

Highlights

- The locations of potential pathogen host areas were identified for each study site
- A new Pathogen Infection Level Index is developed to assess relative pathogen abundance
- Local thermal variables were most important to predict airborne *Ganoderma* spore concentrations
- Whilst local sources of *Ganoderma* spores are a major source, long-distance transport is also a factor at some sites
- UK woodlands were more severely infected with *Ganoderma* species than the other sites investigated

Abundance of *Ganoderma* sp. in Europe and SW Asia: modelling the pathogen infection
 levels in local trees using the proxy of airborne fungal spore concentrations

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41 Abstract

42 Ganoderma comprises a common bracket fungal genus that causes basal stem rot in deciduous and coniferous trees and palms, thus having a large economic impact on forestry 43 production. We estimated pathogen abundance using long-term, daily spore concentration 44 data collected in five biogeographic regions in Europe and SW Asia. We hypothesised that 45 pathogen abundance in the air depends on the density of potential hosts (trees) in the 46 surrounding area, and that its spores originate locally. We tested this hypothesis by (1) 47 calculating tree cover density, (2) assessing the impact of local meteorological variables on 48 spore concentration, (3) computing back trajectories, (4) developing random forest models 49 50 predicting daily spore concentration. The area covered by trees was calculated based on Tree Density Datasets within a 30 km radius from sampling sites. Variations in daily and seasonal 51 spore concentrations were cross-examined between sites using a selection of statistical tools 52 53 including HYSPLIT and random forest models.

Our results showed that spore concentrations were higher in Northern and Central 54 Europe than in South Europe and SW Asia. High and unusually high spore concentrations (> 55 90th and > 98th percentile, respectively) were partially associated with long distance 56 transported spores: at least 33% of Ganoderma spores recorded in Madeira during days with 57 58 high concentrations originated from the Iberian Peninsula located > 900 km away. Random forest models developed on local meteorological data performed better in sites where the 59 contribution of long distance transported spores was lower. We found that high concentrations 60 were recorded in sites with low host density (Leicester, Worcester), and low concentrations in 61 Kastamonu with high host density. This suggests that south European and SW Asian forests 62 may be less severely affected by Ganoderma. This study highlights the effectiveness of 63 monitoring airborne Ganoderma spore concentrations as a tool for assessing local Ganoderma 64 pathogen infection levels. 65

67 Keywords

- 68 Aerobiology; backward trajectories; fungal spores; long-distance transport; phytopathogen
- 69 monitoring; random forest model

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72 **1. Introduction**

73 Ganoderma species are wood-decaying basidiomycete fungi with a cosmopolitan distribution, and they comprise common pathogens on deciduous and coniferous trees, as well 74 as palms with stems without secondary growth. The fungi reproduce via airborne spores and 75 grow in the non-living tissues. They usually attack dead, weakened or damaged trees, 76 however, recent surveys of basidiomycete endophytes revealed that wood-decaying fungi, 77 78 including Ganoderma species, can be found in wood of living trees too (Song et al. 2017). Pathogenicity studies revealed that the investigated Ganoderma species are capable of 79 infecting healthy sapwood following trunk wounding, however, they are not usually 80 81 pathogens in young, actively-growing trees that only possess sapwood (Loyd et al. 2018).

Ganoderma sp. enzymes allow them to break down wood components such as lignin 82 and cellulose (Schwarze et al. 2000). Delignification and defibration caused by undisturbed 83 84 rotting extend throughout the interior of the trunk making the tree susceptible to wind damage (Blanchette et al. 1985; Dill and Kraepelin 1986). Based on the type of decay caused by 85 Ganoderma species, they are classified as white rot fungi (the infected wood becomes wet, 86 spongy or stringy and the colour changes to white or yellow). Several approaches are needed 87 to control white rot infection. Most have been trialed in oil palm plantations due to serious 88 economic losses (Flood et al. 2005; Paterson 2007). Some control methods can be 89 extrapolated to the deciduous and coniferous commercial forests, e.g. infection sources should 90 be reduced at the time of clearing old stands by removing infected debris and Ganoderma sp. 91 fruiting bodies (Panchal and Bridge 2005), and all infected plant material should be treated 92 with a specific type of biofungicide (Soepena et al. 2000). An important problem related to 93 white rot control, however, is the lack of sufficient information on variation in *Ganoderma* 94 species associated with disease and their mode of reproduction. 95

Ganoderma is taxonomically considered as the most difficult genus among all those in 96 the Polyporales order and is in a state of taxonomical chaos (Ryvarden 1985; 1991). 97 Taxonomists have described 326 legitimate Ganoderma species and lower taxa (Robert et al. 98 2013). Among these only 7 species are accepted in the European polypore monographs: G. 99 adspersum, G. applanatum, G. carnosum, G. cupreolaccatum (syn. G. pfeifferi), G. lucidum, 100 G. resinaceum and G. valesiacum (e.g. Pegler and Young 1973; Ryvarden and Gilbertson 101 102 1993; Sokół 2000; Wojewoda 2003). G. adspersum occurs on Alnus sp., Fraxinus sp., Carpinus sp., Morus sp., Quercus sp., Juglans sp., Ulmus sp. and very rarely on conifers; G. 103 applanatum occurs mainly on deciduous trees (Alnus sp., Betula sp., Carpinus sp., Fagus sp., 104 105 Quercus sp., Salix sp., Populus sp.), less frequently on coniferous (Abies sp., Picea sp., very rare on Pinus sp.); G. carnosum infects mainly conifers (Abies sp., Taxus sp.) but rarely 106 occurs on deciduous tree species; G. cupreolaccatum prefers to live on Fagus sp. and rarely 107 on a variety of other deciduous trees (Aesculus sp., Acer sp., Fraxinus sp., Prunus sp., 108 Quercus sp.); G. lucidum grows on Quercus sp., Carpinus sp., Salix sp., Corylus sp., Acer sp. 109 and very rarely on conifers; G. resinaceum infects only deciduous trees (Quercus sp., Salix 110 sp.); G. valesiacum is a Central European species occurring predominantly in montane to 111 subalpine regions, in the natural stands of Larix sp. (Ryvarden and Gilbertson 1993; Sokół 112 113 2000; Szczepkowski and Pietka 2003; Papp and Szabó 2013; Lindequist et al. 2015).

Ganoderma spores can be an important component of atmospheric bioaerosols. A single *G. applanatum* basidiocarp can produce 30 billion spores per day, over a period of six months (Meredith 1973; Levetin 1990). Airborne fungal spores are able to travel long distances via air mass transport (Edman et al. 2004; Sesartic and Dallafior 2011), so studying *Ganoderma* spore concentrations in the air may be relevant from the forestry and the economic perspective on a large landscape scale. In England, the Forest Commission

highlighted *Ganoderma* genus as an important pathogen (McKay 2011). Similar negative
impacts on forestry can be expected in other regions, but this has so far not been quantified.

A limited number of aerobiological studies have focused on characterizing daily and seasonal patterns of *Ganoderma* spore occurrence, and relationships between spore concentration and meteorological parameters, often in the context of the allergenic properties of *Ganoderma* spp. (Tarlo et al. 1979; Levetin 1991; Craig and Levetin 2000; Hasnain et al. 2004; Kadowaki et al. 2010; Grinn-Gofroń and Strzelczak 2011; Kasprzyk et al. 2011; Grinn-Gofroń et al. 2015; Jędryczka et al. 2015; Sadyś et al. 2016).

Until now, the only research on the distribution of *Ganoderma* spores among different landscapes was conducted in the UK (Sadyś et al. 2014). Back-trajectories from that study showed export of these spores from forests to agricultural and urban areas, and the results suggested the main sources of this pathogen were located within a 200 km range from the trap site (Worcester, UK). No evidence of long-distance spore transport from the main continent was found.

In the present study, we hypothesize that Ganoderma spores originate from local 134 sources, and therefore that spore concentrations can be predicted using local meteorological 135 data. Moreover, if local fungal sources are crucial for spore concentration, the relative 136 137 pathogen infection level can be determined by combining airborne spore concentrations and the area covered by potential tree hosts. To test these hypotheses we used aerobiological 138 records from seven sites, representing five biogeographical regions and four climate types, 139 and aimed to (1) estimate the host (tree) density at different distances from each trapping site; 140 141 (2) determine the spore season parameters; (3) assess the relationships between primary meteorological variables and airborne spore concentrations (4) calculate the backward 142 trajectories indicating pathways of possible Ganoderma spores transport to sites; and, (5) 143 compute machine learning models for *Ganoderma* spore concentration in the air. 144

145 **2. Material and methods**

146 2.1. Spore identification, sampling and a comparison between receptor sites

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 $Ganoderma \text{ spp. (hereafter Ganoderma) spores have either an egg or ovoid shape and they are approx. 4.5-8 × 8-13 µm in size (Pegler and Young, 1973; Fig. 1). Ganoderma can be distinguished from other bracket fungi because it possesses a double-walled basidiospore. The external spore wall is transparent and smooth, while the internal wall varies from dark brown to golden in colour. The wall layers are connected by pillars, which under the microscope may resemble dots. Another distinctive feature is a flattened basal apiculus, the projection on the spore from where it was attached to the fungus (Southworth 1974).$

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Figure 1. *Ganoderma* spores under ×1000 (*left*) and ×400 magnification (*right*) (phot. A.
Grinn-Gofroń).

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Fungal spore concentrations were sampled as part of long-term air quality monitoring schemes at seven sites in five countries across Europe and SW Asia, located in five different biogeographical regions (Fig. 2, Table 1). A 7-day volumetric spore sampler of the Hirst design (Hirst 1952) was used at each site. Air monitoring and analysis were performed according to the methods described by the Spanish Aerobiology Network (Galán et al. 2007) and the Minimum Recommendations proposed by the European Aerobiology Society Working Group on Quality Control (Galán et al. 2014).

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167 Figure 2. Location of *Ganoderma* spore monitoring sites.

To compare the Ganoderma spore data from the study sites located in different climate 169 and biogeographical zones, traditional spore season parameters were calculated, *i.e.*, start, 170 peak and end of season dates and the seasonal spore integral for each season and study site. 171 To determine the timing of the spore season, the 90% method was used, which begins when 172 5% of the total spore sum was reached, and ends when the cumulative count reaches 95% of 173 the total for that year (Nilsson and Persson 1981). R software, version 3.6.3. (R Core Team 174 2020) and AeRobiology R package (Rojo et al. 2019) were used to calculate the spore season 175 dynamics. 176

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178 2.2 Determination of potential inoculum sources

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As Ganoderma are phytopathogens particularly of trees, the tree density was assessed 180 181 quantitatively in a radius of 30 km from the spore trap. This distance was considered because most of the spores recorded by the Hirst-type trap originate from sources located up to 30 km 182 away, as previously reported (e.g. Skjoth et al. 2016, Olsen et al. 2019). This has also been 183 documented for Ganoderma spores (O'Connor et al. 2014, Grinn-Gofroń et al. 2020). Since 184 not only forest trees are affected but also small tree patches, the Tree Cover Density dataset 185 186 (aggregated to 100 m resolution, Langanke et al. 2018) was used instead of the popular Land Cover Classification map to estimate the area covered by trees surrounding the study sites. 187 The Food and Agriculture Organization map from the Global Forest Resources Assessment 188 2010 (FAO 2013), with spatial resolution of ~250 m, was used for Vinnytsia because the Tree 189 Cover Density dataset did not cover this area. Both datasets show the tree cover density using 190 a 0-100% scale. 191

192 Tree cover was calculated using ArcMap 10.5 (ESRI). The datasets were initially 193 assigned to a class based on the following classes of tree cover: <10%, 10-20%, 20-30%, 3040%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, 90-100%. Then, the area of each class was calculated and weighted according to the tree cover fraction, *i.e.*, area covered by trees in 35% was multiplied by a factor f=0.35 which resulted in a reasonable estimate of the area covered. Subsequently, the area covered by trees was intersected with buffer and sectors (as per Grinn-Gofroń et al. 2020) surrounding the study sites (total 96 sectors for each site). Finally, the total estimated area covered by trees was aggregated to these sectors showing the distribution of tree cover around each site.

We developed a new method to calculate local *Ganoderma* infection levels, which we have called the Pathogen Infection Level Index (PILI). Assuming that spores originating from a 30-km radius of the sampling site dominate the recorded concentrations, this index will show the relative pathogen abundance calculated per 1 km² area covered by potential host plants. The equation to calculate PILI is:

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$$PILI = \frac{\sum_{i=1}^{SL} Spore_conc}{A_H}$$

where *Spore_conc* is the daily airborne *Ganoderma* spore concentration, *SL* is the spore season length and A_H is the area covered by potential host plants – in this study within 30 km.

211 2.3. Meteorological data

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213 Meteorological data were obtained from weather stations located in the vicinity of the 214 air samplers at each study site (Table 2).

The relationship between weather and airborne spore concentrations was assessed for selected meteorological parameters: (1) dew point temperature (*dew*, °C), (2) daily mean air temperature (*tavg*, °C), (3) daily maximum air temperature (*tmax*, °C), (4) daily minimum air

temperature (*tmin*, °C), (5) precipitation (*precip*, mm), (6) relative air humidity (*humidity*, %), 218 (7) air pressure (*pressure*, hPa), (8) daily mean wind speed (*wind speed avg*, m s⁻¹), (9) daily 219 maximum wind speed (wind speed max, m s⁻¹) and (10) wind direction (°). All meteorological 220 parameters were recorded in hourly (or every third hour, depending on the site) resolution and 221 daily mean values were used for the analysis. Hourly data for wind speed and direction were 222 used to analyse the impact of local wind conditions on Ganoderma spore concentration. The 223 data were obtained from the OGIMET database using *climate* R package (Czernecki et al. 224 2020). 225

Normality of distributions were tested with the Kolmogorov-Smirnov and Chi-square 226 tests, then Spearman's rank association was used to examine the effects of the selected 227 228 weather parameters on spore concentration. Kruskal-Wallis test with post-hoc pairwise Wilcoxon tests with Bonferroni correction were used to examine differences in daily 229 Ganoderma concentrations between sites. The relationship between spore concentration and 230 wind conditions was analysed in detail. By combining three variables, spore concentration, 231 wind speed and wind direction, it was possible to partially explain the potential location of the 232 local pathogen inoculum sources (Uria-Tellaetxe and Carslaw 2014). Spore concentration 233 [spore m^{-3}] was used in daily resolution but wind speed [m s⁻¹] and direction [°] were hourly 234 resolution to maximize the potential of wind data, as per Grinn-Gofroń et al. (2020). 235 Combining data in different time resolution was possible using R software 3.6.3. (R Core 236 Team 2020) and bivariate polar plots from the openair R package (Carslaw and Ropkins 237 2012). The colour key scale for spore concentration in the polar plots was different for 238 individual sites because of the variability in spore levels between sites. 239

To indicate transport pathways associated with high *Ganoderma* spore concentrations at study sites, the backward trajectories were computed by the Hybrid Single Particle Lagrangian Integrated Trajectory model (HYSPLIT) (Rolph et al. 2017; Stein et al. 2015). A

vertical velocity model (obtained from meteorological data) was used for trajectory 243 calculations. Global Data Assimilation System (GDAS) meteorological data of 1°×1° spatial 244 resolution were used as this dataset, being available for all the examined years. One trajectory 245 starting height of 500 m above ground surface was chosen because the influence of the 246 surface is minimized at this altitude. The trajectories were calculated every two hours up to 72 247 hours back in time for days with the highest *Ganoderma* spore concentration at particular 248 249 sites. Two threshold values were used to indicate days with high and extremely high spore concentration (90th and 98th percentile of daily spore concentration at particular sites, 250 respectively). Back-trajectories calculated for all days with spore concentration exceeding 251 252 thresholds were subjected to cluster analysis. As a result, four dominant directions associated with the elevated Ganoderma spore concentrations were determined. In the case of 98th 253 percentile counts recorded at Kastamonu, Vinnytsia and Funchal/Madeira, only two dominant 254 255 directions were indicated due to the low number of days with such high values. The method of Sirois and Bottenheim (1995) was used to obtain a site-specific, angle-based distance 256 matrix taking into account distances between each pair of trajectories, as also used in 257 Bogawski et al. (2019). This method is specifically designed to create trajectory direction-258 dependent clusters. R software 3.6.3. (R Core Team 2020) and openair R package (Carslaw et 259 260 al. 2012) were used to perform all calculations. The results from the cluster analysis were plotted on a map formatted using ArcMap (ESRI) software. 261

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263 2.4 Random forest models

264 2.4.1 Models development and validation

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A random forest model (Breiman 2001) was built to predict fungal spore concentration for each study site. Seven meteorological parameters (except for wind direction) from the same day as fungal spores values were used as independent variables. For each site, theaccuracy of the model was obtained using the following steps:

1. Data were split into a training set (75%) and a testing set (25%).

271 2. The training set was split using 10-fold cross-validation (Kuhn and Johnson 2013) into
272 ten groups of approx. equal size (folds).

3. Random forest model was built on nine folds and tested on the remaining fold. Each
time three metrics were calculated: RMSE (*root mean squared error*), and R² (*coefficient of determination*) and SMAPE (*symmetric mean absolute percentage error*). This was repeated
ten times.

4. The final model was applied to the testing set, and R², RMSE and SMAPE were
calculated.

The three accuracy metrics were selected to show different aspects of the models' 279 280 quality. RMSE is the square root of the average of squared errors, where the lower value indicates better model accuracy. This metric is on the same scale as the data being measured, 281 and therefore depends indirectly on the variability of the fungal spore concentrations at each 282 site. R^2 and SMAPE, on the other hand, can be used to compare models between sites. R^2 is 283 the coefficient of determination providing the goodness-of-fit between predicted and observed 284 values and ranges from 0 to 1, where larger values indicate better model accuracy. SMAPE 285 (Makridakis 1993) also measures the models' quality in relative terms. It focuses on the 286 differences between predicted and observed values. Lower values of SMAPE indicate better 287 model accuracy. Models were created and validated using random forest (Liaw and Wiener 288 2002), parsnip (Kuhn and Vaughan 2019), rsample (Kuhn et al. 2019) and yardstick (Kuhn 289 and Vaughan 2019) R packages. 290

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292 2.4.2. Models interpretation

294 The general effect of independent variables on the models was determined using 295 permutation testing (mean decrease in accuracy) (Breiman 2001; Liaw and Wiener 2002). For each model, the three most important variables were selected. The relationship between each 296 pair of the most important variables and the model predictions was presented using the 297 prediction interaction plot (Paluszynska et al. 2019). This type of plot randomly shuffles other 298 299 variables values (except the two selected), and subsequently creates a prediction. The plot shows both variables on an x- and y-axis, while the colour represents prediction value. It 300 facilitates the understanding of how different variables' values impact model predictions. 301

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- **3. Results** 303
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305 3.1. Spore seasons across Europe and SW Asia

306 The shortest spore season was recorded in Kastamonu (109 days) and Vinnytsia (125 days) whereas the longest (282 days) was in Funchal/Madeira. The Ganoderma spore season 307 started earliest in southern Europe and SW Asia, *i.e.*, in Funchal/Madeira (February), then in 308 Adana (March). The Ganoderma spore season started in May in Worcester, Leicester and 309 Vinnytsia, and started latest in in Szczecin and Kastamonu, in June. The difference in spore 310 311 season start dates between Funchal/Madeira and Kastamonu was 103 days (on average, 25 February in Funchal/Madeira and 7 June in Kastamonu). A 70-day difference was recorded 312 when comparing Funchal/Madeira and Kastamonu spore season end dates (on average, 2 313 December and 23 September, respectively) (Fig. 3, Table S1). 314



Figure 4. Comparison of daily mean *Ganoderma* spore concentrations at the study sites.
Boxes show first and third quartiles, bold line is median, black points are mean values and red
points are extreme values (above 90th percentile).

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Spore concentrations differ significantly between sites (p < 0.001) with the exception of 322 the three sites with the highest concentration (Leicester-Worcester: p ~1, Szczecin-Worcester: 323 p~1, Szczecin-Leicester: p=0.68). Overall, daily mean spore concentrations were the highest 324 in Szczecin, Worcester and Leicester (peak values: 522 spore m⁻³ in 2007, 376 spore m⁻³ in 325 2006 and 372 spore m⁻³ in 2008 for these three cities, respectively). Substantially lower peak 326 values were recorded in southern Europe and SW Asia (e.g. Adana: 21 spores m⁻³ in 2009, 327 Kastamonu: 23 spores m⁻³ in 2006, Funchal/Madeira: 35 spores m⁻³ in 2008). A similar 328 pattern was found in daily median spore concentration, *i.e.*, the highest values were seen in 329 Szczecin, Worcester and Leicester reaching 73, 62 and 52 spores m⁻³ of air, respectively 330 (Figure 4, Table S1). 331

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333 *3.2. Source of fungal spores*

The largest areas covered by trees within 30 km radius from the sampling locations was found at Kastamonu (1138.39 km²) and Szczecin (787.64 km²) whilst Funchal/Madeira, Worcester and Leicester had the lowest tree cover density. The trees irregularly surrounded the study sites; the largest area covered by trees was located 0-5 km (Leicester), 15-20 km (Funchal/Madeira and Adana), 20-25 km (Worcester, Vinnytsia and Szczecin) and 25-30 km (Kastamonu) from the sampling sites (Table 3, Fig. 5). Combining potential sources of fungi with recorded spore counts, we showed that the PILI value was highest at the UK sites, reaching 84.18 spore/km² in Leicester. Markedly lower values were observed in the remaining sites with the minimum of 1.61 and 0.64 spore/km² in Funchal/Madeira and Kastamonu, respectively (Table 4).

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Figure 5. Forest density in a 30 km radius of study sites.

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347 3.3. Relationship between spore occurrence, weather and potential sources of fungal spores

348 The only study site which did not have statistically significant relationships between weather variables and Ganoderma levels was Funchal/Madeira. In the remaining sites, all thermal 349 variables significantly correlated with spore concentrations, especially in the three most 350 northern sites, with the strongest relationship being observed at Worcester, r = 0.77, p < 0.001351 (Table 5). The correlation coefficient between thermal variables and spore concentration 352 353 increased along with increasing latitudes and, excluding Funchal/Madeira, with increasing longitudes too (Fig. S1). Although the relationship between 354 spore concentration and wind speed was relatively weak, a clear geographical trend towards 355 356 increasing correlation along increasing longitudes was nonetheless observed (after excluding Funchal/Madeira) (Fig. S2). 357

Despite the relatively low correlation between daily values of spore concentrations and 359 wind speed, wind conditions were important when data were clustered seasonally. The highest 360 concentrations were recorded in summer and autumn and were associated with high wind 361 speed, especially for Szczecin. In this site, winds with at least 8 m s⁻¹ and 6 m s⁻¹ caused 362 extremely high Ganoderma spore concentrations in summer and autumn, respectively (Fig. 363 6). Extremely high wind speed (>10 m s⁻¹ and even 60 m s⁻¹) in Funchal/Madeira were 364 associated with the highest daily Ganoderma spore concentrations locally. Also in Kastamonu 365 and Vinnytsia (only spring and summer), moderate wind speeds (> 4 m s⁻¹) were required to 366 reach the highest spore concentrations in particular seasons at these sites. In Worcester, 367 Leicester (spring to autumn), Vinnytsia (autumn) and Adana (all seasons), markedly lower 368 wind speed was enough to record season-specific high spore concentrations ($< 2 \text{ m s}^{-1}$) (Fig. 369 6). Season-specific high spore counts were recorded regardless of the wind direction (Adana, 370 Worcester, Vinnytsia in autumn), but for the remaining sites wind direction was important 371 (several dominant directions: Szczecin, SW, S, SE, N, NW; Kastamonu NW, N, NE, E; 372 Funchal/Madeira NW, N, NE; Leicester NE, E, SE; Vinnytsia in spring and summer W, NW, 373 N, NE). 374

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Figure 6. Daily *Ganoderma* spore concentrations (spores m^{-3}) in different wind conditions and seasons for all the sites (2006-2010). The radial scale shows wind speed (m s⁻¹), which increases radially outwards from the middle of the plot (scale in right upper corner). Main wind directions are displayed (N, E, S, W) and can be read in degrees (°), when N direction=0° and S direction=180°.

We clustered back trajectories associated with the highest (above 90th and 98th percentile) 382 daily Ganoderma spore concentrations to investigate possibilities of long-distance transport of 383 Ganoderma spores when the concentrations are high (Fig. 7). Two UK cities located close to 384 each other (ca. 100 km) showed a similar pattern of back-trajectories when comparing those 385 calculated for the 90th percentile threshold. However, 60% of back-trajectories associated with 386 extremely high (> 98th percentile) spore concentrations in Leicester originated from the 387 eastern direction compared to 38.8% in Worcester. The primary source of *Ganoderma* spores 388 in Szczecin was located to the East of the city (ca. 45% of trajectories), however, when 389 considering the extreme spore concentrations, the Western direction became equally 390 391 important. In Vinnytsia, it was clear that the high spore concentrations occurred when the air masses came from the North-East. It was even more pronounced when considering the 392 extremely high concentrations (66.7% of back trajectories were associated with the North-393 East direction). In Kastamonu and Adana, the highest concentrations were related primarily to 394 back-trajectories from the North and South-West, respectively. The most interesting 395 phenomenon was observed in Funchal/Madeira. When considering concentrations > 90th 396 percentile threshold, up to 75% of trajectories came from the sea, and only 25% might 397 originate from the continent (Spain, Portugal). However, if only the concentrations > 98th 398 399 percentile are considered, at least 33% of back-trajectories (but probably more) originate from the continent (Iberian Peninsula) (Fig. 7). 400

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402 Figure 7. Clusters of back trajectories associated with high spore concentration at study sites403 (90th and 98th percentile).

405 *3.4. Random Forest model*

406 Three meteorological parameters, *i.e.*, dew point temperature, daily maximum air temperature and daily minimum air temperature had the most substantial impact on the 407 models; this can be seen on the variable importance plots (Fig. 9). The relationship between 408 two of these three variables and the random forest model predictions are presented using the 409 prediction interaction plot (Fig. 8). For example, the second from the bottom left plot shows 410 the relationship between dew point temperature and maximum air temperature in the Adana 411 412 model results. It can be seen that the highest values of Ganoderma daily mean spore concentrations in the air were predicted to occur when dew point temperature was above 8°C 413 and maximum air temperature was above 28°C. 414

In most cases, the plots indicated threshold value(s) above which the predicted spore 415 concentration was relatively high (Fig. 8). For example, the first threshold can be seen for a 416 dew point temperature of 7°C and the second threshold can be seen for a dew point 417 temperature of 8°C in Szczecin. Another example is Kastamonu, with a visible threshold of 418 10°C of daily minimum air temperature. There are some interesting exceptions to this rule 419 (Fig. 8). Firstly, the relationship of daily maximum and minimum air temperature in Adana 420 421 with the highest predicted concentration values were observed when the minimum air 422 temperature values were between 15°C and 20°C. Secondly, plots for Funchal/Madeira were mostly showcasing random noise: the meteorological variables were unable to predict the 423 424 Ganoderma spore concentrations there.

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Figure 8. Variable interactions between values of dew point temperature (dew), daily
maximum temperature (tmax), and daily minimum temperature (tmin) for each of the random
forest models.

430 **Figure 9.** Variable importance for each site model.

431

432 Finally, a random forest model of daily mean concentrations of airborne *Ganoderma*433 spores was created for each study site (Table 6).

The Worcester model showed the highest value of R^2 of 0.71. Medium values of R^2 (0.44-434 0.55) were found for Kastamonu, Leicester and Adana. Worcester and Leicester are located 435 approx. 100 km apart and their daily concentrations of Ganoderma spores were similar 436 (Spearman's rank correlation of r=0.761, p<0.001; for non-zero days); the differences in the 437 models' performance expressed by R^2 are probably due to a few unusually high values in 438 439 Leicester (it is more difficult to predict less frequently occurring values). Kastamonu had two years of observations and Adana three years of observations, therefore, if their models were 440 based on a longer period of time, they could potentially yield in results similar to the 441 Worcester model. The Szczecin model had the value of R^2 of 0.36. The Vinnytsia model had 442 the value of R^2 of 0.21, which is probably due to the very short measurement period. The last 443 model, for Funchal/Madeira, had the value of R^2 of 0.01, where, apparently, daily mean 444 concentrations of Ganoderma spores cannot be explained using the examined here 445 meteorological data. Importantly, models seemed to be stable (not overfitted) as their 446 447 performances for training and test sets were similar (Fig. 10).

448

Figure 10. Comparison of the models' predictions and true values in the testing set for eachsite. Linear regression lines are shown in red, while the dashed lines represent diagonals.

451 The dashed lines represent diagonals and the red color is used for the linear regression452 lines.

454 **4. Discussion**

455

Airborne Ganoderma spore concentrations were recorded in five different biogeographical 456 regions representing four climate types in Europe and SW Asia. The highest airborne 457 concentrations in Europe were found to the North (Latitudes $> 45^{\circ}N$) whilst there were low 458 levels in the South of Europe and the studied part of Asia. This may result from the higher 459 460 humidity levels in the northern part of the study area; previous studies have shown a positive relationship between high humidity and Ganoderma spore concentrations in the air (Kasprzyk 461 et al. 2011; Sadyś et al. 2014). Similarly to our findings, Haard and Kramer (1970) reported 462 463 that increasing temperature, relative humidity or both lead to increased spore release of 464 Ganoderma. Airborne spores of Ganoderma have been documented to positively correlate temperature levels, maximum, minimum and mean temperature altogether (Hasnain 1993, Li 465 466 and Kendrick 1995). Regarding relative humidity, spore concentrations are known to also correlate with it positively (Li and Kendrick 1995), even though such a relationship with 467 precipitation has been proven negative (Hasnain 1993). Overall, Ganoderma produces a vast 468 amount of spores per fruiting body, e.g. G. applanatum can release 30 billion basidiospores 469 day⁻¹ over 6 months (Buller 1922); also, the genus seems to consist of quite tolerant 470 471 sporophores (distinctively among other basiodospores) that seem to be able not only to just survive under very dry conditions, but also to still continue liberating their spores in spite of 472 drought (Ingold 1971). The above show the expansive and infesting potential of Ganoderma, 473 474 as well as its dependence on specific meteorological and climatic regimes.

In this study, thermal variables were most important in explaining daily *Ganoderma* concentrations, however, the correlations with temperature decreased with decreasing latitudes. The spore sampling sites markedly differed in terms of tree density in the

surrounding areas and therefore the density of potential host plants for the fungi. Interestingly, 478 479 Kastamonu was the site with the largest forest area but the spore concentrations were significantly lower than in Worcester and Leicester - sites with small areas covered by 480 forests. One possible explanation could be that trees in the UK are much more heavily 481 infected with Ganoderma species than the other study sites. This confirms the Forest 482 Commission in England report, which indicated Ganoderma to be an important pathogen 483 (McKay 2011). We propose a new measure, the "Pathogen Infection Level Index (PILI)" 484 which combines spore concentrations and the area covered by potential pathogen hosts. The 485 interpretation is that the higher the PILI value, the higher the level of infection. It should be 486 487 emphasized that PILI values for Leicester are ~80 times larger than for the sites in Southern Europe. 488

Combining spore concentrations with potential sources of fungi (location and size of the 489 490 area covered by trees in this case), local wind conditions and back trajectories can be used to indicate primary spore source areas (Fernandez-Rodriguez et al. 2015; Grinn-Gofroń et al. 491 2020). For example, north directions are interesting in Szczecin because elevated spore 492 concentrations were recorded in three different seasons during North winds (Fig. 6). 493 Specifically, in summer, autumn and winter Ganoderma spores were delivered from North 494 North East (wind speed > 8 m s⁻¹), North (2 ms⁻¹) and North North West (~5 m s⁻¹), 495 respectively. This suggests that sources of emission for *Ganoderma* spores recorded in 496 Szczecin were located to the north to the city. The furthest putative source was located NNE 497 of Szczecin and contributed spores in summer, whereas in the autumn sources were located 498 499 closer to the study site, although not in the immediate vicinity. A similar phenomenon was observed in Vinnytsia – spores recorded in autumn originated from a close proximity source, 500 whilst in spring and summer the potential sources were found further away, however, they 501 were responsible for higher spore concentrations. The high number of potential sources of 502

fungi (areas with trees) close to sites such as Szczecin and Vinnytsia can complicate the 503 production of reliable predictive models of Ganoderma spore concentration. In contrast, 504 Worcester, Adana and Leicester, required lower wind speed to achieve higher concentrations 505 of Ganoderma spores in all seasons, which may be explained by the close location of 506 potential fungal sources. However, aerial photographs of Worcester (Google Maps 2019) 507 show that local trees are scattered and there are only two small patches of forests located to 508 509 the East of the study site. It is possible that *Ganoderma* spores trapped in Worcester may have originated from Ganoderma fruiting bodies living on local scattered trees during the summer, 510 however, the two forest patches in the East (distance 3-5 km) may contribute to the autumn 511 512 pool of Ganoderma spores recorded in Worcester. This interpretation is consistent with typical use of bivariate polar plots for assessing the sources of anthropogenic air pollutants 513 (Grange et al. 2016; Uria-Tellaetxe and Carslaw 2014; Carslaw and Beevers 2013). 514

515 Mean daily airborne Ganoderma spore concentrations appear to have a closer relationship with meteorological factors than Alternaria and Cladosporium spore 516 concentrations. In a previous study using four of the study sites from this study (Leicester, 517 Worcester, Szczecin and Vinnytsia, Grinn-Gofroń et al. 2019), symmetric mean absolute 518 percentage error (SMAPE) was used to describe how well the regression models fit the 519 520 Alternaria and Cladosporium airborne spore data. The lower the value of SMAPE, the better the model. The SMAPE values for Leicester were 0.73 for Alternaria and 0.54 for 521 Cladosporium, compared to 0.30 for Ganoderma in this study. Values for Worcester were 522 0.75 and 0.53 for Alternaria and Cladosporium, respectively, and 0.22 for Ganoderma. For 523 Szczecin SMAPE values were 0.69 and 0.60 for Alternaria and Cladosporium, respectively 524 and 0.39 for Ganoderma. Finally, Vinnytsia had values of 0.6 and 0.56 for Alternaria and 525 *Cladosporium*, respectively, and 0.48 for *Ganoderma*, even though there were only two years 526 of Ganoderma daily mean concentrations compared to five years for Alternaria and 527

Cladosporium. The random forest models for Alternaria and Cladosporium (Grinn-Gofroń et 528 al. 2019), and *Ganoderma* (the current study) were developed based on local meteorological 529 data. The lower SMAPE values in this study indicate that Ganoderma spores are more 530 dependent on local atmospheric conditions than Alternaria and Cladosporium, which may 531 indicate that local Ganoderma sources are more important for the recorded spore 532 concentration than for Alternaria or Cladosporium. A local source for the Ganoderma spores 533 534 is suggested in several polar plots (Worcester, Leicester, Adana, Vinnytsia in autumn). Based on our present findings, maximum spore concentrations were observed during very low wind 535 speed conditions. During such mild winds, there is typically limited horizontal atmospheric 536 537 transport, which further supports that local sources and local meteorology together can determine spore concentrations at those locations. Furthermore, differences in Ganoderma 538 spore concentrations between adjacent urban and rural areas (~ 40 km apart) and between 539 urban and suburban sites (~5 km apart) confirm the local source nature of Ganoderma spores 540 (Oliveira et al. 2010; Grinn-Gofroń et al. 2015). Therefore, we recommend that local 541 Ganoderma spore concentration models need to be developed, and emphasize the importance 542 of local studies on airborne Ganoderma spores to assess the risk of pathogen transmission. 543 Nevertheless, although local sources are clearly important, in some cases the extremely high 544 545 spore concentrations recorded at each site could have resulted from additional spores being transported from remote pathogen sources, as suggested in this study by the back-trajectory 546 results for Funchal/Madeira and Leicester sites. The random forest models generated for 547 Funchal/Madeira showed no predictive power, and there was no significant correlations 548 between spore concentration and meteorological variables. This may suggest that the spores 549 can be transported from outside the Madeira Island, although it is noted that the Ganoderma 550 counts from the Madeira site are the lowest of all sites in this study, and lack of significance 551 may be due to a lack of power due to low numbers. Comparing Leicester and Worcester, two 552

sites geographically close to each other, the dominant directions of back-trajectories associated with extremely high *Ganoderma* spore count were different. This suggests that, despite the close location, *Ganoderma* spores recorded in Leicester and Worcester likely have different sources, and this was most obvious during the extremely high spore concentration episodes.

Our results support observations by Sadyś et al. (2014), which state that Ganoderma 558 559 spores may be transported over long distances (due to their substantially lower volumes when compared to well-flying Alternaria spores and Betula pollen) to distant and uninfected areas. 560 This scale includes transport of bioparticles tens to thousands of meters above the surface of 561 562 the Earth and this transport interacts with a suite of abiotic factors e.g. wind, rainfall, and UV light (Schmale and Ross 2015). In the present study, we showed that Ganoderma spores are 563 transported > 900 km from the Iberian Peninsula to Madeira and, possibly also to Leicester 564 565 from central Europe (> 300 km). At such distances, transport models can predict the movement of plant pathogens in the atmosphere, however, that does not mean the spores will 566 be viable. Several studies have shown the radioprotective properties of Ganoderma spores 567 against UV radiation. Some *Ganoderma* species possess peptidoglycans and polysaccharides 568 capable of enhancing the cell repair process after gamma irradiation treatment in various 569 570 cancer cells and animal models (Suárez-Arroyo et al. 2017). Such protective effects induced by UV radiation may ensure a higher viability of Ganoderma spores during long-distance 571 transport, and as such, probably higher infection rates during deposition process on exposed 572 hosts. 573

The study site in Funchal/Madeira is a unique case. Our data suggests that most of the sources of the *Ganoderma* spores were likely to be located on Madeira island, but at least 33 % of the highest values could be attributed to long distance transport from the Iberian Peninsula (trajectories that come directly from the sea, Fig. 7). Several factors support this

statement. The distance between Madeira archipelago and the closest mainland territory in 578 Europe is about 1,000 km, and between the archipelago and the closest point of the Western 579 African coast approx. 600 km. The prevalent wind directions observed during the high 580 Ganoderma spore concentrations in Madeira were coming from the north and northwest 581 directions. The air masses blowing from the southwest originated over the Atlantic Ocean and 582 should be free from any biological material (Urbano et al. 2011). The air masses arriving from 583 the southeast, although they originated along the African continent, would not have 584 contributed significantly towards the airborne spore concentrations in Madeira due to the lack 585 of suitable pathogen host sources. Whilst 33% of the high Ganoderma values could be 586 587 attributable to transport from the Iberian Peninsula, it must be emphasized that the highest concentrations (> 98th percentile threshold; black lines in Fig. 7) should be considered as 588 originating from the continent (Portugal, Spain). Compared to the other study sites, lower 589 590 Ganoderma levels were detected in the atmosphere of Funchal/Madeira, which may be explained by the coastal proximity of the city and its insular position. Airborne particle 591 concentrations tend to be lower in coastal stations compared to mainland sites due to the 592 proximity of the sea (Belmonte et al. 2008), which may be due to less land mass for growth 593 and development of fungi, and the sea preventing resuspension of particles (Sousa et al. 594 595 2015).

596

597 Conclusions

This is the first comprehensive study of airborne *Ganoderma* spores and thus *Ganoderma* pathogen abundance in Europe and SW Asia. We found significant differences in *Ganoderma* spore concentrations and season timing between four different climates and five biogeographical regions, and showed that the concentrations increased with increasing latitudes (up to 55°N). Airborne *Ganoderma* concentrations were positively associated with

thermal variables at all sites apart from Madeira Island, although the correlations were lower 603 in southern compared to northern parts of the study area. Significant correlations with local 604 meteorological variables and high spore concentrations recorded at low wind speed indicated 605 a local source of airborne *Ganoderma* spores, therefore we developed random forest models 606 predicting spore concentration based on local meteorological data. In cases when predicted 607 values substantially differed from observed spore concentrations, spore transport from remote 608 609 source areas was hypothesized to be the cause. Back-trajectories calculated for days with high spore counts revealed the possibility of long-distance transport, as in the case from the Iberian 610 Peninsula to Madeira Island (at least 33% of the concentrations > 98th percentile). These 611 612 long-distance spore transport incidents in Madeira are further supported by the lower performance of the random forest model there and the complete lack of significant 613 relationships of spore concentrations with local meteorology. 614

615 It should be emphasized that, in sites with a substantial contribution of long distance transported spores, relying only on local meteorological data is insufficient to develop high-616 performance models predicting spore concentrations, even if sophisticated and robust 617 methods as random forests are used for analysis. Therefore, before developing models 618 predicting local spore concentrations, the likely origin of the spores should be investigated. If 619 620 the origin of the spores is determined to be local, Ganoderma spore concentrations will significantly contribute as an additional bio-indicator of pathogen abundance and, thus, 621 complement existing bio-indicators of local tree-health. We propose a new Pathogen Infection 622 Level Index (PILI), which shows the relative pathogen abundance per 1 km² of area covered 623 by host plants in a 30 km area of the sampling site. This index is a combination of spore 624 concentration and pathogen hosts density and revealed that forests in the UK are much more 625 infected with Ganoderma pathogens than the other study sites. 626

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- **Table 1.** Climate category of the study sites, based on the Köppen-Geiger climate classification
- 2 (<u>https://en.climate-data.org/</u>), and the sites' respective sampling periods.

City/	Annual	Climate	Sum of	Dominant	Sampler	Sampler	Period of
Country	mean		annual	wind	height	location	sampling
	temperatu		precipitation	direction			
	re						
Worcester/	9.7 °C	temperate oceanic	647 mm	SW	25 m	52°11'N,	Jan 2006 -
United	49.5 °F	climate (Cfb)	25.5 inch		a.g.l.	2°13'W	Dec 2010
Kingdom							
Leicester/	9.7 °C	temperate oceanic	620 mm	SE	12 m	52°38'N,	Jan 2006 -
United	49.4 °F	climate (Cfb)	24.4 inch		a.g.l.	1°05'W	Dec 2010
Kingdom							
Szczecin/	8.6 °C	temperate oceanic	542 mm	W, SW	21 m	53°26'N,	Jan 2006 -
Poland	47.4 °F	climate (Cfb)	21.3 inch		a.g.l.	14°32'E	Dec 2010
Vinnytsia/	7.6 °C ∣	humid continental	623 mm	W, SW	25 m	49°14'N,	Apr 2009
Ukraine	45.7 °F.	climate (Dfb)	24.5 inch		a.g.l.	28°29'E	- Oct 2010
Kastamonu/	10.3 °C	temperate oceanic	508 mm	SW, NE	7 m a.g.l.	41°36'N,	Jan 2006 -
Turkey	50.6 °F	climate (Cfb)	20.0 inch			33°76'E	Dec 2007
Adana/	19.3 °C	hot-summer	673 mm	N, S	15 m	37°05'N,	Jan 2007 -
Turkey	66.8 °F	Mediterranean	26.5 inch		a.g.l.	35°36'E	Dec 2009
		climate (Csa)					
Funchal/	18.8 °C	hot-summer	587 mm	SW, N	10 m	32°39'N,	Jan 2006 -
Madeira,	65.8 °F	Mediterranean	23.1 inch		a.g.l.	16°55'W	Dec 2010
Portugal		climate (Csa)					

- **Table 2.** Meteorological stations' location, and their distance to the spore trap.

Spore sampling	Meteorological	Distance between	Longitude of	Latitude of
station	station	spore monitoring	meteorological	meteorological
		and meteorological	station (°)	station (°)
		station (km)		
Worcester	Pershore	13.9	-2.03	52.15
Leicester	Church Lawford	32.2	-1.33	52.37
Szczecin	Szczecin	6.8	14.62	53.40
Vinnytsia	Vinnytsia	9.2	28.60	49.23
Kastamonu	Kastamonu	6.3	33.77	41.37
Adana	Adana	12.5	35.42	37.00
Funchal/Madeira	Funchal	14.2	-16.77	32.68

24	Table 3. Estimated area	covered by trees [km ² , %]] in the area surrounding the study sites.
		, , , ,	

Monitoring	Buffer zones for monitoring stations											
litering	0-5		5-10		10-15		15-20		20-25		25-30	
station		%		%		%		%		%		%
	km		km		km		km		km		km	
Worcester	5.8	7.2	17.4	10.9	37.2	15.5	41.1	12.9	65.3	16.3	75.8	15.8
Leicester	7.7	9.6	10.5	6.6	18.9	7.9	22.1	6.9	23.9	6.0	36.7	7.6
Szczecin	16.8	21.0	49.4	30.9	102.3	42.6	182.3	57.0	233.0	58.3	203.9	42.5
Vinnytsia *	15.7	19.6	42.6	26.6	59.2	24.7	101.7	31.8	140.1	35.0	122.0	25.4
Kastamonu	28.9	36.1	72.5	45.3	140.6	58.6	224.8	70.2	280.8	70.2	390.9	81.4
Adana	6.7	8.4	28.6	17.9	37.9	15.8	72.2	22.6	86.0	21.5	95.6	19.9
Funchal/Made	3.0	3.7	16.3	10.2	36.4	15.2	49.5	15.5	21.7	5.4	20.5	4.3

26 *: These values were produced using different tree cover density datasets

Table 4. Pathogen Infection Level Index (PILI) showing the relative abundance of airborne *Ganoderma* spores calculated per 1 km of area covered by potential hosts (in descending order of
values)

Sampling site	Pathogen Infection Level Index
	(PILI)
Leicester	84.18
Worcester	65.04
Szczecin	20.24
Vinnytsia	6.19
Adana	3.34
Funchal/Madeira	1.61
Kastamonu	0.64

- **Table 5**. Spearman correlation coefficients between daily mean *Ganoderma* spore concentrations in
- 50 the air and local meteorological parameters.

Meteorological	Worce	ster	Leices	ter	Szczec	cin	Vinny	tsia	Kastan	nonu	Kastar	nonu	Funchal	/
parameters													Madeira	L
	r	р	r	р	r	р	r	р	r	р	r	р	r	р
tavg	0.77	<.001	0.63	<.001	0.56	<.001	0.38	<.001	0.39	<.001	0.39	<.001	0.01	0.867
tmax	0.77	<.001	0.59	<.001	0.52	<.001	0.36	<.001	0.34	<.001	0.34	<.001	0	0.959
tmin	0.77	<.001	0.42	<.001	0.54	<.001	0.41	<.001	0.4	<.001	0.4	<.001	0.02	0.716
dew	0.75	<.001	0.62	<.001	0.59	<.001	0.45	<.001	0.32	<.001	0.32	<.001	0.01	0.894
humidity	-0.15	<.001	-0.13	<.001	-0.22	<.001	0.09	0.133	-0.27	<.001	-0.27	<.001	-0.03	0.434
wind speed avg	-0.28	<.001	-0.24	<.001	-0.09	0.001	-0.12	0.047	0.16	0.01	0.16	0.01	-0.04	0.401
wind speed max	-0.24	<.001	-0.17	<.001	-0.06	0.041	-0.05	0.434	0.2	0.001	0.2	0.001	-0.05	0.208
precip	-0.13	<.001	-0.12	<.001	-0.04	0.207	-0.01	0.869	-0.05	0.413	-0.05	0.413	0.03	0.542

52 Significant correlations (p < 0.05) are shown in bold.

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Table 6. Average performance of the models for the training and testing sets based on calculated values of root mean square error (RMSE), coefficient of determination (R^2), and symmetric mean absolute percentage error (SMAPE).

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Receptor site	Training set	s		Testing sets			
	RMSE	R ²	SMAPE	RMSE	R ²	SMAPE	
Worcester	35	0.67	0.23	33	0.71	0.21	
Leicester	31	0.51	0.30	32	0.51	0.30	
Szczecin	61	0.36	0.39	66	0.36	0.39	
Vinnytsia	17	0.28	0.48	17	0.21	0.48	
Kastamonu	3	0.49	0.36	2.7	0.55	0.33	
Adana	2.8	0.42	0.30	2.7	0.44	0.29	
Funchal/Madeira	2.3	0.01	0.74	2.1	0.01	0.74	



















