

## RESPONSE OF SAWMILL WORKERS TO WORK-RELATED AIRBORNE ALLERGENS

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**Abstract:** The aim of this work was to determine the reactivity of sawmill workers to biological allergens associated with wood dust. Allergological examinations by skin and precipitin tests were performed in 43 workers employed in a sawmill processing coniferous wood (pine), in 90 workers employed in two sawmills processing deciduous wood (oak), and in 32 healthy urban dwellers not exposed to organic dusts (referents). The skin test was performed by the intradermal method with the saline extracts of wood dust and of the cultures of three microbial species (*Rahnella* sp., *Brevibacterium linens* and *Penicillium citrinum*) isolated from the air polluted with wood dust. Sawdust from pine was used for testing of the pine processing workers and referents while sawdust from oak was used for testing of the oak processing workers. Skin reactions were recorded after 20 minutes, 8 hours and 24 hours. The agar-gel test for the presence of precipitins in serum was performed with the extract of pine wood dust and extracts of 17 microbial isolates. The workers processing pine showed a very high frequency of positive skin reactions to the extract of wood dust at all time intervals, significantly greater compared to the workers processing oak and referents ( $p < 0.001$ ). The early skin reactions to the extracts of dust-borne bacteria and fungi were very common among sawmills workers and showed a significant relationship with the degree of exposure. The frequency of reactions to Gram-negative bacterium *Rahnella* sp. was significantly greater in the pine processing workers than in the oak processing workers and referents ( $p < 0.001$ ). By contrast, the oak processing workers reacted significantly more frequently to *Penicillium citrinum*, compared to the pine processing workers and referents ( $p < 0.01$ ). These results conform to the prior study of airborne microflora in which the dominance of Gram-negative bacteria was stated in the pine processing sawmill while mould fungi were most common in the oak processing sawmills. The antibody response of sawmill workers to work-related antigens was much weaker compared to skin reactions. As many as 41 sawmill workers reported the occurrence of work-related symptoms. A significant relationship was found between the occurrence of symptoms and frequency of allergic reactions, but only with a limited number of antigens. The obtained results suggest that early allergic reactions to coniferous wood and to microorganisms associated with wood dust are common among sawmill workers, posing a potential risk of work-related disease in this occupational group.

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**Key words:** sawmills, woodworkers, sensitization, allergens, wood dust, bacteria, fungi, intradermal test, agar-gel precipitation test, work-related symptoms.

## INTRODUCTION

Workers processing wood could be exposed to various allergenic, immunotoxic and cancerogenic substances

originating from wood itself (such as plicatic acid from western red cedar) and from bacteria and fungi growing on timber [1, 7, 8, 13, 23, 27, 29, 31, 34, 45, 56]. The exposure may cause decline in lung function, bronchial

hyperresponsiveness and various diseases, such as: organic dust toxic syndrome (ODTS), allergic alveolitis, asthma, chronic bronchitis, rhinitis, mucous membrane irritation (MMI), contact dermatitis and nasal cancer [4, 7, 8, 9, 26, 29, 33, 42, 44, 45, 56].

Most adverse effects caused by microorganisms associated with wood dust have an immunological background. The best known are those caused by moulds which may abundantly develop in suitable conditions on stored wood products (planks, chips) as a secondary wood infection. The inhalation of large amounts of spores and mycelial fragments of *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Paecilomyces* spp., *Mucor* spp. and other fungi may cause in exposed workers strong antibody response and respiratory disorders, usually allergic alveolitis (wood trimmer's disease) or organic dust toxic syndrome [3, 23, 24, 35, 37, 47, 50, 59, 60]. Less known are health effects of microorganisms developing in stored timber logs as a primary infection of wood. Timber may contain - depending on kind of wood and conditions of storage - large quantities of diverse bacteria and fungi comprising potentially pathogenic species [13, 15, 34, 41, 52]. Cases of allergic alveolitis and asthma were described in woodworkers who were exposed during logging, debarking and sawing to wood dust primarily contaminated with microorganisms. Fungi of the species *Cryptostroma corticale* [25, 58], *Graphium* sp. and *Pullularia pullulans* [6], *Aspergillus* sp. [57], *Penicillium frequentans* [2], *Penicillium* sp. [22], *Trichoderma* sp. and *Scopulariopsis* sp. [32] and actinomycetes of the species *Thermoactinomyces vulgaris* [57] and *Saccharomonospora viridis* [30] were reported as causative agents of the disease. However, in contrast to wood trimmers, only a few allergological surveys have been conducted among woodworkers employed at logging, debarking and sawing of timber [5, 28, 51].

The aim of this work was to determine the immunological reactivity of Polish sawmill workers to a wide spectrum of biological allergens associated with wood dust from coniferous and deciduous timber, as potential disease agents. Allergens for this study were selected on the basis of the earlier microbiological analysis of the air in the examined sawmills [11, 21].

## MATERIALS AND METHODS

**Examined population.** Two subgroups of woodworkers employed in sawmills located in the Lublin region (eastern Poland) were examined: one consisted of workers processing coniferous wood and the other consisted of workers processing deciduous wood. Previously in all these sawmills microbiological studies of the air were performed for selecting the antigens for allergological examinations [11, 21].

The workers processing coniferous wood were employed in sawmill "A" processing wood of Scots pine (*Pinus sylvestris*). The subgroup comprised 43 workers (42 males + 1 female) aged  $35.6 \pm 10.7$  yrs (mean  $\pm$  S.D.).

The workers processing deciduous wood were employed in sawmill "C" processing wood of English oak (*Quercus robur*) (33 persons) and in sawmill "D" processing wood of English oak and white warty birch (*Betula verrucosa*) (57 persons). The subgroup comprised together 90 workers (48 males + 42 females), aged  $34.8 \pm 10.9$  yrs (mean  $\pm$  S.D.).

The total group of sawmill workers comprised 133 persons (90 males and 43 females), aged  $35.1 \pm 10.8$  yrs (mean  $\pm$  S.D.).

Thirty two healthy office workers living in the city of Lublin and not exposed to organic dusts were examined as a reference group. This group comprised 11 males and 21 females, aged  $36.4 \pm 8.6$  yrs (mean  $\pm$  S.D.).

**Table 1.** Skin response of sawmill workers to the extract of wood dust<sup>a</sup>.

Group	Persons showing positive reaction (number, percent)											
	Early reactions (20 min)				Delayed reactions (8 h)				Late reactions (24 h)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
<b>Sawmill workers</b>												
Workers processing coniferous wood (N = 43)	42 <sup>+++</sup> (97.7%)	39 <sup>+++</sup> (90.7%)	31 <sup>+++</sup> (72.1%)	4 <sup>**</sup> (9.3%)	36 <sup>+++</sup> (83.7%)	26 <sup>+++</sup> (60.5%)	17 <sup>+++</sup> (39.5%)	3 <sup>*</sup> (7.0%)	38 <sup>+++</sup> (88.4%)	19 <sup>+++</sup> (44.2%)	12 <sup>+++</sup> (27.9%)	3 <sup>*</sup> (7.0%)
Workers processing deciduous wood (N = 90)	22 (24.4%)	13 (14.4%)	6 (6.7%)	0	8 (8.9%)	4 (4.4%)	1 (1.1%)	0	1 (1.1%)	0	0	0
Total wood processing workers (N = 133)	64 <sup>++</sup> (48.1%)	52 (39.1%)	37 (27.8%)	4 (3.0%)	44 (33.1%)	30 (22.6%)	18 (13.5%)	3 (2.3%)	39 <sup>+</sup> (29.3%)	19 (14.3%)	12 (9.0%)	3 (2.3%)
<b>Reference group (N = 32)</b>												
	7 (21.9%)	7 (21.9%)	4 (12.5%)	0	10 (31.2%)	9 (28.1%)	1 (3.1%)	0	3 (9.4%)	3 (9.4%)	3 (9.4%)	1 (3.1%)

<sup>a</sup>Workers processing coniferous wood were tested with the extract of dust from pine wood, and the workers processing deciduous wood were tested with the extract of dust from oak wood. Members of the reference group were tested with the extract of dust from pine wood. Grade 1 = reactions weakly positive, diameter  $\geq 5$  mm. Grade 2 = reactions positive, diameter  $\geq 10$  mm. Grade 3 = reactions strongly positive, diameter  $\geq 20$  mm. Grade 4 = reactions very strongly positive, diameter  $\geq 40$  mm. + - +++: significantly greater compared to reference group; +p<0.05, ++p<0.01, +++p<0.001. \*\_\*\*\*: significantly greater compared to subgroup of workers processing deciduous wood; \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

All sawmill workers and members of the reference group were examined by skin and precipitation tests with the saline extracts of wood dust and of cultures of microorganisms isolated from the air polluted with the dust. All the subjects were also interviewed with a questionnaire developed in the Institute of Agricultural Medicine in Lublin [20] for the study of work-related symptoms. Human subjects protocols were approved by the Ethics Commission of the Institute of Agricultural Medicine and all subjects gave informed consent.

**Preparation of allergens.** Samples of settled sawdust from Scots pine (*Pinus sylvestris*) logs and English oak (*Quercus robur*) logs, collected in sawmills "A" and "C" respectively, were extracted in Coca's solution [54] in the proportion of 15 grams per 150 ml (w/v). For skin tests the extracts were used in the dilution of 1:100 (v/v) and for the agar-gel precipitation test in the dilution 1:10, using saline (0.85% NaCl) as a diluent. In skin test, the workers processing coniferous wood and members of the reference group were tested with the extract of sawdust from Scots pine (*Pinus sylvestris*), while the workers processing deciduous wood were examined with the extract of sawdust from English oak (*Quercus robur*). In agar-gel precipitation test, all the subjects were tested with the extract of sawdust from Scots pine.

The antigens of the following 17 microorganisms, associated with organic dusts and found in the air of the examined sawmills [21] were used in the study:

- Gram negative bacteria: *Acinetobacter calcoaceticus*, *Pantoea agglomerans* (syn. *Erwinia herbicola*, *Enterobacter agglomerans*), *Rahnella* sp.;
- Gram-positive bacteria: *Arthrobacter globiformis*, *Bacillus subtilis*, *Brevibacterium linens*, *Corynebacterium* sp.;
- Actinomycetes: *Rhodococcus* sp., *Saccharopolyspora rectivirgula* (syn. *Micropolyspora faeni*, *Faenia rectivirgula*), *Streptomyces albus*, *Thermoactinomyces vulgaris*;

- Fungi: *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus clavatus*, *Aspergillus fumigatus*, *Cephalosporium glutineum*, *Penicillium citrinum*.

All these antigens were used in the agar-gel precipitation test, while for the skin test, on the basis of the common occurrence in the air of the examined sawmills and potential pathogenic properties, the following three antigens were selected: *Rahnella* sp., *Brevibacterium linens* and *Penicillium citrinum*. The antigens of *Rahnella* sp., *Brevibacterium linens*, *Corynebacterium* sp., *Cephalosporium glutineum* and *Penicillium citrinum* were prepared from the strains isolated from the air of examined sawmills, while the other antigens were prepared from the reference strains used for the research and diagnostic purposes in the Institute of Agricultural Medicine in Lublin [10].

Both in skin and agar-gel precipitation tests, lyophilised saline extracts of bacterial or fungal mass, produced in the Institute of Agricultural Medicine in Lublin, were used as antigens. In the case of Gram-negative and Gram-positive bacteria the mass was harvested from nutrient agar cultures while in the case of actinomycetes and fungi the mass was harvested from sugar broth cultures. Then, the mass was homogenised and extracted in saline (0.85% NaCl) in the proportion 1:2 for 48 hrs at 4°C, with intermittent disruption of cells by 10-fold freezing and thawing. Afterwards, the supernatant was separated by centrifugation, dialysed against distilled water for 24 hrs, concentrated by evaporation to 0.1-0.15 of initial volume and lyophilised. In skin test, the antigens were used at the concentration of 1 mg/ml and in agar-gel precipitation test at the concentration of 30 mg/ml [46, 53].

**Skin test.** The test was performed by intradermal method. The antigens were dissolved in 0.85% NaCl, sterilised by filtering and checked for sterility and lack of toxicity. The test was performed by intracutaneous

**Table 2.** Skin response of sawmill workers to the extract of *Rahnella* sp.

Group	Persons showing positive reaction (number, percent)											
	Early reactions (20 min)				Delayed reactions (8 h)				Late reactions (24 h)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
<b>Sawmill workers</b>												
Workers processing coniferous wood (N = 43)	38 <sup>+++</sup> (88.4%)	28 <sup>+++</sup> (65.1%)	15 <sup>++</sup> (34.9%)	2 <sup>*</sup> (4.7%)	41 (95.3%)	31 (72.1%)	19 (44.2%)	2 (4.7%)	42 (99.7%)	34 (79.1%)	28 (65.1%)	12 (27.9%)
Workers processing deciduous wood (N = 90)	42 <sup>+</sup> (46.7%)	26 (28.9%)	8 (8.9%)	0	80 (88.9%)	66 (73.3%)	42 (46.7%)	1 (1.1%)	87 (96.7%)	78 (86.7%)	62 (68.9%)	23 (25.6%)
Total wood processing workers (N = 133)	80 <sup>+++</sup> (60.2%)	54 <sup>+</sup> (40.6%)	23 (17.3%)	2 (1.5%)	121 (91.0%)	97 (72.9%)	61 (45.9%)	3 (2.3%)	129 (97.0%)	112 (84.2%)	90 (67.7%)	35 (26.3%)
<b>Reference group (N = 32)</b>												
	8 (25.0%)	6 (18.7%)	4 (12.5%)	0	30 (93.7%)	26 (81.2%)	11 (34.4%)	1 (3.1%)	30 (93.7%)	26 (81.2%)	21 (65.6%)	5 (15.6%)

Grade 1 = reactions weakly positive, diameter  $\geq 5$  mm. Grade 2 = reactions positive, diameter  $\geq 10$  mm. Grade 3 = reactions strongly positive, diameter  $\geq 20$  mm. Grade 4 = reactions very strongly positive, diameter  $\geq 40$  mm. + - +++: significantly greater compared to reference group; +p<0.05, ++p<0.01, +++p<0.001. \* - \*\*\*: significantly greater compared to subgroup of workers processing deciduous wood; \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

injecting 0.1 ml of the antigenic extracts and of saline (as a control) into the forearm of the subject. The test sites were observed at 20 min for immediate reactions, at 8 hrs for delayed reactions and at 24 hrs for late reactions. The wheal and/or erythema reactions of 5 mm or more in diameter (at negative control) were regarded as positive. The intensity of positive reactions was graded on the basis of diameter as follows:  $\geq 5$  mm - grade 1,  $\geq 10$  mm - grade 2,  $\geq 20$  mm - grade 3,  $\geq 40$  mm - grade 4.

**Agar-gel precipitation test.** The test was performed by Ouchterlony double diffusion method in purified 1.5% *Difco* agar. The subject's serum was placed in the central well and antigens, dissolved in 0.85% NaCl, in the peripheral wells. Each serum was tested twice: not concentrated, and three-fold concentrated, for the detection of low levels of precipitins. The agar plates were incubated for six days at room temperature, then washed in saline and in 5% sodium citrate solution (for preventing false positive reactions), and stained with azocarmine B [46, 48, 53].

**Statistical analysis.** The obtained results were analysed by the Student's t-test, assuming  $p < 0.05$  as a significance level.

The study was performed mostly during the years 1982–1986 and continued during 1995–2000. Preliminary results of this work have been reported elsewhere [11, 17, 18].

## RESULTS

**Skin reactions.** The skin responses of sawmill workers to the extracts of wood dust, *Rahnella* sp., *Brevibacterium linens* and *Penicillium citrinum* are presented in Tables 1, 2, 3 and 4, respectively. The workers processing coniferous wood responded to the extract of pine sawdust with a very high frequency, significantly greater

( $p < 0.001$ ) at all time intervals (20 min, 8 hrs, 24 hrs) compared to reference group tested with the extract of pine sawdust and the subgroup of workers processing deciduous wood tested with the extract of oak sawdust (Tab. 1). No significant difference in the response rate could be found between the workers processing deciduous wood and reference group.

Similarly, the frequency of early cutaneous reactions to the extract of the Gram-negative bacterium *Rahnella* sp. indigenous to wood was very high among sawmill workers processing coniferous wood, being significantly greater ( $p < 0.001$ ) compared to the reference group and the subgroup of workers processing deciduous wood (Tab. 2). The workers processing deciduous wood responded to *Rahnella* sp. with higher frequency than referents but the statistical significance of the difference was lower compared to preceding group ( $p < 0.05$ ). The frequency of positive delayed and late skin reactions to *Rahnella* sp. was very high in all tested subjects and no significant differences between the examined groups could be found.

The frequency of positive early and late reactions to the extract of coryneform bacterium *Brevibacterium linens* was similar in both subgroups of sawmill workers and was significantly higher compared to the reference group (Tab. 3). No significant differences in delayed response (after 8 hrs) were observed between examined groups.

The highest frequency of early cutaneous reactions to the extract of *Penicillium citrinum* was found among the workers processing deciduous wood (Tab. 4). The response rate in this subgroup of sawmill workers was significantly greater ( $p < 0.01$ ) compared to the reference group and the subgroup of workers processing coniferous wood.

**Precipitin reactions.** With the majority of antigens tested, no significant differences were found between the sawmill workers and reference group (Tab. 5). The

**Table 3.** Skin response of sawmill workers to the extract of *Brevibacterium linens*.

Group	Persons showing positive reaction (number, percent)											
	Early reactions (20 min)				Delayed reactions (8 h)				Late reactions (24 h)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
<b>Sawmill workers</b>												
Workers processing coniferous wood (N = 43)	29 <sup>+</sup> (67.4%)	18 (41.9%)	5 (11.6%)	0	16 (37.2%)	5 (11.6%)	3 (7.0%)	1 (2.3%)	16 (37.2%)	12 <sup>++</sup> (27.9%)	9 <sup>++</sup> (20.9%)	3 (7.0%)
Workers processing deciduous wood (N = 90)	61 <sup>++</sup> (67.8%)	47 <sup>++</sup> (52.2%)	14 (15.6%)	0	39 (43.3%)	10 (11.1%)	1 (1.1%)	0	52 <sup>+++</sup> (57.8%)	29 <sup>++</sup> (32.2%)	6 (6.7%)	0
Total wood processing workers (N = 133)	90 <sup>++</sup> (67.7%)	65 <sup>++</sup> (48.9%)	19 (14.3%)	0	55 (41.4%)	15 (11.3%)	4 (3.0%)	1 (0.8%)	68 <sup>++</sup> (51.1%)	41 <sup>++</sup> (30.8%)	15 <sup>+</sup> (11.3%)	3 (2.3%)
<b>Reference group (N = 32)</b>												
	12 (37.5%)	8 (25.0%)	3 (9.4%)	0	10 (31.2%)	2 (6.2%)	0	0	7 (21.9%)	1 (3.1%)	0	0

Grade 1 = reactions weakly positive, diameter  $\geq 5$  mm. Grade 2 = reactions positive, diameter  $\geq 10$  mm. Grade 3 = reactions strongly positive, diameter  $\geq 20$  mm. Grade 4 = reactions very strongly positive, diameter  $\geq 40$  mm. + - +++: significantly greater compared to reference group; + $p < 0.05$ , ++ $p < 0.01$ , +++ $p < 0.001$ .

highest percentages of positive reactions were noted with the antigens of Gram-negative bacteria, but only in the case of the extract of *Pantoea agglomerans* the frequency of positive response in both subgroups of sawmill workers was significantly greater compared to the reference group ( $p < 0.01$ ). Among antigens of actinomycetes, the frequency of positive reactions with the extract of *Streptomyces albus* was significantly greater in the subgroup of workers processing coniferous wood compared to the reference group ( $p < 0.001$ ). The workers processing deciduous wood reacted with the extract of *Streptomyces albus* also significantly more frequently than referents, ( $p < 0.05$ ) but only in the case of concentrated sera. Regarding the extract of *Thermoactinomyces vulgaris*, a significant difference ( $p < 0.05$ ) was noted in the case of concentrated sera between the subgroup of workers processing coniferous wood and the reference group. The antibody response rates to the extract of wood dust and to the antigens of Gram-positive bacteria and fungi were low and no significant differences between sawmill workers and the members of reference group were found.

**Relationship between the occurrence of work-related symptoms and allergic reactions.** Forty one out of 133 interviewed sawmill workers (30.8%) reported occurrence of work-related general, respiratory and skin symptoms. Thirty six workers reporting symptoms were employed in the sawmills processing deciduous wood (40.0% of the total workers employed in these sawmills), while only five were employed in the sawmill processing coniferous wood (11.6% of the total). The most common complaint was cough reported by 26 workers (19.5%), followed by headache reported by 23 (17.3%), dyspnoea by 17 (12.8%), hoarseness and eyes itching - each by 16 (12.0%) (Tab. 6). Among 41 workers having work-related symptoms, 13 persons reported only one symptom, 10 - four symptoms, eight - three symptoms, four - two

symptoms, three - five symptoms, two - six symptoms, and one - as many as eight symptoms. Five workers reported onset of symptoms within 30 minutes since beginning of work, 16 - from 30 minutes to 2 hours, 16 - between 2–6 hours, and four - between 6–12 hours.

The frequency of the positive skin and precipitin reactions in the subgroups of symptomatic and asymptomatic workers is presented in Figures 1 and 2, respectively. Only in a limited number of tests a significant relationship could be found between the occurrence of symptoms and response of sawmill workers to particular allergens. Workers reporting symptoms showed a significantly higher frequency of positive skin reactions to the extracts of *Brevibacterium linens* and oak sawdust, compared to asymptomatic ones ( $p < 0.05$ ) (Fig. 1). The number of workers reporting symptoms in a sawmill processing coniferous wood was too small (five out of 43) for performing similar analysis in relation to the extract of pine sawdust. The analysis of the aggregate results for the total sawmill workers examined did not show a significant relationship between occurrence of symptoms and positive response to the extract of wood dust (Fig. 1).

In the agar-gel precipitation test, a significant relationship between the occurrence of symptoms and positive antibody response was found only in the cases of *Saccharopolyspora rectivirgula* ( $p < 0.001$ , only with concentrated sera), *Bacillus subtilis* ( $p < 0.05$ ) and *Aspergillus candidus* ( $p < 0.05$ , only with concentrated sera) (Fig. 2).

## DISCUSSION

The results of this study clearly indicate that the skin response of sawmill workers processing pine to the extract of pine sawdust is much greater compared to the response of the sawmill workers processing oak to the

**Table 4.** Skin response of sawmill workers to the extract of *Penicillium citrinum*.

Group	Persons showing positive reaction (number, percent)											
	Early reactions (20 min)				Delayed reactions (8 h)				Late reactions (24 h)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
<b>Sawmill workers</b>												
Workers processing coniferous wood (N = 43)	19 (44.2%)	10 (23.3%)	5 (11.6%)	0	6 (14.0%)	5 (11.6%)	3 (7.0%)	0	1 (2.3%)	1 (2.3%)	1 (2.3%)	0
Workers processing deciduous wood (N = 90)	62 <sup>++</sup> (68.9%)	41 <sup>+</sup> (45.6%)	16 (17.8%)	0	14 (15.6%)	4 (4.4%)	0	0	2 (2.2%)	1 (1.1%)	1 (1.1%)	0
Total wood processing workers (N = 133)	81 <sup>+++</sup> (60.9%)	51 (38.3%)	21 (15.8%)	0	20 (15.0%)	9 (6.8%)	3 (2.3%)	0	3 (2.3%)	2 (1.5%)	2 (1.5%)	0
<b>Reference group (N = 32)</b>												
	9 (28.1%)	7 (21.9%)	2 (6.2%)	0	1 (3.1%)	1 (3.1%)	0	0	1 (3.1%)	1 (3.1%)	0	0

Grade 1 = reactions weakly positive, diameter  $\geq 5$  mm. Grade 2 = reactions positive, diameter  $\geq 10$  mm. Grade 3 = reactions strongly positive, diameter  $\geq 20$  mm. Grade 4 = reactions very strongly positive, diameter  $\geq 40$  mm. + - +++: significantly greater compared to reference group; + $p < 0.05$ , ++ $p < 0.01$ , +++ $p < 0.001$ . \* - \*\*\*: significantly greater compared to subgroup of workers processing coniferous wood; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

**Table 5.** Precipitin reactions of sawmill workers to antigens occurring in the air of work environment.

Antigen, group	Persons showing positive reaction (number, percent)							
	Workers processing coniferous wood (N = 43)		Workers processing deciduous wood (N = 90)		Total sawmill workers (N = 133)		Reference group (N = 32)	
	Sera not concentrated	Sera 3-fold concentrated	Sera not concentrated	Sera 3-fold concentrated	Sera not concentrated	Sera 3-fold concentrated	Sera not concentrated	Sera 3-fold concentrated
<b>Wood dust</b>								
Extract of wood dust (pine)	0	0	0	3 (3.3%)	0	3 (2.3%)	0	0
<b>Gram-negative bacteria</b>								
<i>Acinetobacter calcoaceticus</i>	9 (20.9%)	16 (37.2%)	15 (16.7%)	33 (36.7%)	24 (18.0%)	49 (36.8%)	5 (15.6%)	15 (46.9%)
<i>Pantoea agglomerans</i>	12 (27.9%)**	21 (48.8%)***	25 (27.8%)**	42 (46.7%)***	37 (27.8%)**	63 (47.4%)***	1 (3.1%)	1 (3.1%)
<i>Rahnella</i> sp.	13 (30.2%)	24 (55.8%)	16 (17.8%)	37 (41.1%)	29 (21.8%)	61 (45.9%)	7 (21.9%)	14 (43.7%)
<b>Gram-positive bacteria</b>								
<i>Arthrobacter globiformis</i>	0	0	2 (2.2%)	8 (8.9%)	2 (1.5%)	8 (6.0%)	0	2 (6.2%)
<i>Bacillus subtilis</i>	0	0	2 (2.2%)	2 (2.2%)	2 (1.5%)	2 (1.5%)	0	0
<i>Brevibacterium linens</i>	0	0	0	0	0	0	0	0
<i>Corynebacterium</i> sp.	6 (14.0%)	8 (18.6%)	3 (3.3%)	13 (14.4%)	9 (6.8%)	21 (15.8%)	1 (3.1%)	7 (21.9%)
<b>Actinomycetes</b>								
<i>Rhodococcus</i> sp.	0	0	0	2 (2.2%)	0	2 (1.5%)	0	1 (3.1%)
<i>Saccharopolyspora rectivirgula</i>	0	1 (2.3%)	1 (1.1%)	4 (4.4%)	1 (0.8%)	5 (3.8%)	0	2 (6.2%)
<i>Streptomyces albus</i>	13 (30.2%)***	25 (58.1%)***	6 (6.7%)	13 (14.4%)*	19 (14.3%)*	38 (28.8%)***	0	0
<i>Thermoactinomyces vulgaris</i>	4 (9.3%)	6 (14.0%)*	1 (1.1%)	5 (5.6%)	5 (3.8%)	11 (8.3%)	0	0
<b>Fungi</b>								
<i>Alternaria alternata</i>	0	0	0	0	0	0	0	0
<i>Aspergillus candidus</i>	1 (2.3%)	1 (2.3%)	0	1 (1.1%)	1 (0.8%)	2 (1.5%)	0	0
<i>Aspergillus clavatus</i>	0	0	0	1 (1.1%)	0	1 (0.8%)	0	0
<i>Aspergillus fumigatus</i>	0	1 (2.3%)	0	1 (1.1%)	0	2 (1.5%)	0	0
<i>Cephalosporium glutineum</i>	0	0	0	1 (1.1%)	0	1 (0.8%)	0	0
<i>Penicillium citrinum</i>	0	0	0	1 (1.1%)	0	1 (0.8%)	0	5 (15.6%)

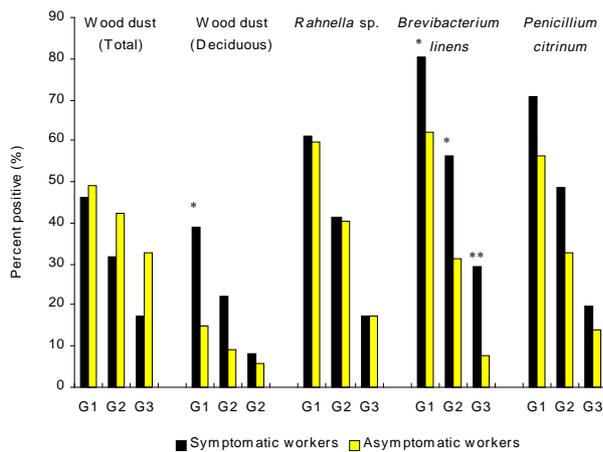
\* - \*\*\*: significantly greater compared to reference group; \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

extract of oak sawdust. It applies to all types of skin reactions: early (20 min), delayed (8 hrs) and late (24 hrs). This finding is in accordance with reports indicating high biological activity of pine dust, which may induce hyperreactivity, decline in lung function and respiratory symptoms in exposed woodworkers [29, 33, 42, 43, 55, 56]. These effects may be explained by sensitising properties of the constituents of pine wood, such as resin acids, rosin and terpenes [8, 14] and by great contamination of pine wood with microorganisms, compared to oak and other woods [13, 49]. The non-specific, irritative properties of pine dust may also contribute to the total effect but certainly not as a main reason, considering the great and highly significant difference between the skin response of sawmill workers and unexposed referents. The frequency of positive skin reactions to wood dust observed in woodworkers examined in this study was higher compared to results obtained by Fasani *et al.* [28] and Carosso *et al.* [5].

The skin response of sawmill workers to antigens derived from microorganisms isolated from the air of

sawmills was in accordance with the results of prior aerobiological examination [11, 21]. The frequency of positive reactions to the extract of Gram-negative bacterium *Rahnella* sp. was greatest among the workers of the pine processing sawmill, who were exposed to large quantities of these organisms which develop abundantly in the sapwood of pine logs [13] and are released in large quantities to the air of the breathing zone during first-cut sawing [11, 13, 21]. The genus *Rahnella* belongs to family *Enterobacteriaceae*, a group characterised by strong allergenic and immunotoxic properties. Thus, exposure to this species in sawmills processing coniferous wood poses a potential health risk to the workers.

Conversely, the frequency of positive reactions to the extract of *Penicillium citrinum* was greatest among the workers of oak processing sawmills who were exposed to the inhalation of large amounts of these moulds growing abundantly on stored parquet boards [11, 21]. *Penicillia* are known as strong aeroallergens [14, 19, 39, 40]. Various *Penicillium* species were reported as a cause of



**Figure 1.** Frequency of the positive skin reactions in the subgroups of symptomatic (N=41) and asymptomatic (N=92) sawmill workers. G1 = grade 1, G2 = grade 2, G3 = grade 3. The grade 4 reactions are not shown because their numbers in the subgroups were very low and the results were inconclusive. \* - \*\* significantly greater compared to asymptomatic workers; \*p<0.05, \*\*p<0.01.

allergic alveolitis in workers exposed to wood dust during logging [22] and other activities [2, 59].

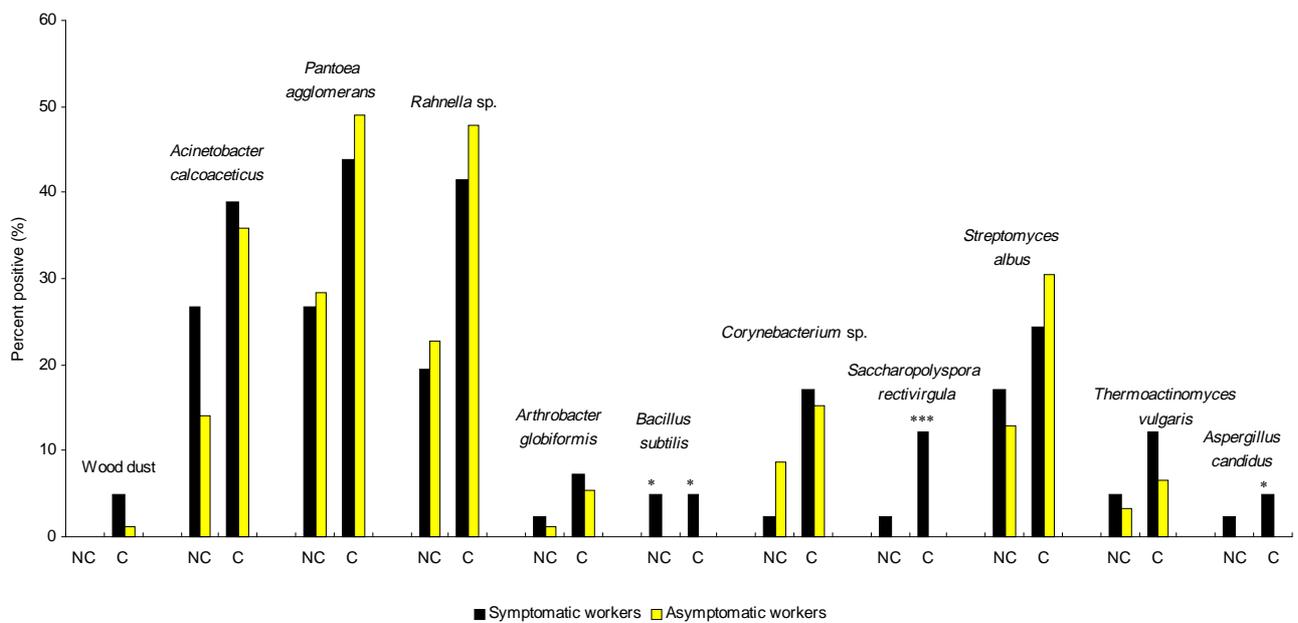
The frequency of positive reactions to the extract of *Brevibacterium linens*, a coryneform species developing in bark and wood, was similar among the workers processing coniferous and deciduous wood. This is consistent with the results of aerobiological examination in which the occurrence of the species was found to be similar in all types of sawmills. *Brevibacterium linens* was reported as a cause of allergic alveolitis in a Polish farmer [46], but generally little is known about the

**Table 6.** Prevalence of work-related symptoms in sawmill workers (N=133).

Work-related symptoms	Sawmill workers reporting symptoms (number, percent)
Cough	26 (19.5%)
Dyspnoea	17 (12.8%)
Chest tightness	4 (3.0%)
Hoarseness	16 (12.0%)
Fever	0
Shivering	1 (0.8%)
Headache	23 (17.3%)
General weakness	6 (4.5%)
Sweating	3 (2.3%)
Eye itching	16 (12.0%)
Body itching	6 (4.5%)
Skin rash	3 (2.3%)
<b>Total symptomatic workers</b>	<b>41 (30.8%)</b>

allergenic and immunotoxic properties of this species, this also applies to other corynebacteria associated with organic dusts.

The relationship between the degree of exposure to particular microorganisms and skin response was best expressed by early reactions. The delayed and late reactions to *Rahnella* sp. were non-specific, occurring with equally high frequency in sawmill workers and members of the reference group. It cannot be excluded that small quantities of endotoxin produced by *Rahnella* sp. [12] might contribute to non-specific irritation and



**Figure 2.** Frequency of the positive precipitin reactions in the subgroups of symptomatic (N=41) and asymptomatic (N=92) sawmill workers. NC = sera not concentrated, C = sera 3-fold concentrated. \* - \*\* significantly greater compared to asymptomatic workers; \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. The results obtained with the remaining seven antigens used in the test (*Brevibacterium linens*, *Rhodococcus* sp., *Alternaria alternata*, *Aspergillus clavatus*, *Aspergillus fumigatus*, *Cephalosporium glutineum*, *Penicillium citrinum*) are not shown because they were inconclusive. The numbers of positive reactions were either equal to zero or very low.

false-positive reactions. The frequency of the positive delayed reactions to *Brevibacterium linens* and *Penicillium citrinum* was much smaller but again no significant differences could be found between the reactivity of sawmill workers and referents. Thus, the delayed skin reactions seem to be useless as an exposure index. The late skin response to *Penicillium citrinum* was very low and inconclusive in all examined groups. By contrast, the frequency of late intradermal reactions to *Brevibacterium linens* was distinctly and significantly higher in sawmill workers than in unexposed referents which suggests that these reactions in particular cases could be useful as an exposure index.

The antibody response of sawmill workers to airborne antigens, as assessed by agar-gel precipitation test, was much lower compared to skin response and in most cases not related to the exposure. This result differed from compared to the results obtained by Scandinavian authors who found a high antibody response [3, 23, 60] and a significant correlation between exposure and antibody levels [23, 24] among wood trimmers exposed to large concentration of moulds, much greater than those found in the present work. In our study, the significant differences between the frequency of positive precipitin reactions in the sawmill workers and referents were found with the antigens of *Pantoea agglomerans* and *Streptomyces albus*. Both species were found in the air of examined sawmills. *Pantoea agglomerans* is a Gram-negative bacterium which is very common on plants [14, 19] and occurs also in stored timber logs [15, 16, 49]. This species possesses strong allergenic and endotoxic properties [10, 12, 14] and has been reported as a common cause of allergic alveolitis in Polish farmers [46]. *Streptomyces albus* is an actinomycete common in soil and dust which may also cause allergic alveolitis [14].

Precipitin reactions in most cases did not show a significant relationship with the occurrence of symptoms which is consistent with the reports of earlier authors who had examined woodworkers with the agar-gel precipitation test [5, 51, 60]. In this study, a significant relationship between the occurrence of symptoms and the presence of precipitins in circulatory blood was found in the case of only three antigens, derived from: a Gram-positive bacterium *Bacillus subtilis*, a thermophilic actinomycete *Saccharopolyspora rectivirgula* and a mould fungus *Aspergillus candidus*. Of these, *Bacillus subtilis* was reported as a cause of the case of allergic alveolitis associated with exposure to wood dust [36]. *Saccharopolyspora rectivirgula* is one of the best known causative agents of farmer's lung, a specific form of allergic alveolitis [39, 40]. The related species of thermophilic actinomycetes, *Thermoactinomyces vulgaris* and *Saccharomonospora viridis*, have been implicated in the etiology of allergic alveolitis in woodworkers [30, 56]. *Aspergillus candidus* is mostly known as a potential respiratory hazard for workers handling grain [38].

A potential bias of the present study is the disproportionately low number of workers reporting

work-related symptoms in the sawmill processing coniferous wood compared to those processing deciduous wood (11.6% versus 40.0%) in spite of greater exposure to airborne microorganisms [21] and much greater skin response to wood dust. One possible explanation is that the workers were unwilling to report symptoms because of fear of losing their jobs, as this sawmill was located within an area of high unemployment. The second potential bias is that the skin response to pine dust could be increased to some extent by non-specific immunologic or irritative mechanisms. The intradermal test has been used by us with good results in testing of agricultural workers with occupational allergens [10, 46], and Wimander & Belin [60] also recommend this test as a relevant tool for allergological testing of woodworkers. However, it cannot be excluded that the workers processing pine show not only an increased bronchial responsiveness, demonstrated by Malmberg *et al.* [42], but also a general hyperreactivity to work-related factors.

Nevertheless, the above-mentioned reservations do not change the essential relationships demonstrated by the present study.

## CONCLUSIONS

- The obtained results suggest that the allergic reactions to sawdust from coniferous wood (pine) are more common among sawmill workers than the allergic reactions to sawdust from deciduous wood (oak).
- The relationship between the degree of exposure of sawmill workers to particular microorganisms associated with wood dust and immunological response is more distinct in the case of the early skin reactions than in the case of precipitin-mediated reactions.
- A significant relationship between the occurrence of work-related symptoms and positive immunological response in sawmill workers could be found only with a limited number of allergens tested.

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