1 Atmospheric Transport Reveals Grass Pollen Dispersion Distances

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11 Abstract

Identifying the origin of bioaerosols is of central importance in many biological disciplines, 12 such as human health, agriculture, forestry, aerobiology and conservation. Modelling sources, 13 14 transportation pathways and sinks can reveal how bioaerosols vary in the atmosphere and their environmental impact. Grass pollen are particularly important due to their widely distributed 15 source areas, relatively high abundance in the atmosphere and high allergenicity. Currently, 16 studies are uncertain regarding sampler representability between distance and sources for grass 17 pollen. Using generalized linear modelling, this study aimed to analyse this relationship further 18 19 by answering the question of distance-to-source area contribution. Grass pollen concentrations were compared between urban and rural locations, located 6.4 km apart, during two years in 20 21 Worcestershire, UK. We isolated and refined vegetation areas at 100 m x 100 m using the 2017 CEH Crop Map and conducted atmospheric modelling using HYSPLIT to identify which 22 23 source areas could contribute pollen. Pollen concentrations were then modelled with source

24 areas and meteorology using generalized linear mixed-models with three temporal variables as 25 random variation. We found that the Seasonal Pollen Integral for grass pollen varied between both years and location, with the urban location having higher levels. Day of year showed 26 27 higher temporal variation than the diurnal or annual variables. For the urban location, grass source areas within 30 km had positive significant effects in predicting grass pollen 28 concentrations, while source areas within 2 - 10 km were important for the rural one. The 29 30 source area differential was likely influenced by an urban-rural gradient that caused differences in the source area contribution. Temperature had positive highly significant effects on both 31 32 locations while precipitation affected only the rural location. Combining atmospheric modelling, vegetation source maps and generalized linear modelling was found to be a highly 33 34 accurate tool to identify transportation pathways of bioaerosols in landscape environments.

35 Keywords

Bioaerosol, Poaceae, HYSPLIT, Atmospheric Transport, Improved Grassland, Urban-Rural
Gradient

38 **1. Introduction**

39 The movement of bioaerosols within the landscape has fundamental impacts for agriculture, forestry, conservation biology and allergy. By identifying natural atmospheric transportation 40 pathways we can quantify how these bioaerosols are likely to be distributed in the landscape 41 42 (Izquierdo et al., 2011; Kurganskiy et al., 2020; Skjøth et al., 2019). This allows us to make informed decisions regarding their spatial and temporal impacts and contribute information 43 that will be relevant to environmental policy. Grass pollen is a prime example where 44 information about the distribution and transportation of a bioaerosol has major impacts not only 45 for society as a whole but also for the individual (Davies et al., 2015). A European-wide study 46 47 found that prevalence of allergic rhinitis in the population varies from 35 - 50%, while specific 48 sensitisation to grasses varies from 10 - 30% (Newson et al., 2014), and is the outdoor aeroallergen causing the highest number of sensitisations (Burbach et al., 2009; Heinzerling et 49 al., 2009). Normal symptoms range from itching involving the eyes and nose, nasal secretion 50 51 and sneezing, but can indirectly also cause general fatigue, lack of sleep, headaches and general discomfort caused by the direct effects (Greiner et al., 2011; Wallace et al., 2008). In addition 52 to being physically debilitating, allergic rhinitis also causes a loss in quality of life (Šaulienė et 53 al., 2016) and a measurable loss of productivity for the entire affected society (Crystal-Peters 54 et al., 2000; Lamb et al., 2006). It has been estimated that up to 90% of patients with allergic 55 56 rhinitis are insufficiently treated (Zuberbier et al., 2014).

57 To assist in treatment a large community of pollen monitors and forecasters exists to provide the public and health professionals alike with accurate and up-to-date information of the current 58 59 situation (e.g. Adams-Groom et al., 2020; Lo et al., 2019). This community is able to provide informed grass pollen warnings to the public by collating information from a wide range of 60 sources, including but not limited to grass pollen monitoring datasets, grass pollen trend 61 analyses (Emberlin et al., 1999; Smith and Emberlin, 2005), local grass flowering observations 62 (Frisk et al., 2021; León-Ruiz et al., 2011), grass maps (McInnes et al., 2017), current weather 63 64 conditions and climatic trends. Previous studies are generally in agreement that temperature, precipitation, and local grass areas are important factors in this dynamic (García-Mozo et al., 65 2010, 2009; Jung et al., 2021; Recio et al., 2010; Ščevková et al., 2020; Skjøth et al., 2013; 66 67 Werchan et al., 2017). This is due both to the growth and maturity of the plants (Charles-Edwards et al., 1971; Förster et al., 2018; Hurtado-Uria et al., 2013) but also the impact on 68 flowering and the release of pollen (Cebrino et al., 2016; Emecz, 1962; Romero-Morte et al., 69 70 2020). However, it is uncertain to what degree all of these factors contribute to the absolute levels and the movement of the grass pollen seen in the atmosphere, with one new modelling 71

72 study suggesting that temperature and CO_2 have important positive and enhancing effects, not only on grass pollen levels now but also in the future (Kurganskiy et al., 2021). 73

It has been suggested that the influence from strong but isolated grass pollen sources is limited 74 75 to just a few hundred metres (Skjøth et al., 2013) and it has several times been shown that there can be substantial variation in the grass pollen concentrations within urban zones such as in 76 77 Aarhus, Berlin (Werchan et al., 2017) and Helsinki (Hjort et al., 2016). On the other hand it has been shown that observations with a pollen trap measured at >10 m are representative for 78 a larger area (Rojo et al., 2019) corresponding to the general assumption in aerobiology that a 79 pollen trap represents an area within 30 km (e.g. Pashley et al., 2009). This conflicting 80 information, especially related to grass pollen, can make it particularly difficult to assess grass 81 pollen concentrations from a single trap. How representative is the trap and from how far away 82 83 does it collect its pollen? In addressing this question we collected grass pollen from two nearby locations over two years and investigated how meteorological variables (temperature and 84 precipitation) and grass pollen source areas contribute to the movement of grass pollen in the 85 landscape. We combined atmospheric transportation modelling with generalized linear mixed-86 modelling approaches to test the following hypothesis: The area with grass pollen sources 87 88 influencing each of the two stations is of equal size. Secondly, we explored whether there was 89 a considerable contribution to the observed grass pollen concentrations from sources further than 30 km away. 90

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2. Material and Methods

2.1. Locations and Pollen Monitoring

Two campus locations belonging to the University of Worcester in Worcester, UK were used 93 in this study: St Johns (52.193N, -2.221E) and Lakeside (52.254N, -2.254E). Both locations 94 95 are situated in the Severn Valley, an extensive flood plain extending from the Bristol Channel

96 to the town of Kidderminster. Lakeside is an experimental site located about 6.4 km NNW of 97 St Johns and it is operated during the grass pollen season. The St Johns pollen trap was located on the roof of the St Johns Edward Elgar building, 10 m above ground (42 m AMSL (Above 98 99 Main Sea Level)). It is part of the UK national pollen-monitoring network and a permanent trap that is active all year. The Corine Land Cover 2018 classifies the St Johns area as 100 101 discontinuous urban fabric, with the surroundings (< 2 km) being mainly comprised of 102 buildings, park landscapes and residential areas. The Lakeside trap (our rural location) was located on the top of a tall container, 4 m above ground and placed on a tripod meaning that 103 104 the inlet is at 5 m (42 m AMSL). The height of the pollen sampler has been shown to play a role in the interpretation of the collected pollen analysis and results (Rojo et al., 2019). The 105 106 reasoning is that sampling should be done elevated from the terrain, with a typical 107 recommendation of about 10 m, while lower elevations can be justified based on the 108 circumstance (Hugg et al., 2020). In our particular case the local circumstance is a flat area with much less atmospheric turbulence compared to an urban area. Rojo et al. (2019) found 109 that the 10 m height was mainly needed for pollen from woody vegetation and commented that 110 this was partly due to the fact that observations were often in urban zones, areas with high 111 turbulence. In contrast, Rojo et al. found that there was much less variation between paired 112 samples of Poaceae obtained at 10 m and below. This is further corroborated by a study where 113 114 it was demonstrated that there is no significant difference in Poaceae pollen concentrations 115 between sampling at ground level in contrast to altitude of 16 m (Fernández-Rodríguez et al., 2014b). Due to this and the special circumstances a lower sampling height is fully justified as 116 stated by Hugg et al. (2020). The Corine Land Cover 2018 classifies the Lakeside area as a mix 117 118 between non-irrigated arable land, pastures and complex cultivation patterns, with the surroundings (< 2 km) being mainly comprised of well-maintained grass areas, lakes, 119 120 agricultural fields, and pastures. The nearest surroundings to both traps (< 200 m) contain amenity grass areas that are regularly mown to keep the grass very short, which severely limits
or completely prevents any flowering making it reasonable to assume that neither of the traps
were primarily influenced by very local sources.

124 Grass pollen were sampled using two Burkard volumetric spore traps of Hirst design (Hirst, 1952), one at each location. All dates between the 1st of May and the 1st of September for the 125 126 years 2018 and 2019 were sampled. Preparation, sampling and identification were conducted according to the standardized pollen monitoring methodology practiced by the UK pollen-127 monitoring network (Adams-Groom et al., 2002; Skjøth et al., 2015), which complies with the 128 129 minimum requirements for national monitoring networks with respects to sampling media, control of sampling, counting method, training and internal validation of counting as proposed 130 by the European Aerobiological Society (Galán et al., 2014). The network uses the transversal 131 132 method by counting twelve transects per microscopy slide and then transforms these values into daily concentrations according to Käpylä and Penttinen (1981). Additionally, the twelve 133 transects from each day were converted into bi-hourly grass pollen concentrations. This 134 allowed for higher resolution grass pollen data time series to be used in the study. All dates 135 were analysed and the numbers of grass pollen were counted using a microscope with x400 136 137 magnification.

138 **2.2. Meteorological Data**

The meteorological information utilized in the study was collected from a set of identical Campbell Scientific meteorological logger stations, one at each location. Both meteorological stations were located close (< 300 m) to the pollen samplers. The stations logged average air temperature (°C) and total precipitation (mm) in intervals of 30 min. Bi-hourly meteorological datasets were calculated to temporally match the grass pollen datasets. Temperature was calculated as the average air temperature from four data points starting at time t min and ending at time t+90 min. Precipitation was calculated in the same manner but as the sum precipitation
over the same time period. Wind speed and wind direction are included as standard in the
atmospheric model described in section 2.4.

148 **2.3. Source Maps**

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2.3.1. Mapping Grids

A generalized grid-based approach was used to create grass maps. The grids were comprised 150 of a circular grid using a square grid definition with a resolution of 100 x 100 m. The circular 151 152 grid approach was utilized to simulate the standard pollen dispersal distance of 30 km (e.g. Avolio et al., 2008; Katelaris et al., 2004; Pashley et al., 2009). To further test the standard 153 pollen dispersal distance a larger than recommended circular grid of 50 km per location was 154 utilized. These two grids were simplified by selecting a point between each location and 155 generating a unified grid with a radius of 53 km. To further narrow down the likely dispersal 156 distance the larger grid was divided into smaller concentric grids based on the atmospheric 157 scale-definition earlier proposed by Orlanski (1975) and contextualized by Smith et al. (2013). 158 This has previously been successfully utilized to identify likely pollen dispersal distances 159 160 (Oteros et al., 2015). The scale-definition was developed to categorize relevant intervals of distances in which similar atmospheric processes occur, which allows for a standardization and 161 comparison of distances in atmospheric research. We have followed the definition by using 162 micro-scale distances (0 - 2 km), meso-gamma-scale distances (2 - 20 km) and meso-beta-163 scale distances (20 - 200 km). Micro-scale grids (0 - 2 km) and smaller meso-gamma grids (2 - 200 km)164 - 10 km) were created for each location (Figure 1). Each meso-gamma grid excluded the 165 current micro-scale location but included the other locations micro-scale grid. One larger meso-166 gamma grid (10 - 20 km) and three meso-beta grids (20 - 30, 30 - 40 and 40 - 50 km) were 167 168 additionally created.

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2.3.2. Grass Source Areas

170 Grass areas present within the 50 km catchment area have been calculated by using the 2017 version of the 'CEH Land Cover® Plus: Crop' dataset (https://www.ceh.ac.uk/services/ceh-171 land-cover-plus-crops-2015), developed by the Centre for Ecology and Hydrology (CEH) and 172 made available to the UK higher education institutions through Digimap 173 (https://digimap.edina.ac.uk/). The CEH dataset contains a wide range of agricultural crops and 174 175 improved grasslands down to field level for England, Wales and Scotland. The dataset is a remote sensing product based on Sentinel-1 and Sentinel-2 data, with a total accuracy (kappa 176 statistic) of 0.82. All improved grasslands were extracted and fused with the abovementioned 177 178 grid definitions to isolate the proportion of grass fields in each 100 x 100 m grid cell. The grass 179 field accuracy is >94% (CEH Land Cover® Plus Crop Map: Quality Assurance). Features smaller than the field-specific map-resolution are not included in the dataset, such as house-180 181 hold lawns, road-verges, and similar features.

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2.4. Atmospheric Modelling

Air mass transport was investigated with the Hybrid Single Particle Lagrangian Integrated 183 Trajectory (HYSPLIT) model (Draxler et al., 2016) using backwards trajectories. The 184 HYSPLIT model was here used to analyse the entire observational record using the 185 meteorological data in the HYSPLIT ARL format with 0.25 x 0.25 degree resolution, 55 hybrid 186 sigma-pressure levels and 2-h temporal resolution. This dataset is available from the year 2016 187 188 and onwards from the HYSPLIT ftp server (ftp://arlftp.arlhq.noaa.gov/archives/gfs0p25/). 189 Trajectories for all bi-hourly pollen records were calculated with HYSPLIT with a 500m receptor height using the same protocol as previous aerobiological studies on both pollen 190 191 (Fernández-Rodríguez et al., 2014a; Plaza et al., 2016) and spores (Grinn-Gofroń et al., 2016; 192 Skjøth et al., 2012), but restricting the model to only 12 hours, thereby excluding potential long 193 distance transport. This is an acceptable compromise because the low release height and relatively high settling velocity of grass pollen makes it less prone to long distance transport in 194 comparison to e.g. birch pollen. Furthermore, modelling studies suggests that only 10 - 20%195 196 of grass pollen SPIn (Seasonal Pollen Integral) is impacted by long distance transport (Sofiev, 2017) and a recent study from Northern Europe found that in this region the severity of the 197 grass pollen season (as measured by SPIn) is a local scale phenomenon (Kurganskiy et al., 198 199 2021). Model output is geographical points dumped along each trajectory with 1-h temporal resolution. The points for both years of data were then further processed in ArcGIS ver. 10.7. 200 201 In order to investigate whether or not air passing over more dense grasslands acquired more grass pollen, or if the distance to the source areas could explain the observed grass pollen 202 203 pattern, the following was done: All the trajectories were compiled to simulate the catchment 204 area for the pollen caught at each location during the time period specified above. This was 205 done by fusing each trajectory dataset with each concentric source map. The spatial fusing method adds all grass pixels passed by each trajectory, allowing trajectories passing pixels with 206 higher percentages of grass areas to gain higher values than trajectories passing pixels with 207 lower percentages of grass areas. This was done for all eight circular and concentric grass maps, 208 209 creating integrated HYSPLIT trajectory-grass variables. It should also be noted that pollen are more likely to originate from areas closer to the pollen traps even if the catchment area is large 210 211 due to the depository nature of (heavier) bioaerosols such as pollen (Adams-Groom et al., 212 2017).

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2.5. Statistical Analyses

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2.5.1. Grass Pollen and Meteorological Analyses

The grass pollen season was defined using the 95% method (Goldberg et al., 1988) for the grass pollen analyses (e.g. Myszkowska, 2014; Smith et al., 2009). The method discards all dates before 2.5% and after 97.5% of the SPIn to avoid long tails of low pollen concentrations. Both 218 locations contributed equally by summing and averaging each cumulative seasonal pollen sum. The datasets for each year were tested for non-normality using the Shapiro-Wilks test (Shapiro 219 and Wilk, 1965), in which all four datasets showed non-normality. The grass pollen time series 220 221 were additionally analysed using a Seasonal Decomposition of Time Series by LOESS (STL Decomposition) analysis (Cleveland et al., 1990), with LOESS being an acronym of Locally 222 estimated scatterplot smoothing (Cleveland, 1979). The STL is divided into three parts: 223 224 seasonal component, trend component and remainder component. The seasonal component represents a moving average, the trend component represents a mean per 12 bi-hourly data 225 226 points (daily mean) and the remainder component represents what remains after the other two components have been removed from the full data series (e.g. Rojo et al., 2017, 2015). Both 227 the full grass pollen time series and each STL component were then analysed using Spearman's 228 229 Rank correlation (Spearman, 1904).

The bi-hourly meteorological time series from each year and location were similarly analysed using the Shapiro-Wilks and Spearman's Rank correlation to investigate the degree of similarity in the meteorology between the locations. Wilcoxon Signed-Rank test was utilized to investigate any general differences in the meteorology between the locations (Wilcoxon, 1945).

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2.5.2. Generalized Linear Mixed-Modelling Approach

Generalized linear mixed-models were created to investigate the contribution of each variable on the bi-hourly grass pollen concentrations from each location. Grass pollen concentrations larger than 30 times the mean value of the variables were considered outliers and removed to improve model performance, and this affected two data points for St Johns and one for Lakeside. The R package *lme4* (Bates et al., 2015) was used to create the models. The models use both fixed and random variables to calculate parameters and minimize model residuals. 242 This model approach utilized a gamma family link-log function to fit the exponential models. To accommodate this, all grass pollen concentrations were increased by one, since the natural 243 logarithm does not allow zero-values. This approach is commonly used in ecological statistics 244 (Fletcher et al., 2005). Three random variables were used in the model, Day of Year (DOY), 245 the time of day (bi-hourly intervals), and year (2018 or 2019). The incorporation of the 246 temporal variables allowed for a model performance and interpretation that includes the 247 temporality, found in many ecological variables (Ryo et al., 2019). Eight fixed variables were 248 used in the model, six trajectory-grass variables (two unique per location and four shared) in 249 250 addition to the two meteorological variables (temperature and precipitation). The trajectorygrass variables were transformed using the natural logarithm (ln), as common practice while 251 using variables with large natural variation. The t-values produced by the model were 252 253 interpretated into p-values using Satterthwaite's method using the R package *lmerTest* (Kuznetsova et al., 2017). Coefficients of determination (R^2) of the model were determined 254 using the R package MuMIn (Barton, 2020). The conservative Trigamma R² estimate was used 255 256 instead of the Delta or Lognormal estimates (Nakagawa et al., 2017). The model was used to predict grass pollen concentrations, these values were back-transformed from the natural 257 logarithm to real numbers by using the base of the natural logarithm (*e*, Eulers number) in order 258 to facilitate an observed-vs-predicted comparison on the same scale. All statistical analyses 259 were performed in the statistical software R (ver. 4.0.3.) (R Core Team 2021; R: A language 260 261 and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online at https://www.R-project.org/.) 262

263 **3. Results**

- **3.1. Spatiotemporal Variation**
- 265 **3.1.1. Grass Pollen**

The grass pollen season in 2018 ranged between 21st of May and 20th of July (lasting 61 days) 266 while the season in 2019 ranged between 1st of June and 5th of August (lasting 66 days) 267 according to the 95% method (Figures 2-3). The daily time series for both years are available 268 269 in the supplementary material (Supplementary Figures 1-2). The Spearman's correlation of the bi-hourly time series between St Johns and Lakeside estimated rho coefficients of 0.683 (p 270 < 0.001) for 2018 and 0.781 (p < 0.001) for 2019. These indicated strong positive spatial 271 relationships between the two locations and years (Table 1). The STL Decomposition indicated 272 that there were strong correlations in the seasonal, trend and remainder components between 273 274 the locations and years (see Supplementary Figures 3 - 6 for the STL Decompositions). The SPIn for the 2018 season was 5941 (pollen*day/m³) for St Johns and 4423 (pollen*day/m³) for 275 Lakeside. This created an estimated SPIn ratio in 2018 for St Johns:Lakeside of 1:1.34. The 276 277 SPIn for the season in 2019 was 4905 (pollen*day/m³) for St Johns and 4164 (pollen*day/m³) for Lakeside. This created an estimated SPIn ratio in 2019 for St Johns: Lakeside of 1:1.18. 278

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3.1.2. Meteorology

280 The temperatures ranged between $5 - 30^{\circ}$ C vs. $4 - 30^{\circ}$ C in St Johns and Lakeside respectively, and $3 - 33^{\circ}$ C vs. $1 - 33^{\circ}$ C for the same in 2019 (Figure 4), with strong positive correlations 281 between the locations and years, with rho coefficients of 0.992 (p < 0.001) in 2018 and 0.993 282 283 (p < 0.001) in 2019 (Table 2). The Wilcoxon Signed-Rank test indicated that the mean temperature was higher for St Johns than Lakeside during both years (16.0°C vs. 15.5°C in 284 2018 (p < 0.001) and 17.5°C vs. 17.0°C in 2019 (p < 0.001) respectively). St Johns received 285 more rain than Lakeside during both years (185.4 - 291 mm vs. 160.2 - 282.2 mm), 286 respectively (Figure 5), with strong positive correlations between the locations and years, with 287 288 rho coefficients of 0.747 (p < 0.001) in 2018 and 0.758 (p < 0.001) in 2019 (**Table 2**). However, there was no significant difference in total amount of precipitation for either 2018 or 2019 289 according to the Wilcoxon Signed-Rank test. 290

3.2. Potential Source Areas

292 The grass maps identified possible grass pollen source areas within 50 km belonging to seven counties in the Midlands area of the United Kingdom: Worcestershire, Gloucestershire, 293 Herefordshire, Shropshire, Staffordshire, West Midlands, and Warwickshire (Figure 6, with 294 the counties being indicated in **Figure 1**). The grass maps successfully highlighted the absolute 295 lower abundance of pollen contributing areas within the major urban areas with the Midlands 296 297 area, where otherwise natural environments have been replaced with urban sprawl. This was 298 especially pronounced in the North-East, which contains the second biggest settlement in the UK, the Birmingham conurbation with connected urban areas. Additionally, the grass map 299 300 highlighted areas where the potential source areas of grass pollen were more highly 301 concentrated than the general matrix. These areas included northern Gloucestershire, central Shropshire, eastern Herefordshire, and north-western Warwickshire. There were also many 302 303 smaller local areas with high concentrations of potential source areas. However, these can be difficult to pinpoint due to being surrounded by areas with fewer sources, potentially obscuring 304 305 their regional importance.

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3.3. Generalized Linear Mixed-Modelling

The model statistics showed that there were varied effects on the bi-hourly grass pollen 307 concentrations from the model variables for the two locations (Tables 3a - 3b). For the 308 meteorological variables, temperature had a strong positive highly significant effect for both 309 locations (p < 0.001) while precipitation had a weak positive non-significant effect for St Johns 310 311 and a weak negative significant effect (p < 0.05) for Lakeside. The effect of the grass source areas differed between St Johns and Lakeside. For St Johns the unique micro-scale (0 - 2 km)312 and the unique small meso-gamma (2 - 10 km) grass maps had weak and strong positive 313 314 significant effects respectively (p < 0.05). The large meso-gamma (10 - 20 km) grass map had 315 a negative non-significant effect while the first meso-beta (20 - 30 km) grass map had a very strong positive highly significant effect (p < 0.001). The second meso-beta (30 - 40 km) had a 316 strong negative very significant effect (p < 0.001) while the third meso-beta (40 - 50 km) had 317 318 a weak positive linear trend (p = 0.087). While for Lakeside the unique micro-scale (0 - 2 km) grass map had a weak negative non-significant effect while the unique small meso-gamma (2 319 -10 km) grass map had a strong positive significant effect (p < 0.05). The large meso-gamma 320 (10 - 20 km) grass map had a weak negative non-significant effect while the first meso-beta 321 (20 - 30 km) had a weak positive linear trend (p = 0.058). The second and third meso-gamma 322 (30 - 40 and 40 - 50 km) had weak negative and weak positive non-significant effects, 323 respectively. The full model had R² values of 49.9% for St Johns and 50.3% for Lakeside. In 324 both locations DOY accounted for a larger proportion of the random variation than the time of 325 326 day, and the same way in regard to the time of day than year (Supplementary Figures 7 - 9). The diurnal grass pollen concentration was found to be the highest around 6 PM 327 (Supplementary Figure 8). The models predicted between 74 - 79% of the bi-hourly grass 328 pollen concentrations to within 30 grains/ m^3 (includes the 'within 10 grains/ m^3 '-category) 329 with regard to the observed values (Figures 7 - 8). Only between 7 - 10% of the predicted 330 values had a difference of above 90 grains/m³. 331

332 **4. Discussion**

Our aim was to investigate the source area contribution to grass pollen concentrations. We found that source areas further than 30 km away did not have a meaningful impact on the pollen concentrations. We also found that the source area contribution is strongly dependent on the surroundings of the pollen sampler. The generalized linear mixed-modelling approach utilizing high resolution source areas, meteorological and temporal variables could predict bi-hourly grass pollen concentrations with high accuracy.

4.1. Spatiotemporal Grass Pollen Variation

340 Our results showed that there were differences in the temporal variation between the two nearby pollen stations and we reject the hypothesis that the area influencing nearby stations is 341 of equal size. The full seasonal grass pollen data correlations were between 0.683 - 0.781, 342 suggesting differences between seasons and locations within a six km range, most likely driven 343 by variations in source distribution as the weather variables between the two stations are highly 344 correlated. There is less overall variation in the seasonal (moving average) and trends (daily 345 mean) component correlations than within the full data between the locations, with the seasonal 346 and trend component correlations between 0.773 - 0.843 and 0.841 - 0.936 respectively. 347 348 However, the remaining variation, that can be likened to residuals, has correlations between 349 0.492 - 0.514, suggesting that there is large unknown variation between the locations that is not included in the seasonal or trend components that nonetheless vary between years. This 350 351 suggests that the differences are caused by temporal factors that vary within and between years. In addition, the SPIn is larger for St Johns than for Lakeside. The SPIn was between 4905 – 352 5941 (mean 5423) for St Johns compared with 4164 - 4423 (mean 4294) for Lakeside, 353 suggesting a large difference in SPIn between the locations. Grass pollen SPIn has previously 354 been shown to vary substantially between the years 2008 - 2016 in Mexico (1267 – 4423 (mean 355 356 2921)) (Calderon-Ezquerro et al., 2018) and the years 1989 – 2018 in France (~3600 - ~6000) (Besancenot et al., 2019). The SPIn ratio between St Johns:Lakeside varied between 1:1.18 and 357 1:1.34, suggesting that the ratio is kept low between closely situated locations. This has also 358 359 been shown in Northern Spain, where grass pollen SPIn ratios have been observed to vary between 1:0.53 and 1:2.06 (Majeed et al., 2018). However, the SPIn can only provide 360 361 information regarding the seasons strength (Lo et al., 2019), while meteorology, atmospheric transportation and source areas likely being able to provide more detailed information 362 363 regarding spatial and temporal variation.

4.2. Representation of Source Areas

365 The grass maps refined from the 2017 Crop Map illustrate the heterogeneity of the potential grass pollen source areas (100 x 100 m resolution) within the larger West Midlands landscape. 366 Previously, the crop map has been utilized to identify local sources of Alternaria spores within 367 West and East Midlands landscapes in the UK (Apangu et al., 2020). In addition, a previous 368 version of the map has produced reliable general grass maps within the larger UK using a lower 369 370 1 x 1 km resolution (McInnes et al., 2017). This is the standard resolution for grass vegetation source maps (Khwarahm et al., 2017; Zerboni et al., 1991), although higher resolutions are also 371 sometimes used (e.g. 0.5 x 0.5 km (Devadas et al., 2018)). One potential improvement of the 372 373 maps would have been to include road-verges, home gardens and other grass containing 374 features, since these are known to contain grassland vegetation capable of contributing grass pollen (Jantunen et al., 2007, 2006). These sources have previously been suggested by study 375 376 from Denmark to be important contributors to localized grass pollen concentrations by using very high resolution grass pollen source areas (0.6 x 0.6 m) (Skjøth et al., 2013). The increased 377 resolution (from 1 x 1 km to 100 x 100 m) of source areas is likely to provide more information 378 and understanding of the source dynamics, and will likely be sufficient until the contribution 379 of local and small-scale source areas (verges etc.) have been thoroughly quantified and made 380 381 available to the public.

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4.3. Temporal Random Effects

The modelled temporal variables highlighted that the variation in the daily progression of the season (DOY) is larger than the diurnal or annual variation. Previous studies have showed that the temporal variation is a major component of the variation seen during the grass pollen season (García-Mozo, 2017; Núñez et al., 2016). Generally, grass pollen concentrations are low during the start of the season, then slowly increase, suddenly peak and then slowly decrease again to 388 background levels (Galán et al., 1995; Norris-Hill, 1995; Plaza et al., 2016; Sabariego et al., 2011). The DOY variation confirms this general pattern, which is collaborated by regional 389 grass pollen calendars (Adams-Groom et al., 2020). We found grass pollen concentrations to 390 391 be low during the night and early mornings, increasing during mid-day and peaking around 6 PM. This is similar to previous studies from Denmark (Peel et al., 2014), Germany (Simoleit 392 et al., 2016) and the UK (Hyde and Williams, 1945; Norris-Hill, 1999). Contrasting studies 393 394 from Poland and Spain have found that grass pollen concentrations are normally high during mid-mornings around 9 - 11 AM, while reducing during the evenings and early mornings 395 396 (Cariñanos et al., 1999; Kasprzyk, 2006; Latałowa et al., 2005). Possibilities for this discrepancy could be the varying climatological and biogeographical factors between North-397 Western Europe and Eastern Europe and Mediterranean locations. This results in different grass 398 399 species diversity between locations, which are known to have different pollen release dynamics 400 (Beddows, 1931; Jones and Newell, 1946). We have observed small differences between the grass pollen mean of the two years, but it is uncertain if this is the true trend since the study 401 402 encompasses only two years of data. Previous studies have suggested that grass pollen concentrations usually fluctuate between years, and that the differences will also depend on 403 sampling durations and locations (Emberlin et al., 1999; Ghitarrini et al., 2017; Karatzas et al., 404 2019; Sabariego et al., 2011; Smith et al., 2014). 405

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4.4. Transportation Distance and Meteorology

The modelling results indicate that the distance to the source areas is of major importance. Grass pollen source areas within 20 - 30 km were most important for St Johns while source areas within 2 - 10 km were most important for Lakeside. Source areas within 10 - 20 km were found to not be important factors for either location. We did not find any evidence of a considerable contribution from grass pollen sources more than 30 km away (within the larger 50 km area). The model found temperature to be an important predictor variable for both 413 locations, while precipitation was only important for Lakeside. Source areas within the first meso-gamma distance (20 - 30 km) had a positive influence on grass pollen levels for St Johns, 414 while areas within the second meso-gamma distance (30 - 40 km) had a negative association. 415 416 This suggests that grass pollen can frequently be transported 20 - 30 km but that areas further away do not contribute grass pollen as the pollen settles sometime before then. Source areas 417 within 30 km were found in general to be important for the grass pollen concentrations for St 418 419 Johns. This is in contrast to Lakeside, where more local source areas (2 - 10 km) were found to be important. Our results are in agreement with previous dispersal studies, suggesting that 420 421 local vegetation is likely responsible for the bioaerosol patterns observed (Apangu et al., 2020; Avolio et al., 2008; Katelaris et al., 2004; Oteros et al., 2015; Pashley et al., 2009; Skjøth et al., 422 2009), although this is not always true (de Weger et al., 2016; Izquierdo et al., 2011; Skjøth et 423 424 al., 2007). However, the concept of 'local sources' is debatable since micro-scale grass pollen 425 source areas (< 2 km) did not have an effect for Lakeside, even if this was the case for St Johns. A study from Spain suggested that this is mostly due to the absence of local source areas close 426 427 to sampling stations (which are normally situated within urban centres) (Oteros et al., 2015). Previous studies from Poland and Germany have suggested that this is due to urban-rural 428 429 gradients in pollen concentrations caused by differential source area allocation (Rodríguez-Rajo et al., 2010; Werchan et al., 2017). Temporal variation is likely to cause part of the 430 431 discrepancy seen for Lakeside. If a few grass species which flower concurrently dominate the 432 micro-scale source areas surrounding Lakeside then the contribution of these grasses will be intensive but short. This phenomenon was not observed, suggesting that mowing of nearby 433 grass areas has been efficient in preventing grasses from flowering. This is supported by 434 435 previous studies which have investigated the connection between grass flowering phenology and grass pollen concentrations (Cebrino et al., 2016; Rojo et al., 2017). This can cause a 436 437 dampening effect on the signal from local vegetation for the season as a whole. However,

vegetation over a regional area is likely to contain a wide range of grass species dispersing
pollen at various intervals, due to different flowering times (Cebrino et al., 2018), localized
micro-climatic factors (Jackson, 1966) and varying management regimes (Theuerkauf et al.,
2015). This efficient mowing could explain the effect of meso-gamma grass source areas (2 –
10 km) and the absence of effects from micro-scale source areas for Lakeside.

443 Another possible explanation for the absence of grass pollen contributions from local source areas (10 - 20 km for both locations) could be attributed to the specific wind-movement 444 patterns present during the investigated years (Smith et al., 2005). Unequal contribution from 445 446 local pollen source areas due to variations in atmospheric transport and wind factors have previously been shown to be major factors for the difference in atmospheric pollen 447 concentrations at closely located pollen sampling stations (Alan et al., 2018; Bilińska et al., 448 449 2019; Maya-Manzano et al., 2017; Maya Manzano et al., 2017; Van De Water and Levetin, 2001). Therefore, the distribution of source areas is only relevant if there is active pollen 450 transport from these sources (Šikoparija et al., 2018) and to what degree the transport is relevant 451 (Adams-Groom et al., 2017). This is possible for Lakeside, since many areas in the near vicinity 452 are either grazed, cut or cultivated, and thus contribute less pollen than would be expected. 453 454 Rojo et al. (2015) suggests that managed areas are a key aspect in reducing source contribution 455 for many pollen types. We found that mowing of nearby grass areas has prevented them from 456 having a major impact on grass pollen concentrations at Lakeside. Furthermore, our study does 457 not include minor grass areas (e.g. roadsides, as specified earlier), which in some cases can cause substantial variations on short geographical scales (Hugg et al., 2017; Skjøth et al., 2013), 458 with one study from Finland showing that land use regression modelling utilizing detailed land 459 460 cover could explain up to 79% of the observed differences in the urban zone (Hjort et al., 2016). 461 Additionally, one previous study has suggested that the quality and resolution of the input data will be important in the atmospheric modelling of the pollen source contribution (Hernández-462

463 Ceballos et al., 2014). In this study the authors concluded that going from HYSPLIT default 464 input to WRF (Weather Research and Forecasting model) generated input while additionally 465 increasing the resolution improved the calculations of the trajectories, which was used to gain 466 increased understanding of the landscape relief. Overall our finding, when positioned against 467 previous ones, suggests that variations in grass pollen concentrations can be both a microscale 468 phenomenon, happening at scales below 2 km, or a meso-scale phenomenon at 30 km or below, 469 depending on the distribution of flowering grass areas.

Temperature and precipitation have been shown by many studies to have strong effects on grass 470 471 pollen concentrations (García-Mozo et al., 2010, 2009; García de León et al., 2015; Khwarahm et al., 2014; Makra et al., 2012; Recio et al., 2010; Sánchez-Mesa et al., 2002). The temperature 472 differential (0.5°C) identified between St Johns and Lakeside is likely caused, at least partly, 473 474 by the Urban Heat Island (UHI)-effect (Kim, 1992). The effect has been shown to have a measurable effect on grass pollen levels in the atmosphere (Ríos et al., 2016). A possible reason 475 for the strong significant effect of temperature on grass pollen levels is not only due to the 476 direct (although complicated) effect (Jung et al., 2021; Myszkowska, 2014; Norris-Hill, 1997), 477 but also from the indirect effect mediated through plant growth, plant maturity, anthesis and 478 479 anther dehiscence (Charles-Edwards et al., 1971; Liem and Groot, 1973). All of the factors 480 mentioned have their own complex relationships to grass pollen release and atmospheric 481 concentrations (Viner et al., 2010). The weak effect of precipitation on grass pollen levels is 482 possibly obscured by the presence of low concentrations of grass pollen during low and high rainfall episodes, making it difficult to discern if the pollen levels were low to begin with, or 483 were lowered due to the precipitation. This might lessen the general effect of precipitation in 484 485 the model estimate. Earlier studies have highlighted that the relationship between pollen 486 concentrations and precipitation is complicated, and rely on factors other than just the presence/absence and abundance of precipitation, such as wind conditions and rain intensities 487

(Kluska et al., 2020; Norris-Hill and Emberlin, 1993; Pérez et al., 2009). Generally lower
intensities of precipitation (< 5 mm/h) have been found to be unlikely to reduce the pollen
levels to any large extent (Kluska et al., 2020). Therefore, the lower abundance of precipitation
during generally higher grass pollen levels is interpreted by the model as not having a large
effect, due to the rain not being intensive enough to reduce the pollen levels.

493 **5.** Conclusion

494 We observed pronounced differences in the bi-hourly grass pollen concentrations between two closely located sampling stations for the two years. These differences were mainly attributed 495 to the higher SPIn for the suburban location of St Johns. The use of a high-resolution grass map 496 497 enabled us to distinguish likely vegetation source areas within the larger West Midlands regional area that contributed atmospheric grass pollen to our two locations. The combined use 498 of atmospheric modelling and grass maps in a generalized linear modelling setting showed that 499 grass pollen source areas beyond 30 km are unlikely to contribute any measurable amount of 500 grass pollen to the seasonal load in each location and that the main sources may be found closer 501 502 to the station, depending on its surroundings. It is likely that an urban-rural gradient exists 503 within these 30 km, that affects the distribution of source areas and atmospheric transportation probabilities of grass pollen. This probably caused an uneven contribution of grass pollen from 504 505 source areas to the two locations based on their immediate surroundings. Temperature had a positive, highly significant effect on grass pollen levels, probably caused by both direct and 506 indirect effects. The overall effect of precipitation was uncertain, possibly due to confounding 507 factors such as precipitation intensity and wind conditions. Generalized modelling approaches 508 509 using atmospheric trajectory modelling and detailed grass vegetation source maps are highly 510 accurate tools in predicting the finer details and differences in high-resolution grass pollen 511 levels between closely located samplers in a heterogeneous landscape environment.

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Table 1

Spearman ρ (r_s) correlations for the STL Decompositions for the 95% seasonal Bi-hourly grass pollen concentration overlap between St Johns and Lakeside for the years 2018-2019.

STL Decompositions	Spearm	an ρ (r₅)	P-Value		
	2018	2019	2018	2019	
Full Pollen Series	0.683	0.781	< 0.001	< 0.001	
Seasonal	0.843	0.773	< 0.001	< 0.001	
Trend	0.841	0.936	< 0.001	< 0.001	
Remainder	0.492	0.514	< 0.001	< 0.001	

Table 2

Model statistics and significance levels for the comparison of bi-hourly temperature and precipitation for the locations St Johns and Lakeside for the combined years of 2018 and 2019.

Variable	Year	Model Statistics							
		Spearman Rank Correlation				Wilcoxon Signed-Rank Test			
		S	rho	P - value	Significance	V	P - value	Significance	
Temperature	2018	4105414	0.992	< 0.001	***	1000781	< 0.001	***	
	2019	3955388	0.993	< 0.001	* * *	1020509	< 0.001	***	
Precipitation	2018	135514067	0.747	< 0.001	***	3728	0.100	NS	
	2019	129536855	0.758	< 0.001	***	12566	0.117	NS	

Significance: P < 0.001 - ' *** ', P < 0.01 - ' ** ', P < 0.05 - ' * ', P < 0.1 - ' · ', P > 0.1 - ' NS '.

Table 3a

Model statistics and significance levels for the Generalized Linear Mixed-Model (GLMER) in regards to the Bi-hourly Grass Pollen concentrations for the location St Johns for the combined years of 2018 and 2019. GM1 and GM3 are SJ specific. **Residual Degrees of Freedom:** 2939. $\mathbf{R}^2 = 49.9\%$.

Variables	Effect	Model Statistics						
				Std.	Std.			
	Туре	Estimate	Variance	Error	Dev.	t - value	P - value	Significance
Intercept	Fixed	-2.251	N/A	0.581	N/A	-3.874	< 0.001	***
Temperature	Fixed	0.133	N/A	0.009	N/A	14.268	< 0.001	* * *
Precipitation	Fixed	0.021	N/A	0.020	N/A	1.054	0.292	NS
GM1 [0 - 2 km]	Fixed	0.034	N/A	0.015	N/A	2.227	0.026	*
GM3 [2 - 10 km]	Fixed	0.145	N/A	0.065	N/A	2.225	0.026	*
GM5 [10 - 20 km]	Fixed	-0.048	N/A	0.051	N/A	-0.946	0.344	NS
GM6 [20 - 30 km]	Fixed	0.331	N/A	0.038	N/A	8.783	< 0.001	***
GM7 [30 - 40 km]	Fixed	-0.158	N/A	0.028	N/A	-5.722	< 0.001	***
GM8 [40 - 50 km]	Fixed	0.031	N/A	0.018	N/A	1.709	0.087	•
DOY [121 - 244]	Random	N/A	2.484	N/A	1.576	N/A	N/A	N/A
Time [00, 02, etc]	Random	N/A	0.173	N/A	0.415	N/A	N/A	N/A
Year [-18, -19]	Random	N/A	0.000	N/A	0.000	N/A	N/A	N/A
Residuals	Random	N/A	1.512	N/A	1.230	N/A	N/A	N/A

Abbreviations: GM - Grass Map,	o, SJ - St Johns,	DOY - Day of Year, N	A - Not Applicable.
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Significance: P < 0.001 - ' *** ', P < 0.01 - ' ** ', P < 0.05 - ' * ', P < 0.1 - ' · ', P > 0.1 - ' NS '.

Table 3b

Model statistics and significance levels for the Generalized Linear Mixed-Model (GLMER) in regards to the Bi-hourly Grass Pollen concentrations for the location Lakeside for the combined years of 2018 and 2019. GM2 and GM4 are LS specific. **Residual Degrees of Freedom:** 2939. $R^2 = 50.3\%$.

Variables	Effect	Model Statistics						
				Std.	Std.			
	Туре	Estimate	Variance	Error	Dev.	t - value	P - value	Significance
Intercept	Fixed	-0.554	N/A	0.627	N/A	-0.884	0.377	NS
Temperature	Fixed	0.117	N/A	0.010	N/A	12.003	< 0.001	***
Precipitation	Fixed	-0.062	N/A	0.024	N/A	-2.552	0.011	*
GM2 [0 - 2 km]	Fixed	-0.054	N/A	0.063	N/A	-0.856	0.392	NS
GM4 [2 - 10 km]	Fixed	0.118	N/A	0.047	N/A	2.521	0.012	*
GM5 [10 - 20 km]	Fixed	-0.009	N/A	0.054	N/A	-0.159	0.874	NS
GM6 [20 - 30 km]	Fixed	0.081	N/A	0.043	N/A	1.898	0.058	•
GM7 [30 - 40 km]	Fixed	-0.034	N/A	0.024	N/A	-1.439	0.150	NS
GM8 [40 - 50 km]	Fixed	0.010	N/A	0.018	N/A	0.538	0.591	NS
DOY [121 - 244]	Random	N/A	2.870	N/A	1.694	N/A	N/A	N/A
Time [00, 02, etc]	Random	N/A	0.189	N/A	0.434	N/A	N/A	N/A
Year [-18, -19]	Random	N/A	0.008	N/A	0.087	N/A	N/A	N/A
Residuals	Random	N/A	1.581	N/A	1.258	N/A	N/A	N/A

Abbreviations: GM - Grass Map, LS - Lakeside, DOY - Day of Year, N/A - Not Applicable.

Significance: P < 0.001 - ' *** ', P < 0.01 - ' ** ', P < 0.05 - ' * ', P < 0.1 - ' · ', P > 0.1 - ' NS '.



Figure 1. Overview of the circular and concentric circular grass maps within the surrounding counties created for the grass pollen investigation of the locations St Johns and Lakeside. The overlapping counties can be found in the United Kingdom overview map.





Figure 2. Bi-hourly concentrations of grass pollen from two locations (St Johns and Lakeside) during the 2018 season in Worcester. Note that the top peak of the season (3359 grains/m³) for Lakeside exceeds the y-axis maximum.



Figure 3. Bi-hourly concentrations of grass pollen from two locations (St Johns and Lakeside) during the 2019 season in Worcester.



Figure 4. Comparison of bi-hourly measurements of temperature from two locations (St Johns and Lakeside) for the years 2018 and 2019.



Figure 5. Comparison of bi-hourly measurements of precipitation from two locations (St Johns and Lakeside) for the years 2018 and 2019.



Figure 6. The circular and concentric circular grass maps within the surrounding counties created for the grass pollen investigation of the locations St Johns and Lakeside. The gridcell resolution is 100 x 100 m. The legend specifies how much of each gridcell is covered by grass areas.



Figure 7. Observed vs Predicted Bi-hourly grass pollen concentrations for St Johns. Modelled using the Generalized Linear Mixed-Model with variables explored in Table 3a.



Observed vs Predicted Bi-hourly Grass Pollen Concentrations for Lakeside

Figure 8. Observed vs Predicted Bi-hourly grass pollen concentrations for Lakeside. Modelled using the Generalized Linear Mixed-Model with variables explored in Table 3b.

Supplementary Material

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