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Exposure to Dust and Endotoxin of Employees in Cucumber and Tomato Nurseries

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Exposure to bioaerosols in occupational settings is associated with a range of adverse health effects. The aim of this study was to investigate the exposure levels to dust and endotoxin of people working in two cucumber nurseries and two tomato nurseries. Exposure was measured for greenhouse workers ($n = 70$) mainly working on harvesting cucumbers and tomatoes and clearing the plants after the harvest season. The people were exposed to between 0.2 and 15 mg inhalable dust m^{-3} (median = 1.6 mg m^{-3}) and between 0.5 and 400 ng inhalable endotoxin m^{-3} (median = 32 ng m^{-3}). The exposure to 'total dust' and endotoxin measured by stationary samplers ($n = 30$) in the greenhouses was low. Endotoxin was present in relatively high concentrations on cucumber leaves compared with leaves on pot plants. The Danish occupational exposure limit (OEL) for total organic dust is 3 mg m^{-3} and 36% and 17% of the cucumber and tomato workers, respectively, were exposed to >3.0 mg inhalable dust m^{-3}. There is no OEL for endotoxin, but 'no effect levels' at ~15 ng m^{-3} have been found. The majority of subjects (65%) were exposed to >15 ng m^{-3}. Significantly higher exposure was found for employees in cucumber nurseries than for employees in tomato nurseries. Clearing tomato plants after the harvest season caused a higher exposure to endotoxin than tomato harvesting. In conclusion, people working in cucumber and tomato nurseries were often exposed to high levels of inhalable dust and endotoxin. Cucumber harvest workers were exposed to significantly more dust and endotoxin than tomato harvest workers. The dust and endotoxin aerosolized during the working processes were only transported to other areas in the greenhouses to a very low degree. Cucumber and tomato leaves were identified as endotoxin reservoirs.

Keywords: cucumber; endotoxin; exposure; greenhouse; harvest; inhalable dust; tomato

INTRODUCTION

Exposure to bioaerosols in occupational settings is associated with a range of adverse health effects. For example, allergic alveolitis is related to exposure to herb dust (Mackiewicz *et al.*, 1999) and to exposure to dust in agricultural environments (Malmberg *et al.*, 1988). Medical examinations of workers at a waste sorting plant and dust analyses showed that workers became ill, predominantly with asthma, and this was probably related to high particulate levels containing bacteria and endotoxin originating from decaying waste (Malmros, 1997). Endotoxin from gram-negative bacteria has been recognized as an important factor in the aetiology of occupational lung diseases, including asthma (Douwes *et al.*, 2003; Williams *et al.*, 2005) and organic dust toxic syndrome (Smit *et al.*, 2005; Smit *et al.*, 2006). Some studies have shown a positive association between endotoxin exposure and health effects (Kennedy *et al.*, 1987; Milton *et al.*, 1996; Michel *et al.*, 1997). In a study of garbage workers, it was concluded that endotoxin was the most potent inducer of inflammation in the nasal mucosa in comparison with other microbial components (Sigsgaard *et al.*, 2000). Inhaled endotoxin and organic dust particles have been shown to have synergistic pro-inflammatory effects in organic dust-induced asthma (Pirie *et al.*, 2003).

Exposure to dust and endotoxin has been measured in different occupational settings and the exposure levels seem to be dependent on many factors. Therefore, very different exposure levels have been found when comparing different farms, plants, crops, tasks

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and seasons (Nielsen *et al.*, 2000; Park *et al.*, 2000; Oppliger *et al.*, 2005; Skórska *et al.*, 2005; Madsen, 2006b). There is a large production of cucumbers and tomatoes in greenhouses in Denmark, and the growing and harvesting season is at least 9 months. The leaves of cucumber and tomato plants are covered with numerous hairs. The leaves of cucumber plants are larger than those of tomato plants. Bacteria, including gram-negative bacteria, are natural residents on leaf surfaces in general (Kinkel, 1997; Beattie and Lindow, 2008). Cucumber and tomato plant leaves may be potential endotoxin reservoirs. In addition, airborne dust may settle on the leaves during the growth season so that the leaf surfaces may also be dust reservoirs. The aim of this study was to investigate whether employees in cucumber and tomato nurseries were exposed to high dust and endotoxin concentrations during harvesting and clearing the plants after the harvest season. Furthermore, we wanted to know whether the bioaerosols were transported to, e.g., passages in the greenhouse. Finally, we wanted to study whether cucumber and tomato plant leaves are potential endotoxin reservoirs. The study was performed in four nurseries.

In order to measure personal exposure, we used Gesamtstaubprobenahme (GSP) samplers. This kind of sampler has been used in other studies of endotoxin exposure (reviewed in Madsen, 2006a). These samplers were chosen as they have a high sampling efficiency for both high and low wind speeds and for particles with aerodynamic diameters <50 μm (Kenny *et al.*, 1997; Kenny *et al.*, 1999a; Aizenberg *et al.*, 2000). However, these samplers also underestimate particles >50 μm (Kenny *et al.*, 1997; Kenny *et al.*, 1999a; Aizenberg *et al.*, 2000), but we expect most dust particles in greenhouses to have aerodynamic diameters <50 μm. This is expected since another study has shown that most airborne microorganisms in occupational settings are present as particles of thoracic size (Kenny *et al.*, 1999b) and because cucumber pollen has a diameter of $\sim$35 μm (Vizintin and Bohanec, 2004) and tomato pollen $\sim$25 μm (Lindstrom and Humphrey, 1933). The occupational exposure limit (OEL) for dust in Denmark is for 'total organic dust', defined as dust sampled by a sampler inlet velocity of 1.25 m s^{-1} (Arbejdstilsynet, 2007). Therefore, we have also measured exposure to dust sampled with a sampler with an inlet velocity of 1.25 m s^{-1}.

MATERIALS AND METHODS

The greenhouses

The measurements were performed in 2007 in two Danish cucumber (*Cucumis sativus*) nurseries and in two Danish tomato (*Solanum lycopersicum*) nurseries (Table 1). In nursery A, employees only worked

Table 1. Average sampling time (minutes) and numbers of samples (*n*) using GSP inhalable dust samplers for personal sampling and Millipore 'total dust' samplers for stationary sampling

Nursery	Crop	Personal samplings			Stationary samplings					
		Summer harvest	Autumn harvest	Clearing of plants	Summer harvest	Autumn harvest	Clearing of plants	Packing department	Potting machine	Outdoor reference
A	Cucumber	353 ($n = 9$)	352 ($n = 4$)	167 ($n = 8$)	250 ($n = 3$)	191 ($n = 3$)	255 ($n = 2$)	340 (n = 1)	($n = 0$)	229 ($n = 3$)
B	Cucumber	339 ($n = 7$)	($n = 0$)	276 ($n = 7$)	298 ($n = 2$)	($n = 0$)	293 ($n = 1$)	145 ($n = 1$)	293 ($n = 1$)	265 ($n = 2$)
C	Tomato	411 ($n = 6$)	($n = 0$)	275 ($n = 9$)	409 ($n = 4$)	($n = 0$)	181 ($n = 4$)	($n = 0$)	($n = 0$)	228 ($n = 2$)
D	Tomato	362 ($n = 9$)	($n = 0$)	277 ($n = 10$)	353 ($n = 4$)	($n = 0$)	209 ($n = 4$)	($n = 0$)	($n = 0$)	292 ($n = 2$)

with cucumbers or activities involving cucumber plants in greenhouses or in the packing department. The cucumber plants in nursery A were younger in the autumn than the plants in the summer, as new cucumber plants were planted in the late summer. During the harvest season in nursery B, employees mainly handled cucumbers in the greenhouses but they also packed cucumbers in a packing department. On the day plants were cleared, employees in nursery B were also potting poinsettia (*Euphorbia pulcherrina*). In nurseries C and D, employees only worked with tomatoes or activities involving tomato plants in greenhouses or in the packing department. On the day the tomato plants were cleared, one of the nine employees in nursery C was also sorting tomatoes. On the day the tomato plants in nursery D were cleared, all employees were only working on clearing the plants and removing equipment (e.g. boxes for bees and strings) in the greenhouse. The cucumber and tomato plants were grown in rock wool media, and most of the medium was covered by plastic. The windows at the top of the greenhouses were open during all periods of exposure assessment. In the two tomato nurseries, bees were used to pollinate tomato flowers in the growth and harvest season.

Personal sampling of inhalable aerosols

Personal dust monitoring was conducted using GSP inhalable samplers (Conical Inhalable Sampler by BGI, Inc., Waltham, MA, USA) as described in Madsen (2006b). The samplers were mounted with Teflon filters (pore size 1 μm) for endotoxin and gravimetric analysis. Results are presented as time-weighted averages (TWAs) and the average sampling periods are presented in Table 1.

Stationary sampling of 'total dust'

Stationary sampling was performed on the same days as the personal sampling and the samplers were set up in greenhouses or packing departments with work activities. Total dust has been defined as the dust collected by a sampler with an entry velocity of 1.25 m s^{-1} (Kenny and Ogden, 2000); we sampled total dust using 25 mm closed-face cassettes (Millipore holder; Millipore, Bedford, MA, USA, flow 1.9 l min^{-1} corresponding to an inlet velocity of 1.25 m s^{-1}) 1.5 m above ground level. The samplers were placed between cucumber plants in passages in the greenhouses, as well as in the packing departments and close to a potting machine. As the employees were moving around over large distances, during the course of the day, the stationary samplers were present at very different distances from the work activities. The samplers were mounted with Teflon filters (pore size 1 μm) for endotoxin and gravimetric analyses. Results are presented as TWA and the sampling periods are presented in Table 1.

Comparison of amount sampled by GSP and closed-face cassettes

We compared results with the closed-face Millipore cassette and the GSP inhalable sampler both as stationary samplers and as personal samplers. For the personal comparison, two people were each equipped on the same day with both samplers. For the stationary comparison, the two times two samplers were mounted side by side in two areas in the greenhouse close to the plants. This was done on the same day as the personal comparison. Of course, as they worked the people wearing the personal samples could be different distances from the stationary samplers. Both types of samplers have been compared with the inhalable dust definition at 0.5 m s^{-1} in a wind tunnel (Kenny *et al.*, 1997), and they both agree reasonably well with the curve up to ~30 μm, but for larger sizes the efficiency of both falls, and at those larger sizes the Millipore sampler has lower efficiency than the GSP. Kenny *et al.* (1999a) compared the two as stationary samplers in calm air in a laboratory and found that the GSP collected more than the Millipore at all sizes.

Outdoor reference measurements

Outdoor references were sampled upwind of the greenhouses on each sampling day, also using Millipore close-face cassettes with Teflon filters. The outdoor references were included in the study to ensure that indoor exposures were not caused by an outdoor source.

Extraction of dust for endotoxin analysis

The dust on the Teflon filters was extracted in 6.0 ml pyrogen-free water with 0.05% Tween 20 by orbital shaking (300 r.p.m.) at room temperature for 60 min and centrifuging (1000 g) for 15 min. The supernatant was stored at −80°C until it was used for the endotoxin assay.

Gravimetric analysis

The mass of the dust collected on the filters was determined by weighing the filters before and after dust sampling. Before weighing, the filters used for collecting the dust were equilibrated at constant air temperature and humidity for 20–24 h. For the GSP samplers, the limit of detection when weighing the filters was 0.031 mg m^{-3} dust and for the filters from the Millipore samplers it was 0.034 mg dust m^{-3}. The detection limit was calculated as three times the standard deviation of 10 blanks and divided by the mean sampled volume.

Determination of endotoxin by the Limulus method

The supernatant was analysed (in duplicate) for endotoxin using the kinetic Limulus Amoebocyte Lysate test (Kinetic-QCL endotoxin kit; BioWhittaker,

Walkersville, MD, USA) with β-glucan blocker. A standard curve obtained from an *Escherichia coli* O55:B5 reference endotoxin was used to determine the concentrations in terms of endotoxin units (EUs) (10.0 EU ≈ 1.0 ng). The limit of detection was 0.005 ng endotoxin ml^{-1}, corresponding to 0.006 ng endotoxin m^{-3} for the air sampling. The data are presented as ng m^{-3}, ng mg^{-1} dust or ng cm^{-2} leaf surface. In the discussion, we present endotoxin measurements from other papers. If these measurements are presented in EU and provided the conversion factor is mentioned, we convert them to nanogram endotoxin. If the conversion factor is not mentioned, we use the conversion factor 10 (10 EU ≈ 1 ng) as a standard.

Endotoxin on leaf surfaces

Concentrations of endotoxin on the surface of cucumber and tomato leaves were measured. We could not find any information on endotoxin concentrations on other leaf surfaces in the published literature. Therefore, for comparison, we measured endotoxin concentrations on the leaf surface of other plant materials. For this, pot plants (*Sansevieria trifasciata*, *Ficus bejamin*, *Kalanchoë blossfeldiana*, *Euphorbia pulcherrina*, *Grassula aborescens*) and barley straw (12% water content, w/w) were included in the study. The pot plants were between 1 and 2 years old and the samples were taken in the late autumn. The areas of plant material were measured using templates of different sizes. Leaf or straw samples ranging between 10 and 20 cm^2 in size (corresponding to between 0.4 and 5.2 g fresh weight) were extracted in 10.0 ml pyrogen-free water with 0.05% Tween 20 by orbital shaking (300 r.p.m.) at room temperature for 60 min. The plant materials were removed from the suspensions and these were centrifuged (1000 g) for 15 min. The supernatant was stored at −80°C and later used for the endotoxin assay.

Treatment of data

The dust and endotoxin exposures were log transformed and the Pearson correlation coefficients between dust and endotoxin exposure were calculated in SAS 9.1. Variance analysis was used to describe the factors (task at the nursery, nursery, plant material) with the assumed effect on the variables using PROC GLM in SAS where $a = 0.05$. The factor 'task at the nursery' covers the nine groups presented in Tables 2 and 3. The person responsible for fastening new plant shoots is not included in the table as he was the only person involved in this task for the whole working day. For statistical analysis of stationary measurements (Tables 2 and 4), data were pooled from the two tomato nurseries from the two cucumber nurseries and from the nine outdoor reference measurements. In the statistical analysis, the four

Table 2. Exposure to dust (median (average) and [range]) in cucumber and tomato nurseries expressed as mg m^{-3}

Nursery	Personal samplings, inhalable dust			Stationary samplings, 'total dust'					
	Summer harvest	Autumn harvest	Clearing of plants	Summer harvest	Autumn harvest	Clearing of plants	Packing department	Outdoor reference
A	2.9^{a} (4.2) [1.6–12]	$1.8^{a,b}$ (1.7)* [0.82–2.2]	7.4^{a} (6.4) [0.31–15]	bd [bd–0.036]	bd [bd–0.039]	0.12 (0.12) [0.087–0.15]	0.047 (0.047)	bd [bd–0.036]
B	1.8^{a} (1.8)* [0.86–2.7]		1.7^{a} (2.1)^{x} [0.56–4.2]	0.066 (0.066) [bd–0.12]		0.054 (0.054)	0.27 (0.27)	bd [bd–0.038]
C	0.43^{c} (0.50) [0.3–0.9]		1.3^{b} (1.2) [0.4–1.8]	bd [bd–0.030]		0.29 (0.29) [0.10–0.50]		0.042 (0.050) [0.042–0.067]
D	0.49^{c} (0.52) [0.2–0.9]		3.3^{a} (4.8) [1.8–8.7]	0.057 (0.056) [bd–0.083]		0.045 (0.076) [0.04–0.17]		bd [bd–0.034]

bd = below detection level. Median exposure values followed by the same letter are not statistically different at the 95% level.
*Part of the day workers also packed cucumbers.
xMost of the day workers also potted poinsettia.

Table 3. Exposure to inhalable endotoxin (median (average) and [range]) in cucumber and tomato nurseries expressed as ng m^{-3} and concentration (ng mg^{-1} dust) of endotoxin in the sampled dust

Nursery	Personal samplings					
	Summer harvest		Autumn harvest		Clearing of plants	
	ng m^{-3}	ng mg^{-1}	ng m^{-3}	ng mg^{-1}	ng m^{-3}	ng mg^{-1}
A	85^{a} (99) [46–171]	33^{a} (33) [4–61]	$75^{a,b}$ (83)* [23–160]	42^{a} (44) [21–72]	118^{a} (149) [15–339]	34^{a} (37) [17–71]
B	27^{b} (24)* [9–43]	14^{b} (14) [7–25]			$43^{a,b}$ (79)^{x} [10–346]	$18^{a,b}$ (29) [16–83]
C	1.3^{c} (1.4) [0.54–2.1]	2.4^{c} (2.8) [1.9–4.9]			13^{b} (20) [7–51]	17^{b} (18) [8–34]
D	2.2^{c} (2.5) [0.7–5.4]	4.9^{c} (5.0) [0.4–8.9]			111^{a} (138) [46–402]	$36^{a,b}$ (37) [5–73]

Median exposure values followed by the same letter are not statistically different. Median endotoxin concentration values followed by the same letter are not statistically different at the 95% level.
*Part of the day workers also packed cucumbers.
xMost of the day workers also potted poinsettia.

cucumber leaf samples were considered as four repeats ($n = 4$). Thus, the factor 'plant material' covers the eight plant materials presented in Table 5. PROC CORR was used to determine the correlation between endotoxin exposure and dust exposure.

RESULTS

Exposure to dust

The median personal exposure to inhalable dust was 1.6 mg m^{-3} and the average 2.9 mg m^{-3}. Significant differences in dust exposure were found between nurseries ($P < 0.0018$), with higher exposure at nurseries A, B and D than at C. The highest individual measurement of exposures was found for one person clearing plants after the harvest season (15 mg m^{-3}) and for a person harvesting cucumbers (12 mg m^{-3}) (Table 2). One person worked on fastening newly developed plant shoots and he was exposed to 1.1 mg dust m^{-3} (not included in Table 2). As seen from Table 2, significantly differing dust exposures were found for the different tasks performed at the four nurseries ($P < 0.0001$).

Five of the stationary measurements were below the detection limit (Table 2), e.g. dust exposure measured close to a potting machine. The median outdoor reference measurement of total dust was below the detection limit. The median exposure measured in the greenhouses with stationary samplers was 0.038 mg total dust m^{-3}. There was no significant difference between the dust exposure measured with stationary samplers inside and outside the greenhouses ($P = 0.136$). Furthermore, no significant difference between the dust exposures measured with stationary cassettes in cucumber nurseries and those measured in tomato nurseries was seen ($P = 0.139$).

Exposure to endotoxin

Personal exposure to inhalable endotoxin was between 0.5 and 400 ng m^{-3} (Table 3) (median = 32 ng m^{-3}, average = 67 ng m^{-3}), and 65% of the subjects included in the study were exposed to >15 ng m^{-3}. One person worked on fastening new cucumber plant shoots and he was exposed to 31 ng endotoxin m^{-3}. Significantly differing endotoxin exposures were found for the tasks performed at the nurseries ($P < 0.0001$); e.g. higher exposures were found for people harvesting in the summer at nursery A than for people involved in harvesting and packing cucumbers at nursery B (Table 3). The highest individual exposures were found for the people who cleared plants after the harvest season, but these exposures were only significantly higher than exposures during harvesting in tomato nurseries. Significantly differing endotoxin exposure between nurseries ($P < 0.0001$) was found, with the highest exposure at cucumber nursery A and with higher exposures at nursery B than at nurseries C and D.

There was no significant difference between concentrations of endotoxin in sampled dust from subjects harvesting in cucumber nurseries compared with those clearing plants. In contrast, the endotoxin concentration in dust sampled during harvest of tomatoes was significantly different from the concentration during clearing of plants (Table 3).

The median endotoxin exposure in the greenhouses measured by stationary samplers (2.1 ng m^{-3}) was significantly higher than the outdoor reference measurements (0.28 ng m^{-3}) ($P < 0.0001$). The highest single exposure measured by stationary samplers was found during the clearing of cucumber plants after the harvest season (Table 4). Measurements by stationary samplers of exposure to endotoxin at the cucumber nurseries were slightly, though significantly, higher than at the tomato nurseries ($P = 0.0249$). Close to the potting machine, the endotoxin exposure may have been underestimated due to loss of the dust deposited on the walls of the sampler. Significantly higher concentrations (ng mg^{-1}) of endotoxin were found in dust from the greenhouses than in dust from the outdoor reference measurements ($P = 0.0126$). In addition,

Table 4. Exposure to endotoxin (median (average) and [range]) measured by stationary Millipore samplers in cucumber nurseries expressed as ng m^{-3} and concentrations in dust as ng mg^{-1} sampled dust

Nursery	Stationary samplings											
	Summer harvest		Autumn harvest		Clearing of plants		Packing department		Potting machine		Outdoor reference	
	ng m^{-3}	ng mg^{-1}	ng m^{-3}	ng mg^{-1}	ng m^{-3}	ng mg^{-1}	ng m^{-3}	ng mg^{-1}	ng m^{-3}	ng mg^{-1}	ng m^{-3}	ng mg^{-1}
A	1.9 (3.6) [1.6–7.2]	80 (108) [49–196]	2.1 (2.2) [1.7–2.7]	69 (75) [69–88]	19 (19) [18–20]	175 (175) [117–233]	8.3 (8.3)	175 (175)			0.28 (0.28) [0.04–0.53]	12 (9.4) [1.3–15]
B	0.86 (0.86) [0.64–1.0]	18 (18) [28–48]			1.7 (1.7)	31 (31)	7.6 (7.6)	28 (28)	11 (11)	nm[a]	0.44 (0.44) [0.37–0.51]	14 (14) [13–15]
C	1.9 (1.9) [1.5–2.4]	67 (12) [55–78]			2.5 (3.8) [1.5–7.7]	19 (16) [0.3–22]					0.25 (0.25) [0.1–0.4]	4.2 (4.2) [2.6–5.8]
D	0.8 (0.8) [0.3–1.3]	12 (12) [7.5–15]			2.1 (3.1) [1.0–2.1]	45 (40) [24–47]					0.07 (0.07) [0.05–0.09]	3.1 (3.1) [2.7–3.6]

[a]The amount of dust was below detection level because the dust behaved differently and some of it was found on the walls of the sampler. Thus, the endotoxin exposure may also be higher.

Table 5. Endotoxin per unit of surface area of cucumber and tomato plants and other plant materials

Plant material	*n*	Endotoxin ng cm^{-2} surface	
		Median	Range
Cucumber			
Old leaves	2	956[a]	678–1233
Young leaves	2	2295[a]	1905–2685
Tomato			
Young leaves	5	16[a,b,c]	3.4–7440
Straw			
Barley	5	262[b]	127–645
Pot plant leaves			
Euphorbia pulcherrina	2	3.5[c]	2.8–4.2
Kalanchoë blossfeldiana	2	4.3[c]	3.0–5.6
Grassula aborescensi	2	32[c,b]	24–40
Ficus bejamin	2	4.1[c]	3.2–5.0
Sansevieria trifasciata	2	228[a,b]	195–260

Median endotoxin concentrations per unit of area followed by the same letter are not statistically different. The four cucumber leaf samples are considered as four repeats ($n = 4$) in the statistical analysis.

significantly higher endotoxin concentrations were found in dust from cucumber nurseries than in dust from tomato nurseries ($P = 0.0009$) (Table 4).

The Pearson correlation (r) between endotoxin exposure and dust exposure in personal samples was 0.81 ($P < 0.0001$, $n = 70$) and for stationary samples 0.44 ($P = 0.0043$, $n = 39$).

Inhalable versus total dust

Dust concentrations of 0.035 versus 0.042 mg m^{-3} and 0.073 versus 0.098 mg m^{-3} were measured using stationary Millipore versus GSP samplers. Using personal Millipore versus GSP samplers, dust concentrations of 0.16 versus 0.19 mg m^{-3} and 6.3 versus 8.9 mg m^{-3} were measured. The mean ratio of the GSP dust to the Millipore dust results was 1.3 for the stationary samplers and 1.6 for the personal samplers. The stationary ratio is consistent with (Kenny *et al.*, 1999a) laboratory measurements. There has not been a laboratory comparison of the two as personal samplers. For endotoxin, the stationary ratio was 0.8 and the personal ratio was 1.6. The ratios for endotoxin per milligram dust were 0.6 for stationary measurement and 1.0 for personal measurement.

Endotoxin on leaf surfaces

Endotoxin concentrations were measured on the leaf surface of cucumber and tomato plants and for comparison also on other plant materials (Table 5). The average endotoxin concentration on cucumber leaves was 6.2 ng mg^{-1} leaf (median = 5.9 ng mg^{-1}) and 1625 ng cm^{-2} (median = 1569 ng cm^{-2}). The average endotoxin concentration on tomato plant leaves was 6.0 ng mg^{-1} leaf (median = 0.06 ng mg^{-1}) and

1602 ng cm^{-2} (median = 16 ng cm^{-2}). The endotoxin concentrations on the pot plant leaves ranged between 0.0017 and 0.082 ng mg^{-1} leaf (average = 0.029 ng mg^{-1}, median = 0.0123 ng mg^{-1}) and between 2.8 and 260 ng cm^{-2} leaf (average = 54 ng cm^{-2}, median = 5.3 ng cm^{-2}) (Table 5).

DISCUSSION

Exposure of cucumber and tomato greenhouse workers to inhalable dust was in general high (median = 1.6 mg m^{-3}). The Danish OEL is 3 mg total dust m^{-3} (Arbejdstilsynet, 2007), and 30% of the people included in the study were exposed to >3 mg inhalable dust m^{-3}. A personal exposure to 3 mg total dust m^{-3} corresponds to an exposure of ~4.7 mg inhalable dust m^{-3} and 20% were exposed to more than this level. Only few studies have investigated the exposure to dust and endotoxin of greenhouse workers. In a study of greenhouse workers (n = 39) working with flowers and ornamental plants in Spain, the workers were exposed to 0.08–0.21 mg total dust m^{-3} (median = 0.09 mg m^{-3}) (Monsó, 2004). In a Dutch study of people working in a cucumber and paprika nursery, the exposure level to inhalable dust was between <0.1 and 2.4 mg m^{-3} (Geometric Mean [GM] = 0.3 mg m^{-3}, n = 14) (Spaan *et al.*, 2006). Consequently, the workers in our study were exposed to higher levels of dust. In other environments, where plant material is handled, both higher and lower exposure levels have been found. For example, citrus fruit (median = 41.8 mg m^{-3}, n = 11) and grape (median = 3.2 mg m^{-3}, n = 10) harvest workers (Lee *et al.*, 2004) and hemp-processing workers (geometric mean = 22.9 mg m^{-3}, n = 7) (Fishwick *et al.*, 2001) are exposed to high levels of inhalable dust. In contrast, mushroom workers are exposed to lower levels of inhalable dust (median = 0.68 mg dust m^{-3}, n = 30) (Simpson *et al.*, 1999). In the study of citrus fruit and grape harvest workers, the citrus fruit workers were exposed to more dust than the grape harvest workers. In this study, cucumber harvest workers were exposed to significantly more dust than the tomato harvest workers. This may be related to the large cucumber leaves, on which the dust can accumulate.

We measured very low levels of exposure to dust when using the stationary samplers compared to the personal samplers. For example, the median exposure to dust during the summer harvest of cucumbers was 2.4 mg m^{-3} for personal measurements using GSP samplers and 0.030 mg m^{-3} for stationary measurements using Millipore samplers. Parallel stationary sampling using GSP inhalable dust cassettes measured a 1.3 times higher dust exposure than the Millipore closed face total dust samplers. Millipore closed face total dust cassettes have earlier been shown to measure lower exposures than inhalable dust samplers (Schlünssen *et al.*, 2001). The concentrations measured in the stationary samples were about 3–8% of the personal concentrations by both samplers. This shows that the dust released during the handling of plant material is not transported to other areas of the greenhouse. Consequently, people walking through a cucumber greenhouse or tomato greenhouse are only likely to be exposed to low amounts of dust.

The exposure to inhalable endotoxin of people working in cucumber and tomato nurseries was in general high (median = 32 ng m^{-3}) and much higher than outdoor reference measurements. We have only found two studies reporting data of endotoxin exposure of greenhouse workers. In one study, greenhouse workers (n = 39) working with flowers and ornamental plants were exposed to 0.17–0.89 ng endotoxin m^{-3} (median = 0.32 ng m^{-3}) (Monsó, 2004). In a Dutch study with 14 measurements of people working in a cucumber and paprika nursery, the exposure level to endotoxin was between 36 and 650 EU m^{-3} (GM = 160 EU m^{-3} = 14 ng m^{-3}) (Spaan *et al.*, 2006). The Danish cucumber plant workers were exposed to higher levels of endotoxin (median = 50 ng m^{-3}). We do not know the age of the cucumber plants in the Dutch study. Due to the high endotoxin concentration found on the leaves, we expect the exposure to endotoxin to increase with increasing leaf area index (LAI, i.e. the ratio of total upper leaf surface of vegetation to the surface area of the land on which the vegetation grows). The high endotoxin exposure in comparison with greenhouse workers handling flowers or ornamental plants may partly be explained by a high LAI of cucumber and tomato plants and the fact that people work between the tall plants. The large leaf surfaces of cucumber plants may be a cause of the significantly higher exposure to endotoxin during cucumber harvest than during tomato harvest. In other environments where plant material is handled, very different exposure levels to endotoxin have been found. In the following environments, the median exposures were lower than for the cucumber workers in this study: mushroom workers (7 ng endotoxin m^{-3}, n = 30) (Simpson *et al.*, 1999), citrus fruit (122 EU $m^{-3} \approx 12$ ng m^{-3}, n = 11) and grape (6.2 EU $m^{-3} \approx$ 0.6 ng m^{-3}, n = 10) harvest workers (Lee *et al.*, 2004) and straw and woodchips workers (55 EU m^{-3} = 4.6 ng m^{-3}, n = 32) (Madsen, 2006b). In Norwegian grain farming, higher exposure to endotoxin has been found (GM = 5900 EU $m^{-3} \approx 590$ ng m^{-3}, n = 104) (Halstensen *et al.*, 2007).

There is no internationally accepted threshold limit value (TLV) or OEL for endotoxin. Suggested TLVs or calculated 'no effect values' for inhalable or 'total endotoxin' exposure are between 3 and 80 ng m^{-3} (Haglind and Rylander, 1984; Rylander

et al., 1985; Castellan *et al.*, 1987; Kennedy *et al.*, 1987; Smid *et al.*, 1992a; Smid, 1993; Michel, 1997; Donham and Cumro, 1999; Donham *et al.*, 2000). In this study, the endotoxin exposure is related to a calculated 'no effect level' for Dutch animal feed workers of 15 ng m^{-3} (Smid *et al.*, 1992a; Smid, 1993). Most (65%) of the subjects in this study were exposed to >15 ng endotoxin m^{-3} and 30% were exposed to >80 ng m^{-3}. Because of these high exposure levels, it would be of great relevance to study potential health effects on the respiratory system of the workers and whether it is possible to reduce exposure through practical measures.

Since there is no internationally accepted TLV or OEL for endotoxin, but there is one for dust exposure, and since calculated no effect values of endotoxin are known, the concentration of endotoxin in dust from different environments is of interest in the evaluation of whether the OEL for dust exposure can also be used for protection against too high endotoxin exposure. If a person is exposed to 2.9 mg total dust m^{-3} (just below the Danish OEL for organic dust) from cucumber nurseries, with a concentration of 82 ng endotoxin mg^{-1} dust (the median concentration in dust from the stationary samplers in the greenhouses), the person would be exposed to 240 ng endotoxin m^{-3}. This exposure level is higher than the calculated no effect values for endotoxin. Consequently, in this environment, the OEL for organic dust would not protect the greenhouse workers from too high endotoxin exposure. Similarly, the endotoxin concentration in mushroom dust (Simpson *et al.*, 1999) and straw and woodchips dust (Madsen *et al.*, 2004) is too high to allow an exposure of 2.9 mg dust m^{-3}.

The endotoxin concentration in the dust from the greenhouses seemed in general to be higher in the dust from the stationary cassettes than from the personal cassettes. This may both be caused by different particle size distribution in the areas and around the working people, the concentrations of endotoxin on particles of different sizes and also by the sampling efficiencies of the two kinds of samplers. The parallel measurement of inhalable and total dust showed higher concentration of endotoxin in total dust than in inhalable dust in stationary measurements but not in personal measurements. In some other environments, endotoxin was present in higher concentrations in particles larger than inhalable size (Smid *et al.*, 1992b; Madsen and Sharma, 2008), but whether this is also the case in tomato and cucumber nurseries cannot be concluded from this study. In Holland, Spaan *et al.* (2006) found that endotoxin concentration in personal dust samples from a cucumber and paprika nursery (275 EU mg^{-1} = 24 ng mg^{-1} of inhalable dust, $n = 14$) was at similar levels to those found in cucumber nurseries in this study (median = 25 ng mg^{-1} dust).

The median outdoor reference level of endotoxin (0.28 ng m^{-3}) was within a published interval of reference exposure in rural areas. Outdoor reference measurements in urban areas are around 0.3–1.4 EU $m^{-3} \approx$ 0.03–0.014 ng m^{-3} (median or GM values) and in rural areas around 1.3–3.6 EU $m^{-3} \approx$ 0.13–0.36 ng m^{-3} (Madsen, 2006a).

The high amount of endotoxin per surface area unit of cucumber leaf in comparison with per surface area unit of pot plant leaf indicates that cucumber leaves are endotoxin reservoirs. The endotoxin concentration on the tomato leaves varied between leaf samples, but in some samples it was high. The median endotoxin concentration on the tomato plant leaves was lower, though not significantly, than on the cucumber plant leaves. The endotoxin concentration (ng mg^{-1} dust) was also higher in dust from stationary cassettes in cucumber greenhouses than in tomato greenhouses. We believe that the endotoxin on the leaves originates from epiphytic bacteria or from airborne dust settling on the leaves. A Dutch study showed very different endotoxin concentrations in different seed extracts (0.04–1000 EU $mg^{-1} \approx$ 0.004–100 ng mg^{-1}) (Smit *et al.*, 2006). The average concentration of endotoxin in extracts from cucumber and tomato leaves was in the middle of this interval of endotoxin concentrations in seed extracts. Older leaves are generally more 'leaky', resulting in greater nutrient concentrations on their surface and this may contribute to enhanced microbial growth. Furthermore, a general pattern of increasing population densities has been recorded for many different bacteria and fungi on leaves during the growing season (Kinkel, 1997). However, in this study, with only few cucumber leaves, we did not see a clear difference in endotoxin concentrations on old and young leaves. This may be because of the size of the study and because epiphytic bacteria on leaf surfaces according to Hirano *et al.* (1982) are unevenly distributed. In the future, it would be interesting to study whether endotoxin exposure increases during the growing season and whether endotoxin accumulates on the cucumber leaves during the growing season.

In conclusion, people harvesting cucumbers and clearing cucumber plants after the harvest season were in general exposed to high levels of inhalable dust and endotoxin. Cucumber harvest workers were exposed to significantly more dust than tomato harvest workers. Significantly higher endotoxin exposure was found for cucumber workers than for tomato workers. Clearing tomato plants caused a significantly higher exposure to endotoxin than tomato harvesting. The dust and endotoxin aerosolized during the work processes was only to a very low degree transported to other areas in the greenhouses. Cucumber leaves and some tomato leaves were identified as endotoxin reservoirs. Because of these high

exposure levels, it would be highly relevant to study the potential health effects of this exposure and to study whether it is possible to reduce dust and endotoxin exposure through practical measures.

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