed at the Department of Wood Science and Wood Protection (Faculty of Wood Technology, University of Life Sciences – SGGW), as a continuation of research into the effects of p-dichlorobenzene in an aerobic atmosphere (WÓJCIK et al. 2014).

MATERIALS AND METHODOLOGY

Nitrogen and argon of technical purity together with 1,4-dichlorobenzene ($C_6H_4Cl_2$) (Fluka) were used in the experiments. The gassing of biological material using p-dichlorobenzene in combination with a low oxygen atmosphere took place in one-litre desiccators with T-joints, each mounted with two multipath taps, sealed with silicone vacuum grease (GE Bayer Silicones). Each tap was joined by rubber tubes to steel bottles containing nitrogen or argon of technical purity. Each variant of the experiment – a different combination of duration, type of non-reactive gas and species of insect – was performed in a separate desiccator. This enabled the content of p-dichlorobenzene in the gas mixture at the end of the experiment to be measured.

Solid p-dichlorobenzene was placed on 55 mm diameter Petri dishes in a glass desiccator, covered with a glass lid and sealed with silicone. The p-dichlorobenzene samples were weighed before and after fumigation on a Radwag-WSP laboratory scale, accurate to 0.01 g, in order to calculate the amount of the substance sublimed.

At the beginning of each variant of the experiment, oxygen was removed from the desiccators using an ILMVAC MPC 052 Z diaphragm pump and flushed three times with technically pure nitrogen or argon. The oxygen content during fumigation was monitored using a Heavy Duty Dissolved Oxygen Meteor (model 407510) oxygen meter (Extech). The oxygen content achieved in this way ranged from 0.3% to 1% at the end of the experiment.

Experiments on the effects of p-dichlorobenzene in nitrogen and argon were carried out at a temperature of 20°C on mature larvae of *Anobium punctatum* (DE GEER, 1774) and *Ptilinus pectinicornis* (LINNAEUS, 1758) (Coleoptera: Anobiidae). The insects were extracted from breeding blocks and weighed. Individual masses ranged from 0.02 to 0.04 g (av. 0.03 g). Each of the three series of experiments included a control variant. In each control 20 larvae were placed outside the wood in an aerobic atmosphere for 48 hours at 20°C and a relative humidity of 55%. The experimental variants are listed in Table 1.

Table 1. Variants of experiments and numbers of Anobiidae larvae in applications of p-dichlorobenzene to low oxygen atmosphere fumigation at a temperature of 20°C.

Atmosphere	Times					
	0 h	3 h	6 h	18 h	24 h	48 h
<i>Anobium punctatum</i>						
Nitrogen	20 larvae	20 larvae	24 larvae	22 larvae	20 larvae	20 larvae
<i>Ptilinus pectinicornis</i>						
Nitrogen	20 larvae	20 larvae	–	22 larvae	20 larvae	20 larvae
<i>Ptilinus pectinicornis</i>						
Argon	20 larvae	–	–	20 larvae	20 larvae	20 larvae

The 20°C ambient temperature in the laboratory was obtained by appropriately setting the air conditioning system. It was controlled in the room and in the desiccators using disc thermometers (Cole Palmer). The humidity of the gases in the laboratory and in the containers used for breeding the wood boring insects was monitored with TA 100 thermo-hygrometers (TFA).

The mortality rate of the larvae in each variant of the experiment was estimated as follows. After the time for implementing a given variant of the experiment had elapsed, atmospheric air was introduced and the larvae were removed from the desiccator, isolated and counted. Larvae were considered dead if they did not move at all when irritated, and also displayed signs of colour change and a complete lack of body turgor. Definitely dead individuals were then removed, while larvae showing evident signs of life (movement), as well as those potentially still alive (no colour change, no lack of skin shell tension) were placed in holes drilled in sapwood Scots pine (*Pinus sylvestris* L.) blocks 15 x 25 x 50 mm in size. The diameters of the holes were adjusted to the size of the individual larvae. These were regarded as living only if after another 30 days they were able to bite into deeper layers of the wood of their own accord. Individuals that had survived the gassing process were treated as being alive, while those that had failed to bite into the wood were regarded as dead. Based on these calculations, the mortality rate of the larvae in the given variant of the experiment was determined.

Chebyshev's inequality was used to evaluate the significance of the mortality in each variant of the experiment. If the absolute difference between the average arithmetic values of mortality of both insect species in a given variant (insect / time / medium) was greater than or equal to three times the standard error of the difference, i.e.,

$$|\bar{x}_1 - \bar{x}_2| \geq 3 \cdot \varepsilon(\bar{x}_1 - \bar{x}_2)$$

then the difference between the averages was taken to be statistically significant. Otherwise, it was treated as accidental.

RESULTS

The mortality rates of *Anobium punctatum* and *Ptilinus pectinicornis* larvae following application of p-dichlorobenzene to low oxygen atmosphere fumigation at a temperature of 20°C are given in Table 2.

In order to simplify the comparison, the results are shown in Fig. 1 together with the mortality rates of both species gassed with p-dichlorobenzene in an aerobic atmosphere, described in detail in an earlier paper (WÓJCIK et al. 2014).

The results of the statistical analysis of the differences in mortality between *Anobium punctatum* and *Ptilinus pectinicornis* larvae following application of p-dichlorobenzene to low oxygen atmosphere fumigation are shown in Table 3.

Table 2. Mortality of *Anobium punctatum* and *Ptilinus pectinicornis* following application of p-dichlorobenzene to low oxygen atmosphere fumigation at a temperature of 20°C. FumT – fumigation time; PDC – content of p-dichlorobenzene in a nitrogen atmosphere at the end of the experiment; MortA – mortality of *Anobium punctatum* larvae in a nitrogen atmosphere; MortP1 – mortality of *Ptilinus pectinicornis* larvae in a nitrogen atmosphere; MortP2 – mortality of *Ptilinus pectinicornis* larvae in a, argon atmosphere.

FumT [h]	PDC [g·dm ⁻³]	MortA [%]	PDC [g·dm ⁻³]	MortP1 [%]	PDC [g·dm ⁻³]	MortP2 [%]
0	0	15	0	10	–	–
3	23	30	30	30	–	–
6	23	45	–	–	–	–
18	76	55	79	50	17	20
24	80	60	82	95	20	45
48	92	100	90	100	80	80

Fig. 1. The mortality of *Anobium punctatum* and *Ptilinus pectinicornis* larvae gassed with p-dichlorobenzene in nitrogen or oxygen atmosphere at 20°C, together with earlier results of the application of p-dichlorobenzene in an aerobic atmosphere at 20°C (WÓJCIK et al. 2014).

Table 3. Statistical analysis of the differences in mortality between *Anobium punctatum* and *Ptilinus pectinicornis* larvae following application of p-dichlorobenzene to low oxygen atmosphere fumigation at a temperature of 20°C.

Times	$ \bar{x}_1 - \bar{x}_2 \geq 3 \cdot \varepsilon(\bar{x}_1 - \bar{x}_2)$	Statistical analysis of the results of the experiment
0 h	$0.05 < 0.321$	Statistically insignificant difference in mortality rates
3 h	$0 < 0.446$	Statistically insignificant difference in mortality rates
18 h	$0.05 < 0.462$	Statistically insignificant difference in mortality rates
24 h	$0.35 < 0.369$	Statistically insignificant difference in mortality rates

In the control variants, mortality rates of the larvae of each species were low, and the difference between them was statistically insignificant. In each fumigation variant of the same duration (except for the 24-hour variant) the differences between the mortality rates of both species were also small and statistically insignificant. The results of the statistical analysis of the significance of differences in mortality rates of a given species for different durations of applying p-dichlorobenzene to low oxygen atmosphere fumigation at 20°C are shown in Table 4.

Table 4. Statistical analysis of the significance of differences in mortality rates of each species for different durations of applying p-dichlorobenzene to low oxygen atmosphere fumigation at 20°C. Comp. – compared variants; *Ap* – *Anobium punctatum*; *Pp* – *Ptilinus pectinicornis*; * statistically significant difference; # statistically insignificant difference.

Comparison of mortality in variants:	Beetle species and the atmosphere (medium) in which it was fumigated with p-dichlorobenzene $ \bar{x}_1 - \bar{x}_2 \geq 3 \cdot \varepsilon(\bar{x}_1 - \bar{x}_2)$		
	<i>Ap</i> – nitrogen	<i>Pp</i> – nitrogen	<i>Pp</i> – argon
0 h – 18 h	$0.4 < 0.415$ #	$0.4 > 0.396$ *	$0.1 < 0.344$ #
0 h – 24 h	$0.45 > 0.417$ *	$0.85 > 0.255$ *	$0.35 < 0.4$ #
18 h – 48 h	$0.45 > 0.138$ *	$0.5 > 0.344$ *	$0.35 < 0.439$ #
0 h – 48 h	–	–	$0.7 > 0.344$ *

This time, several statistically significant differences were obtained, though (as shown in the table) not in all cases.

DISCUSSION

The idea of using p-dichlorobenzene to accelerate the effect of a low oxygen atmosphere for controlling wood boring insects, studied by KIGAWA & YAMANO (1996), probably came from the utilisation of this substance to disinfest fabrics attacked by insects (REGAN 1982, UNGER 1988). As early as 1960, TANNERT (1960) advised the use of p-dichlorobenzene for *Anthrenus* sp. control in entomological collections. However, no information was found regarding the susceptibility of Anobiidae to p-dichlorobenzene, apart from the results of the authors' own study performed in an aerobic atmosphere (WÓJCIK et al. 2014).

The differences in mortality rates of both species for the same duration of the application of p-dichlorobenzene to nitrogen atmosphere fumigation were small and statistically insignificant, as already mentioned above (see Table 3). The authors could not then detect differences in mortality of *Anobium punctatum* and *Ptilinus pectinicornis* larvae by applying p-dichlorobenzene to low oxygen atmosphere fumigation, probably because of the relatively small number of larvae used in each variant of the experiment. The results cannot be related to literature data, as no papers were found that investigated the effectiveness of toxic gases in *Anobium pectinicornis* control. This insect species has been of marginal interest in this regard. Only one article was found that compared the susceptibility of larvae of both species to wood preservatives in a liquid state, which were, as a rule, alimentary or contact toxins. CYMOREK (1970) reported a greater susceptibility of *Ptilinus pectinicornis* larvae than of *Anobium punctatum* larvae to chlorinated hydrocarbons and older wood preservatives such as Combi-Basileum and Basileum SF. The results obtained in the present research seem to confirm CYMOREK's (1970) opinion of the relatively high susceptibility of *Ptilinus pectinicornis* larvae to toxic substances, although following the application of p-dichlorobenzene in an aerobic atmosphere at 20°C, which is close to the optimal temperature of 22°C for *Anobium punctatum*, the differences were not clearly evident (WÓJCIK et al. 2014). No information on the optimal temperature for *Ptilinus pectinicornis* could be found in the literature. Since the abundance of *Ptilinus pectinicornis* beetles peaks in June and July (DOMINIŁ & STARZYK 2004), one can assume that this species is less vulnerable to overheating than *Ptilinus pectinicornis*. When p-dichlorobenzene was used in an aerobic atmosphere, significant differences between the mortality rates of *Anobium punctatum* and *Ptilinus pectinicornis* larvae appeared only after the temperature had been raised to 30°C, definitely unfavourable to *Anobium punctatum* (WÓJCIK et al. 2014). The larvae of both species of Anobiidae turned out to be far more susceptible to p-dichlorobenzene in an aerobic atmosphere than the much larger larvae of *Tenebrio monitor* LINNAEUS, 1758 (Coleoptera, Tenebrionidae) (WÓJCIK & KRAJEWSKI 2013).

All larvae of both species died in the 48-hour variant of p-dichlorobenzene combined with nitrogen atmosphere fumigation at 20°C. A statistically significant difference in the mortality of *Ptilinus pectinicornis* larvae as compared to the control was achieved already after 18 hours of application of p-dichlorobenzene to nitrogen medium fumigation. With regard to *Anobium punctatum* larvae, a statistically significant difference in mortality as compared to the control was achieved only after 48 hours of p-dichlorobenzene treatment in combination with nitrogen fumigation, although if the results were had been rounded up to the first decimal point, the difference could have been classified as significant in the earlier variant. Comparison of the experimental results on larval mortality after the application of p-dichlorobenzene to nitrogen atmosphere fumigation after 24 and 48 hours showed a statistically significant difference for both species.

As far as the application of p-dichlorobenzene to argon atmosphere fumigation is concerned, a statistically significant difference in mortality of *Ptilinus pectinicornis*, as compared to the control variant, was achieved only after 48 hours. What is more, the mortality rate of the test group did not reach the level of 100%. In the research of TAVZES et al. (2001), argon and nitrogen were equally effective in destroying the mycelium of *Coniophora puteana* (SCHUM.: FR.) KARST. and *Antrodia sinuosa* (Fr.) KARST. (in the original version: *Antrodia vaillantii*). However, some authors consider that argon fumigation results in the faster elimination of insects than nitrogen fumigation (VALENTIN 1993, KOESTLER 1995).

Many papers emphasise the slow disinfestation effect of low oxygen atmosphere, as compared to that of toxic gases (REICHMUTH et al. 1991, CONSIDINE et al. 1993, KOESTLER 1993, BROKERHOF 1999, BINKER et al. 2001, UNGER & UNGER 1995, HASEGAWA 2001, KIGAWA et al. 2003). Because of this slowness, attempts have been made to accelerate the process by applying toxic substances such as p-dichlorobenzene (KIGAWA & YAMANO 1996). However, research on the application of p-dichlorobenzene to nitrogen atmosphere fumigation at 20°C, in the case of both *Anobium punctatum* and *Ptilinus pectinicornis* larvae, showed larval mortality to be not significantly quicker than with the application of p-dichlorobenzene to aerobic atmosphere fumigation, which was tested in earlier research (WÓJCIK et al. 2014).

Owing to the incomplete set of duration variants of the experiment, as well as the limited number of larvae used in the tests (20-24 individuals), the results should be treated with caution and only as preliminary. Medium-sized larvae were used, placed outside the wood. The results may provide a basis for further research on the use of p-dichlorobenzene in low oxygen atmosphere fumigation against wood boring insects in wood blocks of various sizes.

However, such research will be much more difficult because of contradictory factors that may appear. Wood, being a porous material, will retain some oxygen, which may slow

down the creation of a low oxygen atmosphere inside the wood. Oxygen is not easily removed from wood at a slightly negative pressure. Combined with the slow penetration of p-dichlorobenzene into the wood, this may slow down larval mortality. On the other hand, wood provides natural conditions for larvae, which lowers their stress levels and may encourage them to open their stigmata more often. This may increase their mortality following the application of p-dichlorobenzene to low oxygen fumigation. Such effects have been observed in research performed by staff of the Department of Wood Science and Protection, Warsaw University of Life Sciences, SGGW. The results of that research have not yet been published.

CONCLUSIONS

The following conclusions can be drawn from the results:

1. The tests did not reveal increased mortality of *Anobium punctatum* and *Ptilinus pectinicornis* larvae following the application of p-dichlorobenzene to low oxygen atmosphere fumigation at 20°C as compared to the results of applying p-dichlorobenzene in an aerobic atmosphere at the same temperature.

2. A 100% mortality of *A. punctatum* and *P. pectinicornis* larvae, placed outside the wood, was achieved after 48 hours of applying p-dichlorobenzene in combination with a nitrogen atmosphere at 20°C. For this reason, it seems that 48 hours should be treated as the default duration for further research on the application of p-dichlorobenzene to a low oxygen atmosphere as a means of controlling wood boring Anobiidae inside wood

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