

# Exposure to airborne microorganisms, hyphal fragments, and pollen in a field of organically grown strawberries

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**Abstract** Many working environments are predisposed for larger than average amounts of fungi and other microorganisms often due to organic material being handled. From 2003 to 2007, the area used for strawberry production in Denmark increased by 62%. The purpose of this study was to determine the levels of exposure to microorganisms, endotoxin,  $(1 \rightarrow 3)$ - $\beta$ -D-glucan ( $\beta$ -glucan), and pollen in a field of strawberries. The study was carried out in eastern Denmark from the middle of June to the beginning of August 2008. The strawberries were grown organically, and microbiological pest control agents (MPCAs) were applied during this and former growth seasons. In order to measure exposure to inhalable bioaerosol components, we used stationary filter samplers. Bioaerosol sampling was performed during 4 working days, and a total of 57 samplings were performed. The filters were analysed for contents of fungi, MPCAs, endotoxin,  $\beta$ -glucan, and pollen. The mean exposure was 6,154 CFU *Cladosporium* sp.  $\text{m}^{-3}$ ,  $1.0 \times 10^5$  fungal spores  $\text{m}^{-3}$ ,  $4.1 \times 10^4$  hyphal fragments  $\text{m}^{-3}$ ,  $5.8 \times 10^3$  pollen  $\text{m}^{-3}$ , 57.3 ng  $\beta$ -glucan  $\text{m}^{-3}$ , and 8.9 endotoxin units (EU)  $\text{m}^{-3}$ . A significant and positive correlation was found between  $\beta$ -glucan and fungal spores and between

CFU of *Cladosporium* sp. and CFU of fungi. We selected specifically for *Metarhizium anisopliae*, *Beauveria bassiana*, and the applied MPCAs *Trichoderma harzianum*, *T. polysporum*, and *Bacillus thuringiensis* but found none of these species. In conclusion, our study shows that berry pickers in this organic strawberry field were potentially subjected to higher levels of fungal spores, *Cladosporium* sp., hyphal fragments, pollen, and thus also  $\beta$ -glucan than is usually seen in outdoor air. Exposure to MPCAs was not seen. The exposure to endotoxin was only slightly higher than e.g. in a town.

**Keywords** Allergens · Biological control · Fungi · Bioaerosols · Horticulture · *Cladosporium* · Working environment

## 1 Introduction

Fungal fragments as well as great quantities of fungal spores may become aerosolized and, given the ubiquity of fungi, inevitably a part of the air we breathe. Exposure to fungi is associated with various allergic diseases such as asthma, rhinitis, allergic bronchopulmonary mycoses, and hypersensitivity pneumonitis, which is highly problematic as up to 6% of the general population is susceptible to fungal allergy (Kurup et al. 2000).

As a component of fungal cell walls,  $\beta$ -glucan has been used as an indicator of fungal exposure, and

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studies have shown that it can elicit respiratory inflammation (Bonlokke et al. 2006; Iossifova et al. 2007; Rylander 1999; Sigsgaard et al. 2000; Thorn et al. 2001). Endotoxins are components of the outer membrane of Gram-negative bacteria (Spaan et al. 2008), which have been found to cause toxic reactions such as organic dust toxic syndrome and are also associated with acute and chronic impairment of lung function (Bünger et al. 2007). Endotoxin exposure has also been linked to a variety of symptoms in sewage workers (Spaan et al. 2008). There is no internationally accepted occupational exposure limit (OEL), though values ranging from 30 to 800 EU m<sup>-3</sup> have been suggested (reviewed in Madsen 2006a).

Many working environments are predisposed for larger than average amounts of fungi and other microorganisms often due to organic material being handled. In order to gain an understanding of the magnitude of these problems, research has been done measuring the levels of fungi as well as other microorganisms and -particles in workplaces such as farms (Radon et al. 2002) and sawmills (Rusca et al. 2008). Most occupational environment research, however, has focused on sampling indoor workplaces with a few outdoor samples for reference. Studies that have looked at outdoor measurements have typically focused on areas such as towns or cities to get a broad sense of the microorganisms in the atmosphere and their seasonal variations (e.g. Mitakakis and Guest 2001; Oliveira et al. 2005).

From 2003 to 2007, the area used for strawberry production in Denmark increased by 62%. Thus, strawberry production is a rapidly expanding industry and employs many people especially during the summer (Kontoret for landbrugs- og transportstatistik 2008). No previous studies have been made of the exposure workers are subjected to in strawberry fields. The purpose of this study was to determine the levels of exposure to microorganisms, endotoxin, (1-3)- $\beta$ -D-glucan (abbreviated  $\beta$ -glucan), and pollen in a field of organically grown strawberries and to study the variation in exposure over an 8-week period. In order to measure exposure, we used stationary GSP (Gesamtstaubprobenahme) samplers. This kind of sampler has been used in other studies of exposure to fungi and endotoxin (Madsen 2006b; Spaan et al. 2008). These samplers were chosen as they have a high sampling efficiency for both high

and low wind speeds and for particles with aerodynamic diameters smaller than 50  $\mu$ m (Aizenberg et al. 2000; Kenny et al. 1997, 1999). However, these samplers also underestimate particles larger than 50  $\mu$ m (Aizenberg et al. 2000; Kenny et al. 1997, 1999), but we expect pollen to be the largest particles sampled and also that most of the common pollen have aerodynamic diameters smaller than 50  $\mu$ m. The aerodynamic spore sizes of common fungi are also within the range sampled reliably by the GSP (Madelin and Johnson 1992; McCartney et al. 2007).

## 2 Materials and methods

### 2.1 Description of the strawberry field

The study was carried out in a strawberry field in eastern Denmark from the middle of June 2008 to the beginning of August 2008. The strawberries were grown organically, and thus no chemical pesticides have been applied. By contrast, different microbiological pest control agents (MPCAs) have at test basis been applied in this and earlier growth seasons. The entire field measured approximately 7.7 ha and was divided into three smaller fields by hedgerows of primarily aspen (*Populus* sp.) and rowan (*Sorbus* sp.) forming a T-shape. Due to an uncharacteristically warm spring, the strawberry plants' flowering was almost over when the sampling started on June 18th and berry picking activity had ended before the final sampling on August 6th. The second and third sampling days were June 23rd and July 3rd.

The average temperatures on, respectively, the first, second, third, and fourth day of sampling were 25, 19, 27, and 20°C. The temperatures were measured using Gemini loggers (Tinytag data loggers). The mean daily variations in temperature for the 4 weeks during which we sampled were 17, 17, 19, and 16°C, respectively. Wind speeds in the area were up to 18, up to 20, up to 13, and up to 21 m/s on, respectively, the first, second, third, and fourth day. There was a very light drizzle on the fourth day and no rain on the other days.

The field was well tended with very few mouldy or smashed strawberries. A layer of straw approximately 10 cm thick had been placed around the strawberry plants keeping the plants damp and the berries dry. In the intersection of the T-shaped hedgerows,

commercial bumblebee colonies were used to deliver the MPCA Binab<sup>®</sup> T Vector as described by Kovach et al. (2000). Binab<sup>®</sup> T Vector is a commercial MPCA product consisting of *Trichoderma harzianum* and *T. polysporum*. However, the bees seemed to be very passive. In the previous growth season, the plants had been treated with the MPCA *Bacillus thuringiensis*.

Usually, a professional berry picking team of about 10 would work in the field in the morning, while designated sections were used for pick-your-own by roughly 30 people in the afternoon.

## 2.2 Sampling of inhalable dust in the field

Conical inhalable samplers called GSP samplers (BGI Inc., Waltham, MA, USA) equipped with polycarbonate filters (pore size 1  $\mu\text{m}$ ) were calibrated and placed in the hedgerow and in the field both up- and downwind of the hedgerow. The positions were noted, and the samplers were placed in the same spots on the three subsequent sampling days. The samplers ran for between 80 and 289 min (average 184 min) each day using a flow of 3.5 l  $\text{min}^{-1}$ . The sampling was performed in the working hours of the berry pickers (between 10:30 and 15:00). At no time were berry pickers within a distance of 10 m of the samplers.

Polycarbonate filters were chosen as the sampling filters as a study by Douwes et al. (1995) has shown the amounts of endotoxin extracted from polycarbonate filters to be two times greater than from cellulose ester filters.

## 2.3 Extraction of dust

About 16 h after sampling, the dust on the 57 polycarbonate filters was extracted in an aqueous solution of 10.0 ml sterile 0.05% Tween 80 and 0.85% NaCl by shaking for a 15-min period (500 rpm) at room temperature. Tween was used in the extraction solution as it increases the extraction efficiency of e.g. endotoxin.

## 2.4 Quantification of microorganisms (CAMNEA)

Microorganisms were quantified in the 57 samples using a modified CAMNEA method (Palmgren et al. 1986).

Dichloran Glycerol agar plates (DG-18 Agar, Oxoid, Basingstoke, England) were incubated at 25°C, and *Cladosporium* sp. colonies as well as the total amount of fungal colonies were counted. In addition to this, DG-18 agar plates were incubated at 45°C to quantify culturable *Aspergillus fumigatus* and plates of media selective for, respectively, *Trichoderma harzianum* (Williams et al. 2003), *Metarhizium anisopliae*, and *Beauveria* sp. (Meyling and Eilenberg 2006) were incubated at 25°C. The colonies on all plates were counted after 3 and 7 days. Plates of *Bacillus cereus* medium (*Bacillus cereus* Agar, Scharlau Chemie S.A., Barcelona, Spain) were incubated at 30°C and counted after 1 and 3 days.

A total count of fungal spores, hyphal fragments, and pollen was done of three samples from each day using samples from the same positions plus another two samples on the fourth day. The sample solutions were stained with 20 ppm acridine orange (Merck, Whitehouse Station, NJ, USA) in acetate buffer for 30 s and subsequently filtrated through a white polycarbonate filter (25 mm, 0.4  $\mu\text{m}$ ; Nuclepore, Cambridge, MA, USA). Bioaerosol components were counted at a magnification of 1,250 times using epifluorescence microscopy (Orthoplan; Leitz Wetzlar). The components were determined in forty randomly chosen fields.

## 2.5 Endotoxin determined by the Limulus method

Endotoxin analysis was performed on one sample from each of the three small open fields on each day ( $n = 12$ ). The dust suspensions were centrifuged (1,000 g) for 15 min. The supernatant was analysed (in duplicate) for endotoxin using the kinetic Limulus Amoebocyte Lysate test (Kinetic-QCL endotoxin kit; BioWhittaker, Walkersville, MD, USA) with  $\beta$ -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  $\approx$  1.0 ng). The limit of detection was 0.05 EU  $\text{ml}^{-1}$ , corresponding to 0.06 EU  $\text{m}^{-3}$  for the air sampling.

## 2.6 Quantification of (1 $\rightarrow$ 3)- $\beta$ -D-glucan

The samples analysed were the same as used for endotoxin but the whole dust suspensions were used.

Thus, dust suspensions were treated with 0.3 M NaOH for 60 min to make the triple-helix structure of the  $\beta$ -glucan water soluble. To quantify  $\beta$ -glucan in duplicate, the kinetic Fungitic G Test (Seikagaku Co., Tokyo, Japan) was used. A standard curve ranging from 4 to 100  $\text{pg ml}^{-1}$  was used to determine the amount of  $\beta$ -glucan.

## 2.7 Statistics

Statistical analyses were performed with SAS (Version 8e, SAS Institute, Cary, NC, USA) on the log-transformed data. The data were log-transformed to approximate normality. Analysis of variance ( $t$  test) was used to test the significance of the differences between the sampling days as well as between the sampling positions with regard to CFUs of *Cladosporium* sp., endotoxin, and the total counts of hyphae and pollen. The content of  $\beta$ -glucan and the total count of fungal spores were only analysed for differences between the sampling days. In some samples, the numbers of pollen were below detection limit ( $4,000 \text{ pollen m}^{-3}$ ) and in most samples the number of *Bacillus* sp. was below detection limit ( $126 \text{ CFU m}^{-3}$ ), and in the comparison between days, the value 50% of the detection limit was used. The multiplicative standard deviation  $s^*$  is calculated according to Limpert et al. (2001) for each day of sampling. Pearson's correlations were calculated between different fungal components and between  $\beta$ -glucan and pollen (only values above detection level were included).

## 3 Results

### 3.1 Culturable *Cladosporium* sp.

The airborne concentration of *Cladosporium* sp. ranged from a mean of  $1,437 \text{ CFU m}^{-3}$  for the first day to a mean of  $8,817 \text{ CFU m}^{-3}$  for the third day, while the mean for all four days was  $6,154 \text{ CFU m}^{-3}$ . The amount of culturable *Cladosporium* sp. differed significantly ( $p \leq 0.05$ ) between the sampling days except for the second and third day (Table 1).

We found no significant differences between the sampling positions neither when looked at independently nor when they were grouped according to whether they were placed in the open field or in the hedgerow.

### 3.2 Culturable mesophilic fungi

Mesophilic fungi other than *Cladosporium* sp. were present on every sampling day (Table 1). *Cladosporium* sp. made up 86–98% of the total amount of CFU of fungi, with the highest proportion of non-*Cladosporium* fungi on days 1 and 3. The fact that a large portion of the cultivable fungi was *Cladosporium* sp. was reflected in a high and significant correlation between CFU of total mesophilic fungi and CFU of *Cladosporium* sp. (Table 2).

None of the MPCAs *T. harzianum*, *T. polysporum*, and *B. bassiana* that we selected specifically for were found. Neither were other *Trichoderma*, *Metarhizium*, and *Beauveria* species found as well as no *A. fumigatus*.

### 3.3 Total counts

The total counts of fungal spores showed a mean of  $1.0 \times 10^5 \text{ spores m}^{-3}$ . The maximum value of  $1.7 \times 10^5 \text{ spores m}^{-3}$  was found on the first day. There was no significant difference between the sampling days. The total spore count per day was approximately tenfold higher than the amount of CFUs we found for all mesophilic fungi collectively, with the exception of day 1, when it was almost 75 times higher.

Hyphal fragments were counted for days 2, 3, and 4 with no significant difference between neither the days nor the sampling positions. A mean of  $4.1 \times 10^4 \text{ hyphal fragments m}^{-3}$  was found.

We found a mean value of  $5.8 \times 10^3 \text{ pollen m}^{-3}$  for the 3 days. The pollen count for day 1 was significantly higher ( $p \leq 0.05$ ) than for the three other days, which showed no significant difference amongst them. Thus, only days 2, 3, and 4 were included when we looked at the data for the sampling positions, which did not differ significantly (Table 1).

### 3.4 $\beta$ -Glucan

The  $\beta$ -glucan content of the twelve samples ranged from 9.3 to  $170 \text{ ng m}^{-3}$  (mean =  $57.3 \text{ ng m}^{-3}$ , median =  $44.0 \text{ ng m}^{-3}$ ). A significant difference ( $p \leq 0.05$ ) between day 3 with a high exposure and day 4 with low exposure was found (Table 1). The  $\beta$ -glucan content correlated positively and significantly with the total number of fungal spores

**Table 1** Exposure (unit  $\text{m}^{-3}$ ) to inhalable pollen, microorganisms, and microbial components in strawberry fields measured by stationary samplers during 4 working days

|                                             | Unit<br>$\text{m}^{-3}$ | Sampling day 1<br>June 18th                                   |                                                 | Sampling day 2<br>June 23rd                                    |                                                 | Sampling day 3<br>July 3rd                                    |                                                 | Sampling day 4<br>August 6th                                  |                                                 |
|---------------------------------------------|-------------------------|---------------------------------------------------------------|-------------------------------------------------|----------------------------------------------------------------|-------------------------------------------------|---------------------------------------------------------------|-------------------------------------------------|---------------------------------------------------------------|-------------------------------------------------|
|                                             |                         | Median<br>(mean)<br><i>n</i>                                  | [Range]<br>(s*)                                 | Media<br>(mean)<br><i>n</i>                                    | [Range]<br>(s*)                                 | Median<br>(mean)<br><i>n</i>                                  | [Range]<br>(s*)                                 | Median<br>(mean)<br><i>n</i>                                  | [Range]<br>(s*)                                 |
| Total count, pollen                         | Number                  | $1.6 \times 10^4$<br>( $1.7 \times 10^4$ ) <sup>a</sup><br>3  | $[1.3 \times 10^4 - 2.3 \times 10^4]$<br>(1.12) | $2.0 \times 10^3$<br>( $5.3 \times 10^3$ ) <sup>b</sup><br>3   | $[\text{bd} - 1.2 \times 10^4]$<br>(2.81)       | $5.1 \times 10^3$<br>( $5.5 \times 10^3$ ) <sup>b</sup><br>3  | $[\text{bd} - 9.5 \times 10^3]$<br>(2.18)       | $2.0 \times 10^3$<br>( $3.5 \times 10^3$ ) <sup>b</sup><br>5  | $[\text{bd} - 7.9 \times 10^3]$<br>(1.83)       |
| Total count, fungal spores                  | Number                  | $1.0 \times 10^5$<br>( $1.2 \times 10^5$ ) <sup>a</sup><br>3  | $[1.0 \times 10^5 - 1.7 \times 10^5]$<br>(1.34) | $9.0 \times 10^4$<br>( $8.3 \times 10^4$ ) <sup>a</sup><br>3   | $[6.1 \times 10^4 - 9.7 \times 10^4]$<br>(1.28) | $8.2 \times 10^4$<br>( $9.8 \times 10^4$ ) <sup>a</sup><br>3  | $[8.1 \times 10^4 - 1.3 \times 10^5]$<br>(1.32) | $1.0 \times 10^5$<br>( $1.1 \times 10^5$ ) <sup>a</sup><br>5  | $[6.3 \times 10^4 - 1.5 \times 10^5]$<br>(1.42) |
| Total count, hyphal fragments               | Number                  | nm<br>0                                                       |                                                 | $3.8 \times 10^4$<br>( $4.0 \times 10^4$ ) <sup>a</sup><br>3   | $[2.1 \times 10^4 - 6.0 \times 10^4]$<br>(1.69) | $3.3 \times 10^4$<br>( $4.4 \times 10^4$ ) <sup>a</sup><br>3  | $[2.6 \times 10^4 - 7.4 \times 10^4]$<br>(1.73) | $3.1 \times 10^4$<br>( $3.1 \times 10^4$ ) <sup>a</sup><br>5  | $[7.9 \times 10^3 - 5.7 \times 10^4]$<br>(2.04) |
| Total count, <i>Cladosporium</i> sp. spores | Number                  | $6.3 \times 10^4$<br>( $6.7 \times 10^4$ ) <sup>a</sup><br>3  | $[4.7 \times 10^4 - 9.2 \times 10^4]$<br>(1.38) | $4.6 \times 10^4$<br>( $5.0 \times 10^4$ ) <sup>a</sup><br>3   | $[3.0 \times 10^4 - 7.2 \times 10^4]$<br>(1.19) | $7.7 \times 10^4$<br>( $8.6 \times 10^4$ ) <sup>a</sup><br>3  | $[6.7 \times 10^4 - 1.2 \times 10^5]$<br>(1.33) | $4.0 \times 10^4$<br>( $5.2 \times 10^4$ ) <sup>a</sup><br>5  | $[3.5 \times 10^4 - 9.8 \times 10^4]$<br>(1.51) |
| Culturable <i>Cladosporium</i> sp.          | CFU                     | $5.0 \times 10^2$<br>( $1.4 \times 10^3$ ) <sup>c</sup><br>13 | $[1.1 \times 10^2 - 8.1 \times 10^3]$<br>(3.17) | $7.5 \times 10^3$<br>( $8.2 \times 10^3$ ) <sup>a</sup><br>12  | $[5.3 \times 10^3 - 1.2 \times 10^4]$<br>(1.20) | $8.3 \times 10^3$<br>( $8.8 \times 10^3$ ) <sup>a</sup><br>15 | $[3.6 \times 10^3 - 2.5 \times 10^4]$<br>(1.63) | $4.8 \times 10^3$<br>( $6.2 \times 10^3$ ) <sup>b</sup><br>17 | $[1.5 \times 10^3 - 1.6 \times 10^4]$<br>(1.90) |
| Total culturable mesophilic fungi           | CFU                     | $1.0 \times 10^3$<br>( $1.7 \times 10^3$ ) <sup>c</sup><br>13 | $[2.2 \times 10^2 - 8.2 \times 10^3]$<br>(2.57) | $7.8 \times 10^3$<br>( $8.3 \times 10^3$ ) <sup>ab</sup><br>12 | $[5.4 \times 10^3 - 1.2 \times 10^4]$<br>(1.24) | $9.5 \times 10^3$<br>( $1.0 \times 10^4$ ) <sup>a</sup><br>15 | $[3.7 \times 10^3 - 3.0 \times 10^4]$<br>(1.69) | $5.6 \times 10^3$<br>( $6.4 \times 10^3$ ) <sup>b</sup><br>17 | $[1.5 \times 10^3 - 1.6 \times 10^4]$<br>(1.90) |
| $\beta$ -Glucan                             | ng                      | 43.4<br>(45.5) <sup>ab</sup><br>3                             | $[38.6 - 54.5]$<br>(1.19)                       | 36.6<br>(39.1) <sup>ab</sup><br>3                              | $[36.2 - 44.5]$<br>(1.12)                       | 95.3<br>(109.3) <sup>a</sup><br>3                             | $[62.6 - 1.7 \times 10^2]$<br>(1.62)            | 28.9<br>(35.1) <sup>b</sup><br>3                              | $[9.3 - 52.6]$<br>(2.22)                        |
| Endotoxin                                   | EU                      | 3.0<br>(3.7) <sup>b</sup><br>3                                | $[2.9 - 5.2]$<br>(1.39)                         | 6.8<br>(12.5) <sup>a</sup><br>3                                | $[2.7 - 27.8]$<br>(3.16)                        | 14.1<br>(16.3) <sup>a</sup><br>3                              | $[9.7 - 25.2]$<br>(1.58)                        | 2.6<br>(3.0) <sup>b</sup><br>3                                | $[2.5 - 3.7]$<br>(1.14)                         |
| <i>Bacillus cereus</i> group                | CFU                     | Bd<br>13                                                      |                                                 | Bd<br>12                                                       |                                                 | 126<br>(435)<br>15                                            | $[\text{bd} - 1.9 \times 10^3]$<br>(2.95)       | Bd<br>17                                                      |                                                 |

Numbers in the same row followed by the same letter are not significantly different,  $p > 0.05$ ; nm, not measured; bd, below detection level. *n*, number of samples analysed for this component

**Table 2** Pearson's correlation coefficients (*r*) between different fungal components and between pollen and  $\beta$ -glucan

|                                             | Total count, pollen                       | Total count, fungal spores                        | Total count, hyphal fragments               | Total count, <i>Cladosporium</i> sp. spores | Culturable <i>Cladosporium</i> sp.                 | Total culturable, mesophilic fungi         | $\beta$ -Glucan |
|---------------------------------------------|-------------------------------------------|---------------------------------------------------|---------------------------------------------|---------------------------------------------|----------------------------------------------------|--------------------------------------------|-----------------|
| Total count, pollen                         | 1                                         |                                                   |                                             |                                             |                                                    |                                            |                 |
| Total count, fungal spores                  |                                           | 1                                                 |                                             |                                             |                                                    |                                            |                 |
| Total count, hyphal fragments               | –                                         | 0.380<br><i>p</i> = 0.248<br><i>n</i> = 11        | 1                                           |                                             |                                                    |                                            |                 |
| Total count, <i>Cladosporium</i> sp. spores | –                                         | 0.506<br><i>p</i> = 0.065<br><i>n</i> = 14        | 0.471<br><i>p</i> = 0.14<br><i>n</i> = 11   | 1                                           |                                                    |                                            |                 |
| Culturable <i>Cladosporium</i> sp.          | –                                         | –0.047<br><i>p</i> = 0.873<br><i>n</i> = 14       | –0.380<br><i>p</i> = 0.250<br><i>n</i> = 11 | 0.181<br><i>p</i> = 0.535<br><i>n</i> = 14  | 1                                                  |                                            |                 |
| Total culturable, mesophilic fungi          | –                                         | –0.077<br><i>p</i> = 0.79<br><i>n</i> = 14        | –0.390<br><i>p</i> = 0.235<br><i>n</i> = 11 | 0.155<br><i>p</i> = 0.597<br><i>n</i> = 14  | <b>0.987</b><br><i>p</i> ≤ 0.0001<br><i>n</i> = 57 | 1                                          |                 |
| $\beta$ -Glucan                             | 0.391<br><i>p</i> = 0.18<br><i>n</i> = 11 | <b>0.708</b><br><i>p</i> = 0.033<br><i>n</i> = 11 | 0.547<br><i>p</i> = 0.16<br><i>n</i> = 8    | 0.625<br><i>p</i> = 0.066<br><i>n</i> = 11  | –0.041<br><i>p</i> = 0.900<br><i>n</i> = 12        | 0.054<br><i>p</i> = 0.868<br><i>n</i> = 12 | 1               |

Significant correlations are written in bold

(Table 2). The  $\beta$ -glucan content correlated positively but not significantly with the total number of *Cladosporium* sp. spores, hyphal fragments, and pollen (Table 2). A mean of 21.2 pg  $\beta$ -glucan per CFU of fungi was found (median = 15.3 pg/CFU of fungi). The maximum of 42.0 pg  $\beta$ -glucan per CFU of fungi was found on day 1.

### 3.5 Bacillus

The MPCA *B. thuringiensis* was not found, but on the third sampling day a *Bacillus* species from the *B. cereus* group was found (mean = 1,055 CFU m<sup>–3</sup>) (Table 1).

### 3.6 Endotoxin

The mean exposure to endotoxin was 8.9 EU m<sup>–3</sup>. The maximum exposure of 27.8 EU m<sup>–3</sup> was found on the second day. We found significantly (*p* ≤ 0.05) higher exposure to endotoxin on the third day than on the first and fourth day (Table 1).

### 3.7 Field versus hedgerow samples

A comparison was made between the samplers placed in the open field and the ones in the hedgerow, but no significant difference was found. Also two groupings were made according to whether the samplers were on the left or the right side of the hedgerow, but they did not differ significantly, either.

## 4 Discussion

*Cladosporium* sp., which had a mean value of 6,154 CFU m<sup>–3</sup>, was in contrast to other culturable mesophilic fungi found in high concentrations. Most other outdoor studies (Beaumont et al. 1985; Larsen and Gravesen 1991; Oliveira et al. 2009; Stepalska and Wolek 2005) have also found *Cladosporium* sp. to be by far the most common in outdoor air. In another Danish study performed in the city of Copenhagen over almost 3 years, Larsen (1981) found the mean *Cladosporium* exposure for June,

July, and August to be 367, 449, and 914 CFU m<sup>-3</sup>, respectively, while the mean exposure to *Cladosporium* CFUs in our study ranged from  $1.4 \times 10^3$  to  $8.8 \times 10^3$  CFU m<sup>-3</sup>. The *Cladosporium* sp. exposure in our study accounted for 93% of the culturable airborne fungi and is also the most frequently found airborne fungus in many other studies (Stepalska and Wolek 2005; Takahashi 1997). The found fraction of *Cladosporium* spores as measured by microscopy per total mesophilic fungi is somewhat high compared to other studies. Thus, in the city of Copenhagen, *Cladosporium* sp. represented 78% of the culturable airborne fungi (Larsen and Gravesen 1991), while in a study from vegetable fields in Turkey *Cladosporium* sp. represented only 43% (Sen and Asan 2001). A reason we found such a high fraction of *Cladosporium* spores may be because straw was tucked around the strawberry plants as *Cladosporium* is a secondary invader of dead plant material (Gravesen 1979). Exposure to *Cladosporium* sp. can be associated with development of both allergy (e.g. Malling et al. 1985) and asthma (Denning et al. 2006).

Hryhorczuk et al. (2001) references several studies on composting facilities, which show fungal exposure levels comparable to ours, e.g. a German composting plant where the median indoor exposure during shredding ranged from  $5.2 \times 10^3$  to  $19.1 \times 10^4$  CFU m<sup>-3</sup> and an Austrian study with a median total fungal concentration of  $6.5 \times 10^3$  CFU m<sup>-3</sup> 3 m from the windrows. Even though other composting plants greatly exceeded those levels, it is still an indication that the fungal exposure is high in the strawberry field. However, the levels are still below the 10,000 CFU m<sup>-3</sup>, which Heida et al. (1995) references as a threat to workers' health.

The total counts of fungal spores showed a mean of 104,307 spores m<sup>-3</sup>, while *Cladosporium* spores had a mean value of 66,292 spores m<sup>-3</sup>, thus accounting for 64% of the total spores. The reason why cultured *Cladosporium* sp. was so dominating over other culturable fungi while constituting only two-thirds of the total amount of spores is unclear. However, *Cladosporium* has earlier been shown to have a higher culturability (33%) than e.g. *Aspergillus/Penicillium* (2%) (Lee et al. 2006). Possible reasons for the high culturability of *Cladosporium* include that the other spores were more easily damaged during the sampling and/or laboratory processes, the growth media used may have favoured some fungi over others, as some

fungi are unculturable, and some fungi may have been in an unculturable state. However, our choice of DG-18 agar plates as growth medium is backed by other studies which found DG-18 to be the superior medium with regard to total fungal concentrations, range of isolated genera, and recovery rates (Horner et al. 2004; Wu et al. 2000). There was no significant and positive correlation between total number of *Cladosporium* spores and CFU of *Cladosporium* spores (Table 2). This is because a higher number of CFU of *Cladosporium* relative to the total number of *Cladosporium* spores found on the second day of sampling compared to the other 3 days. Hyphal fragments of *Cladosporium* may also contribute to the CFU of *Cladosporium*. From this study, we cannot conclude whether weather conditions influenced the cultivability of the spores.

The mean concentration of 104,307 fungal spores m<sup>-3</sup> in the strawberry fields is much higher than found in Portuguese outdoor environments (494–923 spores m<sup>-3</sup> as annual mean values) (Oliveira et al. 2009) and at the level of what is found in biofuel plants (median = 120,000 spores m<sup>-3</sup>) (Madsen 2006b). Thus, our findings seem to be higher than what is found in normal outdoor air and, as with the CFU of fungi, closer to what is found in environments with decomposing organic matter. A study by Eduard et al. (2001) estimated the exposure level where symptoms of the eyes and nose increased to be from 20,000 to 500,000 spores m<sup>-3</sup>. The total *Cladosporium* sp. exposure in strawberry fields is also higher than that found in city environments (Hjelmroos 1993). It has been estimated by Gravesen (1979) that 3,000 *Cladosporium* spores m<sup>-3</sup> is the threshold concentration for evoking allergic symptoms. It is likely that strawberry pickers are exposed to levels greater than what we measured using stationary samplers, as the strawberry pickers are closer to the ground and may cause spores and other microbial components to be released when handling the plants and decomposing straw.

We found no exposure to the MPCAs *T. harzianum* or *T. polysporum* even though these organisms were applied to the environment. We have found no other studies about exposure to the MPCAs *T. harzianum* or *T. polysporum*. The fact that *B. bassiana* and other *Trichoderma* and *Beauveria* species were not found is in accordance with a review study, concluding that *B. bassiana*, *T. harzianum*, and *T. polysporum* infrequently are present in the air and thus people in

general seem to be rarely exposed to these fungi (Madsen et al. 2007). *B. thuringiensis* was applied to the field the previous year but was not found in the air. An earlier study has shown that *B. thuringiensis* can be found in the air 52 days after application to a crop (Hansen et al. 2010). On the third sampling day, another *Bacillus* species from the *cereus* group was found in concentrations up to  $2 \times 10^3$  CFU m<sup>-3</sup> air. This concentration of *Bacillus* sp. is at the same level as what has earlier been found in the air in a cattle feedlot (Wilson et al. 2002).

The exposure of strawberry workers to  $\beta$ -glucan is higher than earlier found for compost workers (Hryhorczuk et al. 2001). A mean of 21.2 pg  $\beta$ -glucan per CFU of fungi was found in this study. The highest content was found on the first day of sampling, probably because the highest pollen exposure was found on that day. In another study, the  $\beta$ -glucan content of *Cladosporium herbarum* spores cultured on an agar medium was analysed and found to be 8.66 pg per spore (Iossifova et al. 2007). An investigation into microbial exposure during grain farming showed that during threshing of grain hyphal exposure explained 72% of the  $\beta$ -glucan exposure and contributed equally as fungal spores to explain the  $\beta$ -glucan exposure (Halstensen et al. 2007).

We found a mean of  $4.1 \times 10^4$  hyphal fragments m<sup>-3</sup>, which appears high when compared with other studies. The exposure is higher than the maximum of 1,811 hyphal fragments m<sup>-3</sup> Pady and Kramer (1960) found during several years of sampling on a roof 46 m above ground at the campus of Kansas State University. Delfino et al. (1997) found a maximum of 416 hyphal fragments 24 h m<sup>-3</sup> with the sampler placed 4 m above ground level. The wind speeds in the strawberry field were generally high, reaching 21 m s<sup>-1</sup>, which may have caused the release of hyphal fragments. The exposure to hyphal fragments is highly important because in a study of the effect of various airborne particles on asthma severity, hyphal fragments caused the most consistently significant effects on the parameters used to define asthma severity (Delfino et al. 1997). Most studies, however, do not include hyphal fragments, but Halstensen et al. (2007) argue that hyphal fragments should be included in microbial analyses as they contain many of the same properties as fungal spores. In this study, hyphal fragments were only quantified in 8 of the samples where  $\beta$ -glucan was also measured, and we

did not find a significant correlation between these two components, probably because pollen and fungal spores also contribute to the  $\beta$ -glucan content. Many of the hyphal fragments were dark and thus probably melanized. As *Cladosporium* species produce melanized hyphae (e.g. Butler et al. 2009) and *Cladosporium* was the dominating fungus in the samples, we expect the hyphal fragments to be *Cladosporium*. The numbers of hyphal fragments correlated positively but not significantly with the total number of *Cladosporium* spores.

The endotoxin levels found (median = 4.5 EU m<sup>-3</sup>) is at the level of earlier measurements of background exposures to endotoxin, e.g. in cities (reviewed in Madsen 2006a). The values, however, are well below the suggested threshold limit values of 30–800 EU m<sup>-3</sup> (reviewed in Madsen 2006a). It is interesting, though, that while the fungal levels were approaching the levels seen in biofuel plants (Madsen 2006b), the endotoxin level found in this strawberry field is far less than the median level (66 EU m<sup>-3</sup>) from the biofuel plants. A reason may be that fungal spores more easily become airborne, a factor that is less noticeable at a biofuel plant, where the organic material generally is handled very roughly.

Compared to other studies the pollen levels in the strawberry field were high. Day 1 was significantly higher than the rest with a mean of  $1.7 \times 10^4$  pollen grains m<sup>-3</sup>, while the other days had a mean value of  $3.1 \times 10^3$  pollen grains m<sup>-3</sup>. A Spanish study measuring pollen in an urban setting for 2 years found a maximum daily concentration of 730 pollen grains m<sup>-3</sup> (Tormo-Molina et al. 2009), and a British and a Turkish study both in cities found a total annual pollen count of respectively  $3.0 \times 10^4$  and  $3.3 \times 10^4$  pollen grains m<sup>-3</sup> (Altintas et al. 2004; Pashley et al. 2009).

In general, only small variations were found in the concentrations of airborne microbial components throughout the period of measurement. Some microbial components were found in higher concentrations on the third day of sampling. On the fourth day of sampling, there was a very light drizzle for brief periods and we found lower concentrations of endotoxin. We have found no study describing the influence of drizzle on the concentration of airborne endotoxin. The discharge of spores from different *Cladosporium* species is affected by many factors (reviewed in Gottwald 1983), two of these being

rising temperatures and declining relative humidity. We found the highest concentration of CFU of *Cladosporium* sp. on the day with the highest temperature (day 3), which also had the largest difference between day and night time temperatures. On the first day of sampling, a higher concentration of pollen was found than on the later days of sampling. This is in accordance with the pollen calendars for grass pollen (Goldberg et al. 1988).

## 5 Conclusion

In conclusion, our study shows that strawberry pickers in this organic strawberry field were potentially subjected to higher levels of fungal spores, *Cladosporium* sp., hyphal fragments, pollen, and thus also  $\beta$ -glucan than is usually seen in outdoor air. The earlier and ongoing treatment with MPCAs did not cause exposure to MPCAs. The exposure to endotoxin was only slightly higher than e.g. in a town.

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