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Building-integrated agriculture: a first assessment of aerobiological air quality in rooftop greenhouses (i-RTGs)

Mireia Ercilla-Montserrat*[1], Rebeca Izquierdo*[2,3], Jordina Belmonte[2,3], Juan Ignacio Montero[4], Pere Muñoz[4], Concepción De Linares[2,3], Joan Rieradevall[1,5] (*These authors contributed equally to the research.)

[1] Sostenipra Research Group (SGR 01412), Institute of Environmental Sciences and Technology (MDM-2015-0552), Z Building, Autonomous University of Barcelona (UAB), Campus UAB, 08193 Bellaterra, Barcelona, Spain

[2] AEROBIOTA Research Group (2014SGR1274), Institute of Environmental Sciences and Technology (ICTA), Z Building, Universitat Autònoma de Barcelona (UAB), Campus UAB, 08193 Bellaterra, Barcelona, Spain

[3] Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona (UAB). Edifici C, 08193 Bellaterra, Spain

[4] Institute of Food and Agricultural Research (IRTA), Carretera de Cabrils, km 2, 08348 Barcelona, Spain

[5] Department of Chemical, Biological and Environmental Engineering, School of Engineering, Building Q, Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Barcelona, Spain.

Corresponding author: Mireia Ercilla-Montserrat. Sostenipra Group. Institut de Ciència i Tecnologia Ambientals, ICTA ICTA-ICP, Edifici Z - Despatx Z/139 Carrer de les columnes Universitat Autònoma de Barcelona. E- 08193 Bellaterra (Cerdanyola del Vallès - Barcelona). Tel: (+34) 935868645. E-mail address: Mireia.ercilla@uab.cat

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Abstract

Building-integrated rooftop greenhouse (i-RTG) agriculture has intensified in recent years, due to the growing interest in the development of new agricultural spaces and in the promotion of food self-sufficiency in urban areas. This paper provides a first assessment of the indoor dynamics of bioaerosols in an i-RTG, with the aim of evaluating biological air quality in a tomato greenhouse near Barcelona. It evaluates the greenhouse workers' exposure to airborne pollen and fungal spores in order to prevent allergy problems associated with occupational tasks. Moreover, it evaluates whether the quality of the hot air accumulated in the i-RTG is adequate for recirculation to heat the building.

Daily airborne pollen and fungal spore concentrations were measured simultaneously in the indoor and outdoor environments during the warm season. A total of 4,924 pollen grains were observed in the i-RTG, with a peak of 334 pollen grains/m^3day, and a total of 295,038 fungal spores were observed, reaching a maximum concentration of 26,185 spores/m^3day. In general, the results showed that the most important source of pollen grains and fungal spores observed indoors was the outdoor environment. However, Solanaceae pollen and several fungal spore taxa, such as the allergenic *Aspergillus/Penicillium*, largely originated inside the greenhouses or were able to colonize the indoor environment under favourable growing conditions. Specific meteorological conditions and agricultural management tasks are related to the highest observed indoor concentrations of pollen grains and fungal spores. Therefore, preventive measures have been suggested in order to reduce or control the levels of bioaerosols indoors (to install a system to interrupt the recirculation of air to the building during critical periods or to implement appropriate air filters in ventilation air ducts). This first evaluation could help in making

decisions to prevent the development of fungal diseases, specifically those due to *Oidium* and *Torula*.

INTRODUCTION

Urban agriculture, particularly rooftop urban agriculture, has increased in the last few years. This is due to the coupled needs of encouraging food sovereignty in urban areas and providing a response to the growing competition for resources associated with conventional agriculture (water, energy, land, etc.) (FAO, 2011). It is thought that the global food system causes between 20% and 50% of total global anthropogenic pressure (directly or indirectly). Most of this pressure is attributable to cities, due to the many people who live there.

Currently, different types of urban agriculture have been developed in urban areas (Fig. 1). Rooftop urban agriculture (RT) is a subcategory within this concept that includes all those crop plants grown on the tops of buildings. RT not only makes it possible to produce food, it also provides environmental and social benefits to surrounding areas. These benefits include boosting local urban markets; making use of empty constructed spaces; creating new sources of employment and support for local economies; allowing savings on the transport of goods and packaging materials; increasing urban biodiversity and naturalizing the environment; etc. (Altieri et al., 1999; Cerón-Palma et al., 2012; Sanyé-Mengual et al., 2013; Specht et al., 2014). RT can be carried out both outdoors and in sheltered environments, which have become known as rooftop greenhouses (Despommier, 2008).

Figure 1. Typologies and nomenclatures used to describe urban agriculture on buildings and rooftop farming

Rooftop greenhouses and the first steps toward integrated rooftop greenhouses

Rooftop greenhouses consist of greenhouses installed on top of buildings to produce food in urban contexts with high yield using soil-free growing systems (to reduce the structural load on the building). They increase the efficiency of resource use and employ constructed spaces that are currently unoccupied (Cerón-Palma et al., 2012). Many of the existing experiments involve greenhouses constructed on roofs that are isolated from the buildings. However, this kind of RT greenhouse can also be integrated (i-RTG) into the building, using residual air flows from the building for the crops (Cerón-Palma, 2012).

One key aspect of i-RTGs is their capacity to make use of the hot and cold air supplied by the building to provide an optimum range of temperatures for the crops, without the need for extra

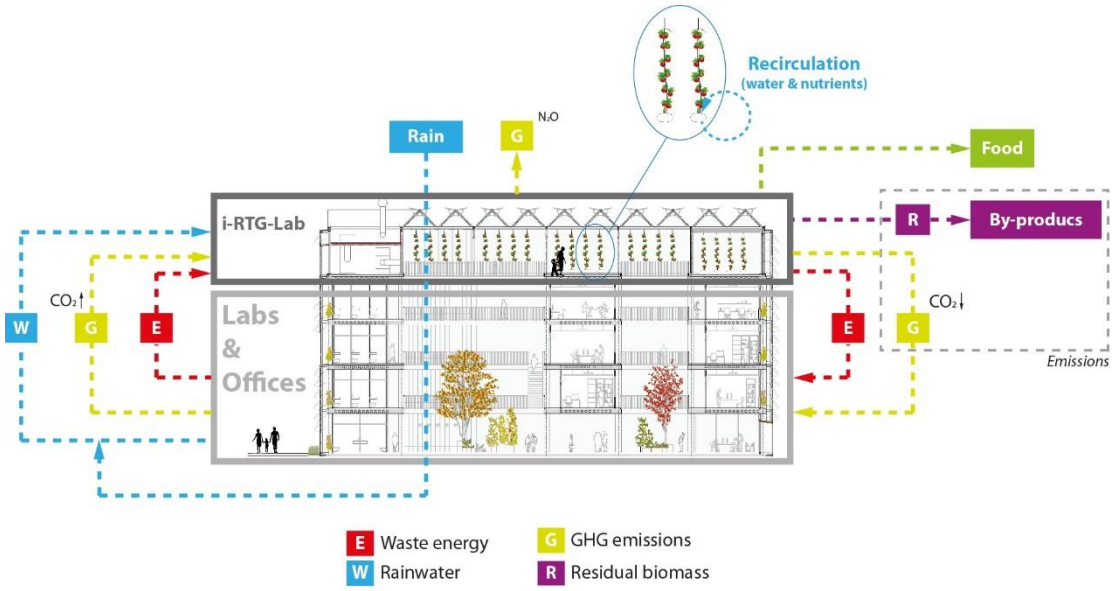
energy. This is true because, first, due to their particular features, i-RTGs provide thermal insulation on the roof of the building. Second, they offer the possibility of supplying air from heated/air conditioned areas to the crops using a pumping system.

Greenhouses used for intensive cultivation of horticultural crops also use the carbon enrichment technique known as carbon fertilization to improve yield. In an i-RTG, this technique consists of increasing the environmental level of CO_2 by injecting waste CO_2 from offices and laboratories inside the building via an air current. Exposure to environments with a high concentration of carbon dioxide generally stimulate crop growth and photosynthetic fixation (i.e., in the case of tomatoes exposed to a CO_2concentration of 900 $\mu mol \cdot m^{-3}$, the rate of growth increased by 30%) (Yelle et al., 1990).

Meanwhile, they also offer the possibility of using greywater or rainwater collected in or on the building for irrigating the crop (Montero et al., 2009).

A new study has recently begun investigating the possibility of creating two-way connections between a building and its greenhouses. Such a greenhouse is known as a bidirectional integrated rooftop greenhouse (Bi-RTG) (Fig. 2). These greenhouses would, among other things, make it possible to use the residual hot air accumulated in the greenhouses, which needs to be ventilated, to heat other areas of the building that do not have a heating system. Previously, the heat was removed by opening the flap ventilators of the greenhouse. The location of the greenhouses on the roofs of buildings promotes higher temperatures inside the greenhouse than in the rest of the building. This occurs because the greenhouse acts as a solar collector and heats up with the incidence of solar radiation through the polycarbonate cover during the day. It does not occur with the same rapidity in the building, which has more thermal inertia and receives less radiation. It enables improving the environmental thermic conditions of the building with minimal energetic and environmental impact.

Figure 2. The bidirectional Bi-RTG concept behind the i-RTG Lab. The exchange of flows between the ICTA-ICP building and the i-RTG is shown.



It is important to evaluate the air quality in these greenhouses, due to the ability of the Bi-RTG system to draw air from the crop area into the building spaces. With regard to the CO_2 concentration, the situation is convenient because the CO_2 is absorbed by the crop, improving the air quality. However, the biological air quality (pollen and fungal spore concentrations) must be studied and taken into account to avoid causing or aggravating allergies and breathing problems, both in terms of the use of the air for space heating and to protect the health of the agricultural workers, who spend large amounts of time inside the greenhouses.

Air quality in greenhouses

It is known that agricultural greenhouse workers are exposed to dust particles suspended in the air. This dust contains bioaerosols, such as spores and pollen, which can cause respiratory problems. These problems include chronic bronchitis, allergies and/or "sensitization and hypersensitivity pneumonitis" (Illing, 1997; Taekhee Lee et al., 2006a; Swan and Crook, 1998) and asthma (Malling et al., 1986; Strachan, 1988).

The conditions of these working environments are characterized by closed spaces, high temperatures, high humidity and high concentrations of microorganisms in the environment. These microclimatic characteristics encourage the growth of fungi and the consequent dispersal of spores (Arny and Rowe, 1991; Jewett et al., 2001). Moreover, restricted ventilation and low light intensity, especially on the bottoms of the plants, are common. This situation is very conducive to the development of several plant diseases, and controlling them is very difficult (Elad et al., 1995).

Exposure levels vary depending on the type of crop, the tasks carried out and the season of the year. The most important periods when pollution occurs are when the plants are senescent, and the pollution is aggravated if the greenhouse is not properly maintained (removing dry leaves, sweeping passageways, etc.) (Hansen et al., 2011).

Most of the vegetable and ornamental plants grown in greenhouses suffer from powdery mildew. Powdery mildew affects all kind of plants, except gymnosperms, and is one of the most common and widespread plant diseases (Agrios, 2005). On tomato crops, the most important fungi causing this disease are *Oidium* sp (Elad et al., 1996). Powdery mildews are characterized by grey or white sporulation colonies on the upper leaf surface. The inoculum consists mostly of conidia. Once infection occurs, it develops rapidly under dry conditions. Dry climates favour dispersal of spores, while humid climates favour spore germination (Agrios, 2005). This is because conidia spores are self-sufficient in terms of water and nutrients and can germinate, grow and infect plants without dew or condensation (Verhaar, 1998).

Air quality inside buildings

In the case of buildings, the situation is different, as they are fairly isolated from the external environment, and the concentrations of pollen and spores detected inside buildings are usually lower than those found outside (Nayar and Jothish, 2013). However, the outside air often continues to be the dominant source of biological pollution inside buildings; therefore, indoor air quality can be interpreted via careful study of the external air (Dassonville et al., 2008; Ghosh et al., n.d.; Shelton et al., 2002). The concentrations detected inside are related to the ventilation of the building and the air conditioning (Flores et al., 2014; Shelton et al., 2002), the opening or closing of windows and doors and the flow of people (Abdulla et al., 2008; Bastl et

al., 2016; D'Amato et al., 1996; Jantunen and Saarinen, 2009; Buss et al. ,2010). Some studies carried out inside buildings point out that allergies persist after the pollination period. Air quality inside a building is a key factor because of the potential health effects, as it is estimated that people spend 80% of their time inside buildings every week (Cariñanos et al., 2004)

The assessment of fungal exposure is complex, considering that fungi are ubiquitous microorganisms that are present in both outdoor and indoor air. There are two main sources of fungal spores in buildings: outdoor sources, which leak rain into or cause flooding in the building, and indoor sources, which are derived from human activity or accidents that cause water damage (Bornehag et al., 2004). Common outdoor fungi include *Alternaria*, *Cladosporium*, and *Epicoccum*, as well as ascospores and basidiospores, though these fungi are often found indoors because they enter through open doors and windows and can be carried indoors (Levetin et al., 2016). Fungi that are more classically associated with indoor water damage or decay include *Penicillium*, *Aspergillus*, *Stachybotrys*, and *Chaetomium* (Levetin et al., 2016). In the case of fungal spores, the indoor-outdoor relationship has not been systematically studied, and most of the available information is based on the simultaneous measurement of culturable fungal spores performed indoors and outdoors (Lee et al., 2006) or on comparisons of the diversity and number of fungal colonies in university classrooms before and during the presence of the students (Buss et al., 2010). However, the total spore count has shown a better exposure to response relationship compared to the culture-based method (Eduard, 2003). Therefore, continuous sampling and spore counting could have a greater potential to generate an adequate database.

European Union legislation currently excludes the monitoring of the concentrations of pollen and spores because they are not considered a consequence of human activity, and Brussels does not compel member States to compile this information. As there are no regulations on this matter, certain limitations as to assessing air quality inside buildings exist (Immunology, 2015)

Current reference values of spore concentrations include those from the American Conference of Governmental Industrial Hygienists (ACGIH 1999), which sets a threshold of 200 CFU/m^3, and Health Canada (Nathanson, 1993), which gives a figure of 150 CFU/m^3. These guidelines recommend that concentrations inside buildings should be no greater than those outside. Although the threshold concentrations required to trigger allergic reactions are unknown, Eduard (2009) suggests that the lowest observed effective level for the appearance of respiratory symptoms for diverse fungal species is 10^5 spores/m^3 per day in non-sensitised populations, while (Santilli and Rockwell, 2003a) consider that the standard for a healthy indoor environment should be defined as 10^3 spores/m^3 per day.

The goals of this study are as follows:

1. To determine airborne pollen and fungal spore spectra indoors (inside an i-RTG) and outdoors in order to identify the diversity, daily concentrations and frequency of each taxon during the cultivation of a tomato crop inside the facility and in the semi-urban area where it is placed, using the same aerobiological monitoring method.
2. To determine the influence of indoor and outdoor meteorological conditions and crop management practices on the biological air quality in the i-RTG.
3. To assess the greenhouse workers' exposure to airborne pollen and fungal spores in order to prevent allergy problems derived from occupational tasks.

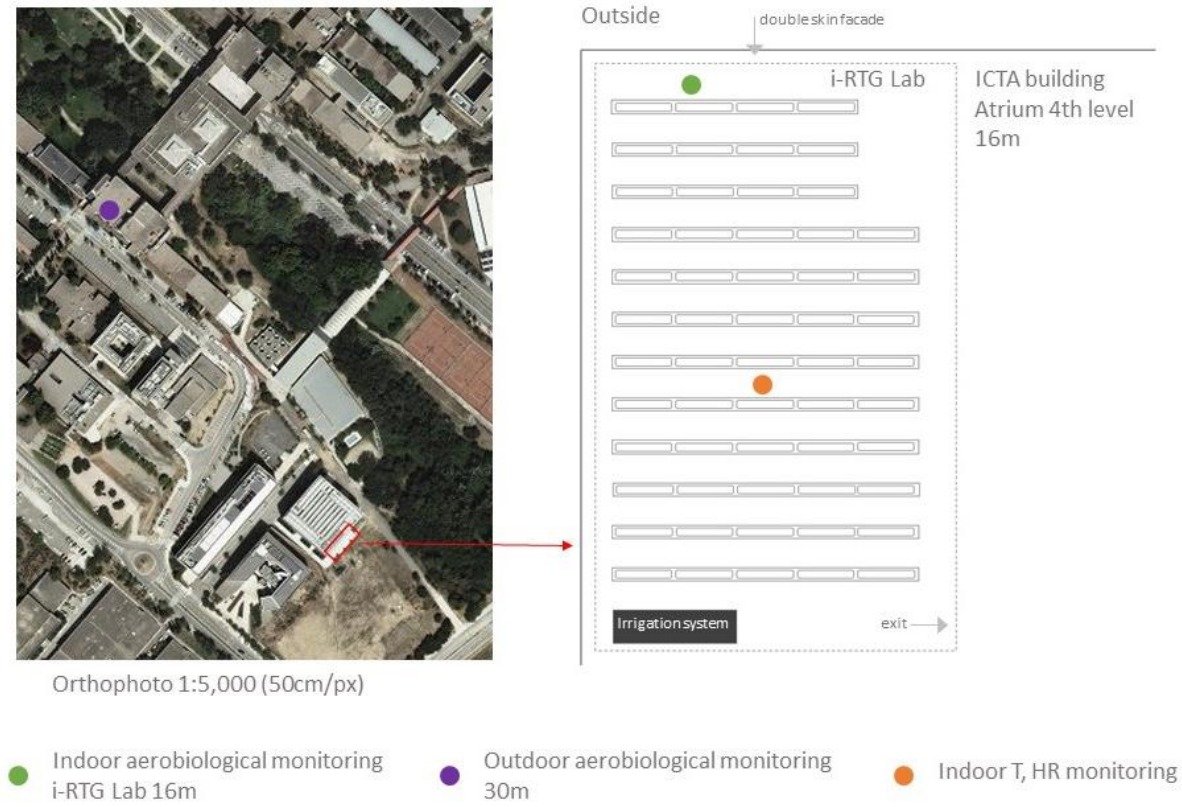
4. To evaluate whether the quality of the hot air accumulated in the i-RTG is adequate for reuse inside the building, and to assess the risk of causing or aggravating respiratory problems.
5. To identify critical stages of the crop production process, and to develop preventive measures to avoid exposing agricultural greenhouse workers and building users to noxious substances if the air is used to heat the building.

MATERIAL AND METHODS

i-RTG Greenhouse Laboratory

The investigation was conducted in a rooftop greenhouse laboratory (the i-RTG Lab) during an experiment involving growing tomatoes *(Solanum lycopersicum* Arawak). The i-RTG is placed on the rooftop of the building hosting the Institute of Environmental Science and Technology (ICTA-UAB) and the Catalan Institute of Paleontology (ICP) at Bellaterra (41°23'40.03"N, 2° 9'50.76"E). The building is on the campus of the Universitat Autònoma de Barcelona, which lies 10 km northwest of Barcelona (Catalonia, Spain). Although the campus is located in a highly populated area between two highways with high volumes of traffic, the university's facilities are surrounded by a forested landscape (Fig. 2). The i-RTG Lab (Fig. 3) is the case study of the "Fertilecity" project (1). Overall, it covers an area of 125 m^2, and part of this area (84,34 m^2) supports a crop including 171 tomato plants grown in a perlite media. The winter planting was carried out on the 15th of September 2016, and the growing season lasted 167 days. The winter crop was planted on the 8th of March, and the growing season was 139 days. The purpose of building the i-RTG Lab was to demonstrate the feasibility of RTGs in Mediterranean urban zones and the potential for their symbiosis with buildings to optimize the efficiency of both the buildings and the greenhouses efficiency (including heating using residual hot air from the RTG, using the increased CO_2 concentration in the building air as a fertilizer in the RTG, and by collecting rainwater from the rooftop for the irrigation system). The water and the nutrient solution needed by the plants were provided through a drip fertirrigation system. Five control schedules administer heating, cooling and window openings to optimize energy use. Each control schedule adjusts the greenhouse control system to adapt it to seasonal temperature requirements (Nadal et al., 2017). Three different ventilation modes were applied in the i-RTG during this study: (1) the winter mode was employed from the 24th of February to the 31st of March 2016 (N days=37), (2) the spring mode was employed from the 1st of March to the 31st of May 2016 (N days=60) and (3) the summer mode was employed from the 1st of June to the 28th of July 2016 (N days=60); the highest ventilation rate occurred during the summer mode and the lowest occurred during the winter mode.

Figure 3. Probe locations within the i-RTG and in the surrounding area.



Airborne pollen and fungal spore sampling

Simultaneous daily i-RTG indoor and outdoor aerobiological monitoring was conducted to test the air quality. The methodology used is standard for outdoor measurements (Hirst, 1952) and represents an innovation in the study of air quality in greenhouses.

According to the Spanish Aerobiology Network working protocol (Galán et al., 2007), the outdoor aerobiological sampler was placed on top of the UAB-C building at 30 m.a.s.l. The indoor sampler was located in the i-RTG Lab, which is 150 m away from the C building.

Samples were obtained using volumetric suction pollen-spore traps based on the impact principle (Hirst, 1952), which is the standard method in European aerobiological networks (Galán et al., 2014). The Hirst sampler is calibrated to handle a continuous flow of 10 L of air per minute, thus matching the human breathing rate. Pollen and spores impact a cylindrical drum covered with Melinex film (Melinex tape, LANZONI s.r.l., Bologna, IT) film and coated with a 2% silicone solution as the trapping surface. The drum is located over a mechanism that causes it to rotate clock at a speed of 2 mm/hour. The exposed tape surface sample was changed weekly at a known time and was cut into one piece per day (0 to 24 h GMT), which was mounted on microscopic slides. Pollen and fungal spore identification was performed by technicians specializing in palynology using a light microscope at 600X magnification, based on morphological characteristics and counting. Daily average pollen concentrations were calculated following the standardized Spanish method (Galán et al., 2007), which consists of analysing

four continuous longitudinal sweeps. Spores were analysed along one continuous longitudinal sweep. Daily pollen and fungal spore concentrations were expressed as the number of pollen grains and spores per cubic metre of air. Finally, the sum and maximum of daily pollen/fungal spore concentrations were used to calculate the indoor/outdoor ratio (hereafter i/o_{sum} ratio and i/o_{max} ratio, respectively).

Study period

The sampling period extended from the 10th of April 2016 to the 28th of July 2016, but the sample analyses were divided into two phases, depending on the intensity of the analyses. Phase I (which extended from the 24th of February to the 10th of April 2016), which coincided with the last month of the winter crop and the initiation of the leaf growth, flowering and fruiting stages of the summer crop was conducted, considering both pollen and fungal spores. Phase II (which extended from the 11th of April to the 28th of July 2016), which included the entire summer crop cycle, was mainly devoted to fungal spores (during the entire period) and to Solanaceae pollen (during the removal of the summer crop, which lasted from the 20th to the 28th of July 2016). This period coincides with the hottest months, when the connection between the greenhouse and the outside is more pronounced. As a result, our study also evaluated the aerobiological air quality in the RTG during the critical period characterized by the greatest degree of outdoor influence.

Indoor and outdoor environmental variables

Indoor temperature and relative humidity were monitored in the i-RTG during the sampling period to determine the effects of environmental conditions on the pollen and fungal spore dynamics. TwoCampbell SCI CS215-Lsensors were used to record the temperature and relative humidity inside the greenhouse. To collect data from the Campbell devices, a CR3000 data-logger was used. The i-RTG Lab has a mechanism to open and close the ventilation system depending on internal and external temperatures, allowing passive acclimatization and ventilation.

The possible influence of plant and fungi phenological stages, agricultural pest incidence, crop treatments and greenhouse maintenance services on indoor aerobiological data were also taken into account as indoor variables. After the growing season, the plants were removed, the long plant stems and support strings were cut down, and they were then bundled together in the aisles. Thereafter, the plants were packaged in industrial garbage bags and transported outside.

Outdoor daily mean temperature, relative humidity, precipitation and wind speed were obtained from the Sant Cugat del Vallès meteorological station (41°28'59.2"N 2°04'46.4"E) belonging to the Catalan Meteorological Service, which was located near the outdoor pollen-spore trap, were used as outdoor variables.

Statistical analysis

Spearman's non-parametric correlation test has been used to explore the relationships between: (1) indoor (inside the i-RTG) *vs.* outdoor daily pollen and fungal spore concentrations; (2) all the indoor and outdoor environmental variables between them; (3) the indoor aerobiological data *vs.* the indoor variables; and (4) the outdoor aerobiological data *vs.* the outdoor meteorological variables. Spearman's rank correlation coefficients (rho) were considered to be

significant when the p-values were <0.05. The statistical analyses were performed using Statistica™.

RESULTS

Airborne pollen diversity and dynamics

In the first part of this paper, we discuss the results of daily airborne pollen concentrations registered indoors and outdoors during phase I, which extended from the 24th of February to the 10th of April 2016. Considering the whole dataset (n=47 daily samples), 47 pollen types were identified. 33 pollen taxa were identified in the indoor environment, and 45 were identified outdoors (see Table 1, Fig. 4). Taxa that were represented by more than 10 indoor pollen grains represented 98% of the total indoor pollen and 97% of the total outdoor. According to this criterion, 17 pollen taxa were selected to study the indoor-outdoor relationship in detail: *Acer*, *Alnus*, Cupressaceae, *Fraxinus*, *Morus*, *Pinus*, *Platanus*, *Populus*, *Quercus* deciduous type, *Quercus* evergreen type, *Salix*, *Ulmus*, *Coriaria*, *Corylus*, *Mercurialis*, Urticaceae and Solanaceae.

Figure 4. Daily Solanaceae airborne pollen concentrations recorded in the i-RTG during phase I of this study (which extended from the 24th of February to the 10th of April 2016) and the last stage of the summer tomato crop (which extended from the 20th of July to the 28th of July 2016) in phase II. The initiation of the leaf growth, flowering and fruiting stages are shown. Crop removal (CR) stages are highlighted for both the winter and summer tomato crops.

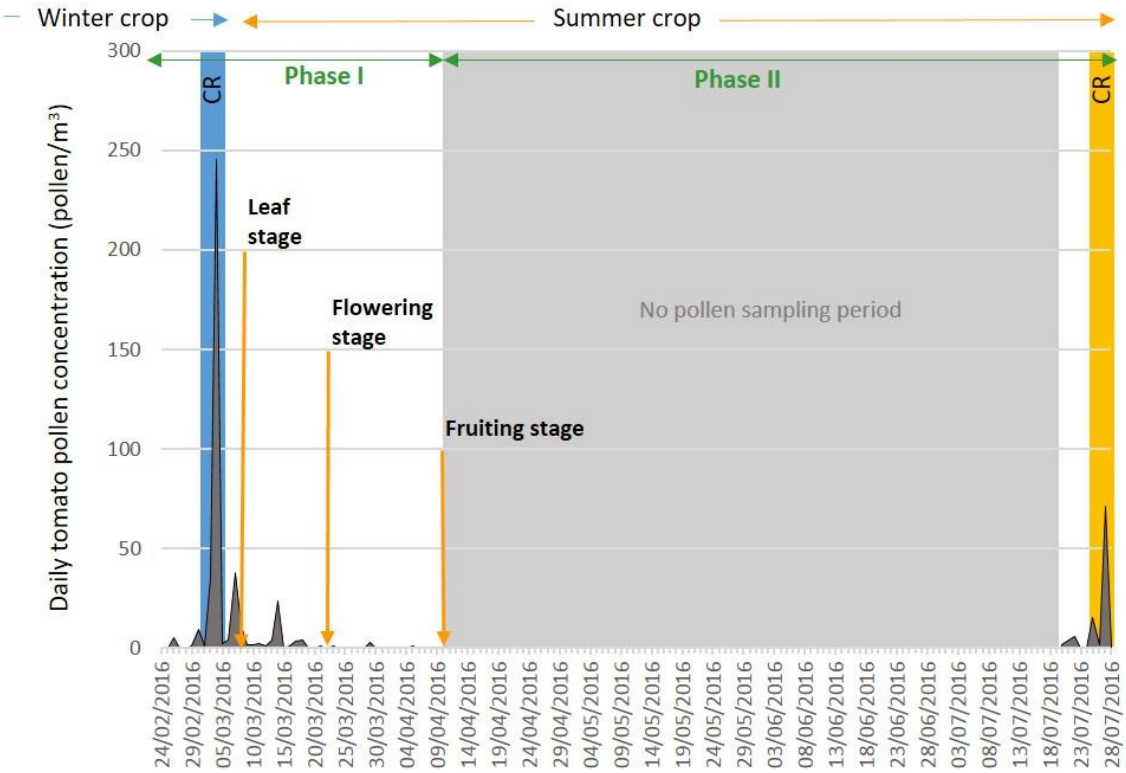


Table 1 shows an overview of the results for the selected pollen taxa and the relationship between the concentrations indoors, in the i-RTG, and the outdoor concentrations. A total of 4,924 pollen grains were counted and identified in the indoor environment versus 17,132 outdoors. The maximum daily concentrations were 334 pollen grains/m^3, which was observed

317 indoors on the 4th of March 2016, and 932 grains/m^3, which was observed outdoors on the 27th
318 of March 2016 (Table 1). The most abundant pollen taxon in both environments was *Platanus*,
319 the pollen sum of which accounted for 1,751 pollen grains indoors and 5,051 outdoors, and its
320 maximum daily concentration was 179 pollen grains/m^3, which was observed indoors on the
321 30th of March 2016 and 460, which was observed outdoors on the 27th of March 2016 (Table 1).
322 The second taxon was *Pinus*, the pollen sum of which ranged between 1,095 and 4,526 pollen
323 grains, and the maximum daily concentrations were 98 pollen grains/m^3, which was observed
324 indoors on the 28th of March 2016, and 319, which was observed outdoors on the 5th of March
325 2016 (Table 1). *Platanus* and *Pinus* accounted for 56 and 58% of the total pollen in both the
326 indoor and outdoor environments, respectively.

327 Solanaceae is the only pollen taxon that was detected exclusively in the indoor environment.
328 Solanaceae was the third most abundant taxon in the indoor environment, with a total of 399
329 pollen grains (8% of indoor total pollen) and a peak concentration of 246 pollen grains/m^3 was
330 observed on the 4th of March 2016 when winter crop removal was carried out (Fig. 4). This
331 concentration was the highest daily pollen concentration recorded in the i-RTG (Table 1). Two
332 other peak concentrations of Solanaceae pollen were observed between the winter crop removal
333 stage and the initiation of the flowering stage, which accounted for 38 pollen grains/m^3 on the
334 7th of March 2016 and 24 pollen grains/m^3 on the 14th of March 2016. Finally, high
335 concentrations of Solanaceae pollen were also measured on the 27th of July 2016 during the
336 summer crop removal, accounting for 71 pollen grains/m^3 (Fig. 4). The i/o ratio and Spearman's
337 rank correlation analysis were not applicable.

338 The i/o_{sum} ratio was 0.11 for total pollen during the phase I sampling period. This quantity
339 ranged between 0.02 and 0.17 for the different pollen taxa, whereas the i/o_{max} ratio was 0.36 and
340 ranged between 0.04 and 1.07. *Ulmus* was the only taxon with an i/o_{max} ratio > 1 (Table 1).
341 Positive and significant correlations between the indoor and outdoor concentrations were
342 observed in 14 cases out of 16 (Table 1).

343

344

345 **Table 1.** Spectra of pollen taxa: (1) mean, maximum and sum of daily pollen concentrations
346 (pollen grains/m^3) recorded in both indoor and outdoor environments from the 24th of February
347 to the 10th of April 2016 (N days = 47); (2) indoor/outdoor (i/o) ratio of using the maximum

Pollen taxa		**Indoor**			**Outdoor**			**i/o ratio**		**Spearman's rank correlation**
		Mean	**Max**	**Sum**	**Mean**	**Max**	**Sum**	**i/o_{max}**	**i/o_{sum}**	
Trees	*Acer*	1.4	16.8	65.1	3.0	26.6	140.0	0.63	0.05	0.76*
	Alnus	0.3	2.1	14.0	1.4	14.0	65.1	0.15	0.02	0.44*
	Cupressaceae	15.1	102.9	711.2	56.0	346.5	2,632.7	0.30	0.04	0.66*
	Fraxinus	0.3	2.8	15.4	1.4	7.7	65.8	0.36	0.04	0.33*
	Morus	3.5	23.1	162.4	16.1	98.0	755.3	0.24	0.04	0.78*
	Pinus	23.3	98.0	1,094.8	96.3	319.2	4,526.2	0.31	0.07	0.61*
	Platanus	37.3	179.2	1,751.4	107.5	459.9	5,050.5	0.39	0.08	0.88*
	Populus	5.3	25.9	249.2	44.9	217.7	2,111.9	0.12	0.02	0.46*
	Quercus deciduous type	2.1	18.9	100.8	14.3	128.1	672.0	0.15	0.02	0.78*
	Quercus evergreen type	0.2	3.5	10.5	0.5	7.0	22.4	0.50	0.03	0.25
	Salix	0.7	7.0	33.6	1.0	9.1	48.3	0.77	0.08	0.32*
	Ulmus	1.4	11.2	65.8	2.7	10.5	128.8	1.07	0.13	0.50*

348 (i/o_{max}) and the total (i/o_{sum}) values; and (3) Spearman's rank correlation coefficient (rho) values
349 between the indoor and outdoor daily pollen concentrations.

Shrubs	*Coriaria*	0.2	2.1	11.2	0.7	5.6	32.2	0.38	0.04	0.42*
	Corylus	0.4	2.8	18.2	1.6	9.1	77.0	0.31	0.04	0.26
Herbaceous plants	*Mercurialis*	0.7	3.5	30.8	2.2	7.7	102.2	0.45	0.09	0.32*
	Urticaceae	2.3	14.7	107.1	4.5	16.1	213.5	0.91	0.14	0.46*
	Solanaceae	8.5	245.7	399.0	0.0	0.0	0.0	-	-	-
Other pollen taxa[1]		1.8	6.3	83.3	10.4	154.7	488.6	0.04	0.17	0.37*
Total pollen		**104.8**	**333.9**	**4,923.8**	**364.5**	**931.7**	**17,132.5**	**0.36**	**0.11**	**0.65***

350 [1]less than 10 pollen grains during the sampling period *p-value<0.05

351

352

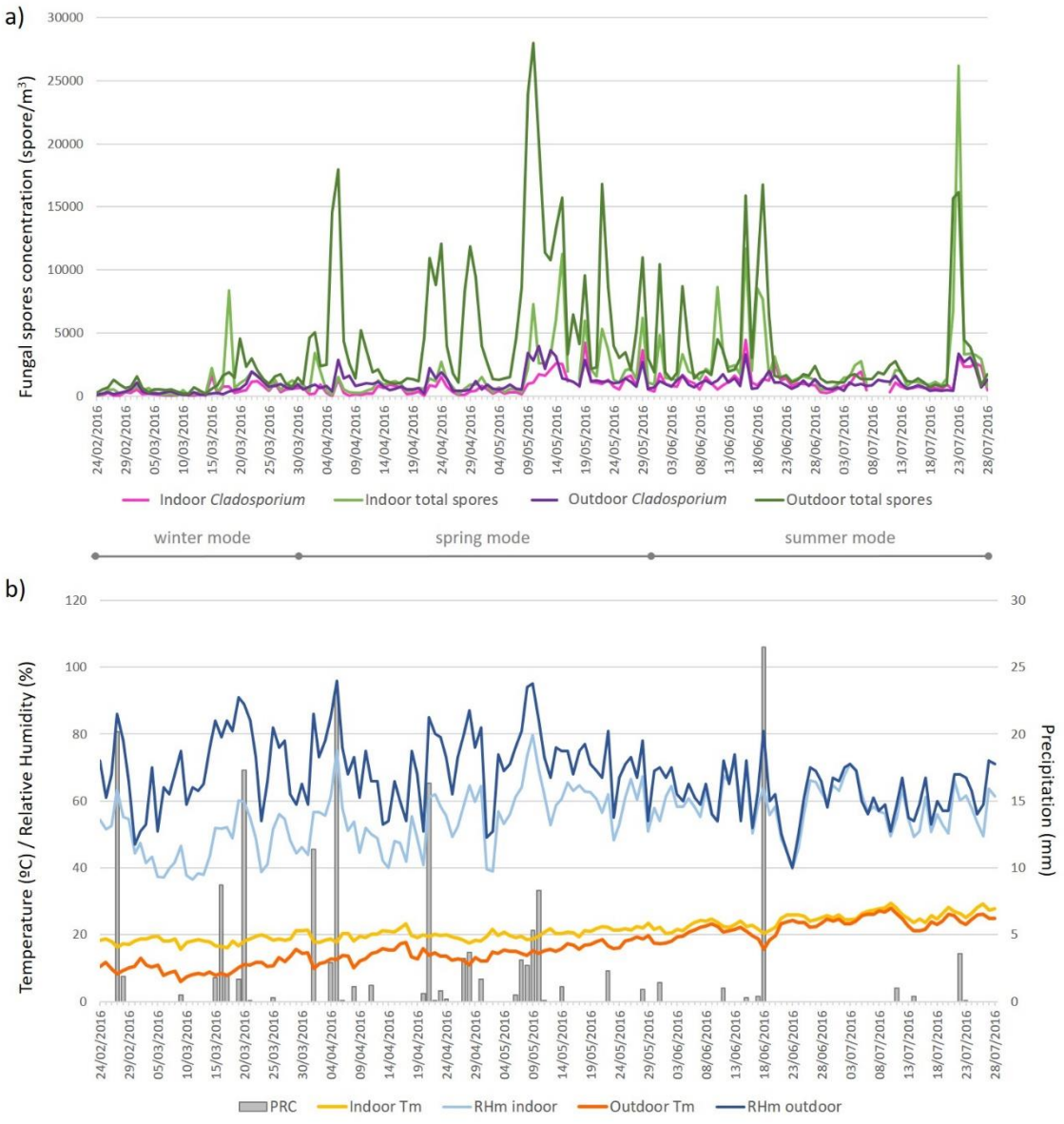
353 **Airborne fungal spore diversity and dynamics**

354 Fungal spore dynamics recorded during the whole sampling period (phases I and II, which
355 extended from the 24th of February to the 28th of July 2016) are analysed in detail in the second
356 part of this paper. The diversity of fungal spores observed included 29 taxa in the indoor
357 environment versus 31 outdoors. Taxa that accounted for more than 10 indoor fungal spores
358 during the study period accounted for 99.9% of the total spores in both environments.
359 Considering this threshold, 26 fungal spore taxa were selected: *Agaricus, Agrocybe, Alternaria,*
360 *Arthrinium, Aspergillus/Penicillium, Chaetomium, Cladosporium,* Coprinaceae,
361 *Drechslera/Helminthosporium, Epicoccum, Ganoderma, Leptosphaeria,* Myxomycota, *Oidium,*
362 *Pithomyces, Pleospora, Polythrincium, Stemphyllium,* Thelephoraceae, *Torula, Ustilago,*
363 Xylariaceae, other ascospores (unicellular, bicellular, pluricellular) and other basidiospores.

364 A total of 295,039 fungal spores were identified from the indoor environment and 606,642 from
365 the outdoor environment were identified during the study period (Table 2). The maximum daily
366 concentration of total fungal spores observed indoors was 26,186 spores/m^3 on the 27th of July
367 2016 during the summer ventilation mode, and 28,000 spores/m^3 was observed outdoors on the
368 10th of May 2016 during the spring mode (Table 2, Fig. 5a). The most abundant fungal spore
369 taxa in the indoor environment (where the total was >10,000 spores and the maximum was >
370 3000 spores/m^3) were *Cladosporium* (129,234 spores), the group of other ascospores (15,277-
371 94,822 spores) and *Aspergillus/Penicillum* (10,055 spores); followed by *Ustilago,* Coprinaceae,
372 *Alternaria, Agrocybe* and *Oidium,* for which the total over the period ranged between 1,000 and
373 10,000 spores (Table 2). The totals of the rest of the taxa accounted for <1,000 spores during the
374 study period (Table 2).

375

Figure 5. Indoor (inside the i-RTG) and outdoor variations in: (a) daily fungal spore concentrations of *Cladosporium* and total spores; (b) daily precipitation and mean temperature and mean relative humidity, from the 24th of February to the 28th of July 2016. Ventilation modes are also indicated.



In the outdoor environment, more than 10,000 spores were recorded for *Cladosporium* (154,314 spores) and the group of other ascopores (33,396-327,827 spores) during the study period; however, *Aspergillus/Penicillum* accounted for 4,281 spores (Table 2). Excluding *Aspergillus/Penicillum*, fungal spore taxa that ranged between 1,000-10,000 spores outdoors were, in decreasing order, Coprinaceae, *Ustilago*, *Alternaria*, *Agrocybe*, *Ganoderma*, *Pleospora* and *Oidium* (Table 2). The totals of the rest of the taxa accounted for <1,000 spores during the study period (Table 2).

Based on the i/o ratios, the fungal spore taxa were classified into three groups: (1) i/o_{sum} and i/o_{max} ratios ≤1 were observed for *Agaricus*, *Agrocybe*, *Chaetomium*, Coprinaceae, *Epicoccum*, *Ganoderma*, *Leptosphaeria*, *Pleospora*, *Stemphyllum*, Thelephoraceae, Xylariaceae, other ascospores (unicellular, pluricellular), other basidiospores and total fungal spores during the

whole study period and the spring ventilation mode; (2) i/o_{sum} ratio ≤ 1 and i/o_{max} ratio >1 for *Alternaria, Arthrinium, Cladosporium, Oidium, Polythrincium, Ustilago*, the other bicelullar ascospores and the total fungal spores during the winter and summer ventilation modes; and (3) i/o_{sum} and i/o_{max} ratios >1 for *Aspergillus/Penicillum, Drechslera/Helminthosporium, Myxomycota, Pithomyces, Torula* and other basidiospores (Table 2). The case of *Aspergillus/Penicillum* should be highlighted; it showed an i/o_{max} ratio of 28, given that its maximum daily concentration indoors was 6986 spores/m^3 versus 252 outdoors (Table 2).

Positive and significant correlations were observed for 17 fungal spore taxa out of 26, as well as the total fungal spores for the whole period and the three ventilation modes (Table 2). The strongest correlations between data from indoors (inside the i-RTG) and outdoors (rho>0.60; p-value<0.05) were observed for *Alternaria, Cladosporium,* Coprinaceae, *Ustilago*, *Ganoderma* and other ascopores (bicellular, pluricellular), as well as for the total fungal spores during the winter and summer ventilation modes. The indoor and outdoor dynamics of the daily fungal spore concentrations of *Cladosporium* and total spores are shown in Fig. 5a. No correlations were observed between indoor and outdoor daily concentrations for 9 taxa. Note that the indoor and outdoor dynamics were independent for *Agaricus, Aspergillus/Penicillium, Chaetomium, Epicoccum*, Myxomycota, *Oidium*, *Pithomyces*, *Polythrincium* and *Torula.*

Table 2. Spectra of fungal spore taxa: (1) mean, maximum and sum of daily fungal spore concentrations (spores/m^3) recorded in both indoor (inside the i-RTG) and outdoor environments during the whole sampling period (from the 24th of February to the 28th of July 2016, N days = 152); (2) i/o ratio of the maximum (i/o_{max}) and the total (i/o_{sum}); and (3) Spearman's rank correlation coefficient (rho) between indoor and outdoor daily fungal spores concentrations. In addition, these data were calculated for total fungal spores during the periods corresponding to the different ventilation modes: winter (from the 24th of February to the 31st of March 2016, N days = 37), spring (from the 1st of March to the 31st of May 2016, N days = 60) and summer (from the 1st of June to the 28th of July 2016, N days = 60).

Fungal spore taxa	Indoor			Outdoor			i/o ratio		Spearman's rank correlation
	Mean	Max	Sum	Mean	Max	Sum	i/o_{max}	i/o_{sum}	
Agaricus	3.4	22.4	518.0	5.6	44.8	854.0	0.50	0.61	0.14
Agrocybe	7.3	47.6	1,106.0	16.2	81.2	2,464.0	0.59	0.45	0.46*
Alternaria	22.6	182.0	3,432.8	24.2	179.2	3,670.8	1.02	0.94	0.70*
Arthrinium	4.9	291.2	747.6	5.0	78.4	753.2	3.71	0.99	0.29*
Aspergillus/Penicillium	66.2	**6,986.0**	10,054.8	28.2	252.0	4,281.2	**27.72**	**2.35**	0.01
Chaetomium	0.1	2.8	14.0	0.3	2.8	39.2	1.00	0.36	-0.06
Cladosporium	850.2	4,449.2	129,234.0	1,015.2	3,956.4	154,313.6	1.12	0.84	0.69*
Coprinaceae	28.0	266.0	4,253.2	47.5	431.2	7218.4	0.62	0.59	0.64*
Drechslera/Helminthosporium	3.2	33.6	492.8	2.0	16.8	310.8	2.00	1.59	0.45*
Epicoccum	1.9	14.0	285.6	1.9	22.4	288.4	0.63	0.99	0.11
Ganoderma	6.2	50.4	935.2	11.6	67.2	1,755.6	0.75	0.53	0.64*
Leptosphaeria	0.7	11.2	106.4	2.3	78.4	347.2	0.14	0.31	0.24*
Myxomycota	0.1	14.0	16.8	0.1	11.2	14.0	1.25	1.20	-0.01
Oidium	7.1	89.6	1,080.8	7.2	42.0	1,100.4	2.13	0.98	0.15
Pithomyces	0.1	2.8	14.0	0.1	2.8	11.2	1.00	1.25	-0.03
Pleospora	3.9	89.6	588.0	7.3	92.4	1,106.0	0.97	0.53	0.36*
Polythrincium	0.4	11.2	61.6	0.4	5.6	64.4	2.00	0.96	0.01
Stemphyllium	1.9	25.2	291.2	2.4	25.2	358.4	1.00	0.81	0.26*
Thelephoraceae	1.7	14.0	263.2	3.7	33.6	565.6	0.42	0.47	0.40*
Torula	1.9	39.2	294.0	1.8	53.2	266.0	0.74	1.11	0.13
Ustilago	35.8	450.8	5,437.6	44.9	229.6	6,820.8	1.96	0.80	0.66*

Xylariaceae	3.8	25.2	582.4	4.7	39.2	714.0	0.64	0.82	0.44*
Other unicellular ascospores	623.8	13,834.8	94,822.0	2,156.8	19,107.2	327,826.8	0.72	0.29	0.41*
Other bicellular ascospores	100.5	5,474.0	15,276.8	219.7	3,494.4	33,395.6	1.57	0.46	0.62*
Other pluricellular ascospores	157.9	3,245.2	24,007.2	375.4	5,684.0	57,061.2	0.57	0.42	0.74*
Other basidiospores	5.4	64.4	817.6	4.8	30.8	725.2	2.09	1.13	0.39*
Other fungal spores taxa[1]	0.1	2.8	16.8	0.4	14.0	53.2	0.20	0.32	0.07
Total fungal spores	**1,941.0**	**26,185.6**	**295,038.8**	**3,991.1**	**28,000.0**	**606,642.4**	**0.94**	**0.49**	**0.58***
Total fungal spores - winter	874.0	8,388.8	32,337.2	1,085.8	4,558.4	40,174.4	1.84	0.80	0.75*
Total fungal spores - spring	1,767.1	11,244.8	106,027.6	6,311.2	28,000.0	378,669.2	0.40	0.28	0.51*
Total fungal spores - summer	2,848.6	26,185.6	156,674.0	3,414.5	16,744.0	187,798.8	1.56	0.83	0.73*

[1]less than 10 fungal spores during the sampling period *p-value <0.05

Effects of environmental variables on airborne fungal spore levels

Strong significant positive correlations were observed between indoor *vs.* outdoor temperatures (rho=0.96, p-value<0.05) and relative humidity (rho=0.55, p-value <0.05) (Table 3). However, temperature values were higher indoors, in the i-RTG, than outdoors, and relative humidity was higher outdoors (Fig. 2b). Temperature and relative humidity indoors (inside the i-RTG) showed a weak significant positive correlation (rho=0.21, p-value <0.05), however, a stronger significant negative correlation was observed outdoors (rho=-0.43; p-value <0.05) (Table 3, Fig. 5b). Outdoor relative humidity was also strongly positively correlated with precipitation (rho=-0.59, p-value<0.05; Fig. 5b) and negatively with wind speed (rho=-0.59; p-value <0.05) (Table 3). In the same way, precipitation also showed weak significant negative relationships with outdoor temperature (rho=-0.29; p-value <0.05) and wind speed (rho=-0.33; p-value <0.05) (Table 3).

Table 3. Spearman's rank correlations between indoor and outdoor environmental daily data from the 24th of February to the 28th of July 2016 (N days = 152): mean temperature (T_m), mean relative humidity (RH_m), precipitation (PRC) and mean wind speed (WS_m).

Spearman's rank correlations		**Indoor variables**		**Outdoor variables**			
		T_m (°C)	**RH_m** (%)	**T_m** (°C)	**RH_m** (%)	**P** (mm)	**WS_m**(m/s)
Indoor variables	**T_m** (°C)	1.00	0.21*	0.96*	-0.52*	-0.36*	0.22*
	RH_m (%)		1.00	0.37*	0.55*	0.31*	-0.36*
Outdoor variables	**T_m** (°C)			1.00	-0.43*	-0.29*	0.14
	RH_m (%)				1.00	0.59*	-0.59*
	P (mm)					1.00	-0.33*
	WS (m/s)						1.00

*p-value<0.05

The relationship between fungal spore concentrations and environmental variables was explored in Table 4. Indoors (inside the i-RTG), daily concentrations of the total fungal spores and 11 out of 26 taxa were positively and significantly correlated with both indoor temperature and relative humidity. These 11 taxa were *Agaricus*, *Alternaria*, *Cladosporium*, *Drechslera/Helminthosporium*, *Ganoderma*, *Pithomyces*, *Torula*, *Ustilago*, Xylariaceae, other ascospores (bicellular, pluricellular) and other basidiospores. *Arthrinium*, *Oidium*, *Epicoccum* and *Stemphyllium* were also positively correlated, but only with indoor temperature; Coprinaceae and *Leptosphaeria* were positively correlated exclusively with indoor relative humidity. The strongest correlations (rho≥0.50; p-value<0.05) were observed between the indoor temperature and *Alternaria*, *Ganoderma*, other basidiospores and total fungal spores.

Outdoor total fungal spores showed significant positive correlations with outdoor temperature, relative humidity and precipitation (Table 4). Depending on the fungal spore taxon, both positive and negative significant correlations were observed for each outdoor variable. Outdoor temperature was correlated with 16 fungal spore taxa. (1) It was positively correlated with *Agaricus*, *Alternaria*, *Arthrinium*, *Cladosporium*, *Drechslera/Helminthosporium*, *Epicoccum*, *Ganoderma*, *Oidium*, *Stemphyllium*, *Torula*, *Ustilago*, Xylariaceae, and other basidiospores; and (2) it was negatively correlated with *Agrocybe*, *Aspergillus/Penicillium* and *Pleospora*. Outdoor relative humidity was significantly correlated with 13 taxa. (1) It was positively correlated with *Agrocybe*, *Leptosphaeria*, *Pleospora* and other ascospores (unicellular, bicellular, pluricellular); and (2) it was negatively correlated with *Alternaria*, *Epicoccum*, *Ganoderma*, *Oidium*, *Stemphyllium*, *Ustilago* and other basidiospores. Precipitation showed a significant relationship with 10 outdoor taxa. (1) It was positively correlated with *Leptosphaeria*, *Pleospora* and other ascospores (unicellular, bicellular, pluricellular); and (2) it was negatively correlated with *Alternaria*, *Chaetomium*, *Epicoccum*, *Ganoderma*, and *Torula*. Finally, wind speed presented weak significant correlations with 4 taxa: (1) positively with *Alternaria* and *Epicoccum;* and (2) negatively with Xylariaceae and other unicellular ascospores. *Alternaria* and *Ganoderma* showed the strongest correlations with both indoor and outdoor temperature (rho ranged from 0.68 to 0.75; p-value<0.05). Strong correlations (with rho≥0.50 and p-value<0.05) were observed for outdoor temperature *vs. Alternaria*, *Ganoderma*, *Ustilago* and other basidiospores, as well as for precipitation *vs.* other ascospores (bicellular, pluricellular).

Table 4. Spearman's rank correlations (rho) between daily: (1) indoor (inside the i-RTG) fungal spore concentrations (spore/m^3) *vs.* indoor mean temperature (T_m) and mean relative humidity (RH_m); and (2) outdoor fungal spore concentrations (spore/m^3) *vs.* outdoor T_m, RH_m, precipitation (P) and mean wind speed (WS_m) from the 24th of February to the 10th of April 2016 (N days = 152).

Spearman's rank correlations	**Indoor variables**		**Outdoor variables**			
Fungal spore taxa	**T_m** (°C)	**RH_m** (%)	**T_m** (°C)	**RH_m** (%)	**P** (mm)	**WS** (m/s)
Agaricus	0.21*	0.29*	0.18*	0.05	-0.02	-0.06
Agrocybe	0.01	0.28*	-0.18*	0.33*	0.12	-0.15
Alternaria	0.69*	0.27*	0.68*	-0.35*	-0.25*	0.25*
Arthrinium	0.34*	0.14	0.26*	-0.10	-0.15	0.01
Aspergillus/Penicillium	0.05	0.11	-0.19*	0.09	0.00	-0.04

Chaetomium	0.13	0.02	0.00	-0.15	-0.19*	0.11
Cladosporium	0.56*	0.25*	0.43*	-0.08	-0.08	0.13
Coprinaceae	0.13	0.24*	0.08	0.16	-0.01	-0.09
Drechslera/Helminthosporium	0.44*	0.23*	0.41*	-0.09	-0.09	0.05
Epicoccum	0.33*	0.07	0.27*	-0.16*	-0.23*	0.20*
Ganoderma	0.75*	0.34*	0.72*	-0.25*	-0.20*	-0.03
Leptosphaeria	0.03	0.27*	-0.14	0.31*	0.42*	-0.11
Myxomycota	0.15	0.04	0.02	0.01	0.05	-0.16
Oidium	0.30*	0.04	0.20*	-0.18*	-0.08	0.09
Pithomyces	0.24*	0.17*	-0.01	0.00	-0.02	0.06
Pleospora	-0.07	0.10	-0.20*	0.19*	0.47*	0.08
Polythrincium	0.05	0.11	-0.02	0.10	-0.04	-0.11
Stemphyllium	0.39*	0.03	0.24*	-0.16*	-0.10	0.08
Thelephoraceae	-0.05	0.07	-0.15	0.13	-0.11	0.03
Torula	0.39*	0.18*	0.22*	-0.15	-0.18*	0.00
Ustilago	0.39*	0.19*	0.57*	-0.25*	-0.11	0.14
Xylariaceae	0.49*	0.42*	0.25*	0.09	0.03	-0.19*
Other unicellular ascospores	0.17*	-0.03	-0.01	0.42*	0.41*	-0.19*
Other bicellular ascospores	0.25*	0.27*	0.08	0.39*	0.51*	-0.14
Other pluricellular ascospores	0.31*	0.47*	0.12	0.37*	0.50*	-0.13
Other basidiospores	0.53*	0.25*	0.53*	-0.26*	-0.18	0.15
Total fungal spores	**0.54***	**0.27***	**0.18***	**0.30***	**0.32***	**-0.13**

*p-value<0.05

Relationship between fungal diseases in the tomato crop and airborne fungal spores

Fig. 6 shows *Oidium* and *Torula* airborne spore dynamics recorded in the i-RTG during the study period. As expected, due to the fact that these fungal spore taxa are related to fungal diseases of tomato crops, indoor concentrations showed no relationship with concentrations outside of the greenhouse (Table 2). For this reason, information about the summer and winter cultivation periods were indicated in Fig. 6, as well as the initiation of leaf, flowering and fruiting stages, the winter and summer crop removal stages and some crop management practices. During the summer cultivation period, a total of 30 sweeps were made, and 29 pollinations were performed by hand (Fig. 6). Additionally, between the 18th of April and the 17th of June, 14 organic pesticide treatments were performed, using wettable sulphur and Costar (80% *Bacillus thuringiensis*) (Fig. 6). However, it was not possible to eradicate the pest, and the treatments were only effective to avoid its expansion.

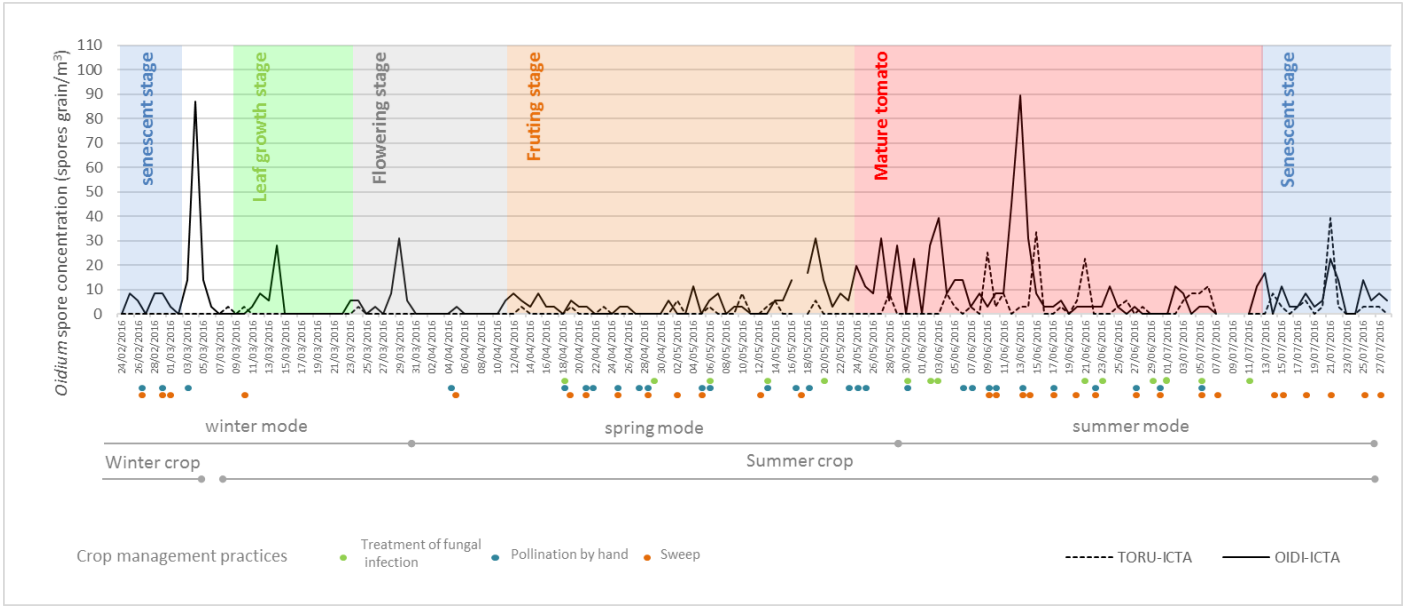
Overall, the number of fungal spores observed was higher for *Oidium* than *Torula*. Both fungal spore taxa showed similar behaviour during the summer cultivation period (Fig. 6). A total of 1,081 and 294 spores, respectively, were counted and identified in the indoor environment (Table 2). Most of the peaks occurred during the harvest period (Fig. 6).

Oidium was one of the most abundant taxa in the indoor environment (>1,000 spores, Table 2), and accounted for the highest indoor daily spore concentration on the 4th of March 2016 at the

time that the winter crop was removed, with 86.8 spores/m^3 (Fig. 6). In addition to the first peak of *Oidium* spore concentration, several episodes of peak concentrations have been detected during the leaf growth stage and the initiation of the flowering stage, accounting for 28.0 spores/m^3 on the 14th of March 2016 and 30.8 spores/m^3 on the 29th of March 2016. Additionally, high concentrations of *Oidium* spores were registered on the 19th, 24th, 27th, 29th and 31st of May and the 2nd of June 2016 during the tomato harvesting period, and these concentrations ranged between 19.6 and 39.2 spores/m^3. Finally, the highest daily concentration of *Oidium* was detected on the 13th of June 2016 during the summer crop, when 89.6 spores/m^3 were observed (Fig. 6). This date coincides with a day when pollination was done by hand and a sweep was done.

Regarding *Torula*, its highest spore concentration was measured on the 21st of July 2016, with 39.2 spores/m^3 (Fig. 6). Moreover, three more peaks of 25.2, 33.6 and 22.4 spores/m^3 were measured on the 9th, 15th and 21st of June 2016, all of them during the harvesting season (Fig. 6).

Figure 6. *Torula* and *Oidium* airborne fungal spore concentrations recorded in the i-RTG from the 24th of February to the 28th of April 2016. Summer and winter cultivation periods are shown, as well as the initiation of leaf growth, flowering and fruiting stages, the winter and summer crop removal (CR) stages and the three different ventilation modes. The visual detection of fungal infection by *Oidium* and the corresponding crop treatment are also indicated.



Discussion

The construction of RTGs on urban buildings has intensified in recent years, as a result of growing interest in the development of new agricultural spaces and in the promotion of food self-sufficiency in urban areas (Sanyé-Mengual et al., 2015). In greenhouses, the growth conditions for plants are optimized to increase crop yields (Hansen et al., 2010). However, indoor handling of large amounts of plant materials occurs in different occupational settings, including greenhouses, and causes exposure to bioaerosols (Madsen et al., 2014). As indoor air

is highly dynamic, studies have been carried out to examine indoor air quality, especially for occupational and public health purposes (Bonetta et al., 2010). Adequate assessment of human exposure to biological aerosols has been recognized as an important need, as many studies have linked adverse health effects to bioaerosol hazards (Burge and Rogers, 2000; Douwes et al., 2003). Fungal spores and pollen grains are the major components of airborne biological contaminants (Nayar and Jothish, 2013). They have the potential to trigger the risk of occupational respiratory diseases, such as allergic rhinitis, asthma, allergic alveolitis, dermatitis, hypersensitivity pneumonitis and bronchopulmonary aspergillosis (Cariñanos et al., 2004; Kim et al., 2007). In this study, we investigated the exposure to airborne pollen and fungal spores in an i-RTG in order to assess the biological air quality and to advise on preventive measures for workers and for users of the building in case the air is used to heat the building. It is appropriate to note that this is the first study that evaluates the diversity and dynamics of bioaerosols in building-integrated agriculture.

The pollen spectra collected both inside the i-RTG and in the outdoor environments are representative of the vegetation of the study area, in which *Platanus* and *Pinus* are the most abundant pollen taxa (Table 1). According to previous studies (Burge and Rogers, 2000; Lee et al., 2006; Tormo-Molina et al., 2009), the i/o ratios <1 and the significant positive correlations observed between indoor and outdoor pollen concentrations for most taxa (Table 1) indicate that the indoor pollen concentrations inside the i-RTG are controlled by those outdoors. Outdoor pollen entered the building through ventilation openings, except for Solanaceae pollen, which was directly introduced into the i-RTG via tomato plants. The level of pollen penetration depends on the aerobiological characteristics of the pollen and their pollination period (Pichot et al., 2015), as well as the distance to the ventilation opening (Jantunen and Saarinen, 2009). It has been recommended that allergic people reduce indoor exposure to pollen by keeping windows and doors closed during the peak flowering season, or by using appropriate air filters in ventilation windows and ducts (Jantunen and Saarinen, 2009). These measures could also be applied to i-RTG air that is to be recirculated into the building.

However, the recirculation of air from the i-RTG to other parts of the building is planned to occur during the winter, when the estimated pollen penetration was one hundred times lower than that in summer (Pichot et al., 2015). This fact is related to the increase of pollen penetration coefficients from winter to summer, which strongly supports the hypothesis that the opening of doors and windows facilitates the penetration of pollen into dwellings (Pichot et al., 2015). Moreover, outdoor pollen concentrations are lower in winter. Therefore, we focused our attention on indoor Solanaceae pollen concentrations. In particular, we considered that immunoglobulin E against tomato pollen has been detected in greenhouse workers (Toorenenbergen and Wijk, 2000). The highest Solanaceae daily pollen concentrations were recorded during both the winter and summer crop removal stages (Fig. 4). Consequently, our recommendation is that i-RTG air should not be recirculated during crop removal unless appropriate air filters in ventilation ducts are installed.

Nearly 10% of people worldwide have a fungal allergy (Burge, 2001), in which the exposure to fungi is associated with rhinitis, asthma, allergic bronchopulmonary mycoses, allergic fungal sinusitis and hypersensitivity pneumonitis (Baxi et al., 2016). Although an official threshold for fungal spore concentrations necessary to evoke allergenic symptoms has been not established, the highest indoor daily concentration of total fungal spores observed in the i-RTG (26185 spores/m^3, Table 2) was lower than the concentration of 10^5 spores/m^3 proposed by (Eduard,

2009), but much higher than the concentration of 10^3 spores/m^3 recommended by (Santilli and Rockwell, 2003b).

In this study, the most prevalent i-RTG indoor fungal spore taxa were, in decreasing order, *Cladosporium* (Fig. 5a), the group of other ascopores, *Aspergillus/Penicillum*, *Ustilago*, Coprinaceae, *Alternaria*, *Agrocybe* and *Oidium* (>1,000 spores, Table 2), of which *Cladosporium*, *Aspergillus/Penicillium*, *Ustilago* and *Alternaria* are classified as allergenic fungi (Levetin et al., 2016). *Cladosporium* and *Aspergillus/Penicillium* were also identified as predominant fungal species in other aeromycological studies carried out in other greenhouse environments (Hansen et al., 2010; Li and Lamondia, 2010; Magyar et al., 2011). In addition, sensitization to *Cladosporium*, *Penicillium*, *Aspergillus*, and/or *Alternaria* has been reported in nearly 20% of greenhouse flower growers (Monsó et al., 2002). In this context, the daily maximum concentration of *Cladosporium* (4,449 spores/m^3) and *Alternaria* (182 spores/m^3) in the i-RTG exceeded both the fungal spore concentration thresholds recommended by Gravesen (1979), namely 3,000 and 100 spores/m^3, respectively. The highest indoor concentration of *Aspergillus/Penicillum* was 6,986 spores/m^3 (Table 2). Although its threshold is unavailable, this value is certainly able to provoke respiratory symptoms if we compare it with the much lower outdoor values, which are known to cause problems for the population at large.

On the other hand, *Cladosporium* was identified as the most abundant outdoor fungal spore taxon (Table 2, Fig. 5a), as has been reported in previous studies performed in Catalonia (Vélez-Pereira et al., 2016) and at other Iberian Peninsula locations (Oliveira et al., 2009; Recio et al., 2012; Reyes et al., 2016). Also, according to the outdoor fungal spore spectrum (Table 2), the airborne concentrations of *Aspergillus/Penicillum*, Coprinaceae, *Ustilago*, *Alternaria* and *Pleospora* were also notable in the study area, and have been noted by the previously cited authors at other Spanish localities (Herrero et al., 2006; Sabariego et al., 2000). For allergic people, indoor exposure to pollen and fungal spores is of particular concern (Nayar and Jothish, 2013). The i/o_{sum} ratio showed that the outdoor concentrations were approximately double those of indoor areas in terms of total fungal spores (Table 2), and the outdoor concentrations were more than triple the indoor concentrations for pollen (Table 1). These results suggest that fungal spores have much higher penetration efficiency than pollen grains (Table 1), which reflects the difference in their particle sizes (Lee et al., 2006). In contrast, the fungal spore concentrations measured by stationary sampling in traditional heated tomato greenhouses reported higher values in indoor environments in Denmark (Hansen et al., 2010, 2012) and concentrations similar to those observed outdoors in Japan (Okushima et al., 2004). The i/o ratio for fungi observed in greenhouses where flowering plants are cultivated in the Midwestern USA in winter was also >1 (Adhikari et al., 2011). In this cases, the introduction of outside air into the greenhouses was recommended in order to dilute the indoor bioaerosol exposure (Madsen et al., 2014).

However, based on previous studies on indoor airborne fungi in buildings (Taekhee Lee et al., 2006b; Nevalainen et al., 2015), the i/o_{sum} ratios ≤ 1 and significant positive correlations registered for most of the fungal spore taxa in this study indicated that the most important source of fungi found indoors was the outdoor environment (Table 2). Within this group, the i/o_{max} ratio was ≤ 1 for *Agrocybe*, Coprinaceae, *Ganoderma*, *Leptosphaeria*, *Pleospora*, *Stemphyllum*, Thelephoraceae, Xylariaceae, other ascospores (unicellular, pluricellular), the total fungal spores during the whole period and during the spring ventilation mode (Table 2, Fig. 5a), which suggests that the indoor concentrations followed the outdoor concentrations,

given that the outdoor levels were greater than the indoor ones (e.g., total fungal spores, Fig. 5a). On the other hand, the i/o_{max} ratio was >1 for *Alternaria, Arthrinium, Cladosporium, Ustilago,* other bicelullar ascospores, and the total fungal spores during the winter and summer ventilation modes (Table 2). These results suggest that, although fungal exposure primarily occurred outdoors, fungi might have colonized the indoor environment under favourable growing conditions after entering the i-RTG through ventilation openings, even reaching the highest daily concentration in the indoor environment (e.g., *Cladosporium*, Fig. 5a). Since the air penetration from the outdoor environment while the winter ventilation mode was in operation was lower than for the other modes, highly favourable growing conditions in the i-RTG during the winter ventilation period could explain this result. Indoor sources also played a significant role in emitting fungal bioaerosols (Faridi et al., 2015) and displayed behaviour independent of that seen outdoors, as indicated by the (1) i/o_{sum} and i/o_{max} ratios >1 that were observed for *Aspergillus/Penicillum, Drechslera/Helminthosporium,* Myxomycota, *Pithomyces, Torula* and other basidiospores; and (2) the non-significant correlations obtained for *Agaricus, Aspergillus/Penicillium, Chaetomium, Epicoccum,* Myxomycota, *Oidium, Pithomyces, Polythrincium* and *Torula* (Table 2).

The most effective way to manage fungi in a building is to remove the conditions that favour the establishment and growth of fungi (Haleem Khan and Mohan Karuppayil, 2012). From the point of view of indoor air quality, prevention of any outdoor particles from entering the indoor environment is considered desirable and can be done most effectively by combining an optimized airflow together with filtration of outdoor air (Hänninen, O. and Asikainen, 2013). Regular cleaning and disinfection of heating, ventilating and air conditioning systems should be conducted to avoid the occurrence of air contamination (Liu et al., 2015). In this context, our attention was focused mainly on understanding the dynamics of the fungal spores identified as characteristic of indoor environments or capable of colonizing it.

Although indoor variables were controlled in the i-RTG in order to provide adequate conditions for growing crops, the results showed that indoor temperature and relative humidity also depended on outdoor environmental conditions, especially in the case of temperature (Table 3, Fig. 5b). Different patterns have been observed between fungal spore taxa *vs.* indoor and outdoor variables (Table 4). In the indoor environment, the daily concentrations of fungal spores were positively and significantly correlated with indoor temperature, relative humidity or both for the total of fungal spores and most taxa, except for *Aspergillus/Penicillium, Chaetomium,* Myxomycota, *Pleospora, Polythrincium* and Theleforaceae, which displayed no correlation (Table 3). Taking into account that *Aspergillus/Penicillium, Chaetomium,* Myxomycota and *Polythrincium* showed their own dynamics indoors, further research is needed to understand which variables determined the increases in fungal spores of these taxa, particularly *Aspergillus/Penicillium*, which is one of the most abundant fungal taxa in the i-RTG (Table 2).

In the outdoor environment, the correlations between the daily concentrations of fungal spores and the outdoor variables were variable, according to specific climatic requirements of each taxon. In general, the relationship with temperature was positive for the total of fungal spores and most of the fungal spore taxa; however, it was negative for *Agrocybe, Aspergillus/Penicillium* and *Pleospora* (Table 3). Fungal spores are commonly described as either "dry" or "wet," according to the type of weather associated with their occurrence in the air. Ascospores generally follow precipitation, whereas spore types such as *Alternaria* and *Cladosporium* are abundant in dry conditions (Troutt and Levetin, 2001). In keeping with the

wet/dry-spore type classification, *Alternaria, Chaetomium, Epicoccum, Ganoderma, Oidium, Stemphyllium, Torula, Ustilago* and other basidiospores probably belong to the dry-spore type, considering that they were negatively correlated with relative humidity and/or precipitation (Table 3). Otherwise, *Agrocybe, Leptosphaeria, Pleospora* and other ascospores (unicellular, bicellular, and pluricellular) were positively correlated with these variables and therefore correspond to the wet-spore type (Table 3). In addition, the total fungal spores were directly correlated with both relative humidity and precipitation (Table 3). This fact explains the substantial increases in the daily concentrations observed during and just after periods of rain (Fig. 5), which were largely produced by the massive proliferation of the group of other ascospores. Bearing in mind that the group of other ascospores was the second most abundant in both environments, in conjunction with the fact that the most important source of indoor spores was the outdoor environment (Table 2), our suggestion is to keep the ventilation openings in the i-RTG closed during rainy periods and for a few days after in order to avoid the entrance of fungal spores from the outdoors. Although the building under study has been programmed to close the ventilation openings during rainy periods, the time span of closure should be longer than just the time when the rain is falling, provided this measure does not affect crop development. This measure would enable us to avoid the increase of indoor fungal spore concentrations, because a smaller number of spores would be able to penetrate and colonize the indoor environment.

The behaviour of *Torula* and *Oidium* inside the i-RTG has been studied in detail, because these fungal spore taxa are related to fungal diseases of tomato crops (Fig. 6). Spores of both taxa were present in the greenhouses throughout the monitored period; however concentrations inside the i-RTG showed behaviour that was independent from that observed in the outside environment (Table 2). The results also suggest that agricultural management tasks, such as pollination by hand, fungal treatments and sweeps, increased the exposure to *Torula* and *Oidium* spores. Previous studies carried out in open-air field crops determined that powdery mildew spore (*Oidium*) release patterns are influenced by wind speed, rainfall, temperature, relative humidity and solar radiation (Byrne et al., 2000; Willocquet and Clerjeau, 1998; Willocquet et al., 1998). Conversely, the primary factor influencing the *Oidium* spore concentrations inside the i-RTG in this study was the indoor temperature (Table 3). Good temperature and humidity management are essential for minimizing disease, particularly for powdery mildew and *Botrytis*, in greenhouse crops (Gullino, 1992). Specifically, powdery mildew develops rapidly under dry conditions once infection occurs (Körner and Challa, 2003). This fact explains the development of *Oidium* disease in the tomato crop in our i-RTG (Fig. 6), which is characterized by higher ventilation and lower relative humidity than are typical in conventional greenhouses. Further research is needed to characterize the release patterns of the fungal spores in the case of the i-RTG, given the special internal environmental (climate) conditions demanded by the building requirements.

Generally, concentrations of fungal spores registered for both taxa (Fig. 6) were in accordance with (Hansen et al., 2011) who suggested that vegetable growers' exposure to mesophilic fungi was found to be highest in environments where tomato plants were being removed, followed by environments in which tomatoes were harvested. However, unlike (Hansen et al., 2011), it was observed in this research that harvesting from mature plants caused higher spore concentration levels than harvesting from senescent or older plants (Fig. 6). The results also suggest that, when plants dry out, the growth of *Oidium* and *Torula* stops (Fig. 6). However, an important fungal spore peak concentration was observed during the last days of the summer crop (Fig. 6).

Previous studies showed that cleaning the aisles after leaf nipping resulted in a significantly higher exposure to endotoxin (Madsen et al., 2014). In this study, days when a sweep was performed did not always reflect an increase in the concentrations of *Oidium* or *Torula* spores (Fig. 6). Finally, the results showed that fungicide applications used were useful to treat powdery mildew. However, in accordance with (Wspanialy and Moussa, 2016), the infection was not eliminated because the treatment started during the last stage of the fungal disease.

CONCLUSIONS

To our knowledge, indoor bioaerosols have not previously been measured in building-integrated agriculture facilities, and even less attention has been paid to urban rooftop greenhouses. In this study, elevated levels of pollen grains and fungal spores were observed in an i-RTG. These levels showed significant seasonal variations. A total of 4,924 pollen grains were observed inside the i-RTG during phase I of this study, which extended from the 24th of February to the 10th of April 2016, arriving at 334 pollen grains/m^3 per day, and a total of 295,038 fungal spores were observed during the whole sampling period, which extended from the 24th of February to the 28th of July 2016, reaching a maximum daily concentration of 26,185 spores/m^3. It is not possible to assess if these values are able to evoke allergenic symptoms, as an official threshold for fungal spore concentrations has not been established, and different authors have proposed different levels, such as 10^5 spores/m^3 (Eduard, 2009) or 10^3 spores/m^3 (Santilli and Rockwell 2003).

In this study, the most important source of indoor fungi was the outdoor environment (i/o_{sum} ratio showed that concentrations outdoors were approximately double those observed indoors). However, it should be noted that the sampling period coincided with the highest ventilation rates in the i-RTG. However, while the winter ventilation mode was in operation, the air penetration from the outdoor environment was lower than when the spring and summer modes were in operation. Consequently, the results suggested that highly favourable growing conditions occurred in the i-RTG during the winter. In the case of pollen dynamics, significant correlations were observed between indoor and outdoor pollen concentrations for most taxa. Our results suggest that fungal spores have a much higher penetration efficiency compared with pollen grains.

Solanaceae pollen and several fungal spore taxa, such the allergenic *Aspergillus/Penicillium*, were likely to have originated within the i-RTG, as indicated by the i/o ratios. Information on the qualitative and quantitative prevalence of airborne pollen grains and fungal spores is an important tool in the management of occupational allergic disorders in workers employed in the i-RTG and users of the building.

Meteorological conditions and agricultural management tasks that could cause high levels of pollen grains and fungal spores inside the i-RTG have also been identified. These results allow the identification of critical moments when the recirculation of residual i-RTG air is not appropriate, such as periods when crops are removed and when tomato plants are harvested; the Solanaceae pollen concentrations increased suddenly during these times. Our results suggest that Solanaceae pollen exposure could be significantly reduced by not drying out the tomato plants in the i-RTG. In addition, some i-RTG conditions and crop management tasks were related to the fluctuations in fungal spores associated with diseases of tomato crops (*Oidium and Torula*). This disease develops rapidly under dry conditions once infection occurs, and it has

been detected in the i-RTG Lab, which is characterized by higher ventilation and lower relative humidity than conventional greenhouses.

To conclude, it is possible to recirculate the air of the i-RTG to the building, and thus to convert the system into an Bi-RTG without posing health risks due to allergies for the building users if the biological air quality is monitored and the corresponding preventive measures are taken. To do so, the most important moments (which are related to the quality of the outdoor air, meteorological conditions and management practices) have to be identified. First, it is necessary to adapt the management of the i-RTG by controlling the opening and closing of the ventilation system. This measure can improve the air quality in the greenhouse for the workers during the summer. Second, when an Bi-RTG (that is, a building-greenhouse symbiosis system) has been installed to heat the building during the winter, it is necessary to identify times at which the residual air circulation should not take place. If it is not possible to stop the air injection, this study provides enough information to implement an appropriate air filter system that guarantees good air quality in the building spaces.

This study was carried out during the warm season, which coincides with the highest ventilation levels inside the i-RTG. As the recirculation of i-RTG air to buildings will be done during winter, further research is needed to elaborate the best strategy to avoid causing or aggravating allergies and breathing problems in the users of the building. Finally, it is necessary to establish internationally accepted occupational exposure limits for airborne fungi concentrations in greenhouses and in buildings.

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