TOWARDS THE INTEGRATED ASSESSMENT OF HUMAN EXPOSURE TO GRASS POLLEN IN URBAN ENVIRONMENTS

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Abstract

Pollen allergy affects a substantial proportion of the European population, and in many European countries the greatest rates of sensitisation are found for grass pollen allergen. Pollen allergy incidence rates tend to be greater in urban than in rural areas, likely due in part to the effects of urban air pollution on the allergy-causing potential of pollen grains. Background pollen concentrations measured at roof level monitoring stations are typically used as a proxy for exposure, but may differ considerably from the exposure experienced by allergy sufferers. In a 2003 report on phenology, the World Health Organisation highlighted the need for an improved understanding of the relationship between pollen monitoring station data and actual personal exposure.

Four studies are presented in this thesis. Three of these address three different aspects of urban exposure to grass pollen, whilst the fourth supporting study concerns pollen sampler efficiency. In Study A, the relative efficiency relationships between three models of pollen sampler were established under field conditions, and efficiency correction factors derived. These factors enable the quantitative comparison of data collected with different samplers, as is often necessary during exposure assessment. The results contribute to Study B, in which background grass pollen concentrations measured at roof level were compared with those at street level within an urban canyon. A tendency for lower concentrations within the canyon was observed, consistent with the deposition of pollen from the recycling component of within-canyon air, and indicating that monitoring station data typically overestimates exposure in the canyon environment. In Study C, grass pollen dose rates estimated through personal sampling were compared with monitoring station data, and dose rate/background concentration ratios determined. These ratios, which as far as the author is aware have not been reported previously, may be used to estimate inhaled pollen dose from monitoring station data. In Study D, diurnal grass pollen concentration profiles were shown to vary systematically throughout the pollen season, with this variation apparently associated with a succession of different grass species with different flowering patterns dominating pollen emission as the season progresses. Profiles averaged over entire seasons are commonly used to advise allergy sufferers on avoidance strategies,

however such systematic intra-seasonal variation is not thought to have previously been demonstrated.

As far as the author is aware, each of these four studies represents a novel contribution to the area of pollen exposure assessment. As a body of work, this thesis furthermore lays foundations for the development of a human exposure model for grass pollen, an important constituent of an integrated pollen exposure assessment strategy.

Statement

I confirm that the work presented in this thesis was performed entirely by myself under the direction of a team of supervisors unless otherwise indicated, with the exception of Study D (Chapter 5), which was a collaboration between myself and a single colleague. The final analysis and write-up of Study D were completed solely by myself, as was the majority of the literature review, data interpretation and preparation of figures. Preliminary data exploration duties were shared equally between myself and my collaborator, and I made only a small contribution to the compilation of the grass species inventory presented in Table 5.3.

Robert Peel

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Preface

The work presented in this thesis was performed at the National Pollen and Aerobiology Research Unit (NPARU), University of Worcester, UK and at the Department of Environmental Science (ENVS), Aarhus University, Denmark, between September 2009 and May 2013. The author was based at NPARU for the first 18 months, and at ENVS thereafter.

Four studies were ultimately completed, all of which relate directly or indirectly to human exposure to grass pollen in urban environments. The first of these is a supporting study, in which the relative efficiencies of three models of pollen sampler were established (Study A, Chapter 2). The results of Study A contribute to Study B (Chapter 3), an examination of pollen concentrations in the street canyon environment. In Study C (Chapter 4), personal exposure to grass pollen was assessed through pollen dose measurements, whilst in Study D (Chapter 5) the seasonality of diurnal variation in grass pollen concentrations was investigated.

This thesis is divided into six chapters. Chapter 1 comprises an introduction to the subject area, an outline of the state of existing research, and an account of the evolution of the project's objectives. The four studies are presented in Chapters 2-5, and an overall discussion of the results can be found in Chapter 6. Supporting materials in the form of non-standard methods, auxiliary results and other miscellaneous items are presented in five appendices.

Chapter 1

Introduction

1.1 Pollen allergy

1.1.1 Allergy

Allergic reactions

An allergic reaction occurs when the body's immune system mistakes a harmless foreign entity for a threat, and takes action against it. The immune system identifies disease-causing microbes such as bacteria or virus cells through a group of very specific chemicals (usually proteins) found on their surfaces, known as *antigens*. Antigens can also found on non-infectious entities, and those that lead to an allergic reaction are known as *allergens* (Brostoff & Gamlin, 1996, pp. 27-48).

Each antigen has its own distinctive chemical 'fingerprint' or *epitope* by which the carrier microbe can be identified. Epitopes are read by *antibodies*, a principal constituent of the human immune system. We each possess millions of different types of antibody, each able to recognise one specific antigen. When they encounter one another, an antibody will bind itself to its target antigen and communicate its presence to other immune cells. Each antibody has approximately eight different variants or *isotypes*, each of which instigates a different kind of immune response by interacting with different groups of immune cells (Brostoff & Gamlin, 1996, pp. 27-48).

The antibody isotype involved in allergic reactions is known as *immunoglobulin E* (IgE). The immune cells that IgE interacts with include *mast cells*, which are packed with granules of potent chemicals including *histamine* and *leukotrienes*. The presence of allergenic material triggers the release of these chemicals, which are responsible for the acute symptoms of allergy. Histamine causes sneezing, itching, rashes, tissue swelling and low blood pressure, whilst leukotrienes cause airway narrowing and swelling, leading to shortness of breath and wheezing. These symptoms are usually expressed within 15 minutes of exposure, however a secondary *late phase reaction* also commonly occurs, typically initiated 4-6 hours after the initial symptoms have ceased. The symptoms of the late phase reaction, typically a persistent blocked nose and ongoing wheezing, are chronic and can

continue for days or even weeks. The late phase reaction is thought to be brought on by a class of immune cell known as *T helper 2* cells, which synthesise inflammation causing proteins (Royal College of Physicians 2003, pp. 3-6; World Allergy Organisation 2003).

Sensitisation and atopy

Under normal circumstances, levels of IgE in the body are low, and initial exposure may not elicit allergic symptoms. Exposure to an allergen stimulates the production of the corresponding allergen-specific IgE antibody in allergic individuals, which then binds to immune cells with which it has a high affinity. This process in known as *sensitisation*. Once sensitised, an individual is liable to experience an allergic reaction upon subsequent exposure (World Allergy Organisation, 2003). Individuals with a tendency to produce IgE antibodies and become sensitised to allergens following low doses are known as *atopic*. The atopic state is genetically transmitted, and individuals with a familial history of allergy are more likely to develop allergies themselves (WHO, 2003b, p. 4).

Allergic diseases and allergy causing agents

Over 20% of the world's population are estimated to suffer from IgE-mediated (atopic) allergic diseases (WHO, 2003b, p. 3) including allergic rhinitis, atopic asthma, food allergy, and the skin conditions atopic eczema and urticaria. Allergic individual's may suffer simultaneously from a number of common allergic diseases (Royal College of Physicians, 2003, pp. 3-6).

Allergy causing substances may invoke a reaction after being inhaled, swallowed, injected, or following contact with the skin, eyes or mucosa. Substances that commonly provoke allergic reactions are found in both indoor and outdoor environments and include pollen, fungal spores, elements of house dust related to dust mites, animal dander (particularly cat and dog), certain foods (e.g. nuts, eggs or fresh fruit) and some chemical agents such as latex. The allergy that an individual is afflicted with may shift between substances over the course of their lifetime (Royal College of Physicians, 2003, pp. 3-6).

1.1.2 Allergy to pollen

The physician John Bostock is usually credited as the first to record the allergic reaction to pollen, when in 1819 he described a recurring summer affection of the eyes, nose and chest (Cecchi, 2013, p. 1). The connection with pollen was not however proved until over 50 years later when Charles Blackley, also a physician, related his own hayfever symptoms to grass pollen grains found in roadside dust thrown into the air by a passing carriage, and confirmed this through experimentation (Blackley, 1873, p. 71). The association between pollen allergy and disorders of the upper and lower respiratory tracts is now well established (Gioulekas *et al.*, 2004), and pollen is today recognised as one of the major causes of allergic disease. In Europe, pollen is the most important source of outdoor allergens both in terms of prevalence (Bousquet *et al.*, 2007) and the clinical relevance of

Table 1.1: The principal pollen taxa from an allergological perspective Skjøth *et al.* (2013a, p. 10).

Scientific name	Taxonomic level	Common name
Ambrosia	Genus	Ragweed
Alnus	Genus	Alder
Artemisia	Genus	Mugwort
Betula	Genus	Birch
Chenopodiaceae	Family	Goosefoot
Corylus	Genus	Hazel
Cupressaceae/Taxaceae	Family	Cypress/Yew
Olea	Genus	Olive
Platanus	Genus	Plane tree
Poaceae	Family	Grass
Quercus	Genus	Oak
Urtica/Parietaria	Genus	Nettle/Wall Pellitory

sensitisation (Burbach *et al.*, 2009), according to the European Community Respiratory Health Survey (ECRHS) and Global Asthma and Allergy European Network (GA^2LEN) respectively.

An important factor in sensitisation to a particular allergen is repeated exposure to a suitably high concentration (Hyde, 1972). The pollen of plants that utilise the entomophilous pollination pathway tends to be large, heavy and slightly sticky, and does not readily become airborne. The pollen of anemophilous (wind dispersed) pollinating taxa on the other hand must be small and light enough to readily become and remain airborne. Furthermore, in order to reproduce effectively, anemophilous plants produce far greater amounts of pollen than those with other reproductive strategies (Brostoff & Gamlin, 1996, pp. 1-14). Ragweeds are considered to be amongst the most prolific pollen producers, with common ragweed (*Ambrosia artemisiifolia* L.) producing up to three billion pollen grains per plant per season (Fumanal *et al.*, 2007), whilst for some grass species a single inflorescence is estimated to produce over 22 million pollen grains (Prieto-Baena *et al.*, 2003). The principal allergy-causing pollen taxa (see Table 1.1) therefore derive from anemophilous plants (Brostoff & Gamlin, 1996, pp. 1-14).

Pollen allergen

Pollen grains are the vector by which a plant's male reproductive cell reaches the female part of a plant, the stigma (Brostoff & Gamlin, 1996, pp. 1-14). The allergenic component is a set of proteins found mainly in the outer wall of the pollen grain (the *exine*), but also in the internal matter (the *contents*). These proteins, whose function is to ascertain whether a compatible host plant has been encountered, are released following contact with a moist surface such as the stigma of a flower, the wall of the respiratory tract or the surface of the eye (Emberlin, 1998).

The number and nature of pollen allergens varies from species to species. Allergens are denoted by the first three letters of the genus and the first letter of the species from which they derive, and an Arabic numeral designating the group to which they belong -

for example the group 1 allergen of the grass species *Lolium perenne* is known as Lol p 1. Allergen groups relate to the chemical structure of the allergen, for example group 1 allergens are glycoproteins. To date, 11 different groups of grass pollen allergen have been identified. *Phleum pratense* thus far appears to be the most prolific allergen producer amongst the grasses, and has been been found to carry allergens belonging to 10 of these groups (Andersson & Lidholm, 2003; Chapman *et al.*, 2007). Different allergens have different levels of allergenicity, and may be designated as major or minor depending on their sensitising potential, with major allergens typically considered to be those to which more than 50% of allergic individuals react (Suphioglu, 2000). Group 1 and group 5 allergens tend to dominate the allergenicity of those grass species in which they are present (Andersson & Lidholm, 2003).

If the epitopes of two allergens are similar, an antigen produced for one can also bind to the other, meaning that an individual sensitised to one allergen may also react to other closely related allergens. This is known as a *cross-reaction*. Cross-reactions commonly occur between the species that make up a plant genus or family[1], whilst there are also some common cross-reactions between these groups (Brostoff & Gamlin, 1996, pp. 153-169). For example, individuals allergic to *Alnus* and *Corylus* pollen also commonly react to *Betula* pollen, a fellow genus of the Betulaceae family (Corden *et al.*, 2000). Cross-reactions also occur between pollen and other allergenic substances, for example grass pollen allergen can cross-react with a number of foods including beans, peas, cereals, peanuts, melon, carrot and celery (Puc, 2011).

The allergenic potency of pollen grains, i.e. the amount of allergen carried by a single pollen grain, is subject to considerable variation. For birch pollen, the amount of Bet v 1 allergen has been found on average to differ within a single year by a factor of up to around three for pollen collected from trees several hundred km apart, and from year-to-year by a factor of up to more than five for pollen collected from trees in the same area (Buters *et al.*, 2008).

Symptoms

The allergic reaction to pollen typically manifests itself in the form of rhinitis, conjunctivitis or asthma, and during the pollen season these often occur simultaneously in the same patient (Cecchi, 2013, p. 2). Most sufferers of asthma (both allergic and non-allergic) also suffer from some form of rhinitis, whilst 10-40% of allergic rhinitis sufferers have asthma (Bousquet *et al.*, 2012). Indeed, it has been suggested that allergic rhinitis and asthma are in fact manifestations of the same disease (one airway, one disease) rather than distinct afflictions of the upper and lower respiratory tracts respectively (Grossman, 1997).

The symptoms of rhinitis include rhinorrhea (discharge of mucus from the nose), nasal obstruction, nasal itching, and sneezing. There are many causes of the disease including infections (viral or bacterial) and hormone imbalance, whilst rhinitis is also a side effect

[1]This is one reason why aerobiologists deal with genus or family groups - see Table 1.1.

of some types of medication. Allergic (IgE mediated) rhinitis occurs in several forms. Where the causative agent is a seasonal outdoor allergen such as pollen allergen, the term *seasonal allergic rhinitis* (SAR) is used (Bousquet *et al.*, 2001). The symptoms of SAR are focused in the nose because particles within the pollen size range show a propensity to deposit predominantly within the nasal cavity (D'Amato *et al.*, 1998). The prevalence of SAR is low amongst children but peaks amongst late adolescents/young adults, the age at which the disease is typically developed. Some sufferers however 'grow out' of SAR, and prevalence consequently declines with advancing age (UCB Institute of Allergy, 2004, p. 6).

Conjunctivitis is the principal eye symptom of pollen allergy. Allergic conjunctivitis is typically induced when allergen comes into contact with the surface of the eye (Ono & Abelson, 2005), and is common amongst pollen allergic individuals because the size of pollen grains means that they easily become trapped in the eyes (Brostoff & Gamlin, 1996, p. 16). Typical symptoms include itching, burning, stinging or redness of the eye, epiphora (the overflow of tears onto the face), and photophobia. As with rhinitis, conjunctivitis resulting from exposure to pollen allergen (or other seasonal allergens such as fungal spores) is known as *seasonal allergic conjunctivitis* (Bielory & Friedlaender, 2008). Conjunctivitis is a common companion of pollen-induced rhinitis (Cecchi, 2013, p. 3).

Asthma is experienced as recurring episodes of wheezing, breathlessness, tightness of chest, or coughing. Asthma is estimated to affect around 300 million individuals and to lead to 250,000 deaths per year worldwide, with national prevalence rates thought to range from 1-18% (GINA, 2011, pp. 2-3). Exposure to allergens is just one possible cause of asthma, and attacks may also be provoked by viral infections, exposure to air pollutants or stress (Royal College of Physicians, 2003, p. 5), although the condition is thought to be atopic in over 50% of adult and in over 80% of child sufferers (WHO, 2003b, p. 3). Asthma may be induced through the presence of pollen in the nose (Brostoff & Gamlin, 1996, pp. 15-26), or through the direct action of pollen allergen on the bronchi, where allergen found amongst pauci- and submicronic particles can penetrate (Emberlin, 1998).

It is difficult to define precisely and unambiguously the condition experienced by a sensitised individual following exposure to allergenic pollen. The term hayfever is frequently used, however it is often not clear exactly what this encompasses (Brostoff & Gamlin, 1996, p. 3). A more precise term, pollinosis, refers to symptoms of both eye and nose induced by exposure to pollen (Gioulekas *et al.*, 2004), but has not fallen into general usage. Seasonal allergic rhinitis, another well defined term is also commonly used but is not specific to pollen and refers only to nasal symptoms. An alternative that includes eye symptoms is seasonal allergic rhino-conjunctivitis.

One method of managing the symptoms of allergy is *allergen avoidance*, whereby the patient takes steps to avoid situations where exposure to the allergy-causing agents is probable (Custovic *et al.*, 1998), however in the case of ubiquitous allergens such as pollen grains this is not straightforward to achieve. Allergy UK (2012) suggest a number of

strategies by which exposure may be minimised, such as remaining indoors when pollen concentrations are likely to be high and keeping doors and windows closed. Astma-Allergi Danmark (2012) advise patients to remain indoors around mid-day, the time of day when grass pollen concentrations are thought likely to be high in Copenhagen.

The impact of pollen allergy

Although allergic rhinitis is not usually a severe disease, it is widely acknowledged to have a significant socio-economic impact on sufferers, their families, and upon society in general. Patients may experience a decreased quality of life, loss of sleep and reduced social interaction (World Allergy Organisation, 2011, pp. 28-29). The economic impact is sustained through direct costs including medical prescriptions and doctors' appointments, and through indirect costs such as time taken of work or school and reduced productivity (Malone *et al.*, 1997).

When left uncontrolled, asthma can interfere with a patients normal day-to-day activities and have a serious affect on their quality of life. The financial impact of the disease is substantial and includes medical costs such as hospitalisation, prescriptions and doctors' appointments, and non-medical costs such as total cessation of or time off work, and premature death (World Allergy Organisation, 2011, pp. 34-37).

One recent Danish study has linked a history of allergy with an increased risk of suicide completion (Qin *et al.*, 2011).

Trends in prevalence

The prevalence of pollen allergy has been steadily increasing over the last two centuries (Brostoff & Gamlin, 1996, p. 49); in particular, the prevalence of respiratory diseases related to pollen allergy has increased in Europe over recent decades, especially in industrialised countries (D'Amato *et al.*, 2007), although this increase is thought to have plateaued amongst older children (13-14 years) in areas where prevalence is already high (Asher *et al.*, 2006). Individuals living in rural areas however show a lower tendency to develop pollen related allergic disorders than do urban dwellers (D'Amato, 2000), even though concentrations of many pollen taxa, particularly grass pollen, might be expected to be greater in the countryside.

Air pollutants have been shown to influence the symptoms of pollen allergy, and to interact with pollen grains in a number of ways that could increase air allergen content (D'Amato *et al.*, 2007), leading to a complex multivariate relationship between air pollution and pollen allergy (this is discussed in more detail in Section 1.4.2). Given the huge increase in emissions of atmospheric pollutants during the 20^{th} century (D'Amato, 2000), one theory suggests that pollen/pollutant interactions are one of the factors mediating pollen allergy prevalence (Emberlin, 1998).

1.1.3 Grass pollen allergy

Grass pollen is the most common cause of pollen allergy in Europe as well as in many other parts of the world, although incidence levels vary from country to country (D'Amato *et al.*, 2007). When comparing data from 35 different centres in 15 developed countries, Bousquet *et al.* (2007) found median sensitisation rates of 16.9% for grass pollen, nearly three times greater than the next most common pollen taxa (birch pollen, 6.4%). Within Europe, grass pollen sensitization rates were estimated to vary from 7.8% (Spain) to 26.3% (Switzerland), whilst rates approaching 30% were recorded in Australia and the USA.

The dominance of grass pollen largely comes down to the ubiquity of grasses, which tend to be widely distributed in and around cities (León-Ruiz *et al.*, 2011) as well as in the countryside, and also to the high degree of cross reactivity between species. Many members of the Poaceae family cross-react, in particular the members of the sub-family Pooideae, which is common throughout Europe (Bousquet *et al.*, 2007).

1.2 Grasses and grass pollen

1.2.1 Grass pollen grains

Pollen grains are produced in the anthers as quadruplet groups known as *tetrads* which for some species, including those of the grass family (Poaceae or Gramineae), become separated from one another during the final stages of development and are released as single pollen grains or *monads* (Pacini & Franchi, 1999; McCormick, 2004). The pollen grains of the species that comprise the Poaceae family are morphologically very similar (Fig. 1.1). They are spheroidal, ovoidal or ellipsoidal in shape with a single circular opening or *pore*, and tightly packed with small starch granules (Wodehouse, 1965, pp. 303-320). Allergen is found both in the pollen grain's outer shell (*exine*) and in the internal starch granules (Emberlin, 1995).

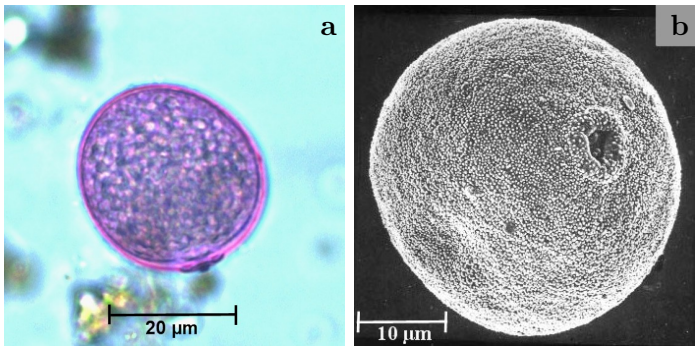

Figure 1.1: Poaceae pollen grains as seen by (a) light and (b) scanning electron microscopes. Photograph (a) was taken by the author, whilst photograph (b) was provided by the National Pollen and Aerobiology Research Unit, University of Worcester.

Table 1.2: The diameter (µm) of pollen grains from several wild grass species. Data taken from the pollen grain size distributions presented by Morrow Brown & Irving (1973).

Species	Overall range	Modal range
Agrostis alba	10-35	20-25
Agrostis gigantea	18-35	23-25
Alopecurus pratensis	15-40	30-35
Anthoxanthum odoratum	15-40	30-35
Bromus mollis	25-45	35-40
Cynodon dactylon	20-35	25-30
Cynosurus cristatus	20-40	25-30
Dactylis glomerata	15-40	30-35
Festuca pratensis	15-40	25-30
Holcus lanatus	10-35	25-30
Lolium perenne	15-35	20-25
Phleum pratense	15-45	30-35
Poa pratensis	13-35	23-25

Size, density and settling velocity

Pollen grain size and density vary, even within a species (Di-Giovanni *et al.*, 1995). Amongst the grasses there are two major groups: *wild* grasses tend to produce smaller pollen grains, whilst *cereals* tend to produce larger pollen grains (Hyde, 1972). Pollen from species within the cereal group, such as *Zea mays*, are not a common cause of pollinosis, probably because large pollen grains do not readily travel far (Brostoff & Gamlin, 1996, p. 14), and in the experience of the author they are rarely captured at urban monitoring stations. Morrow Brown & Irving (1973) measured the size distribution of a number of wild grass species (Table 1.2). Superposing their modal ranges gives an average range of 20-40 µm. Pollen grain density also varies between taxonomic groups and between species. Durham (1946) measured the specific gravity of several species of wild grass, reporting values between 0.90-1.01 (Table 1.3).

The *settling velocity* or rate of fall through still air of a particle depends on its size, shape and density. Knowledge of the settling velocities of pollen grains is important for understanding their aerodynamic properties and for pollen dispersion modelling. Settling velocity can be measured directly, or estimated using Stokes's Law (Di-Giovanni *et al.*, 1995). The settling velocities of several wild grasses are presented in Table 1.3, as measured by (Durham, 1946). Experimentally determined values range from 2.13-3.20 cm s^{-1}, whilst theoretical estimates are a little lower at 1.46-2.80 cm s^{-1}.

Table 1.3: The diameter (µm), specific gravity, and theoretical and experimental settling velocities (cm s^{-1}) of hydrated pollen grains from some species of wild grass. Data taken from Table V of Durham (1946).

			Settling velocity	
Species	Diameter	Specific gravity	Calculated	Experimental
Cynodon dactylon	28.5	1.01	2.50	1.86
Dactylis glomerata	34	0.91	3.20	2.77
Phleum pratense	34	0.90	3.17	2.80
Poa pratensis	28-30	0.90	2.13-2.44	1.46 - 1.74

Influence of relative humidity

Pollen grains are hygroscopic, and their moisture content varies with ambient relative humidity. For some taxonomic groups, including the Poaceae family, this can lead to changes in their size, shape, density and hence settling velocity. Following release from the anther, *Zea mays* pollen grains exposed to air dry out until an equilibrium water content is reached, with the rate of dessication and the equilibrium value itself dependent upon ambient relative humidity. Freshly released pollen exposed to 20% relative humidity was found at equilibrium to have lost 95.6% of its water content after just one hour. Even exposure to 75% relative humidity resulted in an 84.3% loss of water after around four hours. Drying was accompanied by transformation in shape from oblate spheroid to indented prismatic solid, with dry pollen grains 16% denser than when freshly collected from the anther. The net result of these changes was a reduction in settling velocity of up to 45% (Aylor, 2002, 2003). Durham (1943) also found considerable variation in pollen grain density, estimating that hydrated *Zea mays* and *Phleum pratense* pollen grains can weigh up to 76% and 100% as much as dry grains respectively.

1.2.2 Pollen production and flowering in grasses

The grass family is thought to consist of around 10,000 species and 600 genera, with over 400 wind pollinated species growing in Europe (D'Amato *et al.*, 2007). In Denmark there are 218 species recognised as native or naturalised (Schou *et al.*, 2009), whilst in the UK there are thought to be 220 species that are native, naturalised, or introduced regularly and thereby persist (Cope & Gray, 2009, p. 25). Grasses in temperate regions are almost entirely wind pollinated (Adams *et al.*, 1981) although some species self-fertilise, keeping pollen within the flower unless it is disturbed or broken open (Brostoff & Gamlin, 1996, p. 13), or are apomictic and reproduce asexually (Smart *et al.*, 1979). The family includes both annual species that flower only once, and perennial plants that flower over several successive years (Cope & Gray 2009, p. 10; León-Ruiz *et al.* 2011).

Pollen production

The capacity to produce pollen varies hugely between different species of grass. Prieto-Baena *et al.* (2003) assessed pollen production in 38 species growing in the area surrounding Córdoba, Southern Spain, and found average pollen grain production per inflorescence[2] to range from 14,458 (*Vulpia myuros*) to 22,665,145 (*Sorghum halepense*). Typically annual species are low pollen producers and perennial species are high pollen producers, although examples of high producing perennials and low producing annuals can be found (Prieto-Baena *et al.*, 2003; Aboulaich *et al.*, 2009). Pollen production amongst grasses is thought to decrease with altitude, although this has not been verified (Markgraf, 1980).

Pollen productivity data are a key input for dispersion models (Broström *et al.*, 2008),

[2]An inflorescence is an individual stem.

and according to Hyde (1972) the most useful measure of pollen production is number of pollen grains produced per unit area. Four different habitat types in the surroundings of Córdoba were estimated to produce between 85 million and 2.13 billion pollen grains m^{-2} (León-Ruiz *et al.*, 2011), whilst five different habitats around the city of Tetouan in Northwest Morocco were estimated to produce between 227 million and 1.86 billion pollen grains m^{-2} (Aboulaich *et al.*, 2009). Such estimates are of course highly specific to the species composition and population density of a particular habitat and region.

Flowering cycles

Pollen is emitted by grasses when their anthers reach maturity, during flowering. Different species flower at slightly different points during the flowering season, thus grass pollen seasons are the result of a succession of overlapping episodes of flowering. This leads to a relatively long season in comparison with other allergologically relevant taxonomic groups (Aboulaich *et al.*, 2009). León-Ruiz *et al.* (2011) surveyed the phenological development of 24 different species of grass in several different environments around the Spanish city of Córdoba over three years. Species generally flowered in the same relative order from location to location and from year to year. The time elapsed between the onset and the end of flowering varied from 16-46.5 days (median 33.2 days) whilst the central 50% of flowering took place over 4-15 days (median 9 days) for the species studied.

There are two stages to flowering or *anthesis*: the presentation of anthers to the dispersal agent (usually wind in the case of grasses), and anther *dehiscence*. During this latter stage the anther splits open, releasing the pollen it contains (Khanduri, 2011). The number of anthers that dehisce per hour can vary over the course of a 24-hour period, and for many species of grass has been shown to follow a species-specific diurnal pattern (Jones, 1952; Emecz, 1962; Ogden *et al.*, 1969; Liem, 1980; Subba Reddi *et al.*, 1988). Subba Reddi *et al.* (1988) found that of 54 grass species surveyed in India, the majority exhibited active anther dehiscence during a single period of the day, lasting between 2-16 hours, although for most species not more than seven hours. The time of peak anther dehiscence differed between species, with all times of the day and night represented. Other patterns were also observed. Some species showed two discrete periods of anther dehiscence whilst others flowered continuously throughout the 24-hour period. Altogether ten different diurnal flowering patterns were identified, and these are summarised in Table 1.4.

The diurnal flowering patterns of grasses have been found to be consistent provided weather conditions remain constant, but may be advanced, delayed, or otherwise altered or interrupted by variable weather. In this way meteorological factors drive anthesis, whilst the physiology of each individual species determines its response. Emecz (1962) observed that in the temperate Welsh climate, anthesis is dependent on species-specific minimum temperature and sunlight thresholds that must be exceeded for a critical induction period before anthesis is possible. If either threshold was not met, anthesis failed to occur, or already activated anthesis was liable to be interrupted. Two groups of grasses were

Pattern	Hours of greatest release
Middle-night	23:00-01:00
Post middle-night	01:00-04:00
Early morning	04:00-06:00
Forenoon	06:00-11:00
Middle-day	11:00-13:00
Afternoon	13:00-17:00
Evening	17:00-20:00
Pre middle-night	20:00-23:00
Bimodal	-
24-hour	-

Table 1.4: The ten diurnal flowering patterns identified by Subba Reddi *et al.* (1988). Hours of greatest release are those hours during which ≥50% of pollen is emitted. For the bi-modal distributions, emission peaked between 06:00-07:00 and 16:00-18:00.

identified, one requiring a shorter induction (1.5-5 hours) on the day in question, and the other a longer induction (8-10 hours) on the preceding day. For some species, limiting sunlight or wind speed thresholds were observed, above which anthesis ceased. For some species, anthesis interrupted by unfavourable conditions would resume when conditions improved. A minimum temperature threshold was also reported by Jones (1952) for *Bromus inermis* L.

According to Subba Reddi *et al.* (1988), rain on the preceding day or immediately prior to anthesis can have a delaying effect, and in some cases reduce the duration of pollen release. Rain a few hours ahead of anthesis may either delay or advance anthesis depending on the species, whilst heavy rain completely halts blooming. It is generally agreed that conditions promoting intense pollen emission typically reduce its duration (Emecz, 1962; Subba Reddi *et al.*, 1988).

Grass species inventories

Any given region is likely be host to many different species of grass that flower at different times of the season, at different times of day, and that respond in different ways to changes in the weather (as discussed in the above sub-section on flowering cycles). Interpretation of pollen concentration data would therefore be greatly aided by an inventory of local species, including information on relative abundance. This information is however rarely available. Species inventories have been compiled on a national level, for example Pedersen (1974), Frederiksen *et al.* (2006) and Schou *et al.* (2009) for Denmark and Cope & Gray (2009) for the UK, and occasionally at greater spatial resolution, for example the survey of urban nature for Copenhagen of Hald (2011). Information on plant physiology can be found for species of commercial interest (e.g. cereals and grasses grown for seed), but for wild species this information is often patchy or non-existent.

1.3 Exposure to pollen

1.3.1 Theoretical framework

The terminology used in exposure science is often vague and ambiguous, and in order to avoid confusion it is important to define precisely the terms being used. In the most

general sense, *exposure* occurs when an *agent* (usually an entity that produces an adverse health effect) and a *target* (usually a living organism) coincide in space and time (Zartarian *et al.*, 1997). In the context of this thesis, the agent will be defined as grass pollen. The most suitable definition of the target will depend upon one's objective. The target will be defined as the lining of the airways in this thesis, as this is where the respiratory symptoms of pollinosis are provoked[3] (see Section 1.1.2).

With the agent and target established, a clear definition of precisely what is meant by exposure is needed. Zartarian *et al.* (1997) define the basic unit of exposure $\xi(x, y, z, t)$ to a concentration $C(x, y, z, t)$ as the contact between agent and target at a single point in space and time, termed an *instantaneous point exposure*. In practice this will usually be a discrete event such as an individual pollen grain depositing within the respiratory system, though it may often be useful to model exposure as continuous. Exposure can be thought of as the agent concentration at the point of contact, and thus the units of exposure are typically the same as the units of concentration.

In order for exposure to occur, the agent must transfer from the vector medium (in this case air) to the target by crossing a *contact boundary*, defined by Zartarian *et al.* (1997) as 'a surface in space containing at least one exposure point on the target of interest'. As with the target, an appropriate choice of contact boundary will depend upon the context. For the purposes of this thesis, a sensible choice of contact boundary is the union of nasal and oral orifices[4]. The amount of agent taken up by a target through a contact boundary is termed the *dose*. It is important to observe that exposure and dose are not necessarily equivalent - you cannot have a dose with no exposure, but you can have exposure with no dose (Zartarian *et al.*, 1997).

The *contact zone* is a conceptual volume adjacent to a contact boundary within which an agent has a high probability of encountering the contact boundary (Zartarian *et al.*, 1997). In aerosol exposure the concept of a *breathing zone*, a pool of air about a person's face from which they draw their breath, is frequently invoked. The European Committee for Standardization (1998) define the breathing zone to be a 'hemisphere (generally accepted to be 0.3 m in radius) extending in front of the human face, centred on the midpoint of a line joining the ears' where 'the base of the hemisphere is the plane through this line, the top of the head and the larynx'. It is assumed that the air within the breathing zone is to all intents and purposes homogeneous with respect to the inhalant in question (Lidén & Waher, 2010).

[3]This definition only takes into account the component of exposure relating to respiratory symptoms. Where ocular symptoms are of interest the surface of the eyeball would be a suitable target. In extreme cases other organs such as the skin may react to direct pollen exposure (Brostoff & Gamlin, 1996, pp. 15-26), in which case other target specifications may be appropriate.

[4]This is a sensible choice since essentially all pollen-sized particles crossing this boundary are retained within the body, see Section 1.3.2.

1.3.2 The relationship between dose and exposure

The inhalable fraction

The proportion of particles within ambient air that enter the upper respiratory tract is known as the inhalable fraction, defined by the ISO (1995) as the 'mass fraction of total airborne particles which is inhaled through the nose and mouth'. Wind tunnel tests at wind speeds up to 8 ms^{-1} with coal dust (up to 60 µm in diameter) have shown that for both nasal and oral breathing modes, a breathing model head facing into the wind will under-sample particles > 10 µm when inhalation velocity (the velocity of air entering the nose or mouth) is greater than ambient air speed, but over-sample when ambient air speed exceeds inhalation velocity. When facing away from or perpendicular to air flow, the head will under sample particles > 5 µm, increasingly so at higher wind speeds (Armbruster & Breuer, 1982).

The inhalable fraction has been estimated for several bioaerosols in still air for nose breathing (Breysse & Swift, 1990), but as far as the author is aware has never been measured for grass pollen. The ISO (1995) however define an inhalable convention, a curve to be used for calculating the inhalable fraction for a specific particle aerodynamic diameter[5]. For wind speed $u < 4$ ms^{-1}, the percentage E_I of particles of aerodynamic diameter $D < 100$ µm that are inhaled is given by the equation

$$E_I = 50\,(1 + exp[-0.06D]) \tag{1.1}$$

An alternative formulation is tentatively suggested for wind speeds $4 < u < 9$ ms^{-1} and particle aerodynamic diameter $D < 90$ µm:

$$E_I = 50\,(1 + exp[-0.06D]) + 10^{-3}u^{2.75}exp[0.055D] \tag{1.2}$$

These conventions give values averaged over all wind directions, and are plotted for grass pollen-sized particles in Fig. 1.2.

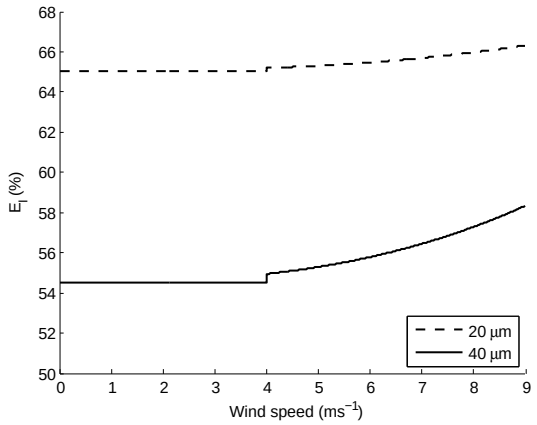

Figure 1.2: The inhalable fraction E_I (equations 1.1 and 1.2 for wind speeds <4 ms^{-1} and 4-9 ms^{-1} respectively) for aerodynamic diameters of 20 µm and 40 µm, the (approximate) lower and upper bounds of the grass pollen grain size range as defined in Section 1.2.1.

[5]A particle's aerodynamic diameter is the diameter of a sphere of unit density that falls at the same speed as the particle in question (Vincent, 2007, p. 27).

Respiratory tract particle deposition

Breathing mode will determine to a large extent where pollen-sized particles are deposited within the airways. During nose breathing, the majority of large sized particles are deposited in the anterior region of the main nasal passage, whereas during oral breathing large particles may be deposited on the soft palate, or failing this in the upper trachea after passing through the larynx (Swift & Proctor, 1988). A fraction of particles entering the nose may be expected to impact against and be retained by nasal hairs, though the author is not aware of any studies that have quantified this effect. The ISO (1995) also present a thoracic convention, a curve that defines the *thoracic fraction* or the mass fraction of inhaled particles that penetrate beyond the larynx. The thoracic fraction declines as particle size increases and is 5.9% for particles with an aerodynamic diameter of 20 µm (ISO, 1995), therefore it can be assumed that almost all inhaled grass pollen grains that reach the main nasal passage are deposited within the nasopharynx.

Breathing rate

Inspiration rates have been reported to vary from 7 l min^{-1} (during sleep) to 113.8 l min^{-1} (a healthy young male during extreme exercise), and can thus have a profound impact on the relationship between exposure and dose. Breathing rates vary with both the individual and the level of activity; furthermore, as breathing rate increases so do the number of breaths per minute and the velocity at which air is inspired, which may in turn alter the inhalable fraction (O'Meara & Tovey, 2000).

Nasal-oral partitioning

Most people breathe through their nose at rest (though in subjects with rhinitis and asymptomatic asthma the likelihood of some degree of mouth breathing increases), but switch from a nasal to an oronasal breathing pattern as their exercise level rises (O'Meara & Tovey, 2000). Becquemin *et al.* (1999) found that the oral component of breathing ranged from 0-30% at rest in a group of 10 adults, rising to 30-67% during vigorous exercise. For a group of 10 children it ranged from 0-45% at rest, and from 20-84% during vigorous exercise. These results show that for some individuals a substantial proportion of air flow is through the nasal route, even during exercise.

1.3.3 Pollen dose and allergy symptoms

Threshold values

The relationship between pollen dose and symptom development is complex, with the *threshold* dose necessary to illicit symptoms varying between individuals and with recent exposure history (Galán *et al.*, 1995). Connell (1968) found that the nasal membrane becomes progressively more hyperreactive upon daily challenge with ragweed pollen, leading to a reduced threshold dose, but recovers in the absence of exposure. This is known

as the *priming effect*. One way of quantifying dose thresholds is through clinical trials. These are conducted outside the pollen season to ensure that exposure can be controlled, however this also means that subjects are not 'primed' at the time of the trial. Davies (1985) exposed 13 individuals to increasing concentrations of grass pollen in an exposure chamber. Exposure lasted in all cases for 30 minutes, however none of the test subjects experienced symptoms at an average concentration of 4,252 grains m^{-3} and in five subjects no response was seen even at a concentration of 35,000 grains m^{-3}. To put this into context, the maximum hour-averaged grass pollen concentration recorded at the Aarhus City Centre monitoring station in Denmark during 2011 was 360 grains m^{-3}. Bousquet *et al.* (1987) took a different approach, challenging a panel of grass pollen allergic individuals with grass pollen grains administered by nasal insufflation and finding that the average dose necessary to illicit symptoms was 332 pollen grains (range 15 - 1215). Without dose rate information it is however not easy to reconcile these values with actual episodes of outdoor exposure.

An alternative approach to the study of dose thresholds is to quantifying the symptoms of a group of allergic individuals and compare these with background pollen concentrations. All grass pollen allergic individuals have been reported to exhibited symptoms when daily average concentration exceed 50 grains m^{-3} in London, UK and Warsaw, Poland (Davies & Smith, 1973; Rapiejko *et al.*, 2007). In Spain, lower values of 37 grains m^{-3} in Bilbao (Antépara *et al.*, 1995) and 35 grains m^{-3} in Ciudad Real (Feo Brito *et al.*, 2010) have been reported, however a 50 grain m^{-3} threshold is often assumed (Galán *et al.*, 1995), as is the case in Denmark.

It is worth noting that the above studies assume uniform allergen content between pollen grains, even though considerable variation is known to occur (see Section 1.1.2). Pollen grain dose is therefore not necessarily proportional to allergen dose.

Dose-response relationships

A *threshold limit value* is the level of an agent that an individual may be exposed to on a daily basis without experiencing adverse health effects. It has been concluded fairly recently that there is currently insufficient information available, including exposure, dose and response information, to define such limit values for bioaerosols (Buttner *et al.* 1997, p. 629; Vincent 2007, p. 474). In a 2003 report on phenology, the WHO (2003a, p. 40) highlighted the need for an improved understanding of the relationship between background pollen concentrations and exposure.

1.3.4 Exposure assessment

Exposure to atmospheric agents may be assessed using either direct or indirect methods.

Direct methods

Actual exposure levels can be measured using personal exposure monitors, small lightweight samplers carried by an individual that measure agent levels in, for example, the breathing zone. These methods are often applied to population subgroups whose exposure may be representative of the wider population or who may alternatively share some common attribute, known as panel and cohort studies respectively. Exposure may also be measured directly through biomarkers, samples of bodily material such as blood or urine that give information on the level of exposure to the agent of interest (Hertel *et al.*, 2008). Biomarkers are not commonly used to asses exposure to pollen.

Direct methods give the most accurate measure of personal exposure (Berglund *et al.*, 2001) but are logistically complex, expensive, labour intensive and time consuming as they typically require the selection and recruitment of suitable sample subjects, the procurement, preparation and maintenance of a number of personal samplers (or the collection of biomarker samples), and the assay of numerous samples in addition to data analysis (Sexton & Barry Ryan, 1988, p. 212). Personal exposure data are however crucial for the development and testing of exposure models (Hertel *et al.*, 2001b).

Indirect methods

Where the application of direct methods is unfeasible, indirect methods may be used. This generally involves combining agent concentrations with information on the amount of time spent in a particular environment. This is often achieved by invoking the concept of a microenvironment, defined as a three dimensional space within which the concentration of the agent of interest may be assumed to be spatially homogeneous or can be otherwise well defined (Hertel *et al.*, 2008). A microenvironment could be for example a garden, a street canyon, or the interior of a building or vehicle. The cumulative exposure ξ_i of individual i as they move between microenvironments, known as *time weighted exposure*, can then be calculated using the formula

$$\xi_i = \sum_{j=1}^{n} C_j t_{ij} \tag{1.3}$$

where C_j is the pollutant concentration in microenvironment j, t_{ij} is the overall time that person i spends in microenvironment j, and n the total number of microenvironments. Indirect methods are based upon a number of assumptions which potentially limit their achievable accuracy (Hertel *et al.*, 2001a):

- The concentration in each microenvironment is assumed to be constant or to have well-defined variation.
- The concentration in a microenvironment and the presence of a person there are assumed to be independent events, which may not always be the case.

- The number of microenvironments must be limited to a manageable number, thus potentially simplifying variation within individual microenvironments.
- Concentration estimates are often based upon hour-averaged data, which may mask short term peak values and lead to the miscalculation of brief exposure episodes.

The concentration data used for indirect exposure assessment may either be measured (for example by a monitoring network) or calculated using transport models (Hertel *et al.*, 2008).

Monitoring networks and integrated monitoring

Monitoring networks provide a continuous record of measurements, usually at fixed sites, and are an important source of exposure data (Hertel *et al.*, 2008). Pollen monitoring networks consist almost exclusively of urban background stations situated at roof level, where the local signal will not dominate[6]. National pollen monitoring networks are typically relatively sparse. For example in 2010, the UK network consisted of only 19 stations[7] and the Danish network of only two (Sommer & Rasmussen, 2010) - by way of contrast, the respective national air quality monitoring networks include around 300 (Defra, 2011) and 19 stations (Hertel *et al.*, 2007). On the European scale however, coverage is far better, with the European Aeroallergen Network consisting of some 521 pollen monitoring stations (Skjøth *et al.*, 2013a, p. 15).

Integrated monitoring is defined as monitoring through a combination of measurements from for example monitoring stations, and calculations performed with models. Measured data may be used to determine actual levels and trends in atmospheric species, to understand processes, and to develop models. Model results may then be used to interpret measured data, to study source apportionment, and to extend spatial coverage of a monitoring network to areas not represented by existing stations. Models may also be used for scenario studies that investigate the potential impact of future changes (Hertel *et al.*, 2007).

1.3.5 Pollen exposure studies

Direct studies

Only a handful of studies have used direct methods to investigate exposure to pollen. Riediker *et al.* (2000) performed a panel study in Zurich, Switzerland, in which a single SKC personal filter sampler was carried on 36 occasions by 10 different individuals as they went about their normal activities. Sampling began at 8 am and typically lasted for 11 1/2 hours. Background pollen levels were obtained from a single roof level 7-Day sampler

[6]The area of coverage of the Hirst-type sampler, the standard monitoring device used in Europe (Emberlin & McCartney, 1996) and the USA (Muilenberg, 2003), is generally assumed to be a disk of diameter 50 km (Rieux *et al.*, 2008).

[7]Of these 19 stations, six were only in operation during the grass pollen season.

situated within 5 km of the home and workplaces of all study subjects. Average total pollen personal exposure and background concentrations were found to be strongly ($r = 0.719$) and significantly correlated. Average concentrations recorded with the personal sampler (i.e. exposure) were on average only 0.31 times as great as corresponding daily average background concentrations, partly due to the fact that during sample collection subjects were outdoors on average for only 14% of the time[8]. A similar study, conducted by Kailin (1964) for *Ambrosia* pollen using passive samplers, also found that exposure related substantially to the amount of time spent outdoors.

There exists a small body of work in which pollen dose has been measured in the outdoor environment. O'Meara *et al.* (2004) recruited a panel of 30 adults who wore Nasal Air Samplers (NAS) for a period of two hours whilst engaged in similar low-level activities. Although always in close proximity to one another, pollen doses varied considerably between subjects, ranging from 1-1699 (mean 53.4) for *Ambrosia* and 2-32 (mean 9.8) for Poaceae. In a similar study by Mitakakis *et al.* (2000), nine family groups each consisting of two adults and two children wore NAS and IOM filter samplers for one hour on four separate occasions (twice indoors and twice outdoors) at two different activity levels, low (e.g. reading or dozing) and moderate (e.g. cooking or gardening). The number of inhaled pollen grains ranged from 0-81 (median 1) grains and 0-72 (median 5) grains for grass and non-grass taxa respectively. When all measured particles were taken into account (pollen plus *Alternaria* and *Cladosporium* spores), both increased activity level and the outdoor environment had a positive effect on the the magnitude of inhaled dose. Correlation between family groups in the same environment were generally low, suggesting that the nature of exposure is intensely personal, even between individuals within the same microenvironment. The correlation between NAS and IOM samplers was also low, suggesting that exposure is not necessarily an accurate proxy for dose.

Indirect studies

Alcázar *et al.* (2004) complemented the single permanent pollen monitoring station in the Spanish city of Córdoba with three temporary stations, thus estimating exposure to *Platanus* pollen in four sectors of the city. Concentrations were compared with the symptom scores of 19 allergic patients. Analysis of the correlation between trap data and symptom intensity suggested that the permanent trap (which ran 24 hours a day irrespective of the weather) provided a general picture of the pollen situation for the whole city, whereas each portable trap (these were run for 12 hours a day on days without rain only) gave a more accurate account of local exposure.

[8]This equates to approximately 6.7% of a 24-hour period, in close agreement with the results of time-activity studies. The US population are estimated to spend on average 7-8% of each 24 hour period outdoors (Klepeis *et al.*, 2001), whilst information complied by Larsen *et al.* (1997, pp. 70-71) implies that the corresponding figure for Denmark is 5.2%.

A couple of studies have used pollen concentration outside a subject's residence as a proxy for personal exposure[9]. O'Rourke & Lebowitz (1984) measured levels of total pollen outside 51 homes in Tucson, Arizona using Rotorod samplers run intermittently for approximately 72 hours. For the 30 cases where pollen was recorded, residence concentrations were on average 20% those recorded at a roof level monitoring station, falling to 10% when all samples were taken into account. Feliziani & Marfisi (1992) took a different approach, recruiting test subjects allergic to either Poaceae or Urticaceae pollen and equipping each with a Burkard Personal Volumetric Air Sampler. Subjects were asked to initiate collection of 30 minute samples when symptoms became severe, the sampler being placed on a balcony outside their home. The personal samplers recorded pollen levels 2-6 times higher than those recorded with a Burkard 7-Day sampler at the local monitoring station. The fact that sample collection was brief in duration and triggered by the onset of severe symptoms will of course skew the picture from the general situation.

When studying the epidemiology of pollen allergy, concentration data from a single pollen monitoring station is often used as a proxy for personal exposure, in particular where the focus is on co-exposure to pollen and other air pollutants. Hajat *et al.* (2001) and Feo Brito *et al.* (2007) found that concentrations of pollen, NO_2, PM_{10}, SO_2 and O_3 were positively associated with asthma symptom intensity and the number of GP consultations for allergic rhinitis respectively. Anderson *et al.* (1998) on the other hand reported no link between daily emergency hospital emissions for asthma and co-exposure to pollen and air pollutants, whilst Momas *et al.* (2003) found no significant difference in exposure to pollen or air pollutants between healthy children and those suffering from allergic rhinitis.

1.4 Atmospheric pollen in urban environments

1.4.1 The urban landscape

Whilst only around 4% of Europe's land surface has been urbanised, around 75% of the European population currently live in cities, and this is expected to increase to 80% by the year 2020 (EEA, 2010). An understanding of the factors governing exposure to atmospheric pollen and the development of pollen allergy in urban areas is therefore of increasing importance. Plants are typically relatively scarce within cities, occurring in discrete patches. Grasses are commonly found in a number of different urban habitats, however their potential to contribute to the atmospheric pollen load is determined by whether they are managed[10] or not. According to Skjøth *et al.* (2013b), grasses growing

[9] One weakness of this method is that although pollen grains do penetrate buildings, the ratio between indoor and outdoor concentrations tends to be small - for example Lee *et al.* (2006) report ratios between 0.001-0.194 with a median value of 0.025. Small indoor/outdoor ratios mean that ambient concentrations outside a residence are unlikely to be a reliable proxy for exposure.

[10] *Managed* grasses are defined as those that are mown regularly, and therefore do not flower or release pollen. *Unmanaged* grasses are defined as those that are mown infrequently or not at all, and thus flower and release pollen. These definitions are taken from Skjøth *et al.* (2013b).

close to buildings and in public areas such as parks and cemeteries are more likely to be managed and contribute little pollen, whilst those found along major roads and railways and in industrial or non-residential areas are likely to be unmanaged and thus more prolific producers.

Urban areas are also characteristic in terms of their physical configuration. One of the basic units of the urban landscape and an environment where outdoor exposure is likely to occur is the street canyon, the volume of air delimited by a road and the buildings standing on either side. Street canyons have their own climate, distinct from that of the overlying urban airmass (Nakamura & Oke, 1988). In particular, air flow patterns within a street canyon typically include a degree of recirculation, mediated by canyon geometry and the speed and relative direction of ambient winds (Oke, 1988). It is well known that street canyons influence street level aerosol concentrations and hence, potentially, exposure (Berkowicz *et al.*, 1997). The influence of the street canyon environment on pollen concentrations has however not previously been investigated, as far as the author is aware.

1.4.2 Urban pollution and pollen allergy

Urban areas typically suffer from high concentrations of gaseous and particulate air pollution, much of which results from vehicular emissions (D'Amato, 2000). A number of studies have reported greater incidence of pollen allergy amongst urban than amongst rural inhabitants, whilst seasonal atopic asthma symptoms (Feo Brito *et al.*, 2007) and GP consultations for allergic rhinitis (Hajat *et al.*, 2001) have been linked to fluctuations in ambient NO_2, PM_{10}, SO_2 and O_3 concentrations. Although urban air pollution is known to contribute to respiratory diseases in its own right (D'Amato & Cecchi, 2008), atmospheric pollutants may indirectly exacerbate the effect of pollen allergen on allergic individuals via a number of different pathways (Emberlin, 1998). Plants growing under stress from pollutants have been linked to more allergenically potent pollen (Emberlin, 1998), whilst SO_2 and NO_2 have been shown to alter pollen protein content (Santra *et al.*, 1991; Rezanejad, 2007; Shahali *et al.*, 2009). Increased levels of CO_2 can lead to greater pollen production in some plants, though its effect on grasses is currently unknown (Ziska *et al.*, 2009). The bioavailability of grass pollen allergen may be increased through the release of cytoplasmic granules due to exine degradation and rupture following exposure to NO_2 or O_3 (Motta *et al.*, 2006), or through the transfer of allergen to particulate matter agglomerated to pollen grains and subsequently liberated (Behrendt *et al.*, 1992). Exposure to O_3 and NO_2 has been shown to induce an increased airway response to pollen allergen in allergic individuals (Molfino *et al.*, 1991; Jenkins *et al.*, 1999), whilst the sensitising potential of allergen may increase following binding to diesel exhaust particles (Emberlin, 1998).

1.4.3 Variation in atmospheric grass pollen concentrations

Emission, transport and deposition

Following release into the atmosphere, pollen grains are dispersed by turbulence, small scale eddies that serve to mix particle laden air with ambient air (Hertel *et al.*, 2009, p. 178). As for all particles emitted close to the earth's surface, pollen is largely restricted to the boundary layer, the lowest layer of the atmosphere, which varies in depth from around 100 - 3000 m (Stull, 1988, pp. 1-25). Relatively small amounts of pollen may however reach the overlying free atmosphere during episodes of deep convection (Gregory, 1961, pp. 22-30). The movement of pollen grains within the boundary layer is thought to be determined primarily by turbulent diffusion (Gregory, 1961, pp. 76-89). Gravitational settling likely also makes a noticeable contribution to the downward component of flux, especially for larger taxa (Hirst *et al.* 1967; Seinfeld & Pandis 2006, pp. 900-901), however the author is not aware of any studies that have quantified this effect.

Pollen leaves the atmosphere when it is deposited from the lowest portion of the boundary layer onto a physical surface. During the day, the boundary layer is characterised by turbulence, and pollen grains are deposited through impaction and a number of other processes. At night, a calm stable boundary layer that may grow to several metres in depth develops below a turbulent residual boundary layer, in response to the cooling earth's surface. Pollen grains that enter the stable layer will settle out under gravity at a rate determined by their settling velocity (see Section 1.2.1). Pollen grains may also leave the atmosphere through rain scavenging, a wet deposition pathway. The efficiency with which rain drops remove pollen from the atmosphere depends on the size of both the raindrops and the pollen grains (Gregory 1961, pp. 76-89; Stull 1988, pp. 1-25).

Although we may only expect in the order of 1% of the pollen released from herbaceous plants to be transported further than 1 km (Raynor *et al.*, 1970), long distance transport of pollen does occur. Erdtman (1938) detected pollen grains, including those from the Poaceae family, in the mid-Atlantic approximately 900 km from land, whilst Hirst *et al.* (1967) found pollen in the air mass over the North Sea up to an altitude of 2 km. Episodes of long-distance transport have been shown to influence *Betula* (Skjøth *et al.*, 2007), *Ambrosia* (Šikoparija *et al.*, 2009) and *Cannabis* (Cabezudo *et al.*, 1997) pollen concentrations on the regional scale, however it is not clear whether the same is true for grass pollen which is typically both larger and denser (Durham, 1946).

Seasonal variation

The period of the year during which grass pollen can be found in the air is known as the *grass pollen season* (Dahl *et al.*, 2013, p. 30). Pollen seasons generally begin and end with extended periods during which atmospheric pollen occurs only in incidental amounts. In order to define a representative pollen season the 'n%' method is often employed, whereby the season is said to have begun when n% of the year's total pollen catch has been detected, and to end at (100-n)%. Commonly n:=1 (Emberlin *et al.*, 1993; Galán *et al.*, 1995) or

Table 1.5: Typical onset and duration of the grass pollen season in London, UK (1% method, Galán *et al.* 1995) and Copenhagen, Denmark (2.5% method, Goldberg *et al.* 1988).

Location	Start	Duration
London	Early May - early June	3-4 months
Copenhagen	Late May - early June	7-9 weeks

n:=2.5 (Goldberg *et al.*, 1988) is used. Alternatively, pollen seasons may be defined as starting and ending on the first and last day that daily average concentrations exceed some threshold value (Antépara *et al.*, 1995).

The start and duration of the pollen season varies from year-to-year (Dahl *et al.*, 2013, p. 30) and from country-to-country (D'Amato *et al.*, 2007). Within Europe, the season tends to start later at higher latitudes, with an average delay of 2.3 days per degree of latitude between Southern Spain (38°N) and Northern Finland (69°N) according to Emberlin *et al.* (2000). A delayed start is also typically observed at higher elevations (Gehrig & Peeters, 2000), whilst the urban heat island can advance the pollen season by several days within cities (Emberlin *et al.*, 1993; Rodríguez-Rajo *et al.*, 2010). The character of the grass pollen season is dependent on temperature and rainfall during the months preceding onset (Dahl *et al.*, 2013, p. 35), with cool, wet conditions leading to earlier and longer flowering seasons, and warm, dry conditions leading to later and briefer seasons (León-Ruiz *et al.*, 2011). The typical start and duration for London (UK) and Copenhagen (Denmark) are shown in Table 1.5. The same factors that determine the start of the grass pollen season also influence its *intensity*, i.e. the number of grass pollen grains captured at a pollen monitoring station over the course of an entire pollen season. The earlier the season begins, the more intense it will be (Dahl *et al.*, 2013).

Average daily grass pollen concentrations are positively correlated with temperature and amount of sunlight (parameters related to flowering, see Section 1.2.2), whilst relative humidity and rainfall tend to have a negative effect (Smart *et al.*, 1979; Galán *et al.*, 1995). Large day-to-day variation in daily average concentrations can occur during the main part of the grass pollen season, however this is a regional phenomenon, and locations showing similar patterns of day-to-day variation are typically grouped spatially (Rieux *et al.*, 2008).

Diurnal variation

In addition to the seasonal variation in pollen levels, atmospheric pollen concentrations show typical patterns of variation over the course of a 24-hour period. For multi-species taxa such as Poaceae, the time of maximum concentrations is very difficult to predict (Käpylä, 1981), however typical peak times can be obtained by averaging diurnal profiles over an entire season. These average profiles differ from location-to-location, with single evening peak (Mullins *et al.*, 1986; Norris-Hill & Emberlin, 1991; Yang *et al.*, 2003) and single morning peak (Galán *et al.*, 1989, 1991; Trigo *et al.*, 1997) profiles, twin peak profiles (Rantio-Lehtimäki *et al.*, 1991a; Kosisky *et al.*, 2010) and invariant profiles reported

(Gassmann *et al.*, 2002). Average grass pollen profiles have been observed to vary from year-to-year, thought to be due to differences in spring weather favouring development of different species with different flowering patterns (Galán *et al.*, 1989), and throughout the year, thought to relate to patterns of rainfall (Norris-Hill, 1999). For London, UK, peak grass pollen concentrations tend to occur between 18:00-22:00 (Norris-Hill & Emberlin, 1991). Existing literature has not established patterns of diurnal grass pollen variation in Denmark, as far as the author is aware.

Vertical variation

Pollen traps are usually positioned on the roof of a tall building, in order to ensure that measurements are representative of a wide area and to avoid the over-contribution of nearby plants (Spieksma *et al.*, 2000). Exposure on the other hand overwhelmingly occurs at street level. An understanding of vertical pollen concentration profiles is thus important when estimating exposure levels. For most herbaceous plants, including grasses, existing literature shows that in open areas pollen concentrations tend to decrease with height where source plants are found locally, whilst in the absence of local sources little or no vertical gradient is typically observed (Raynor *et al.*, 1973b; Rantio-Lehtimäki *et al.*, 1991b; Alcázar *et al.*, 1999; Spieksma *et al.*, 2000). Urban areas are however characterised by a dense distribution of buildings that control airflow and thus dispersion close to the ground. Vertical concentration profiles about tall buildings have been found to depend upon relative wind direction. The leeward side has typically been associated with a decrease in concentration with increasing elevation, and the windward side with an increase (Käpylä, 1983; Alcázar *et al.*, 1999; Alcázar & Comtois, 2000).

Lateral variation

Immediately following emission, particle concentrations decline rapidly in what is generally assumed to be a logarithmic fashion (Lanner, 1965). Raynor *et al.* (1970) found that the centreline concentration of ragweed pollen plumes, emitted from discrete sources that ranged in size from a point source to an annulus of outer diameter 27.4 m, were reduced to 0.3-12.5% of their initial concentration approximately 60 m from the source, with the relative reduction declining as source area increased (Raynor *et al.*, 1970). The rate at which concentrations decline is also dependent on the dispersion conditions, which determine the rate of mixing (Gregory, 1961, pp. 47-51).

Spatial variation in pollen load across a city has been found in general to submit to source distribution, although urban topography is a secondary influence as it may limit lateral dispersion (Emberlin & Norris-Hill, 1991; Alcázar *et al.*, 2004; Gonzalo-Garijo *et al.*, 2006). For grass pollen however, little variation has been reported. Seasonal roof-level grass pollen deposition across an area of roughly 4 km $\times$ 5 km within central London (UK) was found by Emberlin & Norris-Hill (1991) to be fairly homogeneous. Gonzalo-Garijo *et al.* (2006) similarly found noon street-level concentrations of grass pollen to vary little

across central Badajoz, Spain, although city centre pollen levels were typically 20% lower than concurrent concentrations measured on the outskirts of the city.

Other aspects

Coastal cities[11] have been found to incur lower annual pollen loads than inland cities, with high concentrations in coastal areas linked to land breezes and lower concentrations to sea breezes (Morrow Brown & Jackson, 1978). In the coastal city of Mar del Plata, Argentina, the land-sea breeze system characteristic of summer months in the region has been associated with a rise in grass pollen concentration around midnight, as pollen carried out to sea in the afternoon is returned to land (Gassmann *et al.*, 2002).

It is often stated in aerobiological literature that pollen resuspension contributes to atmospheric pollen concentrations (Potter & Rowley, 1960; Markgraf, 1980; Sánchez Mesa *et al.*, 2003), however the magnitude of this contribution does not appear to have been established. Yli-Panula & Rantio-Lehtimäki (1994) found *Betula* pollen in settled dust both during and up to two months after the end of the birch pollen season, although the residence time appeared to be fairly brief - in fact 15 days after atmospheric concentrations had peaked, only background levels of pollen were detected in the dust reservoir. Resuspension can be affected by wind or mechanical stress, however these processes are highly complex and resuspension rates are difficult to predict (Sehmel, 1980).

Precipitation can have a rapid and dramatic effect on atmospheric pollen concentrations through the direct removal of pollen grains from the air. The proportion of pollen grains scavenged depends on the amount of precipitation and on pollen grain and raindrop size, with smaller drops removing pollen grains more efficiently than larger drops. McDonald (1962) estimated that for *Juniperus communis* pollen, which at 26 µm in diameter is towards the lower end of the grass pollen size range, 1 mm of fine (0.2 mm diameter) and intermediate (1 mm diameter) raindrops may be expected to remove 99% and 72% of pollen grains from the atmosphere respectively, whilst 10 mm of large raindrops (4 mm diameter) should remove 80%.

1.5 Project objectives and development

This project was initially conceived with the general aims of advancing our understanding of allergenic pollen concentrations in urban areas, and developing a human exposure model for allergenic pollen. These aims were to be achieved by satisfying the following three objectives:

(i) Quantify pollen concentrations at different heights above the ground, including personal exposure measurements made at street level, in the city of Aarhus, Denmark.

[11] Here coastal cities are defined as those bordered on one side by the coast. According to some definitions, all Danish cities should be classified as 'coastal'.

(ii) Develop a human exposure model for allergenic pollen based on the field data collected during objective (i).

(iii) Validate the exposure model using street level human exposure data collected in London, UK.

In order to satisfy objective (i) and obtain the exposure data for objective (iii), the following two studies were designed and performed in both Aarhus and London[12]:

Street canyon study: An investigation of vertical variation in pollen concentration within the specific environment of an urban street canyon. This was to be achieved by collecting pollen data at street level within an urban canyon, for comparison with concurrent data from a proximate roof level pollen monitoring station.

Exposure study: A personal exposure study conducted in the vicinity of the same pollen monitoring station. Exposure data was to be collected during a short journey on foot, and related to monitoring station data.

Following a thorough literature review and a series of field tests, the Sampling Technologies Rotorod Model 20 sampler (Rotorod) was selected for data collection in the street canyon study, and the Model 1 Nasal Air Sampler (NAS) for the exposure study. Duplicate samples were collected during both studies using a Burkard Personal Volumetric Air Sampler (PVAS), however these data were ultimately not used due to issues relating to sampler performance[13].

The data collected for the street canyon and exposure studies were to be compared with pollen monitoring station data. In both the UK and Denmark, pollen monitoring is performed with the Burkard 7-Day Recording Volumetric Spore Trap (7-Day sampler). Sampler efficiency can vary between instruments and with environmental conditions, thus data collected with different instruments can only be meaningfully reconciled if the efficiency relationship between the two devices is known for the environment of interest. Information on bioaerosol sampler efficiency is typically patchy and incomplete, and in particular the efficiency relationships between the samplers used in this study could not, in the opinion of the author, be reliably determined for outdoor conditions from existing published literature. An additional field study was therefore performed, in which the relative efficiencies of the Rotorod, PVAS and 7-Day samplers were established. The NAS was not included in this study since it is not volumetric.

[12] Objectives (i)-(iii) only require that the first of these studies (the street canyon study) be performed in Aarhus. In order to facilitate an inter-locational comparison, it was however performed in both Aarhus and London.

[13] For the street canyon study, the data collected with the Rotorod was considered to be of superior quality as Rotorod efficiency is less strongly influenced by wind speed, see Chapter 2. For the exposure study, the strength of the relationship between wind speed and PVAS efficiency coupled with the fact that only relative wind speed data were available at the point of sample collection meant that an efficiency correction could not reliably be applied.

Although grass pollen is the most common source of pollen allergy across Europe, in Scandinavia birch pollen is arguably of equal importance, and similar rates of sensitization are found for the two in Norway and Sweden (Bousquet *et al.*, 2007)[14]. The street canyon and exposure studies were initially planned for both birch and grass pollen, however differences between their respective flowering seasons mean that this would necessitate performing four rounds of data collection for each study (two pollen taxa in each of two different cities). Such a large workload was clearly unfeasible given the objectives of the project, thus grass pollen was selected as the more widely relevant of the two. During the grass pollen season, the only other pollen taxa present in significant amounts was Urticaceae pollen. Other taxa such as *Pinus*, *Taxus* and *Tilia* were also observed, though only in incidental quantities. When assaying the samples collected in London both Poaceae and Urticaceae pollen were counted, however this proved to be a time consuming approach and, given the ambitious objectives of the project, the author eventually elected to focus only on grass pollen. All of the work in this thesis is therefore limited to grass pollen, with the exception of the sampler efficiency study (the first study to be completed) in which Urticaceae pollen is also included.

Data on emissions are a key input of the atmospheric transport models that often form the basis of exposure models. Whereas many of the plant taxa of interest to aerobiologists consist of just a handful of species, several hundred native or naturalised grass species with a range of different flowering and thus pollen emission patterns are found in both the UK and Denmark. Grass species inventories are rarely available at geographical resolutions greater than national level, and furthermore the flowering behaviour of many species does not appear to have been established. Knowledge in both of these areas is a prerequisite of accurate emissions modelling. This lack of supporting information was, given the additional work performed for objective (i) in the shape of the sampler efficiency study, deemed to render objectives (ii) and (iii) beyond the scope of this project. In order to set some of the foundations for model development in place, a further study not covered by the original objectives was performed, in which a species inventory was compiled and the flowering behaviour of grasses investigated using diurnal grass pollen concentration profiles. A development plan for the exposure model, which the author hopes to complete in the future, is described in the overall discussion of Chapter 6.

The research included in this thesis is thus presented as four separate studies, named respectively Studies A-D. The sampler efficiency study is presented in Chapter 2 (Study A), the street canyon study in Chapter 3 (Study B), the exposure study in Chapter 4 (Study C), and the study on the diurnal periodicity of atmospheric grass pollen concentrations in Chapter 5 (Study D).

[14] Comparable statistics are not thought to exist for Denmark.

Chapter 2

Relative efficiencies of the Burkard 7-Day, Rotorod and Burkard Personal samplers for Poaceae and Urticaceae pollen under field conditions[1]

Abstract

In aerobiological studies, it is often necessary to compare concentration data recorded with different models of sampling instrument. Sampler efficiency typically varies from device to device, and depends on the target aerosol and local atmospheric conditions. To account for these differences, inter-sampler correction factors may be applied, however for many pollen samplers and pollen taxa such correction factors do not exist and cannot be derived from existing published work. In this study, the relative efficiencies of the Burkard 7-Day Recording Volumetric Spore Trap, the Sampling Technologies Rotorod Model 20, and the Burkard Personal Volumetric Air Sampler were evaluated for Urticaceae and Poaceae pollen under field conditions, and the influence of wind speed and relative humidity on these efficiency relationships assessed. The three devices were found to record significantly different concentrations for both pollen taxa, with the exception of the 7-Day and Rotorod samplers for Poaceae pollen. Under the range of conditions present during the study, wind speed was found only to have a significant impact on inter-sampler relationships involving the vertically orientated Burkard Personal sampler, whilst no interaction between relative efficiency and relative humidity was observed. Data collected with the three models of sampler should therefore only be compared once the appropriate correction has been made.

[1]A revised version of this study has been accepted for publication by the journal *Annals of Agricultural and Environmental Medicine*, under the title of this chapter (accepted for publication 21 May 2013).

For each pollen taxa, inter-sampler correction factors for the three sampler pairings are presented.

2.1 Introduction

The Burkard 7-Day Recording Volumetric Spore Trap (7-Day sampler, Appendix A.1) is a single stage slit impactor based on the classical design of Hirst (1952), and as such is one of several models of 'Hirst-type' sampler. It has become the industry standard pollen and fungal spore monitoring device in Europe and the USA (Emberlin & McCartney, 1996; Muilenberg, 2003), and has been adopted by many European national pollen monitoring networks. However, the size, weight, power requirements and design of the 7-Day sampler render it unsuitable for many field situations, where portability is often a limiting factor. The Sampling Technologies Rotorod Model 20 (Rotorod, Appendix A.2) and the Burkard Personal Volumetric Air Sampler (PVAS, Appendix A.3) are small, lightweight, battery operated bioaerosol samplers that can be easily deployed in many environments. The efficiency of all three devices is known to vary with aerosol aerodynamic characteristics including size, shape, and density, as well as with ambient wind speed (Di-Giovanni, 1998; Solomon, 2003).

In pollen exposure studies, it is common practice to compare monitoring station data recorded with a Hirst-type sampler with exposure data collected in the microenvironment of a study subject using a portable device (O'Rourke & Lebowitz, 1984; Feliziani & Marfisi, 1992; Mitakakis *et al.*, 2000; Riediker *et al.*, 2000; O'Meara *et al.*, 2004). However, data collected with different instruments can only be meaningfully reconciled if the efficiency relationship between the devices is known. Theory concerning the efficiency of generic aerosol sampling heads related to the 7-Day sampler and PVAS is well established for idealised conditions (Vincent, 2007, pp. 93-127), however in the case of the 7-Day sampler, theory does not agree with experimental data (most likely because it does not account for the influence of the sampler's bulky housing on local air flow, Hirst (1952)), whilst there appears to be no empirical PVAS data with which to validate existing efficiency models. Commonly used theoretical models also fail to fully account for the effects of particle size and wind speed on the efficiency of rotating-arm samplers such as the Rotorod (Di-Giovanni, 1998). Whilst the efficiency of Hirst-type and rotating arm samplers has been established for *Phleum* pollen through wind tunnel studies (Ogden *et al.* 1974, p. 93, Frenz 2000), results obtained under laboratory conditions do not necessarily translate to the outdoor environment. Turbulence is thought to affect the efficiency both of rotating arm samplers (Di-Giovanni, 1998) and, through the misalignment of inlet and mainstream air flow, also Hirst-type samplers (May *et al.*, 1976; Aylor, 1993). The current body of published work is thus incomplete; in particular it does not, as far as the author is aware, establish the relative field efficiency of the three devices for either Poaceae or Urticaceae pollen, two of the taxa responsible for pollen allergy in Europe (D'Amato *et al.*, 2007).

Pollen grains are hygroscopic and following emission have been shown to dehydrate at

a rate related to ambient relative humidity, leading to changes in their size, shape and density (Aylor, 2002, 2003). Ambient relative humidity thus potentially influences sampler efficiency, however this does not appear to have been taken into consideration in previous investigations.

The objectives of this study were to investigate the efficiency of the 7-Day sampler relative to those of the Rotorod and PVAS for Poaceae and Urticaceae pollen under field conditions, to assess the influence of wind speed and relative humidity on these relationships, and to derive appropriate correction factors. For completeness, the efficiencies of the two mobile samplers were also compared.

2.2 Method

2.2.1 Experimental sites

The study was performed at two different sites over three years, however the methods employed were equivalent. Urticaceae data were collected at the University of Worcester, UK, between 15^{th}-19^{th} August 2010, during the second peak of the Urticaceae pollen season[2]. The three samplers were set up in a linear array on the large (approximately 17 $\times$ 51 m^2) flat roof of the Institute of Science and the Environment, 9.5 m above ground level, with the 7-Day sampler in the middle and the two mobile samplers approximately 1 m to either side (Fig. 2.1a). All three instruments were a minimum of 4.5 m from the edge of the roof. The 7-Day sampler was part of the UK national pollen monitoring network, and in continuous operation all year round. It stands on a concrete plinth with its orifice

Figure 2.1: Data collection at the University of Worcester (a) and Danish Meteorological Institute (b). In both cases the PVAS stands on the right, the 7-Day sampler in the centre and the Rotorod on the left.

[2]In the UK the Urticaceae pollen season has two peaks, the first in June/July and the second in mid-August (Corden & Millington, 1991).

1.22 m above the roof. The Rotorod was mounted on a vertical stand and the PVAS in a specially designed cup-shaped holder on top of a tripod, with the sampling points of both devices at the height of the 7-Day sampler's inlet. Weather data were collected with a Davis Vantage Pro 2 weather station (anemometer sensitivity 1 ms^{-1}), positioned approximately 10 m from the sampler array and 2 m above the roof.

Poaceae data were collected at the Danish Meteorological Office (DMI) in North-West Copenhagen, Denmark, between 31^{st} May-14^{th} June 2011 and 11^{th}-21^{st} June 2012. The samplers were set up on the flat platform that constitutes the roof's Eastern corner (approximately 9 × 9 m^2), 15 m above ground level (Fig. 2.1b). Due to space restrictions the samplers were set up in a triangular array, approximately 1 m apart and at least 1.2 m from the edge of the roof. The 7-Day sampler was part of the permanent Danish pollen monitoring network, and typically in continuous operation from January to September. It was mounted on a stand with its orifice 1 m above the roof. The two mobile samplers were mounted in the manner described above. Wind speed data were collected with a switching anemometer (Vector Instruments A100R, sensitivity 0.2 ms^{-1}) mounted above the Rotorod such that the anemometer cups were 30 cm from the sampler's rotating arm, and relative humidity was recorded at the start and end of each sample collection using a hand held thermo-hygrometer (Omega RH82).

2.2.2 Data collection and processing

The Rotorod and PVAS samplers were run concurrently for periods of 58 minutes[3]. Corresponding data were obtained from the 7-Day sampler trace, thus sets of concurrent, approximately hour-averaged data were acquired for the three sampler models. Hour-averaged pollen data can be usefully compared with meteorological data, which varies from hour-to-hour.

Samples were collected on selected days on which concentrations of the target pollen taxa were expected to be high (i.e. warm, precipitation free and during the flowering season of the target plant taxa), and at times of the day when peak concentrations were anticipated (between 09:30 and 23:30 local time in both locations). Sample collection was in all cases halted if rainfall occurred and the affected sample declared void, on the basis that precipitation removes pollen sized particles from the air with great efficiency (McDonald, 1962). In all, 41 sets of Urticaceae samples and 128 sets of Poaceae samples were collected. Many of the Poaceae samples were collected when ambient concentrations were low[4]. To ensure results were of a high quality, sets of samples corresponding to concentrations <85.5 grains m^{-3} according to the 7-Day sampler were rejected (equivalent to a count of <10 pollen grains), leaving only 45 sets of Poaceae data for statistical analysis.

[3]Approximately equal to the averaging period of the 7-Day sampler.

[4]The number of hours each year during which Poaceae pollen concentrations recorded at the DMI monitoring station are high is typically small. As an example, during 2011 the routine bi-hourly concentration data exceeded 100 grains m^{-3} on only 41 occasions. Predicting high concentrations is not easy, thus a large proportion of the Poaceae data were collected under low concentrations.

One set of Urticaceae samples was rejected due to rainfall during sample collection, leaving 40 sets for analysis.

The Rotorod was battery powered (Yuasa NP7-12) in both locations, whilst the PVAS was battery powered in Worcester but mains powered in Copenhagen. All batteries were fully charged at the start of each day. At DMI, the Rotorod rotation and PVAS flow rates were measured at the start and end of each day. These data were not collected at the Worcester site, since rate measuring devices were not available at that time. The results of flow rate measurements, along with details of additional tests concerning the effects of battery decay on Rotorod and PVAS performance, are presented in Appendix D.1. The flow rate of the 7-Day samplers was verified every time the sampling substrate was changed (on a weekly basis in Worcester, and on a daily basis in Copenhagen).

Whilst in the field, all samples were carried in airtight containers and exposed to ambient air as little as possible. A number of Rotorod and PVAS control samples were collected at random times, by mimicking sample exposure to ambient air during normal sample collection. This was done in order to check for contamination during sample preparation, transport to and from the field, and post processing. The results of these controls can be found in Appendix D.2. In order to maximise the temporal accuracy of pollen monitoring station data, 7-Day Sampler sample traces were marked at a known time on each sample collection day in both locations. This was done by making two point marks on the right side of the sampler orifice, one at the top and one at the bottom[5].

In Worcester the 7-Day sampler was fitted with a standard seven-day drum, and samples collected on Melinex tape coated with a 9:1 petroleum jelly/paraffin wax adhesive, the standard adhesive of the UK pollen monitoring network. In Copenhagen the 7-Day sampler was fitted with a 24-hour head assembly, with samples collected directly onto a slide coated with silicone solution (Lanzoni s.r.l.), the standard adhesive of the Danish pollen monitoring network. These two adhesives have been shown to have statistically equivalent trapping abilities (Comtois & Mandrioli, 1997; Warner *et al.*, 2000), thus do not introduce bias. In both locations, PVAS samples were collected on 18 mm pieces of Melinex tape coated with the petroleum jelly/paraffin wax adhesive and mounted centrally on a standard microscope slide. Samples collected with the 7-Day sampler and PVAS were prepared for assay by mounting them on standard microscope slides with a stain-bearing glycerine jelly mountant[6]. The 7-Day sampler samples were prepared and post processed according to the methods of the British Aerobiology Federation (1994), whilst collection substrate preparation and post processing methods for the PVAS can be found in Appendix B.

Rotorod collector rods were coated with the standard silicone grease adhesive. After exposure, rods were stained with Calberla's solution and mounted on a specially designed microscope stage adapter, using the methods described by the manufacturer (Sampling

[5]It is important that the mark is a point. A horizontal line causes disruption to the entire sampling trace, whilst a vertical line risks a slight movement of the collection substrate.

[6]The stain used was basic fuchsin in all cases except for the 7-Day sampler samples from the Copenhagen monitoring station. The Danish pollen monitoring network use the stain safranin (J. Sommer, Danish Asthma & Allergy Association, personal communication, 14^{th} August 2012).

Technologies, 1998). The two positions within the Rotorod rotating arm were marked as positions 1 and 2, and the position inhabited recorded for each collector rod. For the Urticaceae data all rods were assayed, whilst for the Poaceae data only one rod from each pair was assayed.

Samples from all three samplers were assayed by the author under a light microscope at ×400 or ×640 magnification, and counts were converted to concentrations in pollen grains m^{-3} by dividing the number of pollen grains by the volume of air sampled using the conversion factors presented in Appendix C. Samples from the 7-Day sampler were assayed by counting the number of pollen grains deposited along transverse transects, as described by the British Aerobiology Federation (1994). Counts were conducted for time periods corresponding to mobile sampler sample collection, and thus sets of concurrent, approximately hour-averaged data were acquired for the three sampler models. For Rotorod samples, a 22 mm section of each rod was assayed, in accordance with the method outlined by the manufacturer (Sampling Technologies, 1998). For PVAS samples, the entire area of particle deposition was assayed. The numbers of Urticaceae pollen grains caught by the two Rotorod collector rods were found to be very strongly correlated (Spearman's coefficient $r_s = 0.98$, one-tailed $p \leq 0.0005$) whilst the sign test gave no evidence of bias between rods ($z = -0.320$, two-tailed $p = 0.749$), therefore for both pollen taxa concentrations were calculated based on only one rod from each pair[7] (the rod in position 1 unless this was compromised). Meteorological data were aggregated into mean hourly values corresponding to pollen data averaging periods.

2.2.3 Analysis and statistical methods

The efficiency relationships between pairs of samplers were investigated by comparing concurrent concentration measurements, whilst the influence of meteorological parameters on these relationships was investigated using ratios of concurrent concentrations. The divisor in these ratios was the measurement made with the 7-Day sampler or (when comparing

Table 2.1: Descriptive statistics for the Urticaceae (n=40) and Poaceae (n=45) data sets. 'Ratio' means the ratio of concentrations recorded by the two indicated samplers.

		Urticaceae		**Poaceae**	
Variable	Units	Range	Median	Range	Median
7-Day sampler concentration	grains m^{-3}	30.3 - 575.6	190.9	85.5 - 461.5	145.3
Rotorod concentration	grains m^{-3}	13.5 - 479.8	142.8	45.4 - 565.0	156.0
PVAS concentration	grains m^{-3}	5.2 - 215.5	51.7	22.4 - 234.5	48.3
Rotorod/7-Day sampler ratio	-	0.36 - 1.56	0.73	0.44 - 1.55	1.05
PVAS/7-Day sampler ratio	-	0.08 - 0.84	0.28	0.14 - 1.25	0.35
PVAS/Rotorod ratio	-	0.12 - 0.96	0.37	0.19 - 0.99	0.39
Wind speed	ms^{-1}	0.65 - 4.25	2.33	0.74 - 3.45	1.88
Relative humidity	%	45.5 - 76.5	60.4	40.2 - 80.6	52.0

[7] A single rod samples air at 21.7 l min^{-1}, over twice the rate of the 7-Day sampler and PVAS (both 10 l min^{-1}).

the two mobile devices) the Rotorod. Correlation analysis was used to assess the strength of these relationships. The Kolmogorov-Smirnov test for normality indicated that not all pollen datasets could be considered normally distributed, therefore Spearman's correlation was used. Results were considered significant at the 95% level.

Inter-sampler conversion factors were determined by fitting regression lines through data scatter plots. Where wind speed was found to have a significant effect on the efficiency relationship, the dependent concentration ratio was regressed onto the independent wind speed variable using the least squares method. Relationships found to be independent of wind speed were parametrised by fitting geometric mean regression lines forced through the origin to concentration data, as described by Leng *et al.* (2007). Conversion factor performance was assessed using the root mean square relative error (RMSE), with errors scaled by the concentration according to the 7-Day sampler or (when comparing the two mobile devices) the Rotorod. All analysis was performed using version 7.7.0.471 of MATLAB (2008).

2.3 Results

Range and median values for pollen and meteorological variables are presented in Table 2.1. Concentration data for the three sampler pairings and two pollen taxa are plotted in

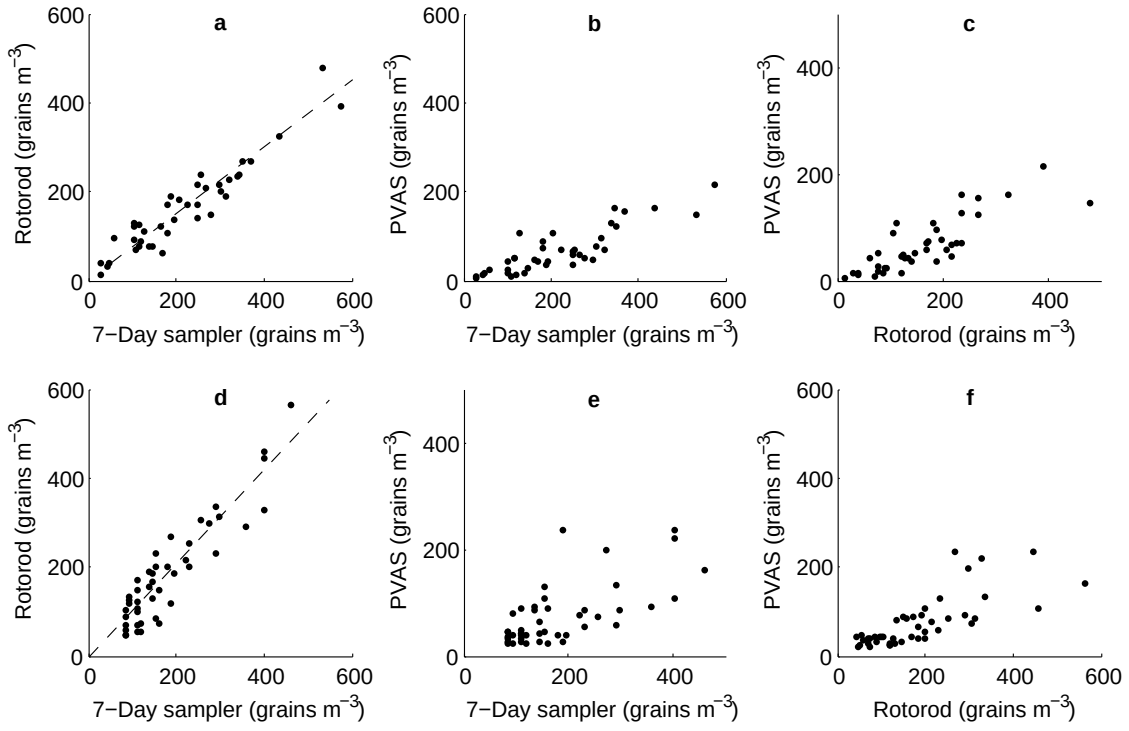

Figure 2.2: Scatter plots of concentration data (grains m^{-3}) for the three sampler pairings and two pollen taxa. Urticaceae data are presented in figures a, b and c, and Poaceae data in figures d, e and f. Geometric mean regression lines are plotted for the Rotorod/7-Day sampler pairings. Line equations are (a) $y = 0.7505x$ and (d) $y = 1.0498x$.

Pollen taxa	Comparison	Test statistic	p-value
Urticaceae	Rotorod & 7-Day	5	<0.0001*
	PVAS & 7-Day	0	<0.0001*
	PVAS & Rotorod	0	<0.0001*
Poaceae	Rotorod & 7-Day	21	0.7660
	PVAS & 7-Day	1	<0.0001*
	PVAS & Rotorod	0	<0.0001*

Table 2.2: Results of the two-tailed sign test on pollen concentration data for the different sampler pairings and pollen taxa. * indicates sampler pairings for which median concentrations were found to differ significantly.

	7-Day	Rotorod	PVAS
7-Day	-	*0.916*	*0.835*
Rotorod	**0.846**	-	*0.829*
PVAS	**0.622**	**0.787**	-

Table 2.3: Spearman's correlation coefficients between pollen concentration measurements for all sampler pairings and both pollen taxa. *Italic* font indicates Urticaceae pollen and **bold** font indicates Poaceae pollen. All relationships were significant, with one-tailed p-values <0.001.

Fig. 2.2. The sign test indicates that the median concentration values recorded by the three sampler models differ significantly for both pollen taxa, with the exception of the Rotorod and 7-Day sampler for Poaceae pollen (Table 2.2). Concentrations recorded with the PVAS tend to be lower than those recorded with the 7-Day sampler or Rotorod for both pollen taxa. Concentration data are significantly correlated for all sampler combinations

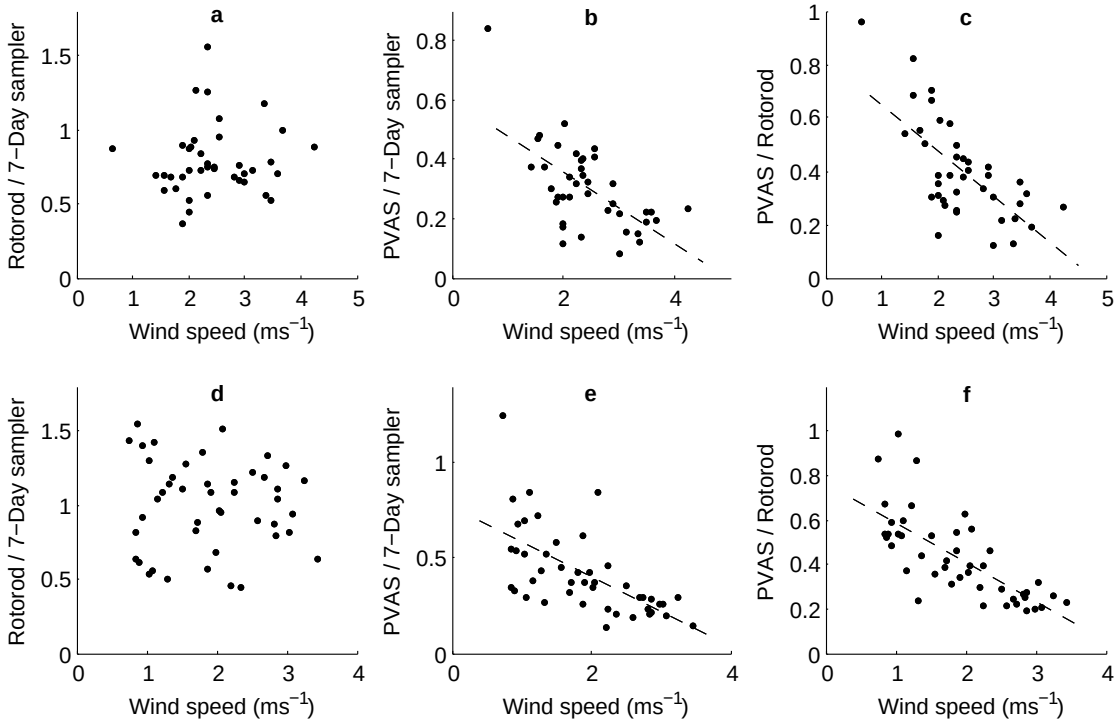

Figure 2.3: Scatter plots of wind speed against pollen concentration ratio for the three sampler pairings and two pollen taxa. Urticaceae data are presented in figures a, b and c, and Poaceae data in figures d, e and f. Ordinary least squares regression lines are plotted for the significant relationships (those involving the PVAS). Line equations are (b) $y = -0.1218x + 0.6001$, (c) $y = -0.1732x + 0.8232$, (e) $y = -0.1828x + 0.7658$, and (f) $y = -0.1792x + 0.7683$. Figures b, c, e and f all hint at a non-linear relationship, however there was judged to be insufficient range in the wind speed data for a curve to be reliably fitted.

Table 2.4: Spearman's correlation coefficients for pollen concentration ratios and meteorological variables. * indicates a significant two-tailed p-value (in all such cases p$\leq$ 0.001).

Pollen taxa	Variable	Rotorod/7-Day	PVAS/7-Day	PVAS/Rotorod
Urticaceae	Wind speed	0.193	-0.517*	-0.613*
	Relative humidity	0.065	-0.048	-0.121
Poaceae	Wind speed	-0.063	-0.705*	-0.782*
	Relative humidity	0.121	0.254	0.157

and both pollen taxa. For both taxa the strongest correlation occurred for the Rotorod/7-Day sampler pairing. For each sampler pairing, a stronger correlation coefficient was found for Urticaceae pollen than for Poaceae pollen (Table 2.3).

Relative efficiencies for sampler pairings involving the PVAS (PVAS/7-Day sampler and PVAS/Rotorod) were for both pollen taxa significantly negatively correlated with wind speed (Fig. 2.3, Table 2.4). These relationships were stronger for Poaceae pollen ($r_s = 0.71$, 0.78) than for Urticaceae ($r_s = 0.52$, 0.61), and for both taxa this relationship is stronger for the PVAS/Rotorod pairing than for the PVAS/7-Day sampler pairing. No significant correlations were found between concentration ratios and relative humidity.

The regression equations presented in Figs. 2.2 & 2.3 were rearranged to produce mathematical relationships that convert concentrations measured with the Rotorod ($C_{rotorod}$) and PVAS (C_{pvas}) to the value expected from a 7-Day sampler operating under identical conditions ($\hat{C}_{7\text{-}day}$), and for converting PVAS concentrations into the value expected from a Rotorod operating under identical conditions ($\hat{C}_{rotorod}$). For Urticaceae pollen these inter-sampler conversion factors (RMSE) were

$$\hat{C}_{7\text{-}day} = 1.3324 \times C_{rotorod} \qquad (32.16\%) \tag{2.1}$$

$$\hat{C}_{7\text{-}day} = \frac{C_{pvas}}{0.6001 - 0.1218u} \qquad (44.21\%) \tag{2.2}$$

$$\hat{C}_{rotorod} = \frac{C_{pvas}}{0.8232 - 0.1732u} \qquad (46.52\%) \tag{2.3}$$

where u is wind speed in ms^{-1}. The conversion factors (RMSE) for Poaceae pollen were

$$\hat{C}_{7\text{-}day} = 0.9525 \times C_{rotorod} \qquad (29.45\%) \tag{2.4}$$

$$\hat{C}_{7\text{-}day} = \frac{C_{pvas}}{0.7658 - 0.1828u} \qquad (37.67\%) \tag{2.5}$$

$$\hat{C}_{rotorod} = \frac{C_{pvas}}{0.7683 - 0.1792u} \qquad (28.73\%) \tag{2.6}$$

All concentrations are in units of grains m^{-3}.

2.4 Discussion

2.4.1 Relative efficiency

The three samplers compared in this study may be classified as *impaction* devices, so called because particles are removed from the air following impaction against an adhesive surface. The efficiency of impaction samplers depends upon how closely target aerosol particles follow air streamlines during changes in speed and direction. This varies with a number of the particle's physical properties, namely size, shape, density (Mandrioli, 1998, p. 9) and surface roughness (Solomon, 2003). Both Urticaceae and Poaceae pollen grains are smooth and approximately spherical (Wodehouse, 1965, pp. 303-304, p. 382) but differ considerably in size, with Urticaceae typically 12-17 µm (Hyde & Adams, 1958, pp. 92-94) and the pollen grains of common Poaceae species typically 20-40 µm in diameter (Section 1.2.1). Furthermore, Poaceae pollen tends to be denser than Urticaceae pollen (Durham, 1946). The larger, denser Poaceae pollen grains are less responsive to the airflow speed and direction changes that effect sampler efficiency (Solomon, 2003), which accounts for the stronger correlation coefficients for Urticaceae than for Poaceae pollen.

The vertical orientation of the PVAS means that as horizontal wind speed increases, air is forced to turn more violently as it enters the sampler's inlet. The proportion of particles that deviate from air streamlines enough to evade capture will also increase, causing aspiration efficiency to decline (Ogden *et al.*, 1974, p. 47). This explains why the PVAS was associated with weaker correlations between concentration data and with stronger correlations between concentration ratios and wind speed.

After release from the anthers, pollen grains exposed to air dry out and undergo a change in shape and a reduction in density and settling velocity (see Section 1.2.1). A consequent decline in the ability of pollen grains to follow air streamlines is expected as relative humidity decreases, which would have a positive effect on the efficiency of the two suction devices and a negative effect on Rotorod efficiency (Solomon, 2003). No evidence for such a phenomenon was however found, indicating that relative humidities within the range observed during this study have a negligible effect on sampler efficiency.

Frenz (2000) published an equation that relates Hirst-type and Rotorod sampler efficiencies for *Phleum* pollen (a member of the Poaceae family) and wind speeds up to 10 ms^{-1}, based on wind tunnel data. For the wind speeds present during this study, this equation predicts a median Rotorod/7-Day sampler concentration ratio of 1.27. A considerably lower value of 1.05 was obtained for grass pollen during this study, indicating similar efficiencies for the two devices. McCartney *et al.* (1997) on the other hand report that rotating arm and 7-Day samplers have comparable efficiencies for *Brassica napus* pollen (25 µm). At 35 µm, *Phleum* pollen is towards the upper end of the grass pollen size range (see Section 1.2.1) and considerably larger than *Brassica napus* pollen. Within the pollen size range, an increase in pollen grain size has opposing effects on Rotorod and 7-Day sampler efficiency, leading to an increase in Rotorod efficiency and decrease in 7-Day sampler efficiency (Solomon, 2003) and therefore an increase in the Rotorod/7-Day

sampler ratio. The higher ratio value obtained using the equation of Frenz (2000) may therefore be accounted for by the relatively large size of *Phleum* pollen, which may not be representative of the grass pollen grains encountered during this study. It should however be noted that the specifications and therefore potentially the efficiency of the rotating arm device used by McCartney *et al.* (1997) were not identical to those of the sampler used in this study.

Such a validation is not possible for Urticaceae pollen due to a lack of published data. There are similarly no published studies using Poaceae or Urticaceae pollen with which to validate the PVAS/7-Day sampler or PVAS/Rotorod relationships, however qualitatively equivalent relationships have been reported for equivalent sampler pairings using birch (Michel *et al.*, 2012) and Pinaceae (Banks & Di Giovanni, 1994) pollen respectively.

2.4.2 Inter-sampler conversion factors

Equations 2.1-2.6 may be used to convert concentration measurements made with one type of sampler to the value expected from another type operating under identical conditions, thus allowing data collected with different instruments to be compared directly.

Although at 29-47% the RMSEs for these conversions are not small, the inhomogeneity of the atmosphere and random sampling effects mean that even two identical samplers standing next to one another would not be expected to record identical concentrations. Even for high volume instruments these discrepancies can be large, for example two Chemvol samplers, which process air at 800 l min^{-1}, have been found to record grass pollen concentrations that differ by 8.7% when standing side-by-side, although a paired T-test showed that this difference was not statistically significant at the 95% level (J. Buters, Center of Allergy & Environment (ZAUM), unpublished observation, 2012). For low volume instruments such as those used in this study, these errors tend to be greater. RMSEs of 38% and 57% have been reported for pairs of 7-Day samplers and vertically orientated Air-O-Cell samplers for birch pollen (Michel *et al.*, 2012), whilst the RMSE between the collector rod pairs in the Urticaceae study was 12.73%. The errors associated with the corrections presented in the current study are therefore acceptable when compared with the errors expected for the individual instruments.

2.5 Conclusions

The 7-Day sampler, Rotorod and PVAS collect Poaceae and Urticaceae pollen with different efficiencies under field conditions, and these differences are statistically significant except in the case of the 7-Day sampler/Rotorod combination for Poaceae pollen. Relative efficiencies involving the PVAS show a significant relationship with wind speed, however for the range of conditions present during this study relative humidity does not appear to effect efficiency relationships. Data collected with different devices must be adjusted before a direct comparison is possible using inter-sampler conversion factors obtained under

field conditions, such as those presented in this study. Existing correction factors for the 7-Day sampler and Rotorod based on wind tunnel *Phleum* pollen data do not accurately reflect the field situation.

Chapter 3

Do urban canyons influence street level grass pollen concentrations?[1]

Abstract

In epidemiological studies, outdoor exposure to pollen is typically estimated using rooftop monitoring station data, whilst exposure occurs at street level. In this study, the relationship between street level and roof level grass pollen concentrations was investigated for city centre street canyon environments in London, UK and Aarhus, Denmark during the grass pollen seasons of 2010 and 2011 respectively. For the period mid-day to late evening, street level concentrations in both cities tended to be lower than roof level concentrations, though this difference was only statistically significant in London. The ratio of street/roof level concentrations was compared with temperature, relative humidity, wind speed and direction, and solar radiation. Results indicated that concentration ratios are influenced by wind direction through effects relating to relative canyon orientation and local source positioning. In the London study, an increase in relative humidity was linked to a significant decrease in the street/roof level concentration ratio, and a possible causative mechanism involving moisture mediated pollen grain buoyancy is proposed.

3.1 Introduction

Pollen monitoring stations are typically situated on the roof of a 10-30 m tall building, in order to measure regional background pollen concentrations (Lacey & Venette, 1995, pp. 424-425). Monitoring station data are frequently used in epidemiological studies as a proxy for outdoor exposure, for example Hajat *et al.* (2001), Momas *et al.* (2003), and Feo Brito *et al.* (2007). Exposure, on the other hand, occurs overwhelmingly at street level. In areas or environments where a vertical concentration gradient exists, roof level data will not accurately reflect street level exposure.

[1] A revised version of this study has been accepted for publication by the *International Journal of Biometeorology*, under the title of this chapter (accepted for publication 27 August 2013).

The relationship between roof level and street level pollen concentrations has previously been investigated in open areas (see Section 1.4.3). Urban areas are characterised by building-delimited street canyons that form semi-continuous barriers to horizontal airflow. Urban grass pollen sources generally lie outside these street canyons, meaning that pollen occurring at street level within a canyon must be transported above roof level and enter the canyon from above. It follows that the vertical concentration trends outlined for open areas in existing literature do not necessarily apply to the specialised case of the street canyon.

The objectives of this study were to establish the relationship between grass pollen concentrations at roof level and those at breathing height within a street canyon, and to investigate which weather parameters influence this relationship. This was achieved by measuring grass pollen concentrations within a street canyon and comparing these with concurrent data from a nearby roof level monitoring station. Two separate studies are presented here, conducted in London (UK) and Aarhus (Denmark) during the 2010 and 2011 grass pollen seasons respectively.

3.2 Method

In both Aarhus and London, roof level samples were collected with a Burkard Seven-Day Recording Spore Trap (7-Day sampler, Appendix A.1) after the design of Hirst (1952), and street level samples were collected with a Sampling Technologies Model 20 Rotorod (Rotorod, Appendix A.2) mounted 1.5 m above the ground on a tripod (Interfit Cor750 light stand). Street level sampling sites were selected as a compromise between the following criteria: they were required to be (i) as close as possible to the roof level monitoring station; (ii) in a street canyon with a pavement spacious enough to accommodate equipment without obstructing pedestrians, and; (iii) so far as was practically possible clear of side roads and other openings that might produce unusual air flow patterns. Data were collected between mid-day and late evening, covering the period of the day when grass pollen concentrations are most likely to be high in both London (Norris-Hill & Emberlin, 1991) and Aarhus (See Chapter 5).

3.2.1 Experimental locations

Aarhus, Denmark's second largest city, lies on the Western coast of the Jutland Peninsula and has a population of around 250,000 (Statistics Denmark, 2012). Within the city, unmanaged grasses[2] can be found along large roads, railways and streams and in industrial areas, whilst the immediately surrounding countryside consists largely of farmland including pasture and crops such as rye and grass seed (Skjøth *et al.*, 2013b), all of which are potential sources of grass pollen. The study was conducted in the city centre, in an area surrounded by a mix of residential and industrial buildings and railway lines, interspersed

[2]A definition of 'unmanaged grasses' is given in Section 1.4.1.

with small parks and gardens. To the East, the Kattegat sea was around 1.5 km or more distant, whilst it was a minimum of 3 km to open countryside. The roof level sampler was situated at the Central Aarhus monitoring station (Fig. 3.1a), on top of an elevator shaft on the roof of the Department of Nature and Environment at Aarhus Municipality, a minimum of 1.4 m from the edge of the shaft and with its air inlet 1 m above the roof's surface. The building sits on a reasonably steep incline, but where the sampler stands the surface of the lift shaft is around 11 m above the ground. Air flow towards the monitoring station is impeded for the sector 350-50° by a series of tall buildings 100-175 m away, and for the sector 90-130° by the municipality building roof, around 60 m away. The sampler is not part of the permanent Danish pollen monitoring network, but is one of three temporary monitoring stations in the city that are part of a large interdisciplinary study into pollen exposure (Skjøth *et al.*, 2013b). It was in continuous operation during the entire 2011 grass pollen season. Street level samples were collected on the West pavement of Eckersbergsgade, 2 m from the adjacent building and around 80 m from the roof top sampler (Fig. 3.1b, Fig. 3.2a). Eckersbergsgade is around 16 m wide and is delimited on both sides by four and five story buildings approximately 20 m in height.

The London study was conducted in Islington, a heavily urbanised and densely populated area of North London that has been home to one of the UK pollen network's monitoring stations since 1997 (Stach *et al.*, 2008). According to unpublished site data compiled by NPARU, there are few local sources of grass pollen in the surrounding areas (the nearest being Hampstead Heath, approximately 3.5 km to the NW), and very few grass verges along nearby roads. It is around 10 km to the nearest area of open countryside. The monitoring station was situated on the roof of Islington Town Hall, a four-storey building on Upper Street (Fig. 3.3a). The sampler was mounted on a metal frame a minimum of 5 m from the edge of the roof, with its orifice approximately 2.5 m

Figure 3.1: (a) the Central Aarhus pollen monitoring station and urban background weather station; (b) data collection on Eckersbergsgade, Aarhus, summer 2011.

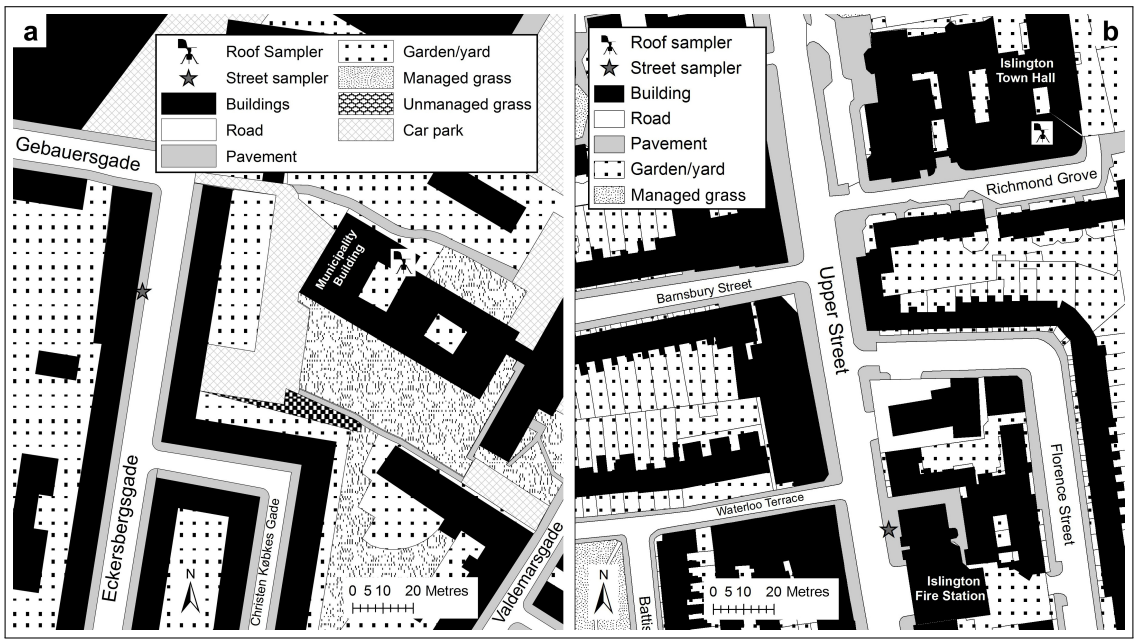

Figure 3.2: Maps of the Aarhus (a) and London (b) study areas. The London background map layer was obtained from Digimap's Ordnance Survey Collection (Edina, 2011). Aarhus map layers were adapted from the Kort 10 data collection provided by the Danish Geodata Agency (2012), obtained in September 2012 via Aarhus University.

above the roof surface and 18 m above ground level. There were no notable obstructions to horizontal airflow at sampler height. The monitoring station was in operation from March to September during 2010. Street level sampling was performed in front of Islington Fire

Figure 3.3: The Islington pollen monitoring station (a) and sample collection at the Upper Street site in Islington, London, summer 2010 (b).

Station on the Eastern pavement of Upper Street, 2.5 m from the wall of the adjacent building and around 142 m from the pollen monitoring station (Fig. 3.2b, Fig. 3.3b). At the point of sample collection, Upper Street was around 16 m wide. The building on the East side of the street was approximately 11 m high, and the building on the West side was slightly taller.

3.2.2 Data collection and processing

In both cities, street level samples were collected at two-hour intervals, with sample collection performed to precisely the same schedule every day. In Aarhus five samples were collected on a full day between 12:00 and 22:00, and in London four samples were collected per day between 14:00 and 22:00. The sampling duration was 60 minutes in Aarhus and 50 minutes in London[3], corresponding approximately to the averaging period of the 7-Day sampler. Collection was suspended if rain began to fall and abandoned for the day if rainfall proved to be heavy or persistent, on the basis that precipitation removes pollen sized particles from the air with great efficiency (McDonald, 1962). Data collection was performed on days when grass pollen concentrations were expected to be high, i.e. warm dry days during the main grass pollen season.

The roof level samplers in Aarhus and London were both operated with a standard seven-day sampling drum, and the standard adhesive of the local pollen monitoring network was used (silicone solution in Aarhus and petroleum jelly wax in London, see Section 2.2). Only one from each pair of Rotorod rods was assayed, since the two rods in each pair were found to produce statistically equivalent concentrations (see Section 2.2). The Rotorod rotation rate was checked at the beginning and end of each sampling day in Aarhus, but not in London, since the necessary equipment was not available at that time. The results of these rate checks can be found in Appendix D.1. The flow rates of the 7-Day samplers were checked on a weekly basis. In all other respects, the particulars of sample collection, sample pre- and post-processing and sample assay were as described in Section 2.2 for both the Rotorod and 7-Day sampler.

Roof level data were obtained for periods corresponding to street level sample collection, such that street/roof data pairs were aligned by their medial time stamps. All data collected with the Rotorod were adjusted using equation 2.4 (Section 2.3) to account for bias introduced by differences in Rotorod and 7-Day sampler efficiency, i.e. street level concentrations C_{street} were calculated using the relationship

$$C_{street} = 0.9525 \times C_{rotorod} \tag{3.1}$$

where $C_{rotorod}$ is the concentration measured with the Rotorod sampler.

Temperature and relative humidity measurements were made at the street level sampling site at the start, middle and end of each sample collection using a hand held thermo-

[3]The briefer sampling period in London was due to the collection of additional data sets not presented in this thesis.

hygrometer (Omega RH82) held approximately 1 m above ground level. In Aarhus, street level wind speeds were measured approximately 2 m above ground level with a switching anemometer (Vector Instruments A100R) mounted above the Rotorod such that the anemometer cups and Rotorod rotating arm were 30 cm apart.

For the Aarhus study, roof level wind direction, wind speed and solar radiation data were obtained from a city background weather station run by the Atmospheric Chemistry and Physics Section at the Danish Centre for Environment and Energy, Aarhus University, for use in the Danish air quality monitoring programme (Hertel *et al.*, 2007). The weather station was located at the Central Aarhus monitoring station (Fig. 3.1a), with wind data collected approximately 10 m and solar radiation 2 m above the roof of the lift shaft (21 m and 13 m above ground level respectively). For the London study, mean hourly roof level wind speed and direction data were obtained from the Olympic Park North weather station (UK Meteorological Office, 2010) which lies approximately 5.9 km due North of the Islington pollen monitoring site. Weather observations were recorded 25 m above ground level and 4 m above roof level (A. Guillory, British Atmospheric Data Centre, personal communication, 13^{th} Jan 2011).

For all meteorological data, hourly averages corresponding to pollen data averaging periods were calculated. For the London wind data this was achieved through time weighted averages. Times are reported in Central European Summer Time (GMT+2) for the Aarhus study, and British Summer Time (GMT+1) for the London study.

3.2.3 Data analysis

After missing or corrupted data and very low concentrations (<20 grains m^{-3}) had been discarded, 46 roof level and street level data pairs were available for analysis for Aarhus, and 32 pairs for London. The Aarhus data were collected over 12 days between 20^{th} June - 7^{th} July 2011, and the London data were collected over eight days between 12-26^{th} June 2010. The Kolmogorov-Smirnov normality test indicated that not all pertinent data sets could be considered normally distributed, therefore non-parametric statistical methods were employed, with results considered significant at the 95% level. The ratio street level concentration/roof level concentration was used to investigate the relationship between street and roof level pollen concentrations. Spearman's correlation coefficients were used to assess the linear association between street and roof level pollen concentrations and between street/roof concentration ratios and weather variables, whilst the sign test was used to test for significant differences between concurrent roof and street level concentrations. The Wilcoxon rank-sum test was used to test whether ratio magnitude was significantly influenced by relative humidity at the London site. All analysis was performed with version 7.7.0.471 of MATLAB (2008), and maps were produced using ArcGIS 10.0 (ESRI, 2011).

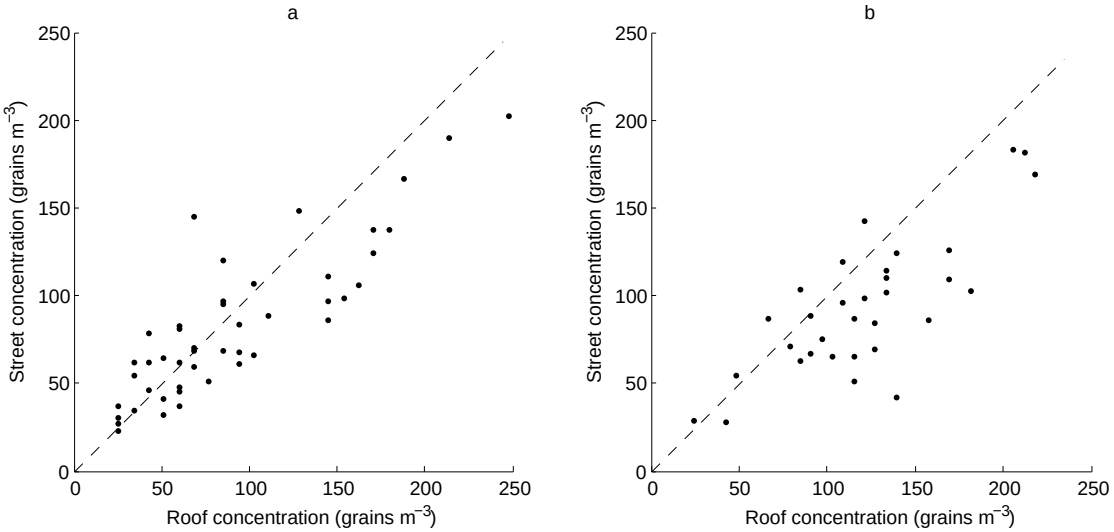

Figure 3.4: Comparison of grass pollen concentrations at roof and street level for (a) Aarhus and (b) London. The line $y = x$ along which roof level and street level concentrations are equal is plotted on each graph.

3.3 Results

Fig. 3.4 shows roof and street level grass pollen concentrations plotted against one another for Aarhus and London. Spearman's correlation coefficients indicate that data are significantly correlated in both cases, with a stronger relationship for Aarhus ($r_s = 0.840$, one-tailed p< 0.001) than for London ($r_s = 0.654$, one-tailed p< 0.001). Median street/roof ratios of 0.89 in Aarhus and 0.77 in London imply that in both cities street level concentrations tend to be smaller than roof level concentrations (Table 3.1), however the sign test indicates that whilst for London the median value differs significantly from unity (sign=6, two-tailed p=0.001), in Aarhus it does not (sign=21, two-tailed p=0.659).

Spearman's correlation coefficients between the street/roof concentration ratio and temperature, relative humidity, solar radiation and wind speed data are presented in Table 3.2. The strongest and only significant relationship occurs with relative humidity for the London data set ($r_s = 0.357$, two-tailed p=0.045).

Fig. 3.5 shows the street/roof level concentration ratio plotted against wind direc-

Table 3.1: Descriptive statistics for the Aarhus (n=46) and London (n=32) data sets.

		Aarhus		**London**	
Variable	Units	Range	Median	Range	Median
Street level pollen concentration	grains m^{-3}	22.7 - 202.2	69.6	29.5- 192.0	92.3
Roof level pollen concentration	grains m^{-3}	25.6 - 247.9	72.6	24.2 - 218.2	118.2
Street/roof concentration ratio	-	0.59 - 2.12	0.89	0.30 - 1.31	0.77
Temperature	°C	15.7 - 25.2	20.3	14.6 - 27.3	21.7
Relative humidity	%	35.6 - 81.5	58.1	27.8 - 56.9	43.9
Solar radiation	Wm^{-2}	6.8 - 837.8	300.0	-	-
Street level wind speed	ms^{-1}	0.53 - 2.98	1.62	-	-
Roof level wind speed	ms^{-1}	1.13 - 5.97	3.46	1.47 - 5.66	4.12

Table 3.2: Spearman's correlations coefficients (r_s) and two-tailed p-values for the relationship between the street/roof grass pollen concentration ratio and meteorological variables. * indicates significance at the 95% level.

		Temperature	Relative humidity	Solar radiation	Wind speed (Street)	Wind speed (Roof)
Aarhus	r_s	-0.078	-0.122	-0.013	0.059	-0.040
	p	0.605	0.419	0.933	0.697	0.793
London	r_s	-0.260	0.357	-	-	0.236
	p	0.150	0.045*	-	-	0.193

tion for the two cities. For the Aarhus data, almost all ratios greater than one belong to two clusters: winds from the sector 127-148°, and winds from the sector 265-289°. After excluding data collected under winds from these two sectors, the median street/roof concentration ratio is 0.80 with the sign test indicating that street level concentrations are significantly smaller than roof level concentrations (sign=6, two-tailed p=0.0227, n=24).

Median Aarhus street/roof ratios averaged by time of day are presented in Table 3.3. There is substantial variation over the course of the afternoon and evening, however this appears to relate to the proportion of samples collected under winds from the two sectors associated with high ratios. The highest and lowest median values (1.09 and 0.73), which occur at 19:00 and 21:00 respectively, have respective contributions of 70% and 22% from these two areas of the compass.

The plot of London median concentration ratios and relative humidities against time of day shown in Fig. 3.6 indicates that the significant relationship between these two variables (Table 3.2) may be restricted to relative humidities below approximately 45%. Between 14:40-18:40 median relative humidity is $< 45\%$ and the initial decline and subsequent

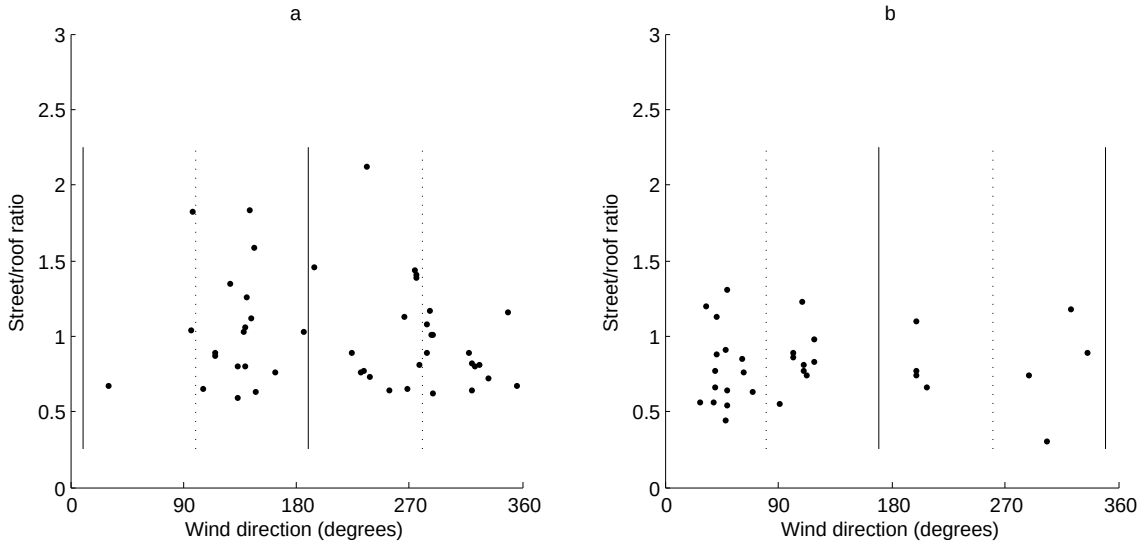

Figure 3.5: Street/roof grass pollen concentration ratio plotted against wind direction for (a) Aarhus and (b) London. Solid lines indicate the bearing of and dashed lines the direction perpendicular to the respective street canyons.

Time	13:00	15:00	17:00	19:00	21:00
Median	0.85	0.96	0.82	1.09	0.73
n	6	10	11	10	9

Table 3.3: Aarhus street/roof grass pollen concentration ratio averaged by time of day. Note that sample size (n) ranges from 6-11.

increase in median relative humidity is repeated in the concentration ratio, however as relative humidity increases above 45% between 18:40-20:40 the concentration ratio does not vary. Street/roof ratios relating to data collected when relative humidity was <45% ($n = 19$) were on average smaller than those relating to relative humidities >45% ($n = 13$), with median values of 0.74 and 0.86 respectively, however the Wilcoxon rank-sum test indicates that this difference is not statistically significant ($W = 257.5$, $p = 0.101$).

3.4 Discussion

3.4.1 The street/roof level ratio

Existing literature shows that for most herbaceous taxa such as Poaceae, the vertical pollen concentration gradient depends primarily on the local availability of source plants. Where local sources exist concentrations generally tend to be greater at ground level than at roof level, whilst in the absence of local contributions little variation is typically observed (Raynor *et al.*, 1973b; Rantio-Lehtimäki *et al.*, 1991b; Alcázar *et al.*, 1999; Spieksma *et al.*, 2000). This trend likely reflects the time it takes for pollen to become well mixed in the lower boundary layer. City centres typically feature a network of street canyons, with the buildings that define them forming continuous barriers to horizontal airflow. Although some pollen producing species such as ornamental trees are commonly found lining urban streets, grasses generally grow outside the street canyon network. Grass pollen found at street level within the canyon must therefore have been transported above roof level before migrating downwards into the canyon airspace, irrespective of distance travelled (Fig. 3.7), thus for the specific case of a street canyon the vertical trend outlined by previous work does not necessary apply.

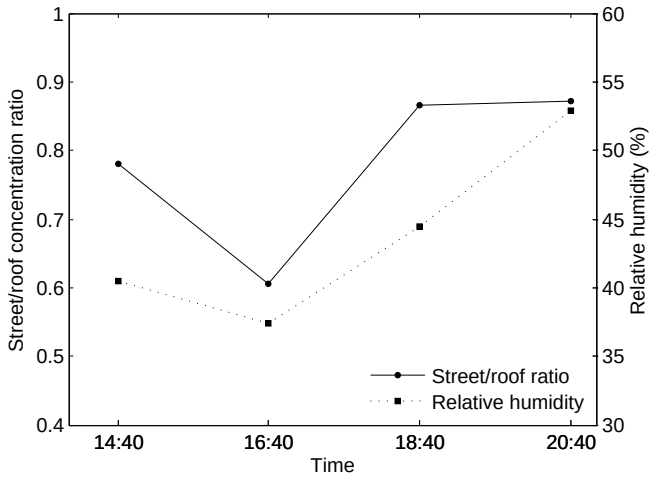

Figure 3.6: Median London street/roof grass pollen concentration ratio and relative humidity averaged by time of day. The sample size is eight for each average.

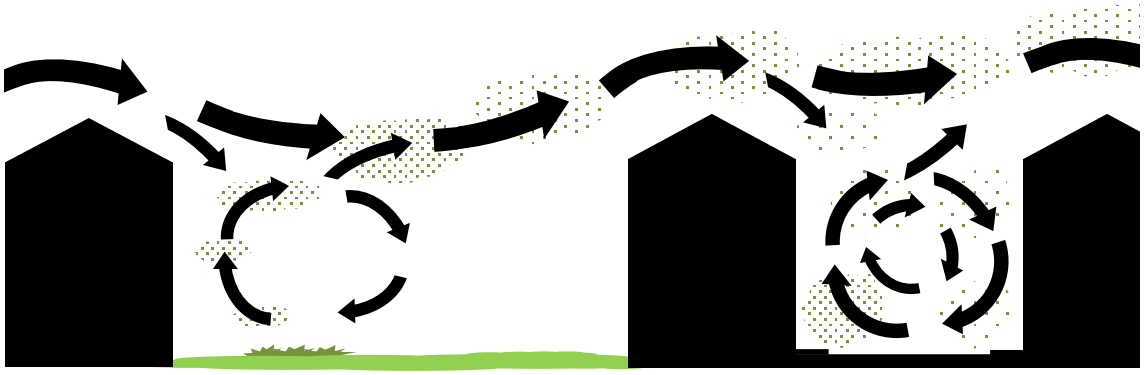

Figure 3.7: Conceptual diagram showing how pollen extraneous to the street canyon must be transported above roof level before entering the canyon environment from above. Wind flow above roof level serves to generate a circulating vortex within the canyon airspace. In the special situation where winds are approximately perpendicular to the canyon, this can lead to increased aerosol concentrations on the windward side of the street (Berkowicz *et al.*, 1996).

Airflow patterns within a canyon depend upon canyon geometry and synoptic (i.e. above roof) wind direction. Street canyons may be defined in terms of their height/width (H/W) ratio. For canyons with H/W ratios >0.7, such as Eckersbergsgade in the Aarhus study, winds approximately perpendicular to the canyon generate a vortex within the canyon (Oke, 1988). The H/W ratio of the Upper Street canyon in the London study is close to this critical value[4]. For smaller H/W ratios and winds approaching the direction of the street, a smaller vortex that does not span the width of the canyon is generated (Berkowicz *et al.*, 1997). These vortices recirculate air within the canyon, which one would expect to gradually become depleted of its pollen load as pollen settles onto the ground or is removed from the air through filtration and impaction against buildings (Emberlin & Norris-Hill, 1991) and other surfaces within the canyon such as trees and parked cars. It follows that the concentration of pollen within the fresh air entering the canyon from above will become diluted as it mixes with this recirculating air, leading to reduced pollen concentrations within the canyon compared with those found above roof level, in accordance with the results of this study.

The air within a street canyon is protected by the delimiting buildings. Wind speeds close to the ground have been found to be approximately $^2/_3$ those at roof level for a canyon with a H/W ratio of one, though a value somewhat closer to unity is expected for the smaller H/W ratio of the canyons in this study (Nakamura & Oke, 1988). This calmer environment will likely have the effect of enhancing pollen deposition.

The rate of exchange between within- and above-canyon air varies with canyon geometry (Palmgren *et al.*, 1996), therefore different street/roof level concentrations ratios would be expected for different street canyon configurations. This exchange rate furthermore determines how rapidly within-canyon pollen concentrations respond to changes

[4]The H/W ratio of the Upper Street canyon depends on wind direction because the height of the buildings on either side differ. For winds from the East the ratio is 0.69. The exact height of the buildings on the West side is not known however it is slightly taller, thus the H/W ratio will be slightly greater.

in ambient pollen abundance. Atmospheric grass pollen concentrations commonly incur rapid change, and a decline may result in a street/roof level concentrations ratio greater than unity. However, it seems unlikely that this effect would significantly impact the hour-averaged data of this study.

3.4.2 Wind direction

In the Aarhus study, street/roof concentrations ratios greater than one were restricted almost exclusively to two areas of the compass: the sector 265-289° (i.e. approximately perpendicular to the street canyon such that street level samples were collected on the windward side of the street with respect to synoptic wind direction, see Fig. 3.2a), and the sector 127-148°.

For winds travelling approximately perpendicular to a street canyon, the recirculating vortex has been shown to lead a local concentration maxima on the windward side of the street, with concentrations typically greater than those on the leeward side by a factor of two or more (Berkowicz *et al.*, 1997). This would account for the large street/roof concentrations ratios that accompanied Westerly winds in Aarhus. In the case of regionally sourced pollen, several authors have associated a similar phenomenon with tall buildings. Käpylä (1983), Alcázar *et al.* (1999) and Alcázar & Comtois (2000) all report that street level pollen concentrations close to a building's windward side[5] tend to be smaller than those at roof level, whilst those on its leeward side tend to be greater than or similar to those at roof level. Very few samples were collected for winds travelling perpendicular to the street during the London study.

Around 50 m from the Aarhus street level sampling site there was a grass covered parcel of land approximately 199 m^2 in area that, according to local residents, was permanently unmanaged (Fig. 3.2a). Winds from the sector 121-150° passed directly over this area but did not travel past the roof top pollen sampler. Under these conditions, the pollen signal from this parcel of land would contribute to street level concentrations but not to roof level concentrations. Very few samples were collected under the reciprocal scenario (i.e. winds passing over the grass-covered parcel and the roof level sampler).

3.4.3 Relative humidity

The only significant relationship between the street/roof pollen concentration ratio and weather variables was a positive correlation with relative humidity for the London study, implying that street level concentrations tend to increase relative to roof level concentrations as humidity increases. Average diurnal profiles for these two variables suggest that this association may only hold for relative humidities below a threshold close to 45%, which would explain why the relationship is restricted to the London data set. The Aarhus relative humidity range is notably higher than that of London, indeed only 13% of Aarhus values fall below 45%.

[5]In the context of a street canyon, this is equivalent to the leeward side of the street.

Pollen grains are hygroscopic and their moisture content, size and settling velocity have been shown to decrease with ambient relative humidity (Aylor, 2002, 2003). The likelihood of a pollen grain transferring from the above canyon air mass into the canyon environment may thus be reduced at lower relative humidity values. The change in size of hygroscopic particles occurs suddenly at a threshold humidity value, and thresholds from 15% to 81% have been reported for salt compounds (Fitzgerald, 1975; Pryor & Barthelmie, 2000). As far as the author is aware such thresholds have not been determined for pollen grains, however a sharp change in pollen grain aerodynamic behaviour at around 45% is clearly plausible. This phenomenon could explain why a relationship appears to occur only for relative humidities under 45%, and why this relationship was not observed in the Aarhus study. Further studies on the physical relationship between pollen grains and relative humidity are however needed before this theory can move beyond speculation.

It is well known that temperature and relative humidity are inversely related, thus it is possible that the correlation between relative humidity and the street/roof ratio may be an artefact of or amplified by a temperature related process. The temperature range and median of the Aarhus study are very similar to those of the London study (see Table 3.1). If temperature were a direct driver, we would expect the relationship to be present in the Aarhus as well as the London data.

3.4.4 Diurnal trends

Differences in the mean street/roof ratio do occur over the period of study for both cities. In London the ratio is fairly consistent other than a minima at 16:40 which is thought to occur in response to the diurnal relative humidity pattern. In Aarhus, the average street/roof ratio peaks at 19:00, and this peak appears to be related to the influence of wind direction which is thought to be partially related to local emission. A study by Alcázar & Comtois (2000) in Montreal found that the street/roof level *Ambrosia* pollen concentration ratio varied with time of day, and related these to the proximity of contributing sources. In the early morning, when pollen was thought to come mainly from small nearby sources, a subtle decrease with height was observed whilst a definite increase with height was observed later in the morning, when pollen was thought to be arriving from further afield. For a canyon environment one would however expect only local sources within or with direct access to the canyon to have any impact, and no such trend is expected or detected in this study. It is of course possible that outside the period of the day studied here other patterns and effects are in operation.

3.5 Conclusion

This study finds that the street canyon environment has an influence on street level grass pollen concentrations, with concentrations at street level within the canyon tending to be smaller than concurrent concentrations at nearby roof level monitoring stations. This

relationship does not hold for the windward side of the street in the special situation where winds are approximately perpendicular to the canyon, whilst nearby pollen sources may complicate the relationship. These findings suggest a tendency for roof level monitoring station data to overestimate exposure in the street canyon environment.

Chapter 4

Personal exposure to grass pollen: relating inhaled dose to background concentration[1]

Abstract

Very few studies on human exposure to allergenic pollen have been conducted using direct methods, with background concentrations measured at city centre monitoring stations typically taken as a proxy for exposure despite the fact that concentrations in different parts of the same city can differ considerably. Furthermore, the relationship between dose rate and allergy symptoms is not well understood. A 2003 WHO report on phenology highlighted the need for an improved understanding of the relationship between monitoring station data and actual exposure. In this study, grass pollen dose rates were measured in a suburban area of the city of Aarhus, Denmark, in a district where unmanaged grasses were prevalent, using Nasal Air Samplers. Data were collected at two-hour intervals between noon and mid-evening under moderate exercise. A median dose rate/background concentration ratio of 0.018 was recorded, with higher ratio values frequently occurring at 12:00 and 14:00 when grass species likely to be present in the area are expected to flower and release pollen. For the period 16:00-20:00, dose rate and background concentration data were found to be strongly and significantly correlated ($r_s = 0.81$). Diurnal averages of dose rate and background concentration data showed opposing trends, each of which motivates a different allergen avoidance strategy.

4.1 Introduction

In a 2003 report on phenology, the World Health Organisation (WHO, 2003a, p. 40) highlighted the need for an improved understanding of the relationship between background

[1] A revised version of this study has been published in the journal *Annals of Allergy, Asthma & Immunology*, under the title of this chapter (volume 111, pages 548-554).

pollen concentration measurements made at monitoring stations, and the actual exposure that allergic individuals experience. In spite of this, only a handful of direct exposure studies have been performed for bioaerosols. Existing pollen exposure studies have largely used breathing zone concentrations as a measure of exposure (Kailin, 1964; Leuschner & Boehm, 1979; Gautrin *et al.*, 1994; Riediker *et al.*, 2000; Myszkowska *et al.*, 2007), however it is difficult to know the relationship between such exposure measurements and the pollen grain dose that an individual receives (O'Meara & Tovey, 2000). Whilst pollen dose has been studied (Mitakakis *et al.*, 2000; O'Meara *et al.*, 2004), dose data does not appear to have previously been related to background concentration data.

In typical direct exposure studies, a panel or cohort of test subjects is recruited and equipped with exposure monitors that they wear as they go about their normal daily routine. This methodology is commonplace in air pollution science. Exposure data can then be related to monitoring station data, and thus the relationship between the two established. However, each individual will typically inhabit numerous microenvironments (Hertel *et al.*, 2008), both indoor and outdoor, thereby rendering the interpretation of results very complex. In this study, grass pollen dose data were collected using NAS samplers (Appendix A.3) in a city centre location characterised by substantial areas of unmanaged grass[2], and related to concurrent background pollen concentrations. The aims of the study were to evaluate dose rates, and to assess monitoring station data as a proxy for exposure.

4.2 Method

4.2.1 Study location

The study was conducted in central Aarhus, in the vicinity of the Central Aarhus pollen monitoring station. A description of Aarhus can be found in Section 3.2. NAS samples were collected whilst walking a single lap of a 2.7 km circuit in an area close to the city centre (Fig. 4.1). The circuit began at the Northern end of P. Hiort-Lorenzens Vej, and proceeded West along Søren Frichs Vej before bearing South-Southwest, first along Åbrinkvej then along the Brabrandstien footpath. Where the footpath and railway lines converge, the circuit turned and headed Northeast along the railway line, first by footpath and finally along P. Hiort-Lorenzens Vej, concluding at the starting point.

To the Northwest of Åbrinkvej and the Brabrandstien footpath lies the Aarhus stream, and there is a 20-50 m wide strip of unmanaged land overgrown with a variety of herbaceous plants (mainly grasses) and trees dividing the two. This nature reserve represents the only substantial potential grass pollen source area along the sample collection route (see Fig. 4.1). The locality is otherwise characterised principally by residential housing, private gardens, parks, railway infrastructure and industrial wasteland, with areas of unmanaged grass either small and discrete or lightly populated.

[2]A definition of 'unmanaged grass' is given in Section 1.4.1.

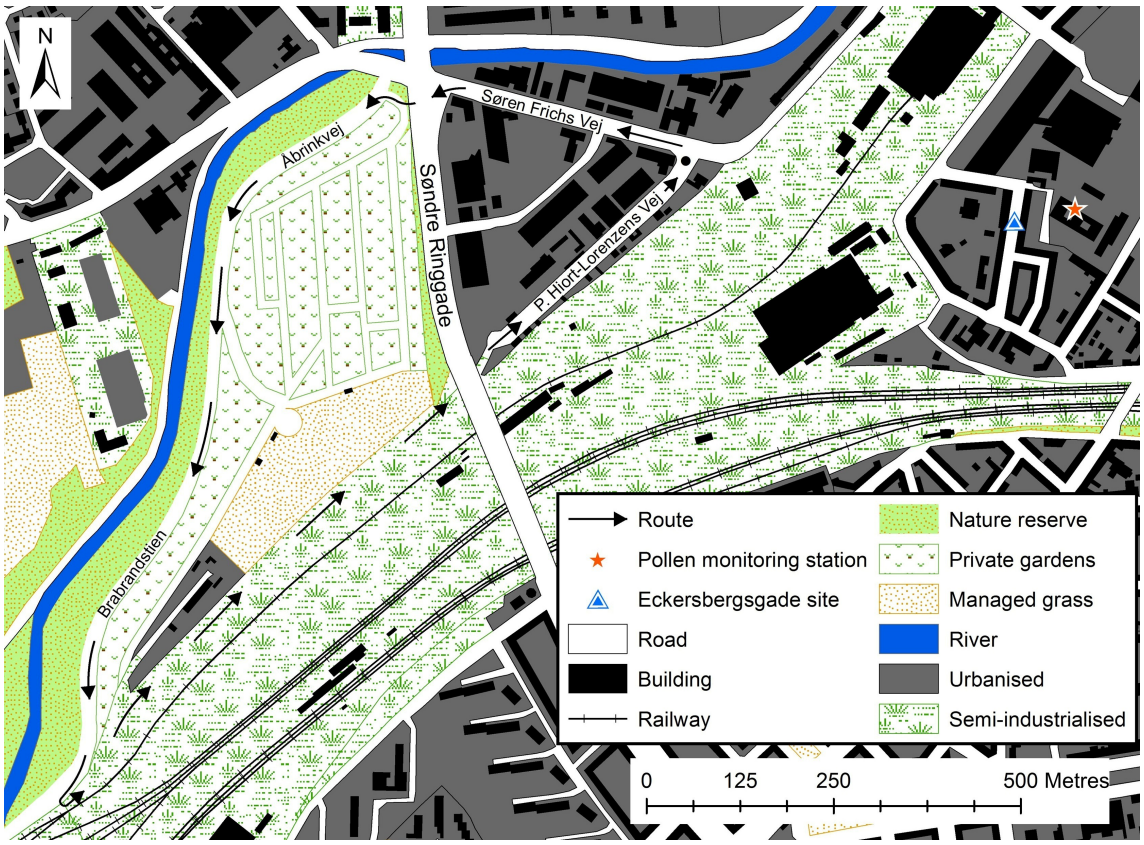

Figure 4.1: Map showing the route along which samples were collected. *Nature reserve* denotes unmanaged areas where grasses are allowed to flower; *semi-industrialised* areas are characterised by low density unmanaged grasses; areas of *managed grass* are mown regularly and assumed not to flower; *urbanised* land cover denotes residential and industrial brownfield sites with little or no vegetation; the characteristics of *private gardens* is unknown. Map layers were adapted from the Kort 10 data collection provided by the Danish Geodata Agency (2012), obtained in September 2012 via Aarhus University.

Background pollen concentration data were collected at the Aarhus City Centre pollen monitoring station, situated within 0.5-1.5 km of the circuit. A description of the monitoring station can be found in Section 3.2.

A similar study was performed in London in 2010. The results are however not discussed here, since the pressure sensitive tape collection substrate used was found to retain grass pollen grains with poor efficiency, rendering data unreliable. For data collection in Aarhus, the tape was coated with silicone grease, which greatly improves its adhesive properties (E. Tovey, University of Sydney, personal communication, 29^{th} October 2010). The results of the London study are presented and compared with those of the Aarhus study in Appendix E.

4.2.2 Data collection and processing

Data from 64 NAS samples collected on 13 separate days between 20th June and 7th July 2011 (within the main grass pollen season) were analysed. Sample collection was performed by two individuals on days on which the weather was expected to favour pollen emission (i.e. warm and rain-free) between mid-day and mid-evening, the period of the

day when background grass pollen concentrations were most likely to peak in the area (see Chapter 5). Sampling lasted for approximately 25-30 minutes and began on all occasions at precisely 15 minutes to the hour, thus the time of sample collection was identified by a medial time stamp on the hour. On each day five samples were collected, at 12:00, 14:00, 16:00, 18:00 and 20:00, with the exception of one day where the final sample was not collected due to rain (times are in Central European Summer Time, i.e. GMT+2).

Whilst using the NAS, air was inhaled through the nose and exhaled through the mouth, as exhaling through the nose occasionally caused the sampler to work loose. NAS samples were transported to and from the field in small airtight containers, pre- and post-processed using the methods described in Appendix B, and assayed under a light microscope at $\times$ 400 or $\times$ 640 magnification by the author. The number of grass pollen grains captured on the left and right nostril NAS adhesive strips were counted and summed, to produce a total grass pollen dose for each sample collection episode. Average dose rates in units of grains min^{-1} were then calculated by dividing the dose by the collection period, to account for differences in the duration of sampling. Monitoring station data were obtained for periods corresponding to NAS sample collection, such that NAS/monitoring station data pairs were aligned by their medial time stamps. Details of the methods relating to monitoring station data can be found in Section 3.2.

Background temperature, wind direction, wind speed and solar radiation data were obtained from the city background weather station described in Section 3.2, and hourly averages corresponding to background pollen concentration data were calculated. Surface temperature and relative humidity readings were taken approximately 1 m above ground level on Eckersbergsgade (Fig. 3.1b), 80 m from the City Centre monitoring station, using a hand held thermo-hygrometer (Omega RH82). Readings were taken approximately 15 minutes before and after each NAS sample collection, and an average calculated (except for the first collection of the day, when only the post-collection reading was taken). Surface wind speed was measured approximately 2 m above ground level during NAS sample collection with a switching anemometer (Vector Instruments A100R) mounted on the side of a rucksack, and averaged over each collection period.

4.2.3 Data analysis

The volume of air inhaled whilst walking the sample collection route was measured for each of the two individuals involved in sample collection, and observed to differ considerably between the two. However, no effect on dose could be detected, most likely because the opposing effects of inhaled volume and inhalation rate cancelled one another out (see Appendix E). It was therefore considered appropriate to pool data collected by the two individuals without making any adjustment. This appears to be standard practice when using the NAS (Mitakakis *et al.*, 2000; Renström *et al.*, 2002; Gore *et al.*, 2006; Renström *et al.*, 2006). Analysis was based upon the ratio dose rate/background concentration. This parameter can be interpreted as the average number of pollen grains inhaled per minute,

per unit of concentration recorded at the monitoring station. The four instances where the monitoring station count was zero were deleted, leaving a ratio dataset of size 60. The Kolmogorov-Smirnov test indicated that pollen data were not normally distributed, thus non-parametric statistical methods were applied with results considered significant at the 95% level. Analysis was conducted using version 7.7.0.471 of MATLAB (2008), and Fig. 4.1 produced with ArcGIS 10.031.

4.3 Results

4.3.1 Dose rate

Daily averaged grass pollen concentrations on data collection days, as measured at the monitoring station, ranged from 13-95 grains m^{-3} with a high count ($\geq$50 grains m^{-3}) recorded on six occasions, whilst hour-averaged background values corresponding to NAS data collection periods ranged from 0 - 311 grains m^{-3}. Grass pollen doses recorded with the NAS ranged from 6-127 grains (median 34 grains), with dose rates between 0.23 - 4.83 (median 1.20) grains min^{-1} (Table 4.1).

The dose rate/background concentration ratio ranged from 0.006 - 0.120 (Table 4.1). Fig. 4.2 shows that whilst 85% of ratio values were $\leq$ 0.037 (median 0.018), there was a group of nine unexpectedly high ratios of 0.063 or greater. Furthermore, of these nine outliers, seven corresponded to samples collected at 14:00 or earlier, and five to 12:00 collections.

Dose rate and background concentration data are presented in Fig. 4.3, grouped by collection time. A large proportion of the disparity between the two clearly lies within the 12:00 - 14:00 data. Spearman's correlation coefficients for the periods 12:00 - 14:00 and 16:00 - 20:00 are 0.518 (two-tailed $p = 0.007$) and 0.814 (two-tailed $p < 0.001$) respectively. For the entire data set the correlation coefficient is 0.644 (two-tailed $p < 0.001$).

Table 4.1: Ranges and median values of data used in this study (n=64, except for dose rate/background concentration ratio where n=60).

Variable	Units	Range	Median
Dose	grains	6 - 127	34
Dose rate	grains min^{-1}	0.23 - 4.83	1.21
Background hour averaged concentration	grains m^{-3}	0.0 - 311.0	56.2
Background daily mean concentration	grains m^{-3}	13 - 95	45
Dose rate/background concentration ratio	m^3 min^{-1}	0.006 - 0.120	0.018
Exposure duration	minutes	25.67 - 31.15	27.56
Average walking speed	ms^{-1}	1.44 - 1.75	1.63
Background temperature	°C	15.2 - 23.3	19.8
Background solar radiation	Wm^{-2}	20.8 - 855.0	459.8
Background wind speed	ms^{-1}	1.5 - 6.5	3.6
Surface temperature	°C	16.4 - 25.3	21.13
Surface relative humidity	%	33.4 - 81.7	58.0
Surface wind speed	ms^{-1}	1.0 - 3.0	2.1

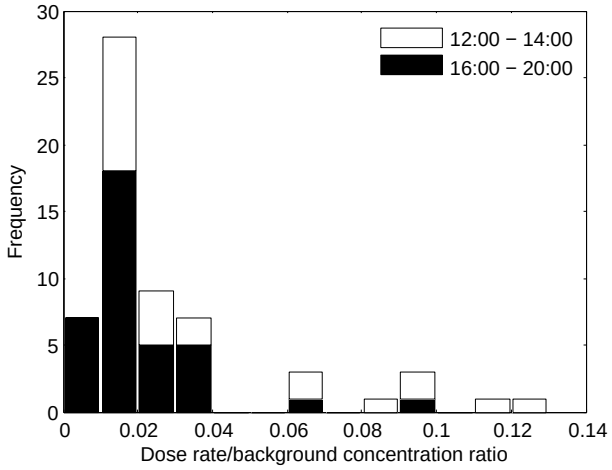

Figure 4.2: Frequency distribution of the dose rate/background concentration ratio (n=60, bin width=0.01). White denotes samples collected at 12:00 or 14:00, and black denotes samples collected at 16:00, 18:00 or 20:00.

4.3.2 Influence of meteorological variables

Fig. 4.4 shows the dose rate/background concentration ratio plotted against weather data. Spearman's correlation analysis indicates significant positive relationships for solar radiation and ground level wind speed (Table 4.2). Fig. 4.4 however reveals that data collected at 12:00 and 14:00 are almost entirely restricted to high solar radiation and surface wind speed values.

Dose rate and the dose rate/background concentration ratio are plotted against wind direction in Fig. 4.5. During sample collection, winds came principally from two areas of the compass - the Southeast (i.e. approaching over the railway lines and private gardens), and the Southwest to Northwest (i.e. from the direction of the Brabrandstien nature reserve), see Fig. 4.1. There is no apparent relationship between dose rate and these two wind direction groups, however the majority of high ratio values (i.e. ≥ 0.063) belong to the former (Southeast) and only two to the latter (Southwest-Northwest).

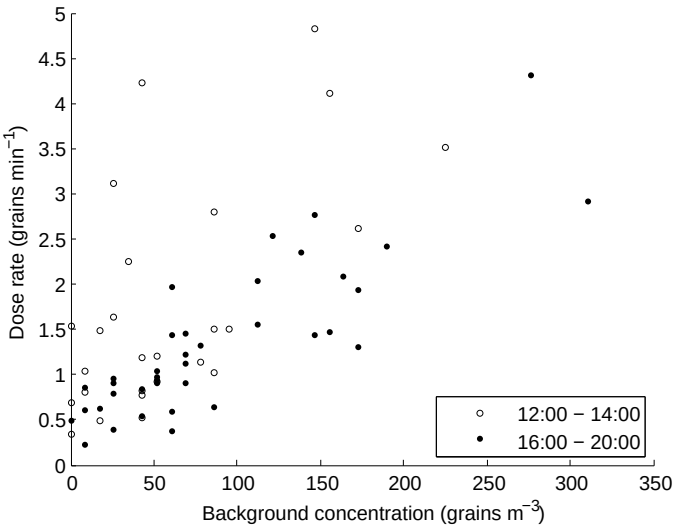

Figure 4.3: Grass pollen dose rates measured with the NAS plotted against concurrent background concentrations measured at the monitoring station. Data are grouped by collection time (12:00 - 14:00, n=26 and 16:00 - 20:00, n=38).

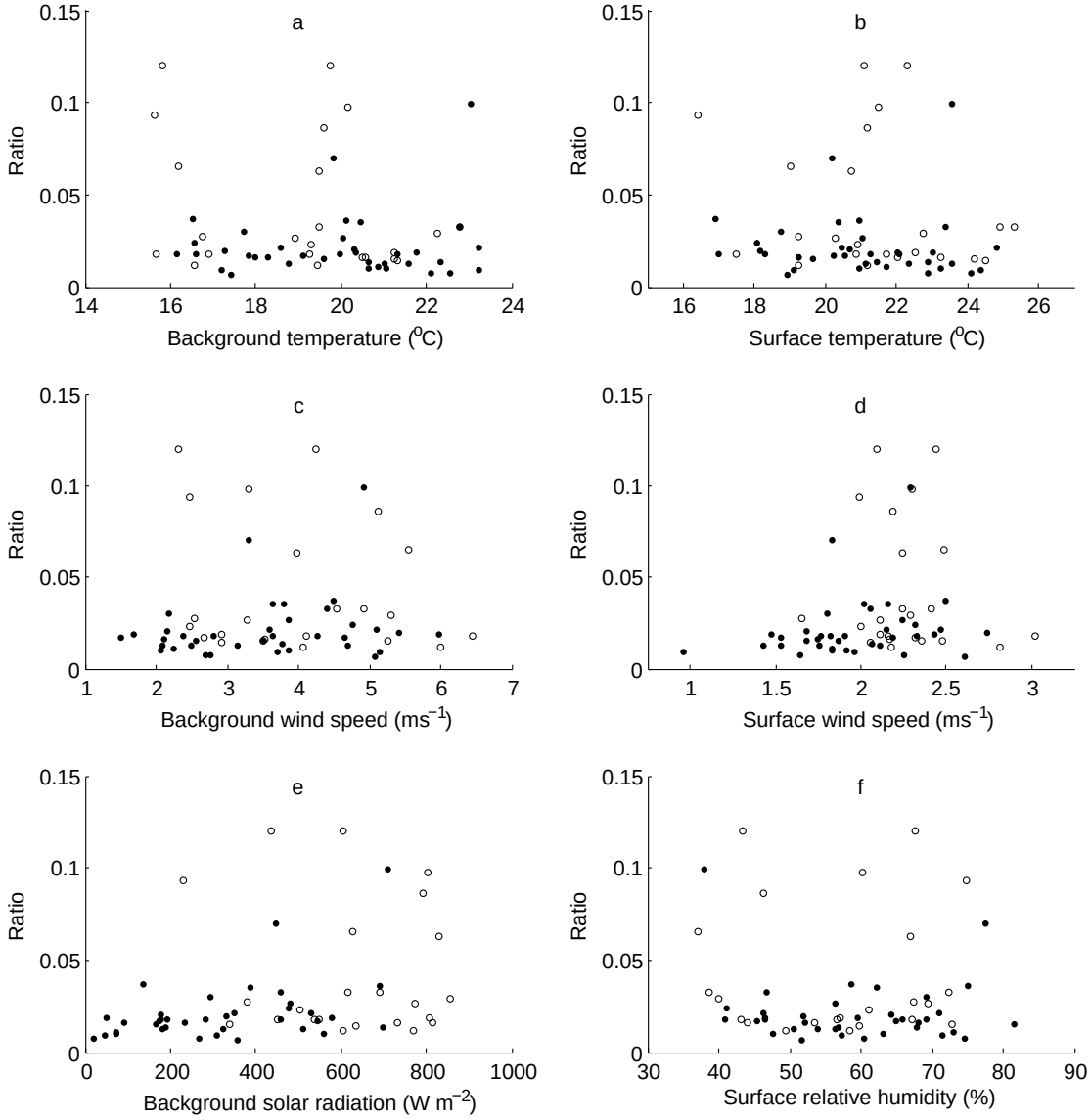

Figure 4.4: Dose rate/background concentration ratio versus meteorological parameters (n=60). Circles represent data collected at 12:00-14:00 and dots data collected at 16:00-20:00. *Background* weather data come from the background weather station, whilst *surface* weather data were collected manually.

Table 4.2: Spearman's correlation coefficients and two-tailed p-values for dose rate/background concentration ratio and weather variable relationships (n=60). * indicates significant result.

	Background temperature	Background solar radiation	Background wind speed	Surface temperature	Surface relative humidity	Surface wind speed
r_s	-0.197	0.372	0.139	-0.157	-0.072	0.274
p	0.131	0.004*	0.290	0.231	0.587	0.034*

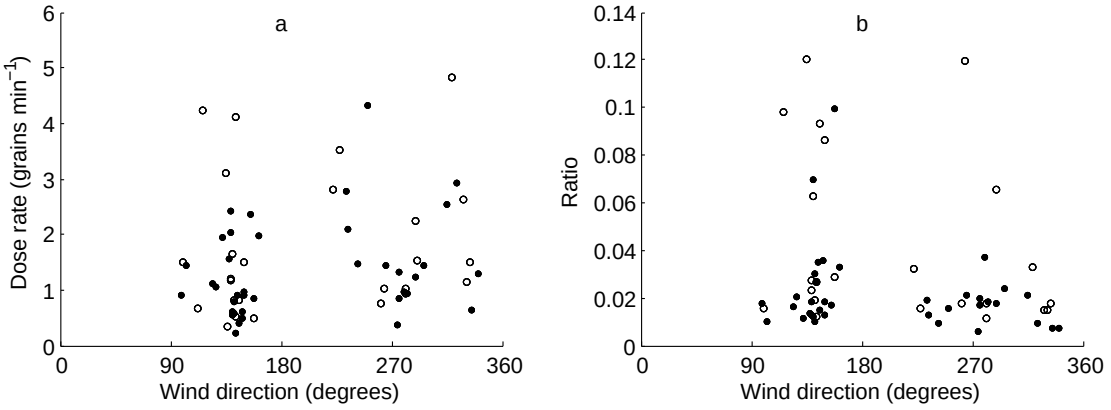

Figure 4.5: (a) dose rate (n=64) and (b) dose rate/background concentration ratio (n=60) plotted against wind direction. Circles indicate the period 12:00 - 14:00, dots indicate the period 16:00 - 20:00.

4.3.3 Temporal trend

Dose rate and background concentration data averaged with respect to time are presented in Fig. 4.6. Whilst averaged background concentrations increase from midday to mid-evening, as expected for the Aarhus area, averaged dose rates show the opposite trend, declining sharply between 12:00 and 16:00 but remaining approximately constant thereafter, with an overall reduction of around 35%. The group of high ratios do not account for this decline alone. The mean 12:00 dose rate is 2.05 for all data and 1.98 when the high ratios are omitted, whilst at 14:00 the two corresponding values are 1.57 and 1.50 respectively.

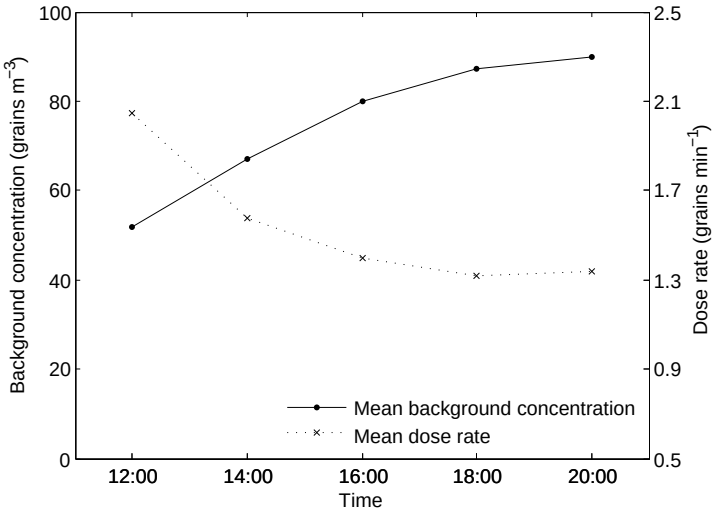

Figure 4.6: Mean dose rate and background concentration data averaged by time of day (n=12 for each time stamp). Only complete daily sets where all five samples were collected are included.

4.4 Discussion

4.4.1 High dose rate/background concentration ratios

In this study, inhaled grass pollen dose data collected in an area where unmanaged grasses were known to be growing were compared with background concentrations measured at a rooftop monitoring station. Pollen concentrations are known to decline very rapidly as distance from source increases (Raynor *et al.*, 1970, 1973a), thus whilst only background levels of pollen will register at a background monitoring station, an individual in the vicinity of flowering plants will be exposed both to background and to freshly emitted pollen. Given that sampling was conducted in an area where unmanaged grasses were abundant, the most likely explanation for the disproportionately high dose rate/background concentration ratios is that they were collected at times when grasses in the locality were flowering.

There are 218 species of grass recognised as indigenous or naturalised in Denmark (Schou *et al.*, 2009). Grasses usually follow a fixed diurnal flowering cycle, however the time of the day that anthesis occurs can differ dramatically from species to species (Subba Reddi *et al.*, 1988). Pollen grains from the Poaceae family are morphologically very similar, and species differentiation is therefore not feasible through light microscopy. No data on the spectrum of grass species specific to Aarhus is thought to exist, however the major species known to have a presence in Denmark are listed by Frederiksen *et al.* (2006) with an estimation of their prevalence. The species *Lolium perenne* and *Festuca arundinacea* are both listed as 'very common'. In a study in Aberystwyth, UK, Emecz (1962) found that for *Lolium perenne* anthesis typically occurred between 10:00 - 14:00, whilst for *Festuca arundinacea* anthesis occurred between 12:00 - 15:00 (and again between 20:00 - 21:00). This shows that at the time of day that dose rates were commonly high compared with background concentrations, grasses likely to be in the area are expected to have been flowering.

4.4.2 Influence of meteorological variables

Whether grasses flower and emit pollen or not depends upon the weather conditions, and temperature, sunlight, wind speed and relative humidity have all been related both to anthesis (Emecz, 1962) and atmospheric pollen incidence (Smart *et al.*, 1979). Different species respond in very different ways to changes in the weather (Subba Reddi *et al.*, 1988), however what they all appear to have in common is that minimum temperature and light intensity thresholds must be exceeded before anthesis is initiated (Emecz, 1962; Liem & Groot, 1973). According to Emecz (1962), both *Lolium perenne* and *Festuca arundinacea* belong to a group of grasses for which anthesis is governed by conditions in the hours immediately preceding and during anthesis, and is possible only if activation thresholds of 14°C and 17°C respectively are met or exceeded. Relative humidities of 40% or greater were furthermore observed to have no direct effect on the flowering behaviour

of either species. During data collection the minimum surface temperature was 16.4°C, whilst relative humidity dropped below 40% on only four occasions. This could explain why no relationship was found between the dose rate/background concentration ratio and either of these variables. Although light intensity thresholds were published by Emecz (1962) for both species, a comparison with the radiation data of this study is not feasible due to unit incompatibility.

In wind tunnel experiments with Bermuda grass (*Cynodon dactylon*), Lu *et al.* (2005) found that upon anther dehiscence only 5-20% of pollen grains was initially emitted, with the remaining pollen released only if wind speed exceeded approximately 2.2 ms^{-1}. In this study, disproportionately high dose rates occurred only for street level wind speeds over a threshold of 1.83 ms^{-1}. Street level wind speed measurements were made whilst in transit, and therefore carry an error of up to walking speed (1.63 ms^{-1} on average). The threshold reported by Lu *et al.* (2005) falls comfortably within this potential error range, although it seems sensible to assume that different thresholds may exist for different grass species.

Whilst the above results could account for the apparently significant influence of solar radiation and surface wind speed on the dose rate/background concentration ratio, the fact that 12:00 and 14:00 data all occur for high solar radiation and surface wind speed values means that the significant relationships may merely reflect the coincidence of pollen emission with the diurnal peak in these two parameters.

That high dose rate/background concentration ratios almost all occurred under winds from the Southeast was somewhat unexpected, and suggests that the species growing in the nature reserve area to the Northwest did not flower between 12:00 and 20:00. This is perfectly plausible, since other grass species likely to occur in the area such as *Dactylis glomerata* and *Phleum pratense* have been shown to flower outside this period of the day (Emecz, 1962; Frederiksen *et al.*, 2006).

4.4.3 Temporal trend

By avoiding exposure to allergens, allergic individuals can reduce the probability both of becoming sensitised and of developing symptoms in the present (Custovic *et al.*, 1998). Indoor pollen concentrations are typically much lower than outdoor concentrations (Jantunen & Saarinen, 2009), therefore it is often advised that patients minimise exposure by remaining indoors at times when background pollen levels are expected to be high (see Section 1.1.2). For the period of the day covered by this study, background concentrations peaked at 20:00 whilst dose rate achieved its maximum at 12:00. For the area where NAS samples were collected, background concentration and dose rate data thus suggest contradictory allergen avoidance strategies.

From 16:00 to 20:00, when grasses in the NAS sample collection area were thought not to be flowering, dose rate and background concentrations show a strong linear relationship ($r_s = 0.814$). This implies that in the absence of an active local source, monitoring

station data may be considered to be a reliable proxy for exposure. The median dose rate/background concentration ratio of 0.018 is tentatively proposed as a parameter for estimating inhaled dose from monitoring station data when local emissions are negligible. This could be verified by conducting a similar study in an area with no unmanaged grasses.

4.4.4 Comparison with previous studies

There exists a small body of work in which pollen dose has been measured using the NAS in the outdoor environment. O'Meara *et al.* (2004) recruited a panel of 30 adults, who wore NASs for two hours whilst engaged in similar low-level activities. Although individuals were always in close proximity to one another, the pollen doses recorded varied considerably between subjects, equating to dose rates of 0.017 - 0.267 grains min^{-1} for Poaceae pollen and 0.008 - 14.158 grains min^{-1} for *Ambrosia* pollen. In a similar study performed by Mitakakis *et al.* (2000), nine family groups each consisting of two adults and two children wore NASs for one hour on four separate occasions (twice indoors and twice outdoors) at two different activity levels, low (e.g. reading or dozing) and moderate (e.g. cooking or gardening), with measured pollen grain doses equating to dose rates of 0 - 1.35 grains min^{-1} for grass pollen and 0 - 1.2 grains min^{-1} for non-grass taxa. Neither study however reports concurrent hour-averaged background concentrations, and thus a direct comparison with the results of this study is not possible.

The relationship between pollen exposure and symptom development is complex, with the dose threshold above which symptoms are experienced varying between individuals and with recent exposure history (Connell, 1968). One way of establishing these thresholds is to quantify the symptoms of a group of allergic individuals and compare these with background concentrations (Davies & Smith, 1973; Antépara *et al.*, 1995; Rapiejko *et al.*, 2007; Feo Brito *et al.*, 2010), however this method does not take into account differences in exposure between individuals. Another approach is to investigate the dose-response relationship directly through clinical trials (Davies, 1985; Bousquet *et al.*, 1987). These are conducted outside the pollen season to ensure that exposure can be controlled, however this also means that subjects are not 'primed' at the time of the trial which can lead to unrealistic results (see Section 1.3.3). The current study presents an alternative method for estimating dose-response relationships. Applying dose rate/background concentration ratios to background concentration and activity data, an individual's dose can be estimated and related to symptom scores.

4.5 Conclusion

The relationship between grass pollen dose rate and background concentration was found to be stronger between 16:00 - 20:00 than between 12:00 - 14:00, when grass species likely to be growing in the area are expected to flower. For the period of the day studied, monitoring station and exposure data suggest different allergen avoidance strategies, with

the highest dose rates occurring between 12:00 and 14:00. These results suggest that exposure may have a high dependence on the local species spectra.

Chapter 5

Seasonal variation in diurnal atmospheric grass pollen concentration profiles[1]

Abstract

Although patterns in the diurnal variation of atmospheric grass pollen concentrations can differ greatly from day-to-day, it is common practice to establish the time of day when peak concentrations are most likely to occur using seasonally-averaged diurnal profiles. The atmospheric pollen load is highly dependent upon emissions, and different species of grass are known to flower and emit pollen at different times of the day and during different periods of the pollen season. Pollen concentrations are also influenced by meteorological factors - directly through those parameters that govern pollen dispersion and transport, and indirectly through the weather-driven flowering process. In this study, different profiles are found to characterise the early, middle and late grass pollen seasons in the city of Aarhus, Denmark. Whilst this variation could not be explained by meteorological factors, it was consistent with the theory that as the season progresses, grass pollen emissions are dominated by a succession of different grass species with different flowering patterns.

5.1 Introduction

Atmospheric grass pollen concentrations typically fluctuate over the course of a 24-hour period, and patterns of variation can differ greatly from day-to-day (Käpylä, 1981). Pollen concentrations are influenced both by pollen emission and by the meteorological parameters that determine dispersion, transport and deposition (Galán *et al.*, 1995). Different grass species release their pollen at different times of the season (León-Ruiz *et al.*, 2011) and at different times of the day (Emecz, 1962). Diurnal flowering patterns are further-

[1]A revised version of this study has been published in the discussion journal *Biogeosciences Discussion*, under the title of this chapter (volume 10, pages 14627-14656).

more known to change in a species-specific manner, in accordance with meteorological factors (Subba Reddi *et al.*, 1988). The changing character of the diurnal grass pollen profile may thus be driven by the weather, the flowering patterns of local grasses, or both.

It is common practice in aerobiology to produce average diurnal pollen concentration curves in order to establish typical patterns of variation, however these profiles usually relate to an entire pollen season (Galán *et al.*, 1989, 1991; Norris-Hill & Emberlin, 1991; Rantio-Lehtimäki *et al.*, 1991a; Trigo *et al.*, 1997; Gassmann *et al.*, 2002; Yang *et al.*, 2003; Kosisky *et al.*, 2010). Intra-seasonal variation has been considered for grass pollen by only a few authors. Mullins *et al.* (1986) compared the average profiles for two months (June and July) but found no difference, whilst Norris-Hill (1999) noted different profiles for the four quarters of the season, proposing that these related to rainfall frequency.

A thorough understanding of diurnal variation in atmospheric pollen abundance contributes to the accuracy of pollen dispersion models (Viner *et al.*, 2010), and the quality of the avoidance advice given to allergy sufferers (Käpylä, 1981). In this study, systematic seasonal variation in the diurnal grass pollen concentration profile was shown to occur in the Danish city of Aarhus. The hypothesis that this variation was driven by meteorological conditions was tested against the alternative hypothesis that it was related to a progression of different grass species dominating pollen emission as the season developed.

5.2 Methods

5.2.1 Site description and data provenance

For the years 2009-2011, three temporary research monitoring stations were operational during the grass pollen season in the city of Aarhus, Denmark (Fig. 5.1). The three stations, situated within 8 km of one another, each consisted of a 7-Day sampler (Appendix A.1) installed on a 15-20 m high roof. The *Central Aarhus* monitoring station is located in the centre of Aarhus, and is described in detail in Section 3.2. The *TV-2* monitoring station lies in the northern outskirts of Aarhus, on the roof of the building housing the TV station TV-2 Østjylland, less than 100 m from an unmanaged grass[2] field and close to open countryside. The *Rundhøjskolen* monitoring station is situated on top of a school building in the southern suburbs of Aarhus. A description of Aarhus can be found in Section 3.2.

Uniform materials and methods were used at the three monitoring stations, and these are detailed for the Central Aarhus station in Section 3.2. Bi-hourly grass pollen concentration data were obtained for each station and each year from the Danish Asthma and Allergy Association. Three-hour averaged wind speed, wind direction, surface air temperature, dew point temperature and precipitation data were obtained from the Flyveplads Kirstinesminde weather station (WMO Station ID 06074), situated just north of Aarhus (Fig. 5.1), courtesy of the UK Meteorological Office (2012). Medial time stamps are

[2]A definition of 'unmanaged grass' is given in Section 1.4.1.

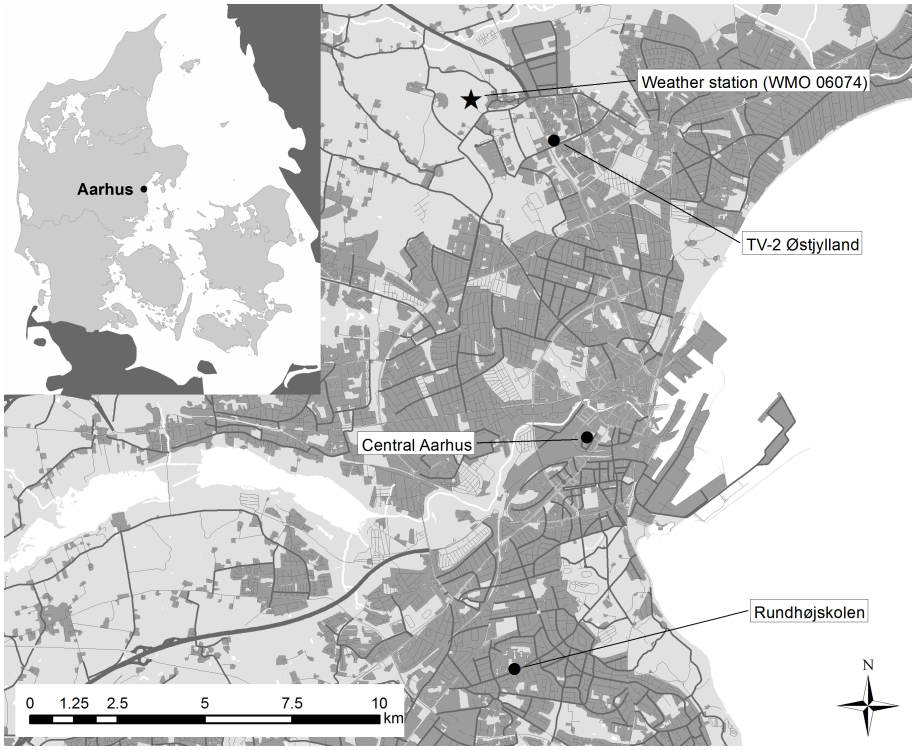

Figure 5.1: Map showing the locations of the three temporary pollen monitoring stations and the weather station in Aarhus.

reported for all data (both pollen and meteorological), as is the convention in aerobiology.

5.2.2 Data reduction and processing

Pollen data

For each monitoring station and each year, the grass pollen concentration time series was divided into 24-hour 'profiles' (midnight-midnight). All profiles with a mean concentration of <20 grains m^{-3} were rejected, on the basis that at low concentrations the resolution of data becomes poor[3]. All profiles that coincided with precipitation were also rejected, since rain removes pollen grains from the air with great efficiency (McDonald, 1962) and may thus strongly influence profile shape. Peaks in concentration were identified for each of the remaining 157 profiles, according to the following criteria:

- All peaks were required to have a minimum[4] concentration of $\geq$ 50 grains m^{-3}. Profiles where concentrations failed to exceed this threshold were considered to have no peaks.

[3]Profiles with daily averages < 20 grains m^{-3} generally failed to show a well defined diurnal pattern, with the effects of random sampling errors apparently dominating. An alternative (and relaxed) criterion of a daily maximum concentration >50 grains m^{-3} was considered, but ultimately rejected for the same reason.

[4]The minimum peak value of 50 grains m^{-3} was chosen because this is generally considered to be the average concentration above which individuals sensitised to grass pollen experience symptoms (Galán *et al.*, 1995).

- Overall maximum concentrations were designated primary peaks.
- Local concentration maxima occurring ≥ 6 hours before or after the primary peak were designated secondary peaks, provided that the trough between the two was at least 50 grains m^{-3} deep.
- Two candidate peaks of equal magnitude ≤ 4 hours apart were considered a single peak and given an intermediary time stamp, e.g. a maximum daily concentration of 90 grains m^{-3} occurring at both 17:00 and 19:00 was defined as a single 18:00 peak.
- Two candidate peaks of equal magnitude occurring six or more hours apart were considered separate peaks.
- Apparent peaks close to midnight that were associated with a greater peak occurring on the preceding evening were rejected, e.g. a peak at 01:00 was rejected if a greater concentration occurred at 23:00 on the previous evening.

In this manner each profile was characterised in terms of the number (zero, one or two) and time at which peaks in concentration occurred. Profiles were then grouped by site and year, and each group arranged in chronological order. Three characteristic profile types were observed to dominate at different points during the grass pollen season, meaning that the season could be divided into three distinct periods:

Period 1: The beginning of the season, characterised by a twin morning and evening peak profile.

Period 2: The middle of the season, characterised by a single evening peak profile.

Period 3: The end of the season, characterised by a single late morning/early afternoon peak profile.

Dates of transition between periods were determined for each monitoring station and each year, and all profiles were thus assigned to Period 1, 2 or 3. Concentration data were then standardised by dividing all bi-hourly concentration values by their respective daily maxima, in order to neutralise day-to-day differences in magnitude and isolate their qualitative shape. For each period, an average diurnal profile was produced from this standardised data.

Meteorological data

Three-hour averaged saturated and actual vapour pressures were calculated from ambient and dewpoint temperatures respectively using Equation 3 of Henderson-Sellers (1984). Vapour-pressure-deficit (VPD) was then computed using Method 1A of Howell & Dusek (1995). VPD, temperature and wind speed data for days corresponding to the pollen dataset were selected, and divided into three groups corresponding to the three periods of

the pollen season described above. Data for days where periods overlapped between the different monitoring stations were omitted. Data from one further day was omitted due to an incomplete record.

Statistical methods

To test for differences between the grass pollen concentrations profiles of different years and seasonal periods, the k-sample Anderson-Darling goodness-of-fit test with adjustment for ties was used (Trujillo-Ortiz *et al.*, 2007). The significance of differences in meteorological variables between the seasonal periods were tested by grouping data by period and time of day, and applying the Wilcoxon rank sum test. Results were considered significant at the 95% level. Analysis was conducted using version 7.7.0.471 of MATLAB (2008).

5.2.3 Grass species inventory

In order to support the interpretation of pollen data, an inventory of grass species likely to be common in Aarhus was produced. The inventory comprises grass species defined by Frederiksen *et al.* (2006) as 'common' or 'very common' along roads and railways and in parks (the habitats where unmanaged grasses are likely to be found in Aarhus, according to Skjøth *et al.* (2013b)), and species found to be common in Copenhagen by Hald (2011). It seems likely that species that are abundant in Copenhagen will also be well represented in other large Danish cities. Information available in existing published literature relating to pollen production and flowering was collated for each constituent species.

5.3 Results

5.3.1 Pollen concentration data

Diurnal variation in time of peak

For each of the three seasonal periods, peak-time distributions from the three monitoring stations were compared using the Anderson-Darling two-sample test (Table 5.1). For Periods 1 and 3 no differences were found between the three stations. For Period 2 the peak-time distribution for TV-2 data was found to differ significantly from those of the Central Aarhus and Rundhøjskolen stations, however inspection of the data showed that distributions were very similar except that the modal peak time for the TV-2 station was 17:00 whilst for the Central Aarhus and Rundhøjskolen stations it was 19:00. It was therefore considered appropriate to pool data collected at different monitoring stations for each of the three seasonal periods.

The peak-time frequency distributions of the pooled data are presented in Fig. 5.2. Period 1 shows a bimodal tendency, with morning peaks common at 09:00 and evening peaks common at 17:00 and later. Period 2 shows a uni-modal distribution, with the majority of peaks occurring at 17:00-19:00 and otherwise background peak levels between

Table 5.1: Anderson-Darling test for differences in peak-time distributions between the three monitoring stations for each seasonal period. * and ** indicate significant differences at the 95% and 99% levels respectively, D is the Anderson-Darling rank statistic and p the associated probability.

		Central/ Rundhøjskolen	Central/ TV-2	Rundhøjskolen/ TV-2
Period 1	p	0.650	0.147	0.435
	D	0.476	1.604	0.832
Period 2	p	0.592	0.004^{**}	0.030^{*}
	D	0.562	4.535	2.855
Period 3	p	0.783	0.663	0.606
	D	0.234	0.443	0.540

07:00-23:00. Period 3 shows a uni-modal distribution, with peaks common between 09:00-17:00 and the maximum occurring at 13:00. The Anderson-Darling test finds that peak-time distributions for the three periods differ significantly at the 99.9% level (D=12.20, $p < 0.001$).

Diurnal variation in concentration

Median diurnal profiles of standardised concentration data for the three periods are presented in Fig. 5.3. Average concentration profiles agree qualitatively with the peak distributions of Fig. 5.2, with small morning and large evening peaks during Period 1, a single evening peak during Period 2, and a mid-day peak during Period 3.

Period transition dates

Fig. 5.4 shows how data were assigned to the three periods, for each station and for each year. Within each year, there is a maximum period overlap of four days between the three stations. The transition from Period 1 $\rightarrow$ 2 occurs later in 2010 than in 2009 or 2011; The transition from Period 2 $\rightarrow$ 3 also occurs later in 2010 than in 2009, but cannot be precisely located in 2011 due to sparsity of data. Period duration cannot in general be precisely stated due to the volume of missing data, but was typically in the order of 1-2 weeks and appears to be shorter during 2010.

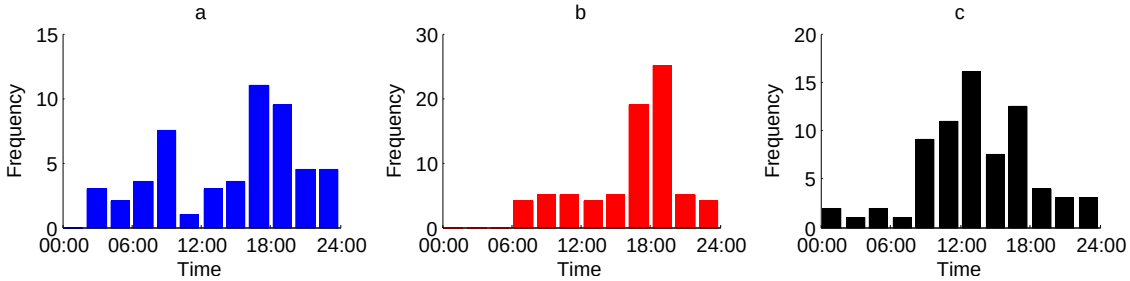

Figure 5.2: Peak-time frequency distributions for (a) Period 1 (n=34), (b) Period 2 (n=58) and (c) Period 3 (n=62). Bin width is two hours.

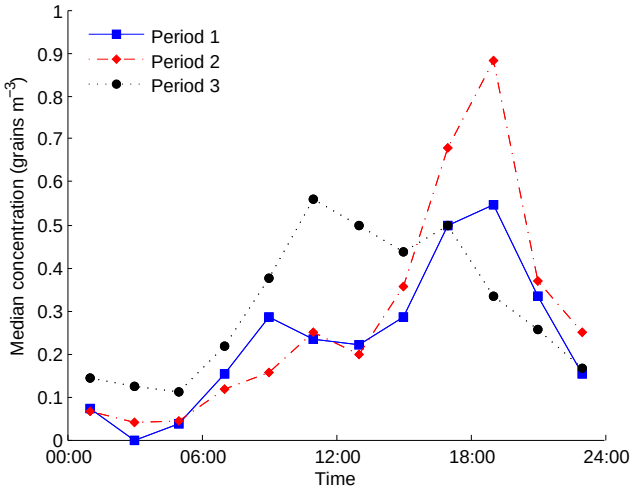

Figure 5.3: Standardised median diurnal profiles for the three seasonal periods.

5.3.2 Meteorological data

Median temperature, wind speed and VPD profiles for each of the three periods are presented in Fig. 5.5, whilst the results of the Wilcoxon rank-sum test for each pair of periods and each three-hour interval of the day are shown in Table 5.2. No significant differences were found between the three periods for wind speed. No significant differences were found between Periods 1 and 2 for temperature or VPD, with the exception of temperature between 22:00-01:00 when Period 2 values tended to be significantly higher. Temperatures during Period 3 were found to be significantly higher than those during Periods 1 and 2 at all times of day. VPD was found to be significantly higher during Period 3 than Period 2 between 07:00-13:00.

Fig. 5.6 shows time of peak plotted against concurrent wind direction. During Period 1, all morning peaks occurred under winds from the West. During Periods 2 and 3 however, morning peaks were associated with winds from all directions of the compass. Fig. 5.7 shows time of peak plotted against the number of days since rain. There was no apparent relationship between the two.

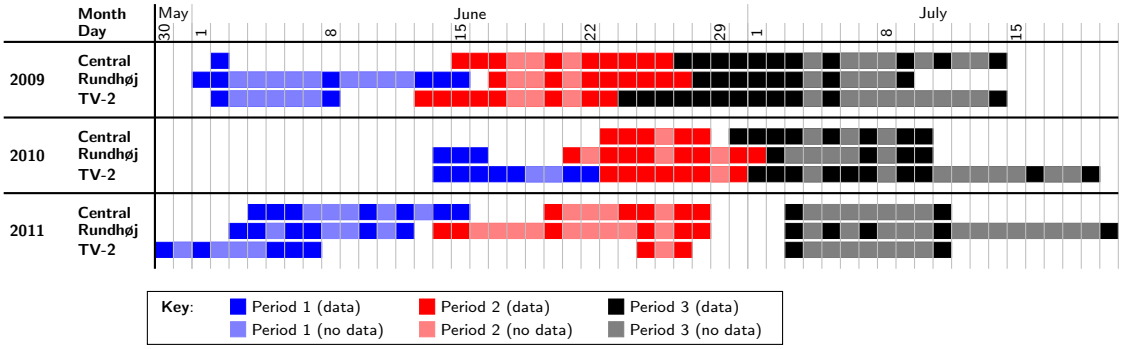

Figure 5.4: Assignment of data to the three seasonal periods for the Central Aarhus (Central), Rundhøjskolen (Rundhøj) and TV-2 monitoring stations for each year. Contributing dates (i.e. dry days with daily average concentrations ≥ 20 grains m^{-3}) are bold, non-contributing dates of known period affiliation are faded.

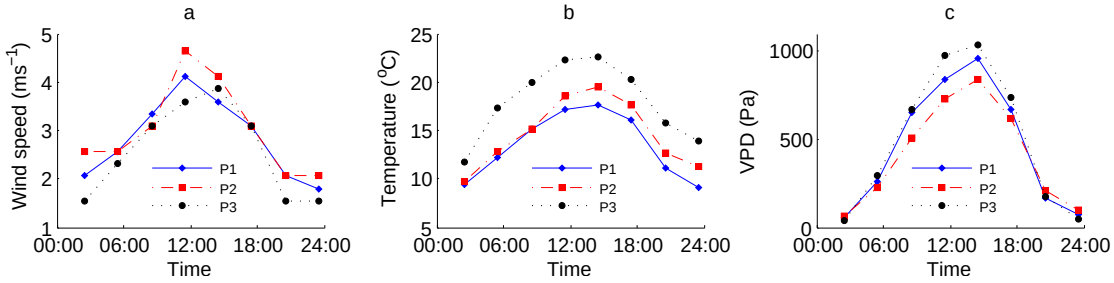

Figure 5.5: Median diurnal profiles for wind speed (a), temperature (b), and vapour-pressure-deficit (c) corresponding to Period 1 (P1, n=16), Period 2 (P2, n=17) and Period 3 (P3, n=24).

Table 5.2: Results of the Wilcoxon sign-rank test (normal approximation applied) for wind speed, temperature, and vapour-pressure-deficit data corresponding to Period 1 (P1, n=16), Period 2 (P2, n=17) and Period 3 (P3, n=24). Two-tailed p-values (p), rank sum (W) and z-score (z) statistics are presented, whilst * indicates $p < 0.05$, ** indicates $p < 0.01$ and *** indicates $p < 0.001$.

Time			01-04	04-07	07-10	10-13	13-16	16-19	19-22	22-01
Wind speed	P1/P2	p	0.514	0.375	0.678	0.537	0.118	0.513	0.942	0.459
		W	253.5	247	260	254.5	228.5	253.5	269.5	251
		z	-0.65	-0.89	-0.42	-0.62	-1.56	-0.65	-0.07	-0.74
	P1/P3	p	0.350	0.667	0.967	0.750	0.956	0.709	0.206	0.266
		W	362	344	330	340	330.5	314	374	368.5
		z	0.93	0.43	0.04	0.32	0.06	-0.37	1.26	1.11
	P2/P3	p	0.130	0.172	0.457	0.294	0.441	0.750	0.162	0.099
		W	414.5	409	385.5	397	386.5	369.5	410	419.5
		z	1.51	1.37	0.74	1.05	0.77	0.32	1.40	1.65
Temperature	P1/P2	p	0.639	0.601	0.773	0.843	0.528	0.438	0.126	0.040*
		W	258.5	257	280.5	266	254	250	229	214.5
		z	-0.47	-0.52	0.29	-0.20	-0.63	-0.78	-1.53	-2.05
	P1/P3	p	0.000***	0.000***	0.001**	0.000***	0.000***	0.000***	0.000***	0.000***
		W	190	174.5	210.5	194.5	196	196.5	185.5	187.5
		z	-3.80	-4.23	-3.23	-3.67	-3.63	-3.62	-3.92	-3.87
	P2/P3	p	0.000***	0.000***	0.001***	0.000***	0.001**	0.003**	0.003**	0.009**
		W	221.5	204	224.5	218.5	233.5	243.5	242.5	258
		z	-3.57	-4.04	-3.49	-3.64	-3.26	-2.99	-3.02	-2.61
Vapour-pressure-deficit	P1/P2	p	0.539	0.397	0.189	0.601	0.957	0.760	0.305	0.460
		W	289.5	296	309	287	274	281	243	251
		z	0.61	0.85	1.31	0.52	0.05	0.31	-1.03	-0.74
	P1/P3	p	0.244	0.301	0.751	0.341	0.209	0.879	0.923	0.180
		W	370.5	290	316	293	282	322	332	377
		z	1.16	-1.04	-0.32	-0.95	-1.26	-0.15	0.10	1.34
	P2/P3	p	0.748	0.115	0.043*	0.017*	0.135	0.741	0.451	0.115
		W	369.5	297	280	266	300	344	386	417
		z	0.32	-1.57	-2.02	-2.39	-1.50	-0.33	0.75	1.58

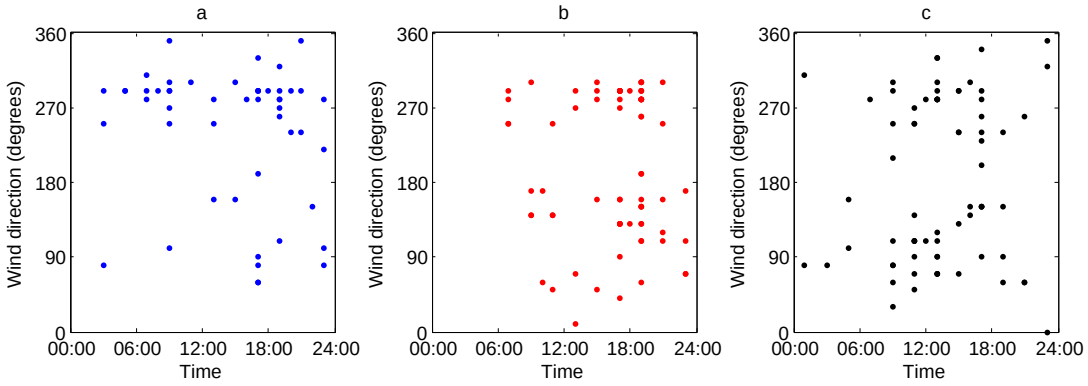

Figure 5.6: Scatter plots showing time of peak against concurrent wind direction for (a) Period 1, n=37; (b) Period 2, n=58; and (c) Period 3, n=62.

5.3.3 Grass species inventory

Table 5.3 lists 18 grass species likely to be present in Aarhus, together with associated information on pollen productivity and flowering behaviour. Data on diurnal flowering behaviour was found for 12 of these species. Amongst these are species that have been reported to flower around the time of the early peak during Period 1 (*Alopecurus pratensis*, *Dactylis glomerata*), around the time of the evening peak during Periods 1 and 2 (*Arrhenatherum elatius* according to Jones (1952)), and during the middle of the day, when peaks commonly occurred during Period 3 (*Festuca arundinacea*, *Lolium perenne*). There are also species that have been reported to flower twice per day at times coinciding more or less with the two peaks of Period 1 (*Anthoxanthum odoratum*, *Holcus lanatus*). Productivity estimates were found for nine species, with the number of pollen grains produced per inflorescence ranging from 0.1×10^6 (*Poa annua*) to 11.7×10^6 (*Festuca arundinacea*).

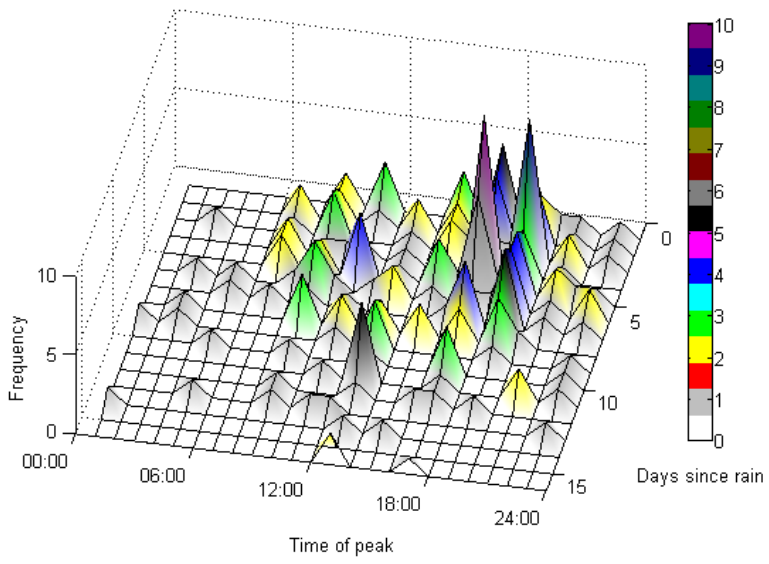

Figure 5.7: Frequency distribution of peak time against the number of days since $\geq$ 1 mm rain.

Table 5.3: Grass species that are likely to be present in and around Aarhus. Constituent species are listed by Frederiksen *et al.* (2006)[a] as 'common' or 'very common' along roads and railways and in parks, and/or were found by Hald (2011)[b] to be common in the city of Copenhagen. Details of productivity (grains/inflorescence[c,e] or spike[d]), time of flowering (range of times gives period of flowering, single time gives time of peak flowering unless otherwise indicated), minimum temperature threshold (°C) that must be exceeded in order for flowering to occur [square brackets indicate temperature that induces maximum liberation], and minimum light intensity (foot candles) and duration (hours) necessary to initiate flowering. Information is derived from the following sources: Prieto-Baena *et al.* (2003)[c], Smart *et al.* (1979)[d], Aboulaich *et al.* (2009)[e], Emecz (1962)[f], Ogden *et al.* (1969)[g], Jones (1952)[h], and Beddows (1931)[i].

Species	Productivity	Time of flowering	Temperature threshold	Light intensity/duration
Agrostis capillaris[a]	-	-	-	-/-
Agrostis stolonifera[b]	2,426,609[c]; 777,058[e]	-	-	-/-
Alopecurus pratensis[a]	-	7:52[f]; 06:00-11:00[i]	11[f]	-/10[f]
Anthoxanthum odoratum[a]	621,363[e]	05:00-10:00 and 17:00[i]	-	-/-
Arrhenatherum elatius[a,b]	-	15:00-19:00[h]; 05:00-11:00 and 18:00-19:00[i]	-	-/-
Bromus hordeaceus[a]	245,176[c]; 407,489[e]	06:00[i]	12[i]	-/-
Dactylis glomerata[a,b]	7,971,347[c]; 3,700,000[d]; 3,419,469[e]	6:25-8:35[f] (peak); 04:00-10:00[h]; 04:00-10:30[i]	15.5[f]	1,600/8[f]
Elytrigia repens[a,b]	-	14:00-16:00[h]; 16:30[i]	23[i]	-/-
Festuca arundinacea[a]	11,697,131[c]	15:08[f]; 06:00[i]	17[f] [14[i]]	3,600/5[f]
Festuca brevipila[a]	-	-	-	-/-
Festuca pratensis[a]	-	10:00[f]; 06:00[i]	15[f] [14[i]]	1,200/2[f]
Festuca rubra[a,b]	-	06:00; 9:45-14:30 (peak from 12:00-13:00)[i]	-	-/-
Holcus lanatus[a,b]	875,715[c]; 4,500,000[d]	Typically ~06:00-07:00 and ~18:00-19:00 though other patterns also reported[i]	-	-/-
Lolium perenne[a,b]	2,300,000[d]	11:45-15:20[f] (peak); 09:00-12:00[i]	14-17[f]	2,000-5,200/1.5-3[f]
Phleum pratense[a,b]	-	5:48-8:54[f] (peak); mostly 06:00-08:00[g] (peak); 04:00-09:00[h] 4:30-10:00[i]	16-17[f]	2,200-3,000/10[f]
Poa annua[b]	115,511[c]; 142,911[e]	04:30[i]	11[i]	-/-
Poa pratensis[a,b]	-	03:00-08:00[h]	-	-/-
Poa trivialis[b]	2,088,492[e]	-	-	-/-

5.4 Discussion

Diurnal variation in atmospheric pollen concentrations is typically assessed by averaging diurnal concentration profiles over an entire pollen season. For grass pollen, these average diurnal profiles have been found to vary with location. A single evening peak has been reported for London (Emberlin & Norris-Hill, 1991) and Cardiff (Mullins *et al.*, 1986) in the UK and Taipei (Yang *et al.*, 2003) in Taiwan, a single morning peak for Córdoba (Galán *et al.*, 1989, 1991) and Málaga (Trigo *et al.*, 1997) in Spain, and two-peak profiles have been reported by Rantio-Lehtimäki *et al.* (1991a) for Turku, Finland (08:00-12:00 and 16:00-18:00) and by Kosisky *et al.* (2010) for Washington DC, USA (09:30-12:30 and 21:30-00:30). Gassmann *et al.* (2002) report no notable daily variation for the city of Mar del Plata in Argentina.

In this study, the possibility of systematic seasonal variation in the diurnal grass pollen profile was investigated. Using statistical methods, different diurnal patterns were shown to dominate during different periods of the season: twin morning and evening peaks characterised the early part of the season, a single evening peak the mid season, and a single mid-day peak the late season. That the grass pollen season can be divided into several periods with different characteristics has previously been observed. In their 7-day ahead grass pollen forecast model for the UK, Smith & Emberlin (2005) used different parametrisations for the pre-peak, peak and post-peak periods of the grass pollen season, whilst Sánchez Mesa *et al.* (2003) obtained greater correlation between meteorological parameters and daily average grass pollen concentration by isolating the pre-peak period from the remainder of the season.

On a day-to-day basis, atmospheric pollen concentrations are determined by two sets of variables - those that mediate pollen release into the atmosphere, and those that mediate its dispersal from source to receptor (Galán *et al.*, 1995). Pollen emission is regulated by biological and meteorological factors which restrict it to a limited range of weather conditions and a specific portion of the day (Raynor *et al.*, 1970). Atmospheric transport is dependent on turbulence. For plants that flower during turbulent weather, which can be crudely approximated as the hours of daylight, we would expect flowering and increased atmospheric concentrations typically to coincide. This is especially true for taxa with smooth pollen grains that are relatively easily removed from the anther (Subba Reddi & Reddi, 1985) or relatively large pollen grains whose residence time in the atmosphere is limited (Skjøth *et al.*, 2013b). For Poaceae pollen, which is both smooth and relatively large, the timing of peaks in atmospheric concentration can thus be expected in general to follow patterns of local emission.

Pollen primarily enters the atmosphere directly from the anthers, following flowering. For grasses, flowering intensity generally follows regular diurnal cycles that differ from species to species, and furthermore vary with changing weather conditions (Jones, 1952; Emecz, 1962; Subba Reddi *et al.*, 1988). Different species of grass flower at different points during the pollen season (León-Ruiz *et al.*, 2011), meaning that as the season

progresses, different subsets of the local grass flora are likely to be contributing to the atmospheric pollen load. The systematic variation between the three periods of the grass pollen season observed in this study could therefore potentially be driven by two different factors: a difference between the weather conditions in the three periods of the season, or a succession of different grass species dominating pollen emission as the season develops.

Flowering amongst grasses is in general dependent on a species-specific temperature threshold being exceeded (Emecz, 1962). It is possible that higher temperatures could lead to thresholds being exceeded earlier in the day, bringing the time of flowering forward. Higher temperatures were recorded during Period 3 than during Periods 1 and 2, however whilst morning peaks were largely absent during Period 2, they were common during Period 1 as well as during Period 3. Temperature was also significantly higher during Period 2 than Period 1 between 22:00-01:00. This may be expected to lead to earlier peaks during Period 2 than during Period 1, however in fact the opposite was seen.

Anther dehiscence, the process of splitting open of anthers to release pollen, occurs following dehydration (Stanley & Linskens, 1974, p. 24). VPD may be considered a proxy for the drying power of the air, and greater VPD earlier in the day could lead to earlier drying, emission and concentration peaks. VPD was found to be significantly lower during Period 2 than during Period 3 from 07:00-13:00, which could account for the shift from an evening to a midday peak, however there is no significant difference with Period 1 at any time of day, even though a morning peak occurs during Period 1.

Horizontal transport is dependent on wind direction (Stull, 1988, pp. 3-5), thus a discrete source may produce concentration peaks if emission coincides with winds that carry pollen from source to monitoring station. Although during Period 1 all morning peaks were recorded under Westerly winds, during Periods 2 and 3 early morning peaks occur under winds from all directions of the compass.

Wind speed is associated both with the primary emission of pollen from the anthers (Emecz, 1962; Lu *et al.*, 2005) and with secondary emission through resuspension (Sehmel, 1980; Sánchez Mesa *et al.*, 2003), however no significant difference in wind speed was found between the three periods of the grass pollen season. Norris-Hill (1999) proposed that the timing of diurnal grass pollen peaks may be related to the time elapsed since rainfall due to the availability of pollen for resuspension, however no such relationship was detected during this study.

Pollen production can vary hugely between species, indeed Table 5.3 shows that pollen production amongst the species common in Aarhus may be expected to vary over at least two orders of magnitude. Clearly species that are prevalent and also prolific pollen producers will exert greater influence on atmospheric pollen concentrations, thus it is possible that the diurnal pattern of pollen concentration variation is determined by only a handful of species. León-Ruiz *et al.* (2011) identified only four species as likely to contribute significantly to atmospheric pollen concentrations in Córdoba, Spain.

The date that an individual grass begins to flower is determined by a number of environmental criteria. The transition to the flowering phase is initiated primarily by

photoperiod. So-called *long-day* grasses are initiated once a critical species-specific day length is reached, which typically occurs between mid-April and 21st June. *Short day* plants are initiated when a critical night length is reached, and thus initiation occurs after 21^{st} June (Manske, 1999). This inter-species variation in photoperiod requirement explains why different species flower at different times of the pollen season. Once the day length criteria has been met, the time necessary to reach the flowering phase is dependent on environmental factors, with greater temperatures and more precipitation both serving to advance the onset of flowering (Dahl *et al.*, 2013). The flowering periods of individual species thus vary from year-to-year, which accounts for the difference in period transition date observed over the three years of this study.

It seems probable that the period during which an individual grass species has the potential to dominate atmospheric pollen emission will largely be limited to the 'full flowering' phase of the flowering cycle, the period during which the central 50% of anthers dehisce. León-Ruiz *et al.* (2011) found that the length of the full flowering phase varied between species, but was typically in the range 1-2 weeks, i.e. comparable with the typical lengths of the three periods identified in this study. The full flowering phase was also found to be briefer during years where flowering began late. In Aarhus, the start of the grass pollen season, here defined as the first day with an average concentration ≥ 10 grains m^{-3} at any of the three monitoring stations, occurred later in 2010 (6^{th} June) than in either 2009 or 2011 (20^{th} and 21^{st} May respectively). This coincided with a tendency for later transition dates between the three periods, and also with apparently briefer periods.

It is well known that local-scale variation in micro-climate can cause the onset of flowering to vary by several days. The urban heat island can for example advance the flowering of grasses within a city compared with on the outskirts (Emberlin *et al.*, 1993; Rodríguez-Rajo *et al.*, 2010) whilst the onset of flowering in *Platanus* trees has been reported to differ between different areas of the same city (Alcázar *et al.*, 2004). In the present study, the dates of transition between the three periods were found to differ between monitoring stations by up to four days, meaning that differences in diurnal pattern were found over distances of under 5 km. The resulting overlap between Periods at the different monitoring stations could be explained by small differences in the onset and culmination of the main flowering phase of specific species.

In this study, it was shown that the typical diurnal grass pollen concentration profile for the city of Aarhus changes as the pollen season progresses. The theory that this variation is driven by a progression of different grass species dominating grass pollen emissions is consistent both with the characteristics of the three seasonal periods, and with the flowering behaviour of the grass species thought to have a significant local presence. No evidence to the contrary was found.

5.5 Conclusions

In this study it was shown that the typical diurnal grass pollen concentration profile for the city of Aarhus changes as the pollen season progresses, leading to three distinct periods of the season with different profiles. The early season is characterised by twin morning and evening peaks, the middle of the season by a single evening peak, and the late season with a single peak around midday. This seasonal variation is most likely driven by a succession of different grass species with differing diurnal flowering patterns dominating atmospheric pollen concentrations at different times of the season.

Chapter 6

Further discussion and future perspectives: towards an integrated assessment capability

Atmospheric concentrations of allergenic pollen are monitored in order to better understand the dynamics of their variation from a health perspective. The knowledge gained from a monitoring network can be used to produce a forecast of when the pollen season will begin and when high concentrations will occur, for dissemination to allergy sufferers and other interested parties; to advise allergy sufferers on allergen avoidance strategies; and to improve the quality of the monitoring network itself. In an integrated assessment programme, data from monitoring stations and exposure models are used in combination, with models used to extend coverage of and better understand monitoring station data (Hertel *et al.*, 2007).

The original aims of this project, as set out in Section 1.5, were to advance our understanding of allergenic pollen concentrations in urban areas, and to develop a human exposure model for allergenic pollen - in other words, to move towards an integrated assessment capability for pollen. Each of the four studies presented in this thesis represents an advance in our understanding of pollen concentrations and exposure to pollen in urban areas, either directly (Studies B-D) or indirectly (Study A), satisfying the first of these aims. The results of these studies are discussed in Section 6.1. The second aim was ultimately not completed. This is discussed in Section 6.2.

6.1 Further discussion

6.1.1 Relevance of findings

In Study A, the relative efficiency of three bioaerosol samplers was assessed. Knowledge of sampler efficiency is of great importance when studying pollen exposure or pollen concentration dynamics, in particular when comparing data collected with different models

of sampler. With the exception of variable flow isokinetic devices (Ogden *et al.*, 1975), which as far as the author is aware have never entered into common usage, the efficiency of suction and rotating arm samplers such those compared in Study A varies with particle aerodynamic size and environmental conditions, in particular wind speed (Ogden *et al.* 1974, p. 93, Di-Giovanni 1998). Ideally, a comprehensive efficiency profile should be produced for every sampler model at the development stage, detailing efficiency over the full range of particle sizes and environmental conditions for which the sampler is designed. This approach was proposed over a decade ago by Di-Giovanni (1998), but is yet to become common practice. Establishing the actual efficiency of a sampler is not straightforward. It requires specialist apparatus such as a wind tunnel, an aerosol source, an instrument for dispersing the aerosol and a reference device of known efficiency (Mark & Vincent, 1986), and was considered to be outside the scope of this project. Instead, relative efficiency relationships for converting concentration measurements made with a portable sampler into equivalent 7-Day sampler values were derived. It is important to note that, according to the sampler's operating instructions, the 7-Day sampler is only $70 \pm 20\%$ efficient and can thus be expected to underestimate actual concentrations - a fact that has often been overlooked by authors.

The majority of the European population live and work in urbanised areas (EEA, 2010), and much urban transit will take place within urban street canyons. In Study B, a tendency was found for grass pollen concentrations at breathing height within street canyons to be lower than those at a nearby roof level pollen monitoring station. This has the important implication that monitoring stations, which are typically situated at roof level (Spieksma *et al.*, 2000), will tend to overestimate urban exposure. Within the field of air pollution assessment, monitoring networks are designed to decompose the pollution signal into its three major components. Rural monitoring stations measure the regional contribution from rural areas, urban background stations measure city background levels, and roadside stations measure concentrations within the urban canyon environment. This structure is reflected in air pollution modelling systems, with different models handling the rural, urban background and street canyon environments (Hertel *et al.*, 2007). It may be appropriate to design pollen monitoring networks in a similar manner. Rural stations to give information on pollen concentrations in the major source area, urban background stations to measure background concentrations that represent exposure in open areas, and street level stations to measure exposure within the canyon environment. A diversified pollen monitoring network such as this could form the basis for an exposure modelling system similar to those used in air pollution assessment, part of an integrated pollen exposure assessment programme (Hertel *et al.*, 2007).

When performing an exposure assessment, it is important to understand that exposure is not necessarily representative of the dose that an individual experiences (Zartarian *et al.*, 1997; Mitakakis *et al.*, 2000). In study C, the relationship between monitoring station data and inhaled dose was assessed, and a factor for estimating dose derived. In this manner, a better understanding was gained of what concentrations recorded at a monitoring station

actually mean for an individual exposed at street level. The results of Study C indicate that dose cannot be robustly estimated from background concentrations in areas where active sources exist. Resolving this issue is, in the case of grass pollen, probably beyond the scope of a network of monitoring stations. The ubiquitous nature of grasses and the complexity of dispersion within a built up urban area mean that a huge monitoring network would be needed to cover an entire city. However, an advanced model system coupled with a detailed source map may offer a reasonably alternative in such situations.

In Study D, it was shown that the typical diurnal dynamics of atmospheric grass pollen concentrations can vary as the pollen season develops. In Aarhus, three seasonal periods were apparent - an early season period with twin morning and evening peaks, a mid season period with a single evening peak, and a late season period with a single mid-day peak. This variation was linked to a progression of different grass species flowering at different times of the day and at different times of the season, and was in other words a function of the local grass species spectra. The results of Study D are thus not necessarily representative of other cities. Different subsets of grasses could produce different seasonal patterns, whilst if the major contributing species have overlapping flowering periods or common flowering characteristics no seasonal pattern may be apparent. Seasonal diurnal average profiles are used to advise allergy sufferers on the time of day that they should avoid being outside, in order to minimise their exposure (Astma-Allergi Danmark, 2012; Allergy UK, 2012). The result of this study clearly show that this advice should change as the season progresses. The practical relevance of this development is however questionable, since it is unclear whether allergy sufferers will follow complex avoidance advice. A more profitable direct application of Study D can probably be found within emission modelling. An emission model is an important component of an exposure model, as it determines when pollen is released into the atmosphere. Based on the results of this study, emissions in Aarhus should be handled differently at the beginning, middle, and end of the season.

6.1.2 Transferability to other bioaerosols

With the exception of Study A, the work presented in this thesis focuses exclusively on grass pollen. There are however numerous other allergenic pollen (see Section 1.1.2) and fungal spore taxa that are of allergological interest, to which this work may also have relevance. The results of Studies A-D are determined by two principle factors. For Studies A, B and C, the magnitude of the ratios between samplers or locations is substantially determined by the aerodynamic properties of grass pollen, a proxy for which is aerodynamic size or equivalently settling velocity (Solomon, 2003). The outcome of Study D (and to a lesser extent Study C) is on the other hand determined by grass pollen emission patterns, and thus the grass species spectra of the local area is a governing factor.

The efficiencies of the three samplers compared in Study A vary with particle aerodynamic size, and their respective relative efficiency relationships would be expected to follow suite. The results obtained for grass pollen, for which observed settling velocities

range from 1.46-2.80 cm s^{-1} (Section 1.2.1), may for example be expected to apply also to *Ulmus* pollen, which has an observed settling velocity of 2.04 cm s^{-1} (Durham, 1946). On the other hand *Urtica* pollen, which along with *Parietaria* effectively constitutes the Urticaceae taxa (Corden & Millington, 1991), has an observed settling velocity of 0.34 cm s^{-1} (Durham, 1946), which accounts for the different correction factor obtained for Poaceae and Urticaceae. The efficiencies of the three samplers respond in different ways to changes in aerosol size, thus the relationships between size and relative efficiencies are not straightforward.

The ratios derived in Study B would likewise be expected to vary with settling velocity. The street canyon environment can be thought of as a source sink system, with pollen entering from above and leaving through deposition to surfaces within the canyon and loss of air to the overlying above-roof reservoir. The ratio between above-roof and within canyon pollen concentrations is determined by the rate at which particles are deposited within the canyon, with aerodynamically larger aerosols more inclined to impact against objects or settle out of circulation under gravity. The larger the aerodynamic size of a species, the larger the expected difference between roof level and street level concentrations, although there may be some lower size threshold below which the difference between the two becomes imperceptible.

In the same way that suction sampler efficiency varies with particle aerodynamic size, so does the inhaled fraction (see Section 1.3.2), which increases as particle size declines. The dose rate/background concentrations ratio determined in Study C would thus be expected to increase for aerodynamically smaller bioaerosols taxa.

The results of Study D can not necessarily be extended to other cities, as previously discussed, and similarly will not necessarily occur for other pollen taxa. In order for seasonal variation in the diurnal profile to occur, the taxa in question must include species with temporally distinct full flowering phases and different diurnal flowering cycles that make significant contributions to the atmospheric pollen load. In the UK, the genus *Betula* is represented principally by three species, *B. pendula*, *B. pubescens* and *B. nana* (Skjøth *et al.*, 2009). The birch flowering season is however relatively brief, lasting typically for 3-6 weeks (Emberlin *et al.*, 1993), meaning that there is likely to be substantial overlap between their respective flowering periods. The UK Urticaceae pollen season on the other hand has two distinct peaks, in June/July and mid-August, relating to the flowering of the genera *Urtica* and *Parietaria* (Corden & Millington, 1991). If these two genera have different diurnal flowering cycles, seasonal variation in the atmospheric diurnal Urticaceae pollen profile would be expected.

6.1.3 Study limitations

Acquiring pollen data with non-automated samplers such as those used in this project is very resource intensive, whilst the quality of data collected these instruments is poor when concentrations are low due to their relatively low sampling rates. The efficiency of data

collection for Studies A-C was therefore optimised by pre-selecting warm and rain free days on which concentrations of the target pollen taxa were expected to be high, whilst during Study D days with no or insufficient pollen were discarded. This policy however produced a dataset that is not universally representative.

Grass pollen concentrations vary from hour-to-hour, from day-to-day and from year-to-year: the annual pollen load is determined by weather conditions during the preceding autumn and spring (Dahl *et al.*, 2013); day-to-day variation is dependent on weather in the preceding 48 hours hours (Section 1.2.2); and hour-to-hour variation is also influenced by meteorological factors, with for example rain washing pollen grains from the air (McDonald, 1962). Since pollen concentrations are intrinsically linked to weather patterns, the data selection policy described above could mean that the results presented in this thesis cannot be extended to times when pollen concentrations were low.

As discussed above, the results of Studies A-C are governed primarily by pollen grain aerodynamic properties, which are affected by ambient relative humidity (1.2.1). Extending data collection to all weather conditions would likely extend the range of relative humidity and could potentially influence the results of Studies A-C, however since concentrations would likely be low at this time the author does not consider this to be a significant point. The influence of low concentrations themselves is expected to be negligible, since the methods used in studies A-D are (largely) independent of concentration magnitude - the correction factors in Study A and the street/roof ratios in study B are concentration ratios, the dose rate/background concentration in Study C is similarly effectively a ratio of sampling rates (concentration is essentially the number of pollen grains sampled per hour multiplied by some constant), whilst for study D concentration data were standardised to remove the magnitude effect. It is furthermore important to note that by excluding low concentrations we are focussing on the conditions most relevant to the allergy sufferer.

The relationship between minute volume and dose is not straightforward, as discussed in Section 1.3.2 - two individuals exposed to the same concentration will not necessarily incur the same dose. One weakness of Study C is that the relationship between dose rate and background concentration is based upon data collected by only two individuals, meaning that it may not be representative of the general population. Ideally, data should have been collected by a large cohort of individuals covering a variety of ages and fitness levels, however recruiting two such groups in two countries (and two languages) was outside the scope of this project in terms of both logistics and available resources.

The effect of rain on atmospheric concentrations of grass pollen deserves special attention. As has already been discussed, data were not collected during or following episodes of precipitation since the atmospheric pollen load was expected to be considerably depleted - although Norris-Hill & Emberlin (1993) report that pollen concentrations occasionally increase during the first few hours of rainfall, a phenomenon thought to be related with convective storms. Rain may also reduce pollen concentrations on the following day, if grasses have not dried sufficiently to flower (Spieksma & den Tonkelaar, 1986). Although rain reduces the presence of pollen grains within the atmosphere, it can simultaneously

lead to an increase in pollen allergen bioavailability. Grass pollen grains are packed with tiny allergen-carrying cytoplasmic granules that are small enough to penetrate to the deep airways where they can initiate asthma attacks in susceptible individuals. When hydrated, pollen grains can split open due to osmotic shock, releasing these granules (Motta *et al.*, 2006). Asthma epidemics have in fact been associated with thunder storms, with increased humidity preceding or rainfall during a thunderstorm thought to trigger the release of cytoplasmic granules from grass pollen (Newson *et al.*, 1997).

Although aerobiology has until recently focused almost exclusively on the pollen grain unit, from an allergological perspective pollen is merely the vector by which allergen reaches an allergic individual. Whilst atmospheric Poaceae pollen grain and allergen concentrations have been found to have parallel dynamics during the grass pollen season, meaning that periods of high concentrations and concentration peaks tend to coincide (De Linares *et al.*, 2010), the correlation between the two, although significant, has been found to be moderate at best. It appears that the strength of their relationship deteriorates in heavily polluted atmospheres, where pollen grain exines degrade at an increased rate (Rodríguez-Rajo *et al.*, 2011). Furthermore, grass pollen allergen has been observed both before and after the pollen season, whilst air allergen content was found to be more strongly related to symptoms than were pollen concentrations (Feo Brito *et al.*, 2010). One might therefore argue that from a human exposure perspective, it makes more sense to measure air allergen content than pollen grain concentration. As far as the author is aware, air allergen monitoring is currently restricted to small-scale research projects and is not routinely performed anywhere in the world, whilst air allergen levels have never been monitored in Denmark. This means that there is no realistic possibility of comparing allergen exposure with background allergen levels, and no historical data against which to develop the exposure model stipulated by the original project objectives. The research presented in this thesis therefore concerns exposure to pollen grains rather than pollen allergens.

6.2 Future perspectives

As set out in Section 1.5, the original objectives of this project included the development of a human exposure model for allergenic pollen. Given the additional field work found to be necessary in the shape of Study A, and the lack of information on the flowering behaviour of grasses that motivated Study D, this proved ultimately to be beyond the scope of the project. Some of the planning and initial development work was however completed. The model was to be parametrised using data from Aarhus, with the results of Studies B, C and D intended to support model development. A schematic describing the proposed exposure model is shown in Fig. 6.1.

Exposure modelling typically involves estimating the exposure of an individual or population, by combining data on their whereabouts with estimated concentrations of the agent of interest (USEPA, 2013). For atmospheric agents, concentrations are commonly

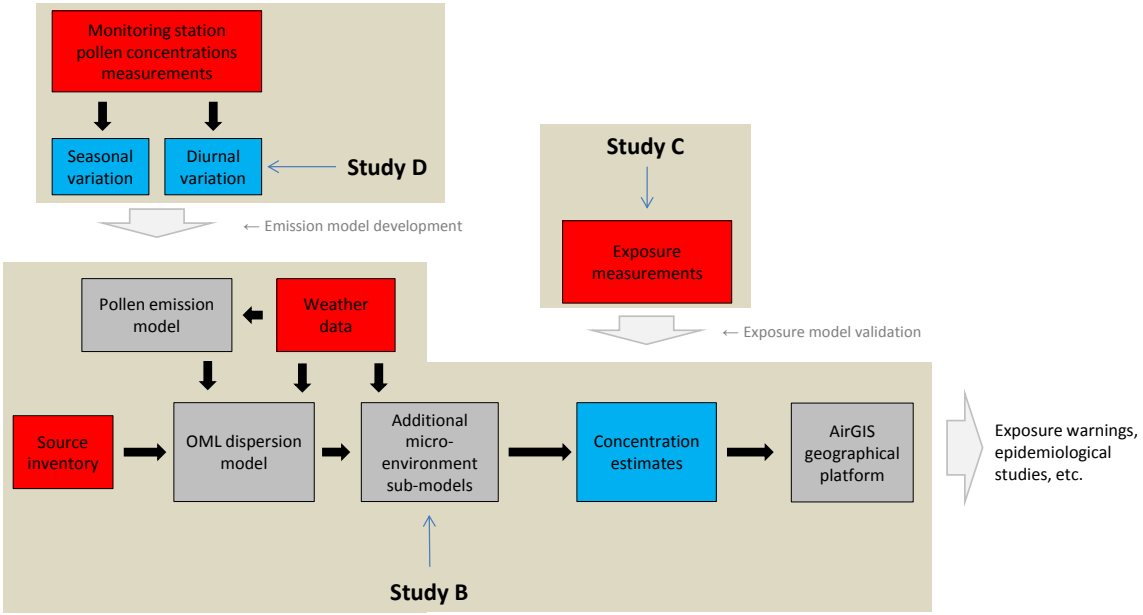

Figure 6.1: Schematic showing how the work of this thesis contributes to the development of an exposure model for grass pollen. Red boxes represent primary input data, blue boxes represent secondary input data (i.e. input generated from primary input), and grey boxes represent model components.

estimated using dispersion models that simulate agent dispersal and transport following emission. For a domain the size of a city and its surroundings, a mesoscale dispersion model is suitable (Seinfeld & Pandis, 2006). The Operational Meteorological Air Quality Model (OML), developed at the Department of Environmental Science at Aarhus University (ENVS, 2013a), is suitable for a domain of up to around 20 km, and was selected as the basis for the pollen exposure model. OML is a modern Gaussian plume dispersion model[1] (Olesen *et al.*, 2007, pp. 11-16). It was originally designed for estimating pollutant dispersion from point sources (e.g. chimney stacks) but a version capable of handling area sources, OML-DEP, was subsequently developed (Olesen, 1995) and has previously been applied to the dispersion of pollen from genetically modified crops (Geels *et al.*, 2004).

During initial model development, the weather data necessary for driving OML was obtained from the weather forecast model MM5 (Grell *et al.*, 1994), the standard input for OML. The *source inventory*, an important model input that defines source size, location and strength within the model domain, was obtained from the high resolution (14.4 m × 14.4 m) grass pollen source inventory that has recently been produced for a 16.7 km × 13.1 km area covering Aarhus and the surrounding region (Skjøth *et al.*, 2013b). The model was developed against the three years of data from the Central Aarhus pollen monitoring station used in Study D.

Reliable emissions input data are essential for dispersion models (Hertel *et al.*, 2001a), including with respect to diurnal emission patterns (Viner *et al.*, 2010). This is particularly

[1] *Modern* Gaussian dispersion models use dispersion rate parametrisations based upon the physical characteristics of the boundary layer that are involved in plume dispersion, in contrast with *traditional* Gaussian models wherein the Pasquill-Gifford-Turner dispersion parameters are typically employed.

challenging in the case of grass pollen due to the multitude of different species potentially contributing to atmospheric pollen loads. Day-to-day and seasonal variation in diurnal emission cycles should be taken into account. Based on the results of Study D, different emission parametrisations are needed for the beginning, middle and end of the grass pollen season in Aarhus. The next step in the development of an emission module would be to determine what drives day-to-day variation within each of these three periods. The species list and associated information relating flowering behaviour to meteorological variables presented in Study D could contribute to this. In addition to emissions within the model domain, it would be necessary to develop a sub-model that accounts for pollen transported into the domain through its lateral boundaries - the rural background model alluded to in Section 6.1.1.

The OML-based model described above, parametrised using background concentration data, equates to the urban background model mentioned in Section 6.1.1. Whilst this model may be appropriate for estimating exposure in open urban areas, the results of Study B show that exposure within the urban canyon environment would typically be overestimated. A sub-model for estimating within canyon concentrations was therefore to be developed using the results of Study B, possibly based on the ENVS Operations Street Pollution Model (ENVS, 2013b) - the street canyon model mentioned in Section 6.1.1.

Once all components are assembled and parametrised, an exposure model must be validated against an independent exposure dataset (WHO, 2001, p. 95). This was to be done with the exposure data used in Study C, by taking into account the inhalable fraction. The original project objectives stated that the model would also be validated using data from London, however given the results of Study D it seems likely that the model's emission module would have to be reformulated before it could be applied to other locations.

Exposure models are a practical and cost-effective way of conducting large scale exposure estimation (Berglund *et al.*, 2001, p. 12). A human exposure model for grass pollen has a number of potential applications - it could be used to study the epidemiology of pollen allergy (WHO, 2005, p. 10), to better understand monitored pollen data (Hertel *et al.*, 2007), to design and validate allergen avoidance strategies (O'Meara & Tovey, 2000), or it might be integrated into a forecast system in order to provide pollen forecast capability. This thesis represents some of the preparatory work necessary to facilitate model development. The author hopes to return to and complete the grass pollen exposure model in the near future.

APPENDICES

Appendix A

Sampling instruments

A.1 7-Day Recording Volumetric Spore Trap

The Burkard 7-Day Recording Volumetric Spore Trap (7-Day sampler) is a single stage slit impactor (Fig. A.1). It is one of several devices known as *Hirst-type* samplers that follow the design of the classical Automatic Volumetric Spore Trap described by Hirst (1952). The 7-Day Sampler has been adopted by the UK and Danish pollen monitoring networks, and is widely used elsewhere in Europe (Emberlin & McCartney, 1996) and the USA (Muilenberg, 2003). Air is aspirated through a horizontally orientated 14 × 2 mm slit at a rate of 10 l min^{-1}, and impacted against an adhesive collection surface that moves past the slit at a rate of 2 mm $hour^{-1}$. Each transverse line across the tape is thus exposed for one hour. The tape is usually assayed by counting the number of pollen grains along a transverse transect, producing approximately[1] hour averaged data. The sampler's inlet is kept facing into the wind by means of a wind vane, allowing for so called *isoaxial* sampling

Figure A.1: Burkard 7-Day Recording Spore Trap (left) and 7-day lid assembly (right).

[1]Depending on microscope field of view width.

(Mandrioli *et al.*, 1998, pp. 55-57). The efficiency of Hirst-type spore traps varies with wind speed and particle size (Ogden *et al.*, 1974, p. 93).

A.2 Sampling Technologies Model 20 Rotorod

Rotating arm impactors have been used for routine monitoring but are perhaps more commonly employed as field instruments, for example O'Rourke & Lebowitz (1984); Sterling & Lewis (1998); Hugg *et al.* (2007); Jantunen & Saarinen (2009). The Sampling Technologies Model 20 (Rotorod) is one of two models currently produced commercially (Fig. A.2a). Particles are collected on a pair of 1.52 mm wide polystyrene collector rods that are mounted vertically in a sampling head and rotated through the air at 2400 rpm. The leading edge of each rod is coated with a user applied adhesive, usually silicone grease, which serves to trap impacted particles. Several different sampling head designs are available. In the present work, a retracting head that protects rods when the sampler is not in operation was used. The sampler may be run from an external 12V battery, or alternatively from mains power (Sampling Technologies, 1998). The efficiency of rotating arm impactors varies with wind speed and particle size (Di-Giovanni, 1998).

A.3 Model 1 Nasal Air Sampler

The Model 1 Nasal Air Sampler (NAS) is a novel personal exposure monitoring device that was developed at the Institute of Respiratory Medicine, University of Sydney, Australia (Fig. A.2b). Each sampler consists of a pair of polyurethane single stage impaction units, connected by a silicone strap, that are worn inside the nostrils. An adhesive tape approximately 2.7 mm wide is placed in each unit, and secured with a soft silicone sleeve. The sleeve is in contact with the inner surface of the nasal vestibule, preventing leakage and holding the sampler secure, whilst a rim on the impaction unit prevents it from being drawn into the nasal cavity. Air inhaled by the wearer accelerates towards the adhesive

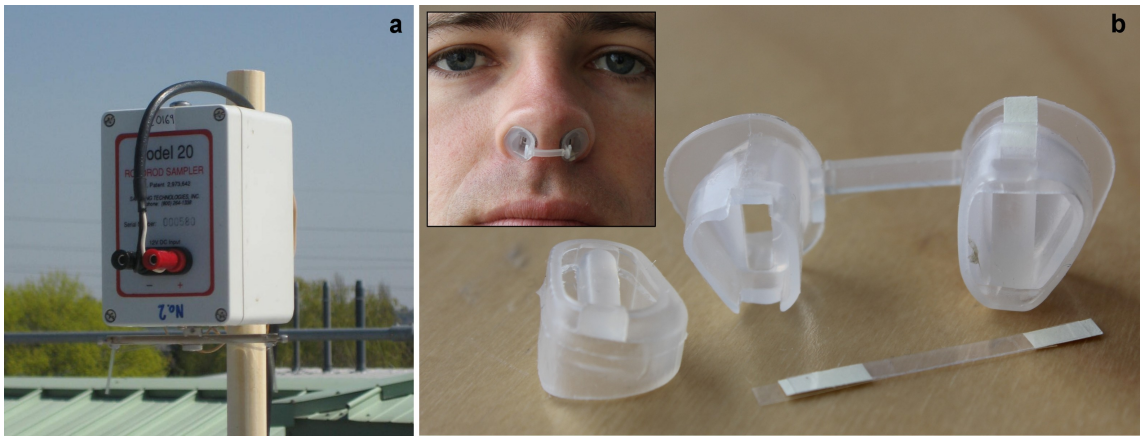

Figure A.2: The Sampling Technologies Model 20 Rotorod Sampler (a) and Model 1 Nasal Air Sampler (b).

tape, before dividing and leaving the sampler through exit ports on either side of the tape. Particles with sufficient inertia impact against the tape and are collected. Exhaled air then follows the same route back through the sampler. Since only those particles inhaled by the wearer may be collected, the NAS gives a measure of pollen grain dose rather than the breathing zone concentrations measured by most personal devices. The sampler was designed such that resistance to flow is low enough not to occasion mouth breathing. For most users, wearing the sampler is comparable to mild congestion, making it suitable for use at rest and during moderate exercise (Graham *et al.*, 2000). The recommended collection medium is an acrylic pressure sensitive adhesive tape developed by Avery Dennison (Mitakakis *et al.*, 2000; O'Meara & Tovey, 2000). The impaction efficiency of the Model 1 NAS depends upon particle size and flow rate (O'Meara & Tovey, 2000), but is thought to be approximately 100% for breathing rates over 15 l min^{-1} and particles >12 µm (Graham *et al.*, 2000).

A.4 Burkard Personal Volumetric Air Sampler

The Burkard Personal Volumetric Air Sampler (PVAS) is a small portable slit impactor (Fig. A.3). Air is aspirated at 10 l min^{-1} through a vertically orientated bell-shaped intake that tapers to a 14 × 1 mm slit. Particles are deposited on a microscope slide, typically coated with an adhesive medium, that is positioned immediately below the slit. The sampler is either run from an internal battery, or is mains powered. An intermittent beep during battery operation warns that the battery requires charging whilst a continuous beep indicates that voltage is inadequate for maintaining the required flow rate. Although designed for indoor use, the compact and uncomplicated design of the PVAS has led to its use in a number of outdoor studies (Feliziani & Marfisi, 1992; Levetin *et al.*, 1995; Alcázar & Comtois, 2000). The efficiency of vertically orientated aerosol samplers is known to vary with particle size, and to decline rapidly as wind speed increases (Armbruster & Breuer, 1982).

Figure A.3: Burkard Personal Volumetric Air Sampler

Appendix B

Sampling media preparation and post-processing methods

B.1 Model 1 Nasal Air Sampler

B.1.1 Adhesive tape preparation and installation

The Model 1 Nasal Air Sampler captures inhaled particles on strips of specially designed pressure sensitive adhesive tape. The adhesive coating is initially protected by a plastic cover sheet that must be removed prior to installation in the sampler. The dry surface of the adhesive tape is not efficient in the capture of grass pollen grains (Razmovski *et al.*, 1998), however efficiency can be improved through the application of a 'wet' adhesive such as silicone grease (E. Tovey, University of Sydney, personal communication, 29th October 2010).

Apparatus

- Nasal Air Sampler
- Scissors
- Pencil
- Adhesive strips × 2
- Forceps
- Latex gloves
- Silicone grease

Method

i. Use the pencil to mark the paper tabs of both adhesive strips with the sample identification code, and in addition mark one strip 'L' (left nostril) and the other 'R' (right nostril). Remove the sleeves from the sampler's left and right nostril inner core units.

ii. Hold the paper tab of one adhesive strip between thumb and forefinger of one hand, supporting the other end on your index finger. With the forceps, carefully peel back and remove the protective cover.

iii. Cover the index fingertip of your free hand with a thin layer of silicone grease, and run this finger along the exposed adhesive surface of the rod in one steady movement. Repeat if necessary until a thin, smooth, barely visible covering is achieved. If the covering of grease is too thick, remove some using a clean finger.

iv. Without touching the adhesive surface take the strip with the forceps and place it adhesive side down into the wall sockets of the appropriate inner core unit (the strip marked 'L' in the left nostril unit, the strip marked 'R' in the right nostril unit). Both paper tabs must lie entirely outside the unit. Press the adhesive strip against the inner core wall where they make contact, using the forceps.

v. Carefully place the appropriate sleeve over the inner core unit (careful - left and right sleeves are not identical). Use the forceps to gently pull on both paper tabs (which should be slightly protruding) to ensure the strip lies flat against the back bar of the sleeve.

vi. Install the second adhesive strip in the same manner.

B.1.2 Sample post-processing

Exposed adhesive tapes are mounted on a standard microscope slide with a stain-bearing glycerine jelly mountant medium in preparation for assay. For efficient storage, two samples can be mounted on each slide. Each sample consists of a pair of adhesive strips, individually referred to as 'left' or 'right' according to the nostril to which the strip corresponds. Details of the glycerine jelly mountant are given by the British Aerobiology Federation (1994).

Apparatus and personal protective equipment

- Boiling water
- Glycerine jelly mountant
- Beaker (1 l capacity suitable for 250 ml Duran bottle of mountant)
- Fume cabinet
- 76 × 26 mm microscope slides (one slide per pair of samples)
- Marker pen
- Small beaker of distilled water
- 1 ml plastic Pasteur pipette × 2
- Exposed samples
- Scalpel
- Cutting block (e.g. Burkard's Perspex cutting block)
- Roll of paper towel

- 22 mm square cover glass (one per sample)
- Razor blade
- Alcohol wipes
- Clear nail varnish
- 12 × 18 mm self adhesive labels (one per sample)
- Ball point pen
- Lab coat
- Nitrile gloves

Health and safety information

A lab coat and gloves should be worn throughout this procedure. Glycerine jelly mountant contains phenol and basic fuchsin in solution, and must be handled inside a fume cabinet when in liquid form. Phenol is corrosive as well as both toxic and harmful by inhalation, through contact with the skin and when swallowed. Prolonged exposure by these three mechanisms poses a serious risk to health. Basic fuchsin is toxic.

Method

The process is described for a single pair of samples. When processing a large number of samples, allow around 10 minutes per sample spread over three separate days.

i. Place the mountant bottle into the beaker. Add boiling water to the beaker until the level of water in the beaker just exceeds the level of the mountant in the bottle. Place the beaker in the fume cabinet and loosen the bottle lid. The boiling water must be replaced every 20 minutes or so[1] otherwise the mountant will become viscous and difficult to handle.

ii. With the marker pen, mark one end of a microscope slide with the identification code of the first sample to be processed and indicate the relative positions in which left and right strips will be mounted (Fig. B.1). Place the slide on the cutting block

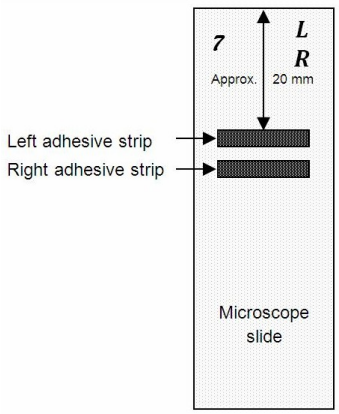

Figure B.1: Positioning adhesive tapes on the microscope slide.

[1]When processed a large number of samples it may be simpler to place the beaker on a hotplate at 80°C (do not allow the water bath to boil).

and put a drop of distilled water on the slide around 2.5 cm from the marked end using a pipette.

iii. This next step should be performed in a draught-free environment (i.e. outside the fume cabinet). Remove the outer sleeve from one of the sampler's inner core units. With the forceps, extract the adhesive strip and place it adhesive side up on the cutting block. Whilst in the sampler, strips are bent permanently into an angular 'U' shape. Without touching the central adhesive portion, hold the strip down with the forceps and cut through the two bends (the corners of the 'U'). This is best done by laying the scalpel blade flat over each bend and exerting pressure. Touch the tip of the scalpel lightly at one end of the central adhesive portion of the strip - it should adhere without difficulty and carefully drag it onto the wet part of the slide, lowering it onto the water. Manipulate the scalpel until it detaches from the strip. Repeat with the second adhesive strip, ensuring that left and right strips are correctly ordered on the slide.

iv. Hold the slide at an angle and allow excess water to drain away from the strips, absorbing it with a small piece of paper towel. Position the strips as shown in Fig. B.1 by gently nudging with the forceps. Place the slide sample side up on top of a piece of paper towelling in the fume cabinet.

v. Using a pipette, apply two drops of mountant to one surface of a cover glass, spreading the mountant over the entire surface with the side of the pipette tip in as few movements as possible. If bubbles result, draw them into the pipette or push them over the edge of the cover glass with the pipette tip. Hold the cover glass vertically for a couple of seconds, allowing mountant to build up against one edge. Lay the cover glass mountant side up on the paper towel.

vi. Resting one of the slide's long edges on the paper towel, slowly lower the adhesive strips onto the cover glass, beginning at the edge with excess mountant. Try to lower the slide at such a rate that the mountant advances across the strips with as linear a front as possible. When the mountant covers both strips, invert the slide. The mountant layer should extend to the margins of the cover glass. Peripheral gaps can be filled by placing additional mountant on the slide directly adjacent.

vii. If necessary, gently adjust the cover slip's position using the forceps until it sits centrally over the adhesive strips. Lay the slide horizontally in the fume cabinet and leave overnight.

viii. Scrape excess mountant from on and around the cover glass and clean away residual traces with an alcohol wipe, taking care not to wipe away the marker pen.

ix. Repeat steps ii - viii with the second sample, mounting it on the empty half of the slide.

x. Seal both samples by painting a layer of nail varnish over the join between cover glass and slide. Allow 10 minutes for the nail varnish to set.

ix. For each sample note the sample number, the date, time, place, duration and mode of collection, the relative positions of left and right strips and type of sampler used on a label with the ball point pen. One sample at a time, clean the marker pen from the end of the slide with an alcohol wipe and apply the appropriate label.

B.2 Burkard Personal Volumetric Air Sampler

B.2.1 Preparing sampling substrata

Burkard Personal Volumetric Air Sampler (PVAS) samples are collected on 18 mm sections of Melinex tape coated with petroleum jelly wax and mounted temporarily on a standard microscope slide. The method for coating the Melinex tape with petroleum jelly wax is described by the British Aerobiology Federation (1994). For each adhesive coated Melinex tape one can produce and mount 16 PVAS sampling substrata in around 45 minutes.

Apparatus and personal protective equipment

- Petroleum jelly wax coated Melinex tape, mounted on Burkard 7-Day sampler drum
- Burkard laboratory stand
- Scalpel
- Forceps
- Perspex cutting block (available from Burkard Manufacturing)
- Scissors
- Distilled water
- 1 ml plastic Pasteur pipette
- 76 × 26 mm microscope slides
- Paper towel
- Template (actual size outline of slide indicating central 18 mm of slides length)
- Marker pen
- Microscope slide box
- Lab coat
- Latex gloves

Method

To minimise contamination a lab coat and latex gloves should be worn, and the entire procedure carried out in a clean laminar flow cabinet. Throughout the procedure ensure that the wax coated surface of the Melinex tape is touched as little as possible, and only close to the edge.

i. Mount the drum on the laboratory stand. With the scalpel, carefully cut through the double sided tape at the point where the Melinex tape is joined. Prise both ends of the Melinex tape away from the drum.

ii. Hold the Melinex tape at one end with the forceps and carefully lift it from the drum before laying it wax side up on the cutting block, taking care not to touch the wax surface.

iii. Lift one end of the Melinex tape with the forceps and remove the untidy end section using the scissors, ensuring the tape is cut square. Repeat at the other end.

iv. Position the Melinex tape straight with respect to the cutting block. Measure an 18 mm section and mark it by puncturing the tape at both edges with the scalpel.

v. Cut off the measured section of Melinex tape with the scissors. This is best done as follows: slide the scalpel blade under the tape and lift it; take hold of the tape at one corner with the forceps; cut straight across the tape through the two puncture marks using the scissors.

vi. With the pipette, place a single drop of distilled water at the centre of a microscope slide. Take the 18 mm tape section with the forceps and hold it wax side up over the drop of water. Beginning with one edge, slowly lower the tape onto the slide such that a continuous film of water is formed between tape and slide. If air becomes trapped, raise the tape slightly and expel before lowering further.

vii. Hold the slide at an angle and allow excess water to drain away from the tape before carefully removing with a paper towel. Do not allow the paper towel to touch the adhesive tape. Place the slide over the template and position the tape square to the slide and as centrally as possible.

viii. Write the sample identification code at one end of the slide using the marker pen. Place the slide in the slide box.

ix. Repeat steps iv-viii until the entire Melinex tape has been mounted.

B.2.2 Sample post-processing

Burkard Personal Volumetric Air Sampler sampling media are prepared for assay by mounting on a standard microscope slide under a protective cover glass, held in place with a stain-bearing glycerine jelly 'mountant'. For efficient storage, two samples may be mounted on each slide. Details of the glycerine jelly mountant are given by the British Aerobiology Federation (1994).

Apparatus and personal protective equipment

- Boiling water
- Glycerine jelly mountant
- Beaker (1 l capacity suitable for 250 ml Duran bottle of mountant)
- Fume cabinet
- 76 × 26 mm microscope slides (one slide per pair of samples)
- Marker pen
- Small beaker of distilled water

- 1 ml plastic Pasteur pipette × 2
- Exposed samples
- Scalpel
- Forceps × 2
- Roll of paper towel
- 22 mm square cover glass (one per sample)
- Razor blade
- Alcohol wipes
- Clear nail varnish
- 12 × 18 mm self adhesive labels (one per sample)
- Ball point pen
- Lab coat
- Nitrile gloves

Personal safety information

A lab coat and gloves should be worn throughout this procedure. Glycerine jelly mountant contains phenol and basic fuchsin in solution, and must be handled inside a fume cabinet when in liquid form. Phenol is corrosive as well as both toxic and harmful by inhalation, through contact with the skin and when swallowed. Prolonged exposure by all three pathways poses a serious risk to health. Basic fuchsin is toxic.

Method

The process is described for a single pair of samples. When processing a large number of samples, allow around 10 minutes per sample spread over three separate days.

i. Place the mountant bottle into the beaker. Add boiling water to the beaker until the level of water in the beaker just exceeds the level of the mountant in the bottle. Place the beaker in the fume cabinet and loosen the bottle lid. The boiling water must be replaced every 20 minutes or so[2] otherwise the mountant will become viscous and difficult to handle.

ii. With the marker pen, mark one end of a microscope slide with the identification code of the first sample to be processed. Place a drop of distilled water around 2.5 centimetres from the marked end of the slide using a pipette.

iii. This next step should be performed in a draught-free environment (i.e. outside the fume cabinet). Detach the tape carrying the first sample from its temporary mount by sliding the blade of the scalpel under the tape. Pick up the tape by the edge with the forceps and hold it sample side up over the drop of water. Beginning with one edge, slowly lower the tape onto the clean slide such that a continuous film of water

[2]When processing a large number of samples it may be simpler to place the beaker on a hotplate at 80 °C (do not allow the water bath to boil).

is formed between tape and slide. If air becomes trapped, raise the tape slightly and expel before resuming.

iv. Hold the slide at an angle and allow excess water to drain away from the tape, mopping it up with a small piece of paper towel. With the forceps, gently nudge the tape into the position shown in Fig. B.2. Place the slide sample side up on top of a piece of paper towel in the fume cabinet.

v. Using a pipette, apply two drops of mountant to one surface of a cover glass, spreading the mountant over the entire surface with the side of the pipette tip in as few movements as possible. If bubbles result, draw them into the pipette or push them over the edge of the cover glass with the pipette tip. Hold the cover glass vertically for a couple of seconds, allowing mountant to build up against one edge. Lay the cover glass mountant-side up on the paper towel.

vi. Resting one of the slide's long edges on the paper towel, slowly lower the tape onto the cover glass beginning at the edge with excess mountant. Try to lower the slide at such a rate that the mountant advances across the tape with as linear a front as possible. When the mountant front reaches the far side of the tape, invert the slide. The mountant layer should extend to the margins of the cover glass. Peripheral gaps can be filled by placing additional mountant directly adjacent.

vii. If necessary, gently adjust the cover slip's position using the forceps until it sits centrally over the tape. Lay the slide horizontally in the fume cabinet and leave overnight.

viii. Scrape excess mountant from on and around the cover glass with the razor blade and clean away residual traces with an alcohol wipe, taking care to preserve the sample identification code.

ix. Repeat steps ii - viii with the second sample, mounting it on the empty half of the slide.

x. Seal both samples by painting a layer of nail varnish over the join between cover glass and slide, taking care not to obscure the tape. Allow 10 minutes for the nail varnish to set.

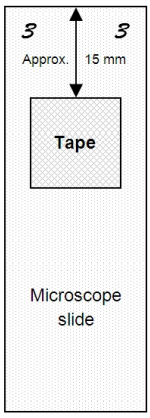

Figure B.2: Positioning 18 mm tape section on the microscope slide.

xi. For each sample write the identification code, the date, time, place, duration and mode of collection, and type of sampler used on a label with the ball point pen. One sample at a time, clean the sample number from the end of the slide with an alcohol wipe and apply the corresponding label.

Appendix C

Deriving concentration conversion factors

C.1 Burkard 7-Day Recording Spore Trap

The collection surface of the 7-Day sampler moves at a rate of 2 mm hour^{-1} past an orifice 2 mm or 2000 µm wide, thus every point on the tape that passes under the orifice is exposed for one hour. Air is aspirated at 10 l min^{-1} or 0.6 m^3 hour^{-1}. On average, the sample collected on a 1 µm wide transverse transect across the tape is therefore derived from $^{0.6}/_{2000} = {}^{3}/_{10^4}$ m^3 of air, and the sample collected on a transverse transect of width d µm from $^{3d}/_{10^4}$ m^3 of air. The number of pollen grains counted on a single transverse transect may thus be converted into a concentration in grains m^{-3} through application of the formula

$$Concentration = \frac{10^4 x}{3d} \tag{C.1}$$

where x is number of pollen grains and d the diameter of the microscope field of view in µm.

C.2 Sampling Technologies Model 20 Rotorod

The Rotorod manual (Sampling Technologies, 1998) states that the Model 40 Rotorod, which has identical performance characteristics to the Model 20, samples 3.12 m^3 of air per rod over a 24 hour period when operating on a 10% duty cycle, assuming assay of the entire rod (22 mm). During operation, each rod therefore samples $^{13}/_{600}$ m^3 of air every minute. The pollen count from a single rod (or the mean count of both rods) may then be converted into a concentration in grains m^{-3} with the formula

$$Concentration = \frac{600x}{13t} \tag{C.2}$$

where x is number of pollen grains and t the duration of sampling in minutes.

C.3 Burkard Personal Volumetric Air Sampler

The Burkard Personal volumetric Air Sampler has a throughput of 10 l or 0.01 m^3 air per minute. Pollen counts may be converted into concentrations in grains m^{-3} with the formula:

$$Concentration = \frac{100x}{t} \tag{C.3}$$

where x is number of pollen grains and t the duration of sample collection in minutes.

Appendix D

Assessing sampling rates and sample contamination

D.1 Sampling rate

Both the Model 20 Rotorod (Rotorod) and Burkard Personal Volumetric Air Sampler (PVAS) are designed to sample air at a set rate, and the factors used for converting pollen grain counts into concentrations (Appendix C) assume these rates to be constant. Variation in sampling rate, due for example to battery decay, may mean that concentrations are not projected correctly. The rates at which the Rotorod and PVAS sample air were checked before and after each days' sampling in Aarhus and Copenhagen. These data were however not collected in London or Worcester, because the necessary instrumentation was at that stage not available. Additional tests were therefore performed in order to establish whether battery decay could be affecting sampler performance.

D.1.1 Performance during field data collection

Rotorod and PVAS sampling rates were measured both before sample collection began, and after it had finished. All tests were performed indoors, in order to avoid wind interference. These data were collected on 16 days in Copenhagen, and on 12 days in Aarhus. The Rotorod was fitted with a pair of blank collecting rods, and was run for five minutes before the rotation rate was measured using a digital tachometer (Farnell AT-6). A blank slide was installed into the PVAS, and it was run for one minute before the volume of air aspirated in one minute was measured using a Wright Respirometer (British oxygen type P.M., Ferraris Development & Engineering). The samplers were powered in the same manner as during sample collection, i.e. the Rotorod was battery powered in both locations whilst the PVAS was battery powered in Aarhus but mains powered in Copenhagen.

Sampling rate statistics are presented in Table D.1. Variation was clearly greater for the PVAS than for the Rotorod. Over the course of a single day sampling rates both increased and decreased, with the magnitude of maximum change similar in both directions.

Table D.1: The range, relative mean difference, and maximum daily change in sampling rate are presented for the Rotorod (rate of rotation in rpm) and PVAS (flow rate in l min^{-1}).

Sampler	Location	Range	Relative mean difference	Maximum daily change
Rotorod	Copenhagen	2,445-2,496 rpm	2.06%	-0.61% / +0.47%
	Aarhus	2,456-2,472 rpm	0.65%	-0.24% / +0.12%
PVAS	Copenhagen	10.03-10.89 l min^{-1}	8.22%	-2.99% / +2.33%
	Aarhus	9.85-10.54 l min^{-1}	6.77%	-2.36% / +4.00%

D.1.2 Effect of battery decay

Rotorod

The Rotorod was tested at the National Pollen and Aerobiology Research Unit at the University of Worcester. The sampler was mounted on a tripod, and two dummy rods were installed so as to reproduce the conditions of sample collection. The battery (Yuasa NP7-12, the model used during field sample collection) was fully charged prior to the test.

The test began at 08:30 on 19^{th} January 2011 and was concluded at 09:00 the following day. Readings were taken every 30 minutes during the first hour, and every hour for the next 10 hours with a final reading taken 24½ hours after the test had begun. During the first eight hours of the test the sampler stood in an environmental test chamber which was in use during the first 5½ hours, meaning that temperature and air flow within the chamber varied. For the remainder of the test, the sampler was stationed in an office.

The results of the test are plotted in Fig. D.1. The maximum rate of 2,464 rpm was recorded at the start of the test, and the minimum of 2,443 rpm was recorded after four hours. The relative mean difference between these two values is 0.86%. After 24½ hours the rotation rate was 2,453 rpm. The relative mean difference between this value and the maximum is 0.33%.

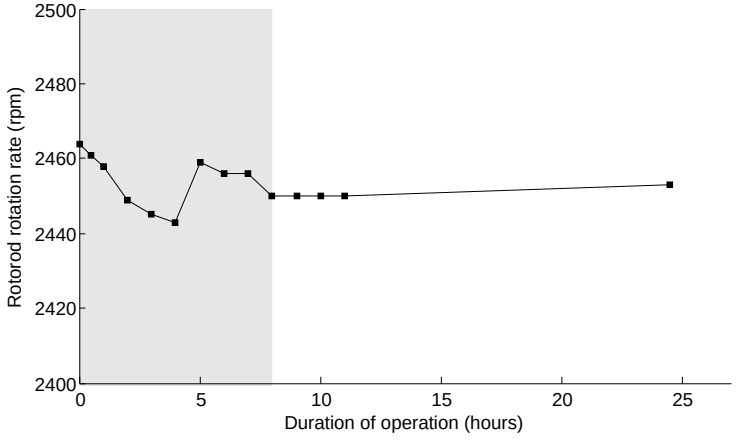

Figure D.1: Effect of battery decay on Rotorod sampling rate. The grey area represents the time that the Rotorod was stationed in the exposure chamber.

PVAS

The PVAS was tested at the Department of Environmental Science, Aarhus University, on 1^{st} April 2013. The sampler was fully charged prior to the test, and set up in an office and a dummy glass slide was installed. The test began at 10:00 with the flow rate checked with Sensidyne Gilian Gilibrator II Air Flow Calibrator every 30 minutes for the first two hours and every hour thereafter. The PVAS sounds an alarm when battery power becomes low enough to affect flow rate. This occurred at 23:22, just over 13 hours after the test began, and the test was immediately terminated.

The results of the test are plotted in Fig. D.2. A maximum flow rate of 8.94 l min^{-1} was recorded after nine hours of operation, and a minimum of 8.13 l min^{-1} after 30 minutes. The relative mean difference between these two values is 9.49%.

D.1.3 Discussion

The respirometer used to measure flow during fieldwork has very low resistance, whilst the air flow calibrator used for the battery decay test was connected to the PVAS through several centimetres of tubing, which introduces some resistance. The flow rate of the PVAS is known to be strongly affected by flow gauge resistance (G. Wili, Burkard Manufacturing, personal communication, 2010), and this likely accounts for the lower flow rates recorded by the later instrument.

The largest relative mean difference in Rotorod sampling rate reported during field work was 2.06%, whilst the greatest change over a single day was a decrease of 0.61%, however both increases and decreases of similar magnitude were observed. Over the course of a 24 hour period, sampling rate was observed to be more influenced by atmospheric conditions than by battery decay, with the greatest change occurring in the test chamber whilst conditions were being manipulated. Very little variation was observed thereafter.

The greatest recorded variation in PVAS flow rate occurred during the battery decay test, with a maximum relative mean difference of 9.49%, however once again this variation cannot be attributed to battery decay with the minimum value occurring after only 30

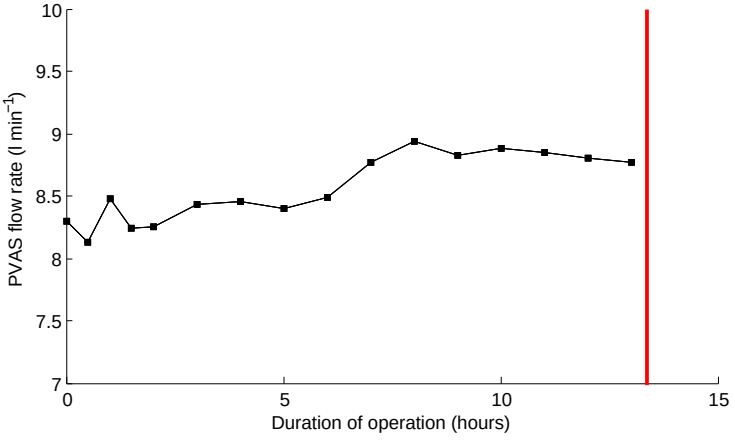

Figure D.2: The effect of battery decay on PVAS sampling rate. The vertical red line shows the time when the battery dropped below the minimum charge level.

minutes of operation and a general increase seen thereafter. During field work, the maximum change over the course of a single day was an increase of 4.00%, although decreases of similar magnitude were also observed.

The maximum period of continuous battery operation during field data collection for the studies presented in this thesis was 13 hours for the Rotorod (in Copenhagen), and seven hours for the PVAS (in Worcester), i.e. well within the periods covered by the battery decay tests.

No evidence was found that either Rotorod or PVAS sampling rates are affected by battery decay, and the observed variation can most likely be attributed to variation in atmospheric conditions.

D.2 Sample contamination

Rotorod, PVAS and NAS control samples were collected to check against contamination during preparation, transport to and from the field, and post-processing. Controls were collected in London, Copenhagen and Aarhus at random times when samplers were not in operation by mimicking sample exposure to ambient air during the collection of ordinary samples. Control samples were prepared, carried to and from the field and post-processed in exactly the same manner as ordinary samples. Whilst in the field, Rotorod and PVAS controls were placed in their respective samplers and left for two minutes with the sampler remaining switched off, before being returned to their respective transport vessels. NAS samplers were placed in the nose under mouth breathing for two minutes before being returned to their storage containers. Rotorod and NAS controls were assayed in the same manner as their ordinary counterpart samples, whilst for PVAS tapes the entire adhesive surface area (18 mm $\times$ 19 mm) was scanned rather than the usual practice of assaying only the area of sample deposition.

Table D.2 shows that in the 51 different control samples collected a total of five grass pollen grains were found, even though ambient concentrations during collection included high values. It was therefore concluded that contamination levels were negligible.

Table D.2: The results of control samples for the Rotorod, NAS and PVAS. 'Ambient concentrations' are those recorded at a nearby pollen monitoring station at the time control samples were collected.

Sampler	Number of control samples	Total number of grass pollen grains	Ambient concentration range (median) in grains m^{-3}
Rotorod	28 rods	2	26-470 (59)
PVAS	15 slides	2	26-470 (67)
NAS	8 tapes	1	43-184 (103)

Appendix E

Exposure data validation

As described in Chapter 4, exposure data were collected in both London and Aarhus using Nasal Air Samplers (NAS). In London, NAS samples were collected on the pressure sensitive adhesive tape recommended by Mitakakis *et al.* (2000) and O'Meara & Tovey (2000). Due to concerns over its collection efficiency, these tapes were coated with silicone grease in Aarhus. The impaction efficiency of the NAS is thought to be approximately 100% for particles over 12 µm (Graham *et al.*, 2000), thus if the collection adhesive is working properly, essentially all inhaled particles should be collected by the sampler.

Data collection in Aarhus was conducted by two individuals. Breathing rates vary from person to person, and as the breathing rate of an individual increases, so does the volume of air they inhale and thus the magnitude of the dose they potentially receive. This could render data collected by different individuals incompatible.

In this appendix, further analysis of the personal exposure data collected with the NAS is presented as two sub-analyses. In the first of these, data collected in London are compared with data collected in Aarhus, in order to examine the effect of changing the sampling substrate on NAS efficiency. In the second of these, data collected by individual 1 was compared with data collected by individual 2, in order to establish whether they were compatible or not.

E.1 Method

E.1.1 Data collection

Twenty-three sets of samples collected in London on the 7^{th}, 12^{th}, 13^{th}, 15^{th}, 16^{th}, 17^{th}, 24^{th} and 26^{th} of June 2010 are analysed. Sample collection began outside Islington Fire station and was conducted whilst walking North, first along Upper Street and then, after bearing left at Highbury & Islington tube station, along Holloway Road, ending at the Camden Road bus stop. The route was approximately 1.95 km in length, lay within 1.8 km of the Islington pollen monitoring station, and typically took some 19 minutes to traverse. A maximum of three samples were collected on each day, since in 2010 only three NAS samplers were available. All sampling in London was performed by the author. Collection

Figure E.1: Device used for estimating breathing rate during exposure sample collection - a Wright Respirometer (British oxygen type P.M., Ferraris Development & Engineering) attached to an anaesthetic mask.

of the Aarhus data is detailed in Section 4.2.

In order to aid the interpretation of NAS data, the volume of air aspirated during personal sample collection was estimated in both cities by walking their respective personal sampling routes whilst wearing a Wright Respirometer fitted to an anaesthetic mask (Fig. E.1). In London this measurement was made on 12 occasions by the author, and in Aarhus on eight occasions by both individuals involved in data collection.

E.1.2 NAS efficiency

The NAS filters inhaled particles from the air, and thus gives a measure of inhaled dose. In order to validate the performance of the NAS adhesive, the parameter α was calculated for each sample collected using the equation

$$\alpha = \frac{\frac{N}{I_e} \times \frac{1}{E_I} \times E_7}{C_7} \tag{E.1}$$

where N (grass pollen grains) is total grass pollen dose as measured by the NAS, I_e (m^3) is an estimate of the volume of air inhaled during sample collection, E_I (no units) is the inhalable fraction as defined in Equation 1.1 evaluated at an aerodynamic diameter of 30 µm (the average size of grass pollen grains according to Section 1.2.1), $E_7 := 0.7$ is the approximate efficiency of the 7-Day sampler for *Phleum* pollen under typical ambient wind speeds (Ogden *et al.*, 1974, p. 93), and C_7 the background concentration recorded by the 7-Day sampler. I_e is defined by

$$I_e = R_i \times t \tag{E.2}$$

where t is the duration of sample collection, and R_i is the estimated breathing rate of individual i based on the inhaled volume data collected with the Wright Respirometer/anaesthetic mask.

The numerator of α gives a crude estimate of the expected breathing zone concentration as measured with a 7-Day sampler. If the retention efficiency of the NAS adhesive is approximately 100% the denominator and numerator should be of reasonably similar size. A value of $\alpha \approx 1$ indicates that NAS retention efficiency appears to be approximately 100%, and a value $\alpha << 1$ indicates that NAS collection efficiency appears to be poor.

Table E.1: Pertinent descriptive statistics. n=23 for the London data and n=60 for the Aarhus data with the exception of the breathing rate data, where n=12 for London and N=8 for Aarhus for both individuals.

Location	Variable	Units	Range	Median
London	Dose	grains	0 - 10	2
	C_7	grains m^{-3}	12.1 - 206.1	97.0
	Exposure duration	minutes	18.28 - 19.72	19.10
	Breathing rate	l min^{-1}	29.69-34.89	32.73
	α	grains m^{-3}	0.00-0.3587	0.0286
Aarhus	Dose	grains	6 - 127	34
	C_7	grains m^{-3}	0.0 - 311.0	56.2
	Exposure duration	minutes	25.67 - 31.15	27.56
	Individual 1 breathing rate	l min^{-1}	20.49-25.79	23.00
	Individual 2 breathing rate	l min^{-1}	27.38-30.27	29.63
	α	grains m^{-3}	0.33-6.28	0.9169

E.1.3 Data compatibility

Data collected in Aarhus between 16:00-20:00 (when local emissions that may complicate the dose rate/background concentration ratio are thought not to occur, see Section 4.4.1) were selected. Dose rate/background concentration ratios were separated into two groups, corresponding to the two individuals, and compared (n=20 for individual 1, n=17 for individual 2). Dose data were then converted into 'inhaled concentrations' by dividing the pollen grain dose by the median inhaled volume of the respective individual, thus correcting for differences in inhaled volume, and the inhaled concentration/background concentration ratios calculated and compared.

E.2 Results

E.2.1 NAS efficiency

Pertinent data are summarised in Table E.1. The parameter α has a median value of 0.029 for the London study and 0.917 for the Aarhus study, i.e. α is approximately one for the Aarhus data and close to zero for the London data.

E.2.2 Data compatibility

The volume of air inhaled whilst walking the sample collection route in Aarhus was estimated to be considerably greater for individual 2 (799-847 l) than for individual 1 (605-657 l). The median dose rate/background concentration ratio was 0.019 for individual 1, and 0.017 for individual 2, meaning that the ratio for individual 2 was 89% that of individual 1. The median inhaled concentration/background concentration ratio was 0.851 for individual 1, and 0.590 for individual 2, meaning that the ratio of individual 2 was 69% that of individual 1.

E.3 Discussion and conclusion

E.3.1 NAS efficiency

The very low value of α for the London data indicates that the retention efficiency of the recommended pressure sensitive adhesive tape used on its own is poor for grass pollen grains. Indeed, subsequent to data collection in London and following consultation with Euan Tovey at the University of Sydney (involved in developing the NAS), it was discovered that a study confirming this had been published. Using a Hirst-type sampler, Razmovski *et al.* (1998) found that the collection efficiency of the recommended adhesive tape was approximately 20% that of the petroleum jelly wax adhesive often used with Hirst-type samplers. This low efficiency was thought to occur because the smooth, spheroidal grass pollen grains were prone to bounce off the hard, dry surface of the tape. In order to improve retention, it was recommended that the tape be coated with a 'wet' adhesive (E. Tovey, University of Sydney, personal communication, 29^{th} October 2010), therefore during sample collection in Aarhus the pressure sensitive tape was coated with silicone grease. The value of $\alpha = 0.917$ obtained from the Aarhus data suggests that retention efficiency and thus overall collection efficiency was in this way elevated to around 100%. The London data were considered highly unreliable, and were therefore omitted from the analysis presented in Chapter 4.

E.3.2 Data compatibility

By inhaling a greater volume of air one potentially incurs a greater pollen grain dose, which would result in the inhaled concentration/background concentration ratio of individual 2 being greater than that of individual 1. The opposite effect is in fact seen, with the correction for inhaled volume resulting in a decrease in the dose proxy/background concentration ratio of individual 2 relative to individual 1.

As minute volume increases, so does aspiration rate, and this leads to a smaller inhalable fraction (Armbruster & Breuer, 1982). Given that correcting for breathing rate appears to render the two data sub-sets less compatible, it was concluded that the positive and negative effects of greater volume and reduced inhalable fraction substantially cancel one another out, and it was considered appropriate to pool data collected by the two individuals without any adjustment. This appears to be standard practice when using the NAS (Mitakakis *et al.*, 2000; Renström *et al.*, 2002; Gore *et al.*, 2006; Renström *et al.*, 2006).

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