

International Journal of Biometeorology

The long distance transport of airborne Ambrosia pollen to the UK and the Netherlands from Central and South Europe --Manuscript Draft--

Manuscript Number:	IJBM-D-15-00221R1
Full Title:	The long distance transport of airborne Ambrosia pollen to the UK and the Netherlands from Central and South Europe
Article Type:	Original Research Paper
Keywords:	Ambrosia; long distance transport, back trajectory analysis, atmospheric movement, Pannonian Plain
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Abstract:	<p>Background: The invasive alien species <i>Ambrosia artemisiifolia</i> (common or short ragweed) is increasing its range in Europe. In the UK and the Netherlands airborne concentrations of <i>Ambrosia</i> pollen are usually low. However, more than 30 <i>Ambrosia</i> pollen grains per cubic metre of air (above the level capable to trigger allergic symptoms) were recorded in Leicester (UK) and Leiden (NL) on 4 and 5 September 2014.</p> <p>Objective: The aims of this study were to determine whether the highly allergenic <i>Ambrosia</i> pollen recorded during the episode could be the result of long distance transport, to identify the potential sources of these pollen grains and describe the conditions that facilitated this possible long distance transport.</p> <p>Methods: Airborne <i>Ambrosia</i> pollen data were collected at 10 sites in Europe. Back trajectory and atmospheric dispersion calculations were performed using HYSPLIT_4.</p> <p>Results: Back trajectories calculated at Leicester and Leiden show that higher altitude air masses (1500m) originated from source areas on the Pannonian Plain and Ukraine. During the episode, air masses veered to the west and passed over the Rhône Valley. Dispersion calculations showed that the atmospheric conditions were suitable for <i>Ambrosia</i> pollen released from the Pannonian Plain and the Rhône Valley to reach the</p>

higher levels and enter the air stream moving to Northwest Europe where they were deposited at ground level and recorded by monitoring sites.
Conclusions: The study indicates that the Ambrosia pollen grains recorded during the episode in Leicester and Leiden were probably not produced by local sources, but transported long distances from potential source regions in East Europe, i.e. the Pannonian Plain and Ukraine, as well as the Rhône Valley in France.

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16 calculations showed that the atmospheric conditions were suitable for *Ambrosia* pollen
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20 **Conclusions:** The study indicates that the *Ambrosia* pollen grains recorded during the episode
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22 distances from potential source regions in East Europe, i.e. the Pannonian Plain and Ukraine,
23 as well as the Rhône Valley in France.

24 **Key words:** *Ambrosia*; long distance transport, back trajectory analysis, atmospheric
25 movement, Pannonian Plain

1. Introduction

Allergic sensitization can result in disorders of the airways such as allergic rhinitis, conjunctivitis and allergic asthma (Zheng et al., 2011). Pollen grains from wind-pollinating (anemophilous) plants are often the causative agents of sensitization (Bousquet et al., 2007). Pollen from *Ambrosia* plants is one of the most relevant allergens in the USA (Oswalt et al., 2015) and is becoming an increasing problem in Europe. *Ambrosia* was accidentally introduced into Europe at the end of the 19th century. Since then, the plant has been steadily conquering Europe causing harm to agriculture and to public health (Smith et al., 2013). The most infested areas of Europe are currently the Rhône Valley in France, Northern Italy, the Pannonian Plain, and large areas in Ukraine and Western Russia (Skjøth et al., 2010; Smith et al., 2013; Thibaudon et al., 2014; Prank et al., 2013; Podberezko et al., 2013; Reznik, 2009). Concomitantly with the increase in plant abundance, there has been an increase in the number of patients sensitized to *Ambrosia*: ~60% in Hungary (Makra et al., 2004); ~ 47% in France, mainly the Rhône Valley (Thibaudon et al., 2010); and an increase from 24% in 1989 to 70% in 2008 was witnessed in Northern Italy (Tosi et al., 2011). In countries like Spain and the UK, the *Ambrosia* sensitization rate is still low (Bousquet et al., 2007), corresponding with the scarcity of the plant in these areas.

Ambrosia seeds are constantly being introduced into Europe via imported grain and animal fodder. Resulting in areas around entry points, such as harbours or airports, being heavily infested by *Ambrosia*. Recent studies suggest a progress of the plant into Germany (e.g. Berlin, (Starfinger, 2008)) and to a lesser extent the Netherlands (de Weger et al., 2009; Smith et al., 2013). In the Netherlands, most of the observations are of single plants or very small populations, often in private gardens, and probably originating from bird seed. However, recent analysis showed that there has been a small increase in the number of larger populations (>50 plants) in public spaces (Beringen et al., 2014; Smith et al., 2013). In the

1 UK, *Ambrosia* is primarily an alien invasive plant of open, ruderal habitats (Essl et al., 2015).
2 *Ambrosia* plants require long-lasting autumns and a late first-frost for their seeds to mature;
3 which limits their northward distribution in Europe. Recent studies based upon climate change
4 prediction models have suggested that habitat suitable for *Ambrosia* range expansion will
5 extend further north and east such that it will become established in Scandinavian countries
6 and Britain by 2050 (Hamaoui-Laguel et al., 2015; Storkey et al., 2014) .

7 In regions that scarcely record any *Ambrosia* pollen, occasional peaks in atmospheric
8 *Ambrosia* pollen concentrations are likely to be caused by long distance transport (LDT) from
9 sources hundreds of kilometres away (e.g. (Belmonte J et al., 2000; Fernández-Llamazares et
10 al, 2012; Makra et al, 2010; Cecchi et al., 2006; Smith et al., 2013)). Studies in Poland using
11 back trajectory analysis showed that peaks in airborne *Ambrosia* pollen recorded during the
12 night and early in the morning were most likely brought by air masses loaded with pollen
13 from the southern areas, like the Czech Republic, Slovakia and Hungary (Smith et al., 2008;
14 Stach et al., 2007). Similarly, Kasprzyk et al. (2011) showed that the Ukraine may be a
15 source area of *Ambrosia* pollen for Poland.

16 Airborne concentrations of *Ambrosia* pollen are usually low in the UK and the
17 Netherlands, generally not exceeding 10 pollen grains per year (de Weger et al., 2009;
18 Pashley et al., 2015). The climatic conditions in these countries are not currently favourable
19 for fulfilling the full life cycle of *Ambrosia*. The late flowering of the plant combined with the
20 early dates of the first frosts in autumn prevent the *Ambrosia* seeds from ripening. However,
21 future climate scenarios for the Netherlands (Klein Tank et al., 2014) and for Europe (Storkey
22 et al 2014) have suggested that *Ambrosia* could spread and persistent as far north as central
23 England by the year 2050, with areas where *Ambrosia* populations are currently classed as
24 casual becoming established. It is important to prevent the plant from becoming established in
25 new regions since examples from other European countries have shown the dramatic increase

1 in *Ambrosia* sensitization once this occurs. It is therefore imperative to routinely monitor for
2 airborne *Ambrosia* pollen as this can be an early warning of invasion by the plant. Such
3 routine monitoring revealed that, at the beginning of September 2014, more than 30 *Ambrosia*
4 pollen grains per cubic metre of air were recorded in Leicester (UK) and Leiden
5 (Netherlands), where there are no known local stands of *Ambrosia* plants. The aims of this
6 study were to: (1) determine whether this episode could be the result of LDT, since local
7 sources are not known to be present; (2) identify the potential sources of these pollen grains;
8 (3) try to describe the conditions that facilitated this possible episode of LDT that resulted in
9 unusually high atmospheric concentrations of *Ambrosia* pollen.

10

11 **2. Materials and Methods**

12

13 *2.1. Pollen data*

14 *Ambrosia* pollen data were collected at ten sites in Europe (Fig. 1) by volumetric spore traps
15 of the Hirst design (Hirst, 1952). Daily average and bi-hourly *Ambrosia* pollen concentrations
16 are expressed as pollen grains per cubic metre of air ($P \text{ m}^{-3}$).

17

18 *2.2. Meteorological data*

19 The overall synoptic weather situation was investigated using analysed weather maps from
20 the UK Met Office, as well as reanalysed meteorological data and meteorological
21 observations obtained from the National Centre for Environmental Prediction (NCEP) using
22 the methodology given by Stach et al. (2007) and Kasprzyk et al. (2011). Synoptic charts
23 were obtained from the website: <http://www.wetterzentrale.de/topkarten/tkfaxbraar.htm>

1 2.3. *Back-trajectory analysis*

2 Back-trajectory calculations were conducted using the HYSPLIT_4 (HYbrid Single-Particle
3 Lagrangian Integrated Trajectory) model (Draxler et al., 2009). **Three dimensional back**
4 **trajectories** were calculated 72h back in time, at five heights above ground level (200m,
5 500m, 1000m, 1500m and 2000m), for bi-hourly periods corresponding to pollen records in
6 Leicester and Leiden on 4 and 5 September 2014. Trajectory calculations involve an amount
7 of uncertainty, and this uncertainty increases exponentially with time. **This is a drawback of**
8 **using individual back trajectories (Stohl and Seibert, 1998).** Therefore, to account for this
9 uncertainty, **clusters** based on nine trajectories with receptor points placed 0.5 degrees apart
10 were calculated. Trajectories of the **cluster** will be closely related until the trajectories reach a
11 certain area, where even small variations in meteorology will create large variations in the
12 transport path of the individual trajectories. All calculated trajectories examined in this study
13 showed little variation with respect to transport path (Stach et al., 2007).

14 Input meteorological data for 1-7 September 2014 came from the Global Data
15 Analysis System (GDAS) dataset provided by the NCEP that covers the period 2006 to
16 present in the form of a 1 degree latitude-longitude grid <https://ready.arl.noaa.gov/gdas1.php>.

17

18 2.4. *Dispersion modelling*

19 **Particle dispersion** calculations were carried out with the HYSPLIT_4 model in order to
20 **determine whether atmospheric conditions during the studied episode would have allowed**
21 **Ambrosia pollen to reach high altitude air masses after release in the source areas and to settle**
22 **down in Leiden and Leicester following atmospheric transport.**

23 In order to verify whether airborne *Ambrosia* pollen released in the source area could
24 reach the altitudinal range of back trajectories arriving at Leiden, the model was set to release

1 2500 particles of 20 μ m at 15m above the ground each hour from 6-12h, which corresponds to
2 the most intensive period of *Ambrosia* pollen release (Barnes et al., 2001; Martin et al., 2010).
3 Sedimentation processes are accounted for in the model by setting the settling velocity of the
4 particles to 0.0156m/s which corresponds to the settling velocity of *Ambrosia artemisiifolia*
5 pollen grains (Raynor et al., 1970), and applying the conversion module that deposits each
6 particle rather than reduce their mass.

7 The starting locations for the particles released into the dispersion model were
8 previously identified as being the most important source areas for *Ambrosia* pollen on the
9 Pannonian Plain (Skjøth et al., 2010) and France (Thibaudon et al., 2014)(Fig. 1). These
10 source areas had been identified by the use of detailed knowledge of *Ambrosia* ecology, land
11 cover information and spatial variations in the annual sum of atmospheric *Ambrosia* pollen
12 concentrations.

13 Simulations of particle deposition using the HYSPLIT_4 were conducted again in
14 order to determine whether *Ambrosia* pollen traveling at the height of airmasses (as described
15 by back trajectories) could settle out from the atmosphere to reach ground level monitoring
16 sites. The dispersion model was set to run so that the released particles arrived at Leicester
17 and Leiden at 12-14h on 5 September, which was the time when the highest *Ambrosia* pollen
18 concentrations were recorded (Table 1). The emission points were selected based on the
19 results of trajectory analysis (9 points for each trajectory in the cluster). Particles were
20 released at an altitude of ~1500m in the path of the air masses travelling to Leicester (Suppl.
21 Table 1) and Leiden (Suppl. Table 2). The model was set to release 500 particles per hour for
22 8 hours (until the end of the period when the highest bihourly *Ambrosia* pollen concentrations
23 were recorded). The total amount of released particles corresponds to approximately 20% of
24 the pollen (19316 P m⁻³) that reached the altitudinal range of air masses that passed over the
25 Pannonian Plain on the way to Leiden.

1 3. Results and Discussion

2 Unusually high daily average concentrations of airborne *Ambrosia* pollen, in excess of 30 P
3 m⁻³, were recorded in Leicester (4-5 Sept 2014) and Leiden (3-5 Sept 2014) (Suppl. Figure 1
4 and Suppl. Table 3). Bi-hourly concentrations of *Ambrosia* pollen began to peak during the
5 night and early morning and continued into the following day in both cities. These diurnal
6 patterns suggested that the pollen grains did not originate from local sources, since studies
7 have shown that *Ambrosia* pollen from local plants is usually recorded in the air from about
8 6.30am to around midday (Ogden et al., 1969). Furthermore, the geographical scope of the
9 episode, recorded in both Leicester and Leiden, suggest that this was not a localised
10 phenomenon caused by emission from local populations (Sommer et al., 2015).

11 Back-trajectory analyses show that air masses arriving at Leicester (Fig. 2) and Leiden
12 (Fig. 3) on the 4 and 5 September came from an easterly direction. The analyses were
13 performed for various altitudes, but only those air masses arriving at Leicester and Leiden at
14 higher altitudes (e.g. 1500m above ground level (=AGL)) passed over potential source areas
15 on the Pannonian Plain (Skjøth et al., 2010) and Ukraine (Kasprzyk et al., 2011). Lower
16 altitude air masses (e.g. 500m AGL) tended to arrive from more northerly regions. It is
17 interesting to note that the back trajectories calculated from Leicester mainly pass over
18 Ukraine, rather than the Pannonian Plain. Whereas, the higher altitude air masses arriving at
19 Leiden spent a considerable amount of time over the Pannonian Plain. However, the air
20 masses arriving at Leicester passed close to Leiden where it is likely that mixing took place,
21 indicating that both the Pannonian Plain and Ukraine were potential sources of airborne
22 *Ambrosia* pollen at the two sites.

23 The idea that the *Ambrosia* pollen grains recorded in Leicester and Leiden were
24 transported by high altitude air masses is supported by the fact that bi-hourly concentrations

1 of *Ambrosia* pollen up to 377 P m^{-3} were recorded on the 2-3 September at Rzeszów, in
2 Southeast Poland, which is located along the path taken by the high level air masses travelling
3 from Ukraine. On the other hand, very little airborne *Ambrosia* pollen (bi-hourly
4 concentrations $\leq 5 \text{ P m}^{-3}$) was recorded at this time in Poznań, in Western Poland, which lies
5 on the path taken by the lower altitude air masses that approached from more northerly
6 regions where notable sources of *Ambrosia* pollen have not been recorded (Suppl. Table 3,
7 Figs 2 and 3).

8 During the period 3-5 Sept 2014, the synoptic situation was dominated by low-
9 pressure systems (993-1012 hPa) residing over the Atlantic to the north of the British Isles
10 and a high-pressure system (1029–1031 hPa) situated over the Baltic and European Russia.
11 An occlusion was positioned over Poland, Denmark, and Germany, particularly during the 1-4
12 September. This occluded front generally ran from east to west and marked the route taken by
13 the pollen. It also helped to direct the warm air masses from Ukraine and the Pannonian Plain
14 up in to the atmosphere. The result was that several different air masses lay on top of one
15 another (the definition of an occlusion) and caused the lower parts of the atmosphere to have a
16 different origin compared to the upper part.

17 Pollen monitoring stations on the Pannonian Plain, i.e. Kecskemét, Debrecen,
18 Nyíregyháza and Sombor, recorded bi-hourly concentrations of *Ambrosia* pollen in the range
19 of 1000 to 4000 P m^{-3} during 1-6 September (Suppl. Table 3). It is likely that these pollen
20 levels were of sufficient magnitude to allow large amounts of airborne *Ambrosia* pollen grains
21 to be transported long distances (Šikoparija et al., 2013; Smith et al., 2008). In order to test
22 the hypothesis that the Pannonian Plain could be a source of the *Ambrosia* pollen recorded in
23 Leicester and Leiden, the HYSPLIT_4 dispersion model was run to determine whether the
24 locally produced *Ambrosia* pollen could reach high enough altitudes to become entrained in
25 high level air flows moving towards Northwest Europe. The calculations were made using the

1 *Ambrosia* pollen source inventory produced by Skjøth et al. (2010) (Fig.1). After release from
2 heavily infested source areas on the Pannonian Plain on 2-3 September (6-12h), an average of
3 36.1% of the particles remaining airborne reached between 316.3 – 3624.7m, which is the
4 altitudinal range of back trajectories arriving at Leiden at the same time when the *Ambrosia*
5 pollen were recorded (Table 1). Interestingly, only *Ambrosia* pollen grains released from
6 sources in northern parts of the Pannonian Plain travelled northward and were able to enter
7 the air stream travelling toward Northwest Europe. Dispersion from sources located on
8 southern parts of the Pannonian Plain tended to go south (Fig. 4). Unfortunately, a detailed
9 inventory for *Ambrosia* pollen sources, as described for the Pannonian Plain (Skjøth et al.,
10 2010) and France (Thibaudon et al., 2014), does not exist for Ukraine and so the analysis
11 could not be repeated for this area.

12 Further investigation showed that towards the end of the episode air masses calculated
13 for 1500m, which arrived at Leiden between 12-22h on the 5 September 2014, veered south
14 and approached from the direction of potential source regions in the Rhône Valley in France
15 (Fig. 3B). At the pollen monitoring station of Roussillon, bi-hourly concentrations of airborne
16 *Ambrosia* pollen between 642-1085 P m⁻³ were recorded during the morning of 4 September
17 (Suppl. Table 3), which is the time period that the air masses dwelled around in the Rhône
18 valley before moving to Leiden.

19 The HYSPLIT_4 dispersion model was run again to determine whether the *Ambrosia*
20 pollen produced in the most heavily infected areas in France (Thibaudon et al., 2014) could
21 reach high altitudes. The particle cloud tended to go south on 3 September, but on the 4
22 September the particles reaching the higher levels went northward (Fig. 5). From particles
23 remaining airborne after release, 2.5% reached between 1471.8 - 2482.1 m, which is the
24 altitudinal range of back trajectories arriving at Leiden when the pollen grains were recorded
25 in the trap (Table 1). Calculations of particle concentration distribution carried out on the 3-4

1 September 2014 confirmed that *Ambrosia* pollen grains could have reached sufficiently high
2 above the ground to enter into the air stream moving towards Leiden (Fig. 5, Table 1).

3 The Rhône Valley has previously been identified as a potential source of *Ambrosia*
4 pollen for Catalonia (Belmonte J et al., 2000) and Switzerland (Taramarcaz et al., 2005), but
5 this is the first time that it has been identified as a potential source of *Ambrosia* pollen in
6 Northwest Europe. The Rhône Valley is a known centre of *Ambrosia* in Europe, and is closer
7 to Leiden and Leicester than the Pannonian Plain, however this study has shown that under
8 these conditions only a fraction of pollen released from France reached Northwest Europe. In
9 addition, the uncertainty resulting from orographically forced meteorology within the Rhône
10 Valley cannot be resolved with default HYSPLIT_4 input data. Focused studies in such a
11 region require much more detailed data, e.g. from the Weather Research and Forecast model
12 as described by Hernandez-Ceballos et al (2014). This suggests that the Pannonian Plain
13 should still be considered to be the main source of the LTD *Ambrosia* pollen in Europe (Table
14 1).

15 HYSPLIT_4 simulations of particle deposition from the high altitude air masses,
16 before they reached Leicester and Leiden, confirm that atmospheric conditions would have
17 allowed for the deposition of airborne *Ambrosia* pollen to ground level in areas where surface
18 pollen measurements took place (Fig. 6).

19 Several aspects of back trajectories are limited in respect to analysing air mass
20 patterns. Earlier *Ambrosia* studies by Stach et al (2007) and Sikoparija et al (2009) used the
21 Danish ACDEP model to calculate trajectories (Skjoth et al, 2002). This was a 2D trajectory
22 model where the air masses followed the σ –level 0.925 wind vectors and 0.25°
23 meteorological input. This approach (e.g. terrain following coordinates or isobaric
24 coordinates) is conceptually simpler, but it neglects the vertical wind component (Stohl et al,

1 1998), which means that errors in the calculation of 2D trajectories can accumulate faster with
2 transport distance than for 3D trajectories. Current practice is therefore to use 3D trajectories,
3 most commonly in relation to *Ambrosia* by using the HYSPLIT model (e.g. Makra et al.
4 (2010), Saulienė et al. (2011), Zemmer et al. (2012) and recently Sommer et al. (2015)).
5 Spatial and temporal resolution in the input data is, however, also very important as
6 demonstrated by Skjoth et al. (2002) and Hernandez-Ceballos et al. (2014). These studies
7 suggest coastal effects and complex terrain often affects the meteorology on scales that are
8 relevant for pollen transport and more detailed input to HYSPLIT or ACDEP provided
9 substantially better output data, thus improving the analytical results. The effect on spatial and
10 temporal resolution, however, depends on the atmospheric physics during the pollen episodes.
11 Simulations of large scale flows will generally be less affected by increased resolution.
12 Conversely, simulations of frontal zones, convective zones and orographic forces flow will be
13 heavily affected (e.g. Hernandez-Ceballos et al, 2014). In our case, there are generally large
14 scale flows over the Pannonian Plain towards Leiden, while the flow in the Rhône Valley
15 could be affected by complex terrain. As such, the findings relating to the Rhône Valley are
16 uncertain due to limitations in resolving complex flows in this area.

17 It is not known whether such episodes of LDT have any consequences for the
18 prevalence of sensitization to *Ambrosia* pollen. Threshold values required for *Ambrosia*
19 pollen to induce symptoms differ among different studies, ranging from 1-3 P m⁻³ for “first
20 symptoms to start” to 50 P m⁻³ for “60-80% of the sensitized patients to show symptoms” (de
21 Weger et al., 2012; Déchamp et al,1997). The public internet platform in the Netherlands
22 (Allergieradar.nl), where sufferers can enter their symptom scores (de Weger et al., 2014), did
23 not show increases in numbers of entries or symptom severity during the studied period.
24 Although it is important to mention that the number of entries was very low during that

1 period. Furthermore, it is a matter of debate whether pollen that have been exposed to extreme
2 circumstances during LDT have preserved their allergenic capacity (Cecchi et al., 2010).

3

4 **5. Conclusion**

5 This study indicates that the *Ambrosia* pollen grains recorded at the beginning of September
6 2014 in Leicester and Leiden were probably not produced by local sources in response to
7 range expansion due to climate change, but transported long distances from potential source
8 regions in East Europe, i.e. the Pannonian Plain and Ukraine, as well as the Rhône Valley in
9 France. As a result, this again confirms that *Ambrosia* pollen can be transported long
10 distances from potential source regions, this time to the Northwest fringes of Europe. In
11 addition, we have shown that, using a dispersion model, *Ambrosia* pollen released from the
12 Pannonian Plain reached high enough altitudes to enter westward moving air masses **and then**
13 **settle out of the atmosphere to reach monitoring stations at ground level where they were**
14 **recorded**. This pollen released from the Pannonian Plain could augment the pollen moving
15 west from more easterly areas such as Ukraine. The occurrence of an occluded front during
16 the period helped to lift the pollen grains high into the atmosphere where they could be
17 transported to Northwest Europe. Furthermore, for the first time, we have identified the
18 Rhône Valley in France as being a potential source of *Ambrosia* pollen in Northwest Europe,
19 albeit only a minor contributor compared to the Pannonian Plain. This study highlights the
20 importance of the HYSPLIT dispersion model as a tool for distinguishing between LDT
21 events and range expansion of an invasive, highly allergenic plant; an important distinction
22 for plant and health management strategies.

23

24 **Acknowledgement**

1 The results presented here address one of the scientific challenges described in the COST
2 Action SMARTER. CHP is supported by the Midlands Asthma and Allergy Research
3 Association (MAARA) and the National Institute for Health Research Leicester Respiratory
4 Biomedical Research Unit. The views expressed are those of the author(s) and not necessarily
5 those of the NHS, the NIHR or the Department of Health

6

7 **Conflicts of interest**

8 The authors have no conflicts of interest to declare.

9

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11

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35

- 1 Table 1. The height of air masses arriving at Leiden after passing through the areas (Pannonian Plain or
 2 Rhône Valley) where the particle clouds were dispersed, and percentage of particles calculated to be present
 3 at each trajectory height range.

Time at which trajectories arrived in Leiden	Pannonian Plain		Rhône Valley	
	Trajectory height (m)	% of particles dispersed in trajectory height	Trajectory height (m)	% of particles dispersed in trajectory height
4th September 02:00*	-	-	-	-
4th September 04:00	1233.9-1965.0	15.3	-	-
4th September 06:00	1211.6-1744.2	13.4	-	-
4th September 08:00	1097.9-1762.5	18.1	-	-
4th September 10:00	1332.1-1975.3	12.2	-	-
4th September 12:00	1164.3-2236.9	19.7	-	-
4th September 14:00	1114.9-2295.2	22.0	-	-
4th September 16:00	1018.3-2391.5	26.4	-	-
4th September 18:00	1112.0-2190.2	21.6	-	-
4th September 20:00	1190.9-1924.7	16.4	-	-
4th September 22:00	1212.9-1945.8	15.9	-	-
5th September 00:00	889.0-1834.3	28.7	-	-
5th September 02:00	785.6-2110.6	37.2	-	-
5th September 04:00	744.6-2300.4	40.5	-	-
5th September 06:00	685.0-2322.7	44.3	-	-
5th September 08:00	519.7-2234.8	55.6	-	-
5th September 10:00	454.9-2348.1	60.6	-	-
5th September 12:00	316.3-2234.5	71.0	1565.9-1648.0	1.1
5th September 14:00	337.5-3624.7	70.4	1573.0-2482.1	2.3
5th September 16:00	495.9-3488.8	58.3	1471.8-2237.2	4.9
5th September 18:00	593.7-3570.2	51.5	1554.2-2316.7	2.6
5th September 20:00	689.2-2981.8	44.8	1583.2-2438.2	2.1
5th September 22:00	591.8-2184.1	50.3	1605.1-2346.4	1.8
AVERAGE	854.2-2348.5	36.1	1558.9-2244.8	2.5

- 4 * Trajectory did not pass over the areas where the particles were dispersed.

Figure 1. Distribution of the aerobiological monitoring stations used in this study and *Ambrosia* pollen source inventories. Dark grey indicates grid cells entered into the dispersion model, corresponding to the areas with the highest *Ambrosia* plant infestation according to the inventories by Skjøth et al (2010) and Thibaudon et al (2014).

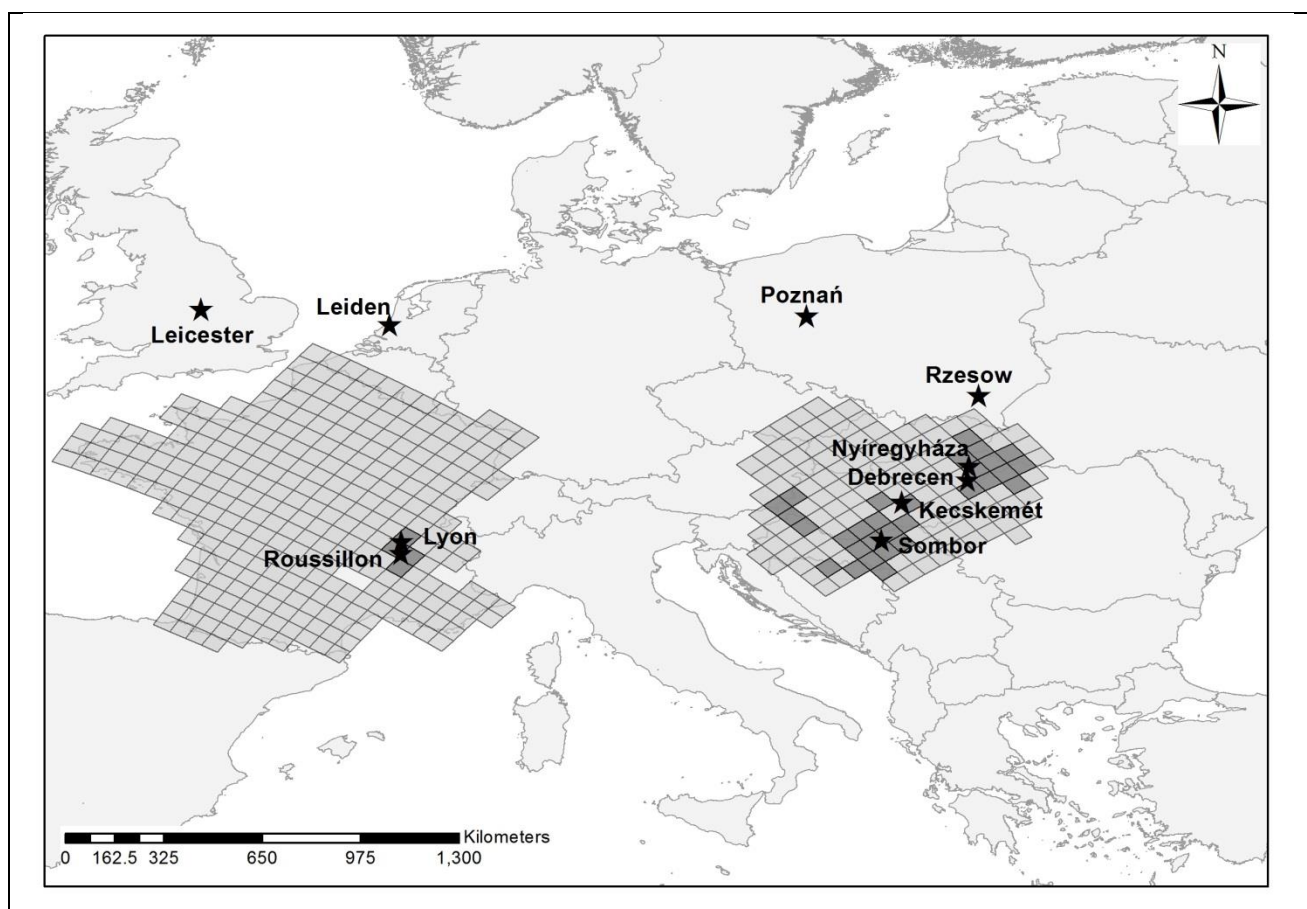
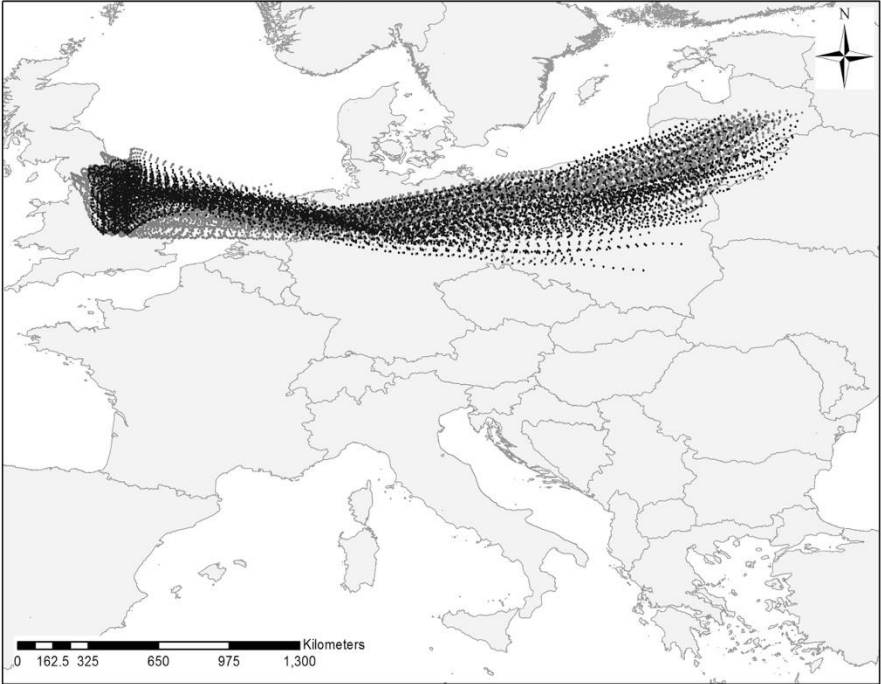


Figure 2. Clusters of 72h backward trajectories calculated every two hours 4-5th September from Leicester at 500m (A) and 1500m (B). The light grey colour indicates trajectories arriving when *Ambrosia* pollen was not recorded.

A



B

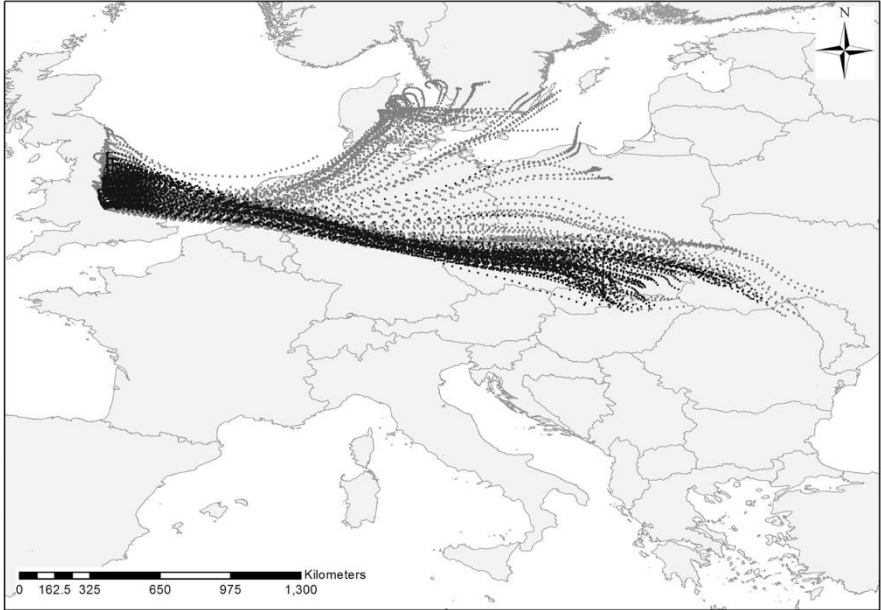
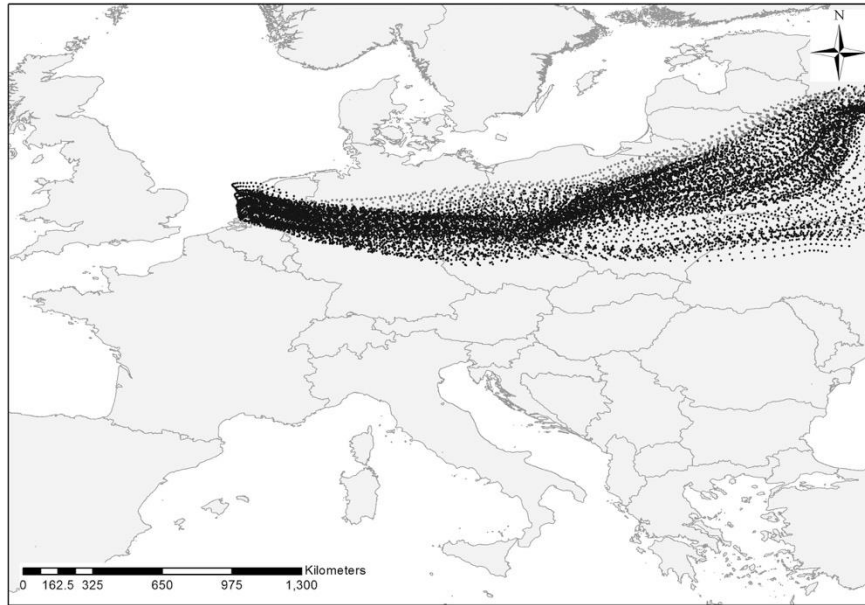


Figure 3. Clusters of 72h backward trajectories calculated every two hours 4-5th September from Leiden at 500m (A) and 1500m (B). The light grey colour indicates trajectories arriving when *Ambrosia* pollen was not recorded.

A



B

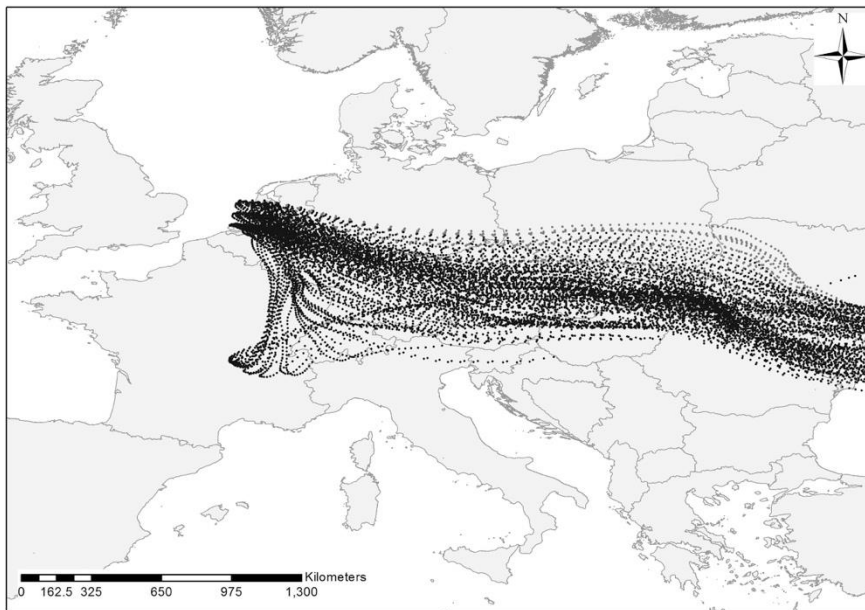


Figure 4. The output of the HYSPLIT model calculations of the distribution of particles released from 6-12am at Pannonian Plain on 2 September (A) and 3 September (B).

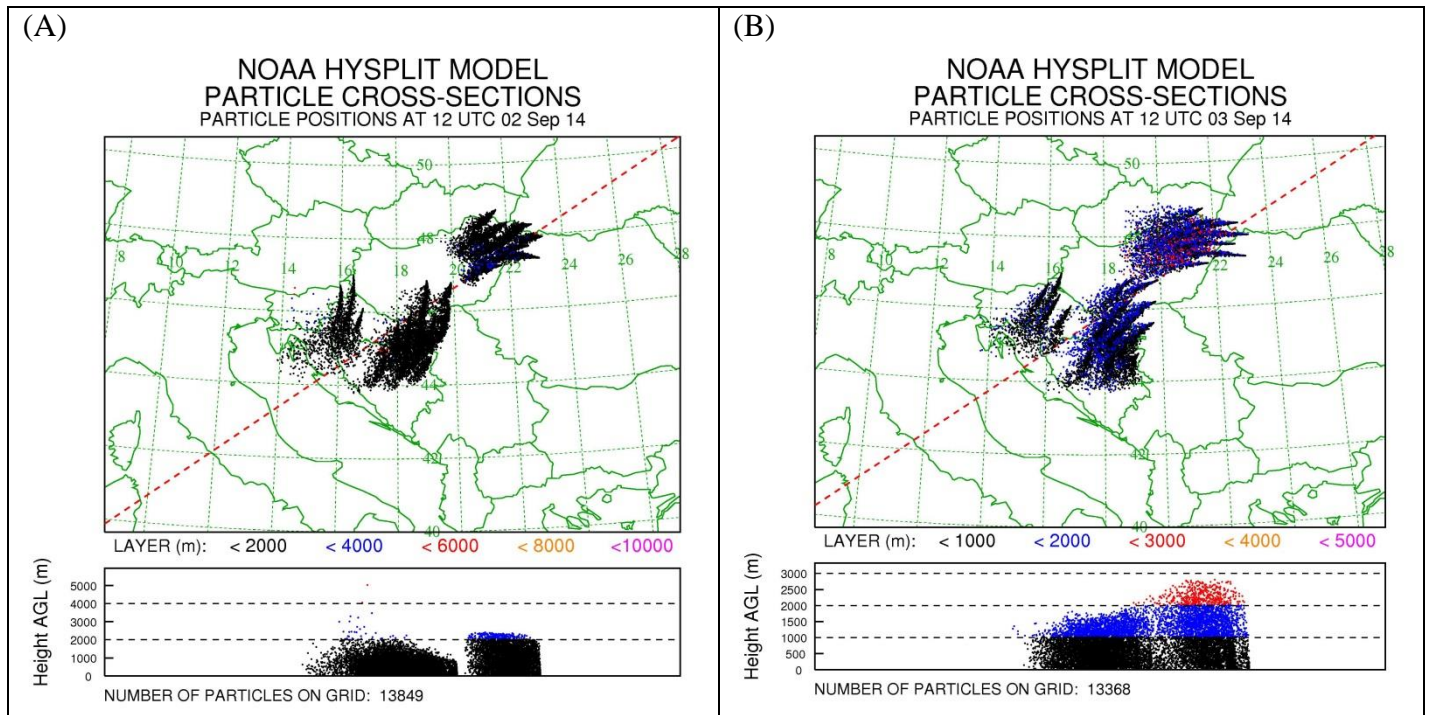


Figure 5. The output of the HYSPLIT model calculations of the distribution of particles released from 6-12am in the Rhône Valley in France on the 3 September 2014 (A) and 4 September 2014 (B).

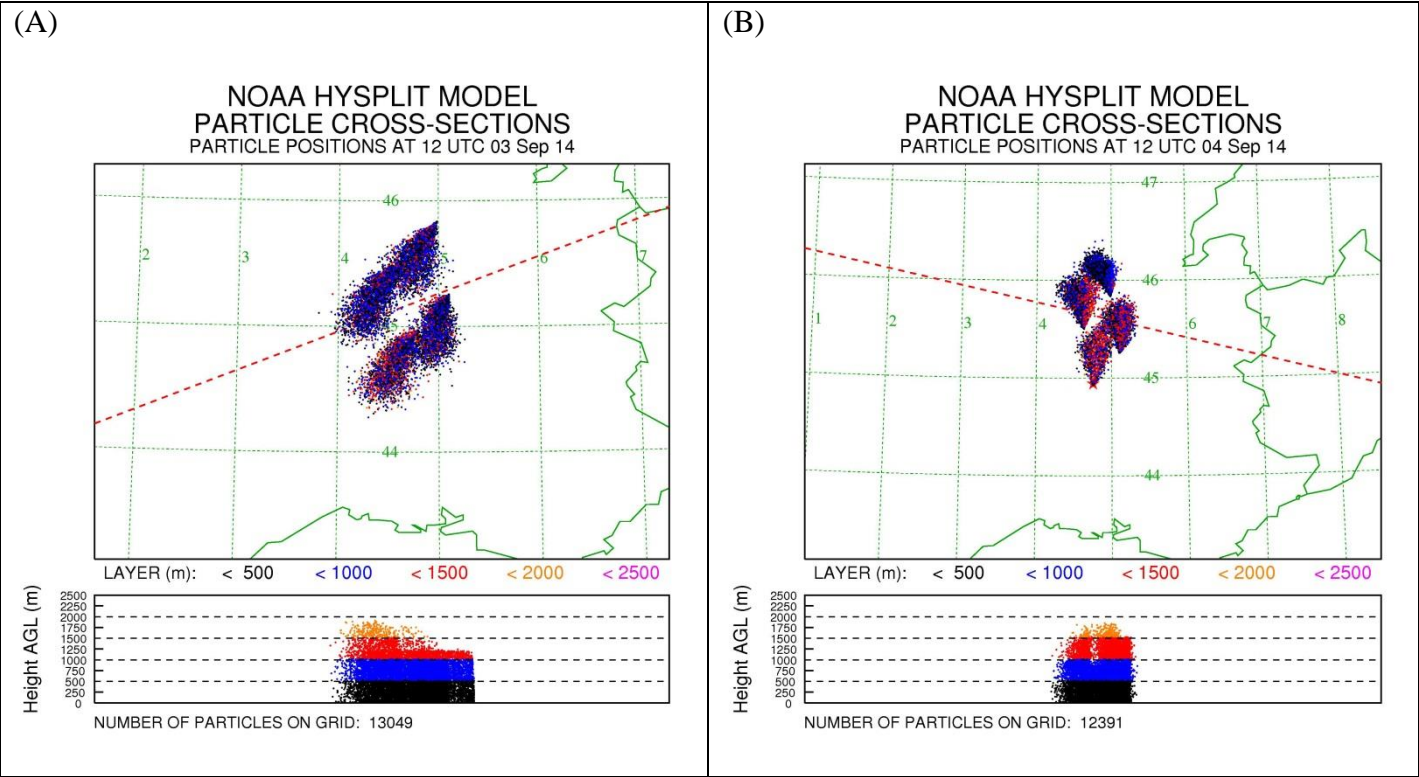
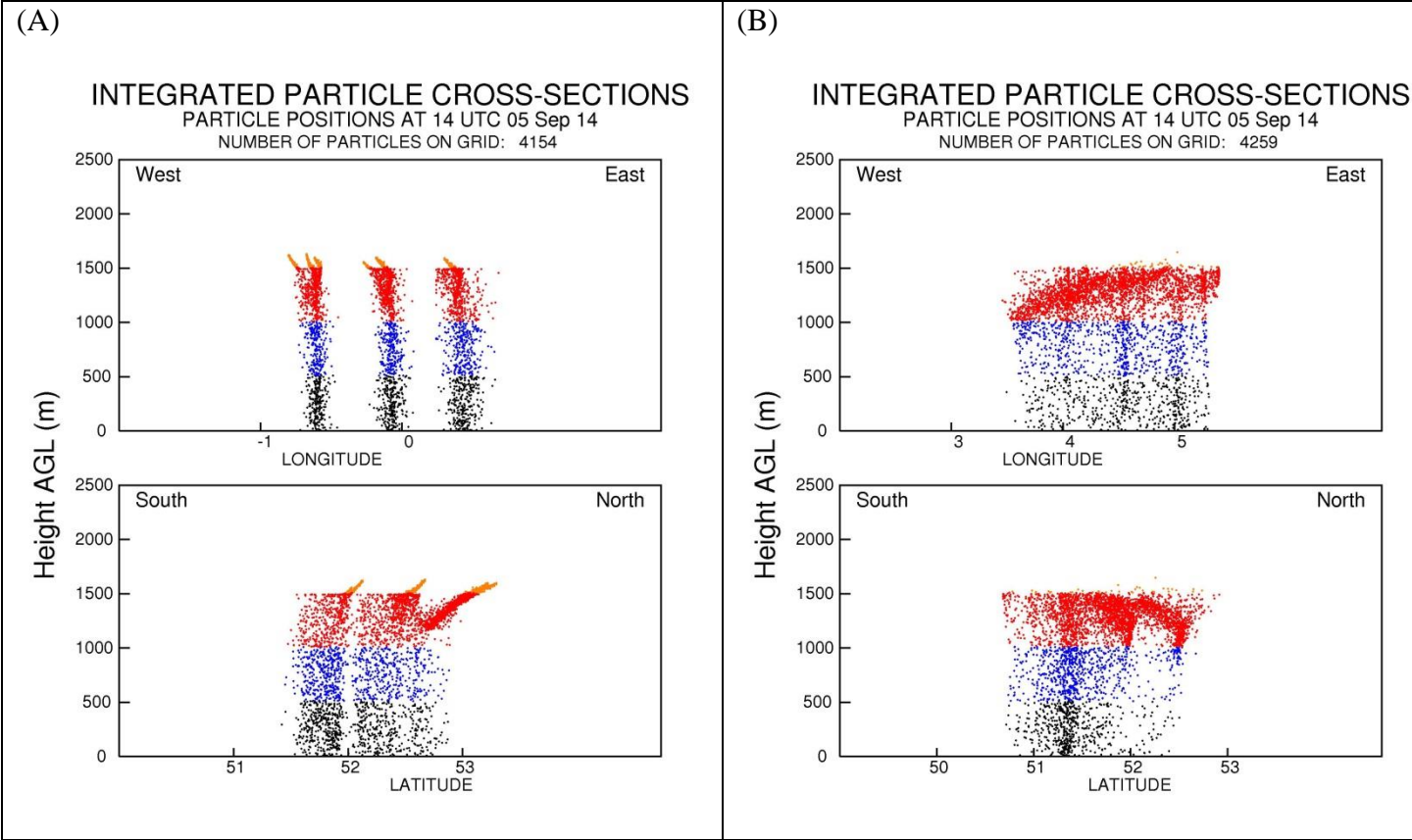
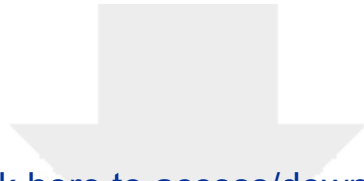


Figure 6. The output of the HYSPLIT model calculations of the distribution of particles released from 06-14h at the location air masses pass on 5 September 2014, six hours before arrival to Leicester (A) and Leiden (B).





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Electronic Supplementary Material

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Answers to the comments to manuscript No. IJBM-D-15-00221

The long distance transport of airborne Ambrosia pollen to the UK and the Netherlands from Central and South Europe

We thank the reviewers for their helpful comments, which we have used to improve our manuscript. Below we answer the questions and indicate the changes we have made to the revised manuscript. Page- and line numbers refer to the revised manuscript. The changes and additions that relate to the comments of reviewer #1 are in brown indicated; those of reviewers #2 in blue.

Answers to the comments of reviewer #1

The authors used trajectory analysis, which has several limitations. Some of them are explored but two are not sufficiently addressed in the paper. Firstly, the authors pointed out that only the high-altitude trajectories pass over the ambrosia sources, whereas the lower ones miss them. This raises two questions: (i) how pollen reached the transport altitude, (ii) how the pollen went down from it. The authors tried to show that almost a quarter of pollens released at the corresponding day climb sufficiently high but I found no indication that sedimentation of pollen is accounted for. The value of 23% sounds pretty large to me, so my suspicion is that settling was forgotten. If true, the results are probably wrong: with sedimentation velocity as high as 1-1.5 cm/sec, the altitude of 1500m may well be unreachable for the vast majority of pollen. At least, the 23% suggested by the authors appears as a strong over-estimation. This must be clarified.

Reply: The aim of the performed test using the HYSPLIT dispersion model was only to check whether physical conditions in the atmosphere over the source region would enable pollen to lift high enough to reach the trajectory altitude. We did not aim to give an indication on the quantity of pollen reaching this height so although we defined particles according to ragweed pollen characteristics we used default settings and did not allow conversion of particles. Furthermore, we did not take into consideration settling velocity of ragweed pollen or the dry deposition processes. We have repeated the analysis accounting for dry deposition processes and known ragweed pollen settling velocity (0.0156 m/s) taken from Raynor et al. (1970). The calculation of numbers of particles reaching the altitude range of trajectories was focused on those particles remaining after the release (12h). Notably less particles remained in the atmosphere compared to the former test (e.g. 13368 compared to 16200 over Pannonian Plain on 3 September). The analysis of particle height confirmed again that a notable amount of particles were able to reach trajectory height range over the Pannonian plain (36.1 %) and some particles reached trajectory height range over the Rhone Valley (2.5%). It is important to note here that back-trajectories starting in Leiden at 1500m travelled over the Pannonian Plain in a wide range of altitudes starting as low as 316m above the ground. This resulted in a high percentage of released particles that could reach the altitude of trajectories travelling towards Leiden. Finally, it should be noted that we only take into account the particles that are released from the

plant and into the atmosphere. It must be expected that a fraction of the pollen production never leaves the plant.

We have replaced Figures 4 and 5 to present results of modeling taking into consideration dry deposition of particles, updated numbers about fraction of particles reaching trajectory height range in Table 1 and updated the methodology (page 6, line 19-24 - page 7, line 1-6):

Particle dispersion calculations were carried out with the HYSPLIT_4 model in order to determine whether atmospheric conditions during the studied episode would have allowed Ambrosia pollen to reach high altitude air masses after release in the source areas and to settle down in Leiden and Leicester following atmospheric transport.

In order to verify whether airborne Ambrosia pollen released in the source area could reach the altitudinal range of back trajectories arriving at Leiden, the model was set to release 2500 particles of 20µm at 15m above the ground each hour from 6-12h, which corresponds to the most intensive period of Ambrosia pollen release (Barnes et al., 2001; Martin et al., 2010). Sedimentation processes are accounted for in the model by setting the settling velocity of the particles to 0.0156m/s which corresponds to the settling velocity of Ambrosia artemisiifolia pollen grains (Raynor et al., 1970), and applying the conversion module that deposits each particle rather than reduce their mass.

and in the result section, page 10, line 1-5:

After release from heavily infested source areas on the Pannonian Plain on 2-3 September (6-12h), an average of 36.1% of the particles remaining airborne reached between 316.3 – 3624.7m, which is the altitudinal range of back trajectories arriving at Leiden at the same time when the Ambrosia pollen were recorded (Table 1).

and page 10, lines 21-25 – page 11, line 1,2

The particle cloud tended to go south on 3 September, but on the 4 September the particles reaching the higher levels went northward (Fig. 5). From particles remaining airborne after release, 2.5% reached between 1471.8 - 2482.1 m, which is the altitudinal range of back trajectories arriving at Leiden when the pollen grains were recorded in the trap (Table 1). Calculations of particle concentration distribution carried out on the 3-4 September 2014 confirmed that Ambrosia pollen grains could have reached sufficiently high above the ground to enter into the air stream moving towards Leiden (Fig. 5, Table 1).

Secondly, the authors did not explain how the particles go down from 1.5km towards the pollen traps in the Netherlands and the UK. They also mention Polish sites but again referred to 1.5km altitude as the transport level. How do these connect with ground observations?

Reply: When interpreting the mechanisms of pollen transport, previous studies using back trajectory analysis assumed that (if pollen is present in the air masses travelling over the area where pollen is recorded) sedimentation processes would have been sufficient to bring some of it down as soon as deposition outweighs the lift from the upward movement of the air (Sikoparija et al., 2013). In this study, we have also assumed that physical conditions in the atmosphere would have allowed some pollen carried in the air masses to be deposited in Leiden and

Leicester where it has been recorded by atmospheric sampler. In order to strengthen this assumption, we have run the HYSPLIT_4 dispersion model set to deal with particles released at the path of trajectories travelling from the Pannonian Plain towards Leiden. The results confirm that particles, having the characteristics of ragweed pollen grains (i.e. 20µm diameter and 0.0156 m/s settling velocity), could have reached low altitudes in the Netherlands and the UK. We have therefore altered the text in the Material and methods (page 7, line 13-25) and added extra tables (Suppl table 1 and 2) to the Supplement.

Simulations of particle deposition using the HYSPLIT_4 were conducted again in order to determine whether Ambrosia pollen traveling at the height of airmasses (as described by back trajectories) could settle out from the atmosphere to reach ground level monitoring sites. The dispersion model was set to run so that the released particles arrived at Leicester and Leiden at 12-14h on 5 September, which was the time when the highest Ambrosia pollen concentrations were recorded (Table 1). The emission points were selected based on the results of trajectory analysis (9 points for each trajectory in the cluster). Particles were released at an altitude of ~1500m in the path of the air masses travelling to Leicester (Suppl. Table 1) and Leiden (Suppl. Table 2). The model was set to release 500 particles per hour for 8 hours (until the end of the period when the highest bihourly Ambrosia pollen concentrations were recorded). The total amount of released particles corresponds to approximately 20% of the pollen (19316 P m⁻³) that reached the altitudinal range of air masses that passed over the Pannonian Plain on the way to Leiden.

We also added Fig.6 and accompanying text in the Result and Discussion (page 11, lines 15-18)

HYSPLIT_4 simulations of particle deposition from the high altitude air masses, before they reached Leicester and Leiden, confirm that atmospheric conditions would have allowed for the deposition of airborne Ambrosia pollen to ground level in areas where surface pollen measurements took place (Fig. 6).

My second comment refers to deficiency of the review in the introduction and the claim that Ukrainian inventory is non-existent. In fact, it exists, published in 2013 in Agriculture and forest meteorology by Prank et al with a reference to a European project on Ambrosia distribution and transport in past and future climate. The project has a thick and detailed publicly available report by Bullock et al, (2012). I was highly surprised not to see any trace of that project and the paper in the author's review and analysis. As of now, the review is incomplete and the information given in the analysis is plainly incorrect.

Reply: The reviewer mentions a deficiency in the introduction with regards to the inventory of Ukraine. In the introduction we mention that Ukraine is one of the most infested areas in Europe and provide now six references (page 3, lines 11). Furthermore, the reviewer questions our claim that the Ukrainian inventory is non-existent. To our knowledge, it is true that a detailed inventory for *Ambrosia* pollen sources for Ukraine, like the pollen source inventories that are available for

the Pannonian Plain and the Rhone valley, does not exist. We have emphasized this point in the text page 10, lines 8-11.

The reviewer suggests 2 papers in this respect. First the paper of Prank et al. 2013 (which is included in the references in the revised MS) supports the statement we made because it mentions "...the distribution data from some of the major pollen sources in Ukraine and Russia are very incomplete, which hindered the modelling of the invasion in these regions". The reviewer also refers to the Bullock report, which was not published in a peer-reviewed scientific journal. The distribution maps within the report are misleading. For example, the map of *Ambrosia* show that the Netherlands is as infested as the Pannonian Plain, when in reality this is not the case and is most likely due to the high incidence of observations (citizen science network) of single plants in back gardens, resulting in the presence of *Ambrosia* in every 10x10km grid in this densely populated country. The number of larger established populations is limited in the NL. Furthermore, the Bullock report shows the Ukraine to be empty of *Ambrosia*. For these reasons the distribution maps from the Bullock report were not used in this manuscript.

Specific comments

Key words: "HYSPLIT" is an empty one. Nobody will be able to use it to locate this paper. Also, "invasive alien species" is not up to the topic of the paper, which actually shows that it is not the invasion but rather atmospheric transport that created the case.

Reply: We deleted HYSPLIT and invasive alien species as keywords and added instead "back trajectory analysis", "atmospheric movement" and "Pannonian Plain"

P.3, line 14 and elsewhere in the introduction section: the reference to the public report Bullock et al and paper by Prank et al should be included.

Reply: The reference of Prank et al is included. However, for the reasons stated above we declined to add reference to Bullock's report to the manuscript.

Introduction section: it is worth mentioning that ambrosia seeds are constantly being introduced in Europe together with seed/weed/corn imports - and this is one of the reasons why big international cargo hubs (including e.g. Rotterdam) are the areas with comparatively strong ambrosia presence.

Reply: This comment has been added to the manuscript as suggested (page 3, lines 18-20), which now reads "*Ambrosia seeds are constantly being introduced into Europe via imported grain and animal fodder. Resulting in areas around entry points, such as harbours or airports, being heavily infested by Ambrosia*".

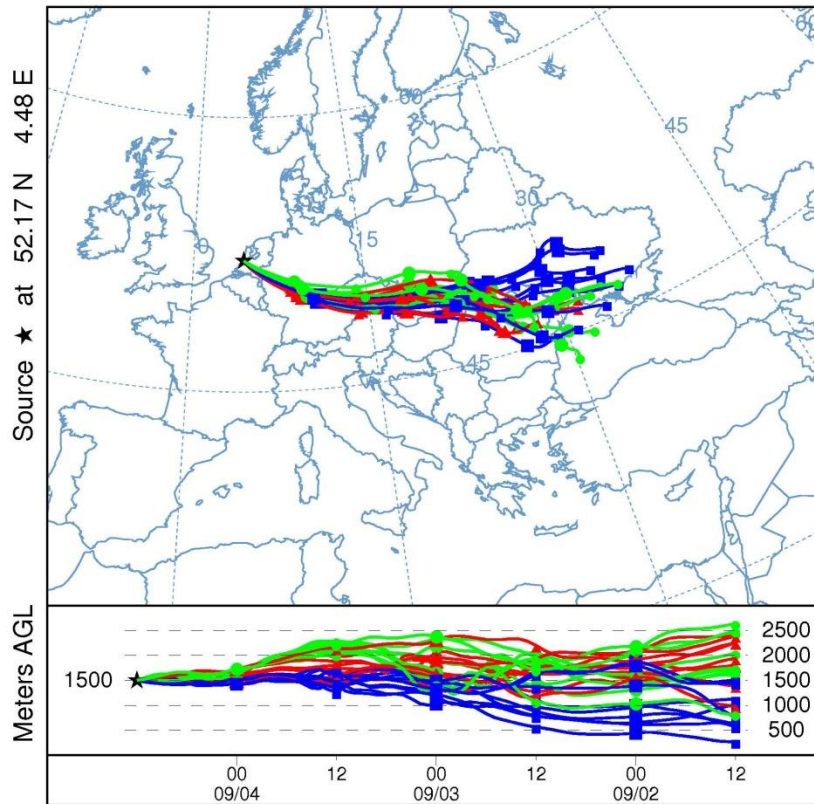
P.5, l.19. What trajectories were used? As it is written in the paper, they were 2D constant-height trajectories but I sincerely hope that this was not the case. It has been over half a century since shown that such trajectories, as well as the isobaric ones, can go to a completely wrong direction in some weather conditions.

Reply: We have used three-dimensional trajectories that are known to be more accurate than any other type of trajectories, including isentropic ones (Stohl and Seibert, 1998). Each trajectory data point, besides geographical coordinates, contains altitude which is used to analyse possible interception between air masses moving towards Leiden/Leicester and particles released over major source regions. In order to make it clearer we altered the text in the Material and methods (page 6, line 2): “**Three dimensional** back trajectories were calculated 72h back in time,

P.6, l.6. With the input data of 1 degree resolution, what is the use of shifting the trajectory starting point to half a degree? The results are then completely determined by the interpolation algorithm. One has to shift by a degree. That calculation has to be redone.

Reply: The wind speed and directions are always interpolated to the exact location of the starting point of the trajectory. This interpolation also occurs for each of the individual trajectory points. In the horizontal direction this always requires input from 4 different grid points. A consequence is that trajectories from two nearby starting points (e.g. within 0.5 degree grid distance) in general will not have the same direction and path. The reason is that the wind speeds are only defined at the centre of a grid cell. An exception for this rule is that if a trajectory point exactly matches the centre of one grid cell. It should be noted that this is a very rare case. In order to confirm that the approach that we applied (Skjoth et al 2007) did not affect the robustness of the methodology, we have calculated a cluster of 72h back-trajectories from Leiden starting 4 September (12h when a notably high ragweed pollen concentration is recorded) at 1500m above the ground level. We have used default HYSPLIT settings for the cluster calculation which results in calculation of 27 trajectories by offsetting end point in 1 degree in horizontal (as requested by the reviewer) and 250m in vertical direction (see figure below). The results confirmed low variation in the transport path for all trajectories calculated within the cluster. It should also be noted, that the cluster methodology since 2012 has been a standard methodology in the on-line version of the HYSPLIT model.

NOAA HYSPLIT MODEL
 Backward trajectories ending at 1200 UTC 04 Sep 14
 GDAS Meteorological Data



P.6, l.11-16. *There seems to be the same message repeated twice with minor alterations.*

Reply: The reviewer is right and this had been adapted

P.7, l.4-5. *I recall a second peak of ambrosia emission in the afternoon, which would need just ~10 hours of travel time to reach the trap in the morning. That would correspond to barely 500km distance from the source and suggest much closer areas as a possible origin (providing that they have ragweed emission).*

Reply: It is not clear to which ‘second peak of *Ambrosia* emission in the afternoon’ the reviewer is referring to. If this is the peak caused by emission facilitated by pistilodium growth (Martin et al., 2010*) then although the pollen emission is extended showing bi-modal character, the majority of pollen is again released before 10am. On the other hand, if the reviewer refers to the second peak driven by resuspension of pollen sediment on leaves and local vegetation after morning emission it would still be notably lower so pollen released in the morning hours would predominate pollen transported over large distances. In this paper we have not argued against the possibility that pollen emitted in the areas closer to Leiden could contribute the records in Leiden and Leicester but this would require notable ragweed pollen sources which would result in more

frequent episodes like this one. It is important to stress here that the wind speeds in the high altitude masses are generally much higher than near the surface. This is clearly seen on the figure above by using the markers. Six hours back the air mass is over central Germany and 12 hours back the air mass is mainly over the Czech Republic reaching as far Slovakia and the most North-Eastern parts of Austria. This suggests that within 12 hours, air masses from the Pannonian Plain can travel all the way to the Netherlands, thus more than 1000km. Furthermore, to our knowledge there are no known highly infested *Ambrosia* areas on the route of the air masses, which could act as a source.

**Martin MD, Chamecki M, Brush GS. Anthesis synchronization and floral morphology determine diurnal patterns of ragweed pollen dispersal. AgricForMeteorol 2010;150:1307–17*

P.9, l.5. “). Unfortunately, an inventory for Ambrosia pollen sources does not exist for Ukraine and so the analysis could not be repeated for this area. “

This is wrong. See general comments

Reply: We mention here an inventory of the sources on ground level that produce the pollen. This has been calculated extensively for the Pannonian plain and the Rhone valley, by making use of the many data that were available for that areas. (Skoth et al. 2010;Thibaudon, et al, 2014). For the Ukraine such detailed data are not available. In the latest assessment(Skjoth et al, 2013) Ukraine had only two observational stations compared to roughly 50 in three neighbor countries Poland, Slovakia and Hungary, thus limiting the possibilities for inventories based on pollen data. It is possible to integrate other sets of data in the Ukrainian area. Observations of pollen using other sampling methods (i.e. not from a Hirst type trap) are available for sites like Zaporosje, but this adds uncertainty and would require new analytical methods to be developed for creating inventories.

P.9, l.9 onwards. This is a shaky statement: the input meteorology is 100km resolution, i.e. the authors discuss the loop made by the trajectories of barely 2-3 meteorological grid cells in diameter. Worse, the topography of Rhone Valley is also resolved with 100km step, i.e. all but missed. As a result, uncertainty of this loop is huge and it should be said explicitly. Same refers to conclusions. In fact, I would not rush in Rhone Valley as a contributor, rather point out at a possibility for this indicated by the (very uncertain) results.

Reply: We agree with the reviewer that the pollen from the Rhône Valley will contribute only to a minor degree and that the uncertainty of the trajectories must be taken into account and that topographical driven meteorology in the Rhone Valley cannot be resolved with this input data. In the manuscript we have been careful in phrasing the role of the Rhône Valley in the origin of the pollen recorded in Leiden en Leicester. (e.g. page 13, lines 12-14: “we have identified the Rhône Valley in France as being a potential source of *Ambrosia* pollen in Northwest Europe, albeit only a minor contributor compared to the Pannonian Plain”).

As suggested by the reviewer we have added a comment to the manuscript that specifically address the issues around the Rhône Valley (pages 11, lines 8-14) . In addition, the uncertainty resulting from orographically forced meteorology within the Rhône Valley cannot be resolved with the default HYSPLIT input data. Focused studies in such a region requires much more detailed data, e.g. from the Weather Research and Forecast model as described by Hernandez-Ceballos et al (2014). This suggests that the Pannonian Plain should still be considered to be the main source of the LTD Ambrosia pollen (Table 1)

Figures. The light grey colored trajectories are indistinguishable.

Reply: The figures have been altered as suggested.

Replies to the comments of Reviewer #2

Analysis of long-range pollen transport based on individual back-trajectories should be considered with reservations due to uncertainties in determining trajectories. Trajectory position error is typically about 20% of the traversed distance (Stohl, 1998). However, the statistical uncertainty will be substantially reduced when using a large number of back-trajectories.

Reply: We are aware of the uncertainties of the trajectory analysis when the study relies on just single trajectories. Therefore we have used a cluster of trajectories where the receptor points are 0.5 degree apart, thus 9 trajectories for each hour and for each height. This method was for the first time used in aerobiology by Stach et al (2007) and has been used with success a number of times. Since the entire series of trajectories result in a similar path it is likely that the air masses have moved to Western Europe.

If next time, or in a 2nd part of this research you decide to analyze a relationship of locally measured daily pollen levels vs long-range pollen transport, then I encourage you taking for example the daily pollen levels exceeding the upper quartile and you can associate these days with long-range pollen transport. In this way you can get quite lots of days fulfilling the above requirement for an e.g. 5-year or maybe longer data set. If you have a sufficiently large number of days then you can use cluster analysis and the reliability of the results using cluster analysis increases with increasing number of the backward trajectories (Stohl, 1998; Borge et al., 2007).

Reply: This is indeed a very nice suggestion. In the Leiden *Ambrosia* pollen are present in the dataset since 1992 and in the past 23 years 3 years have counts barely above the upper quartile (2006, 1997 and 1996) and only one year 1996 had counts comparable to our study year 2014. These annual counts until 2008 have been published and cited in the manuscript (de Weger et al. 2009).

The researchers at Leicester have pollen datasets for their region of the UK that date back to 1970. Of these only one year does not have data generated during the *Ambrosia* season, resulting in 45 years (including 2015) of data for *Ambrosia*. Ragweed pollen has been observed during 8 of the 45 years for which data is available. Other than 2014 (the subject of this study), the maximum daily average concentration on any given day, or in any given year, was 5 (To clarify, in one year 5 grains were detected in a day, but no pollen on other days that year. In 2 years a peak of 2 grains were detected in a day (which includes 2015), and in the other 4 years counts never went above one grain). This means, whilst theoretically interesting, it would be impossible to perform the sort of analysis the reviewer suggests, and highlights the uniqueness of the situation that arose in 2014. Other than the data from 2015, this data has already been published and the study cited in our manuscript (Pashley et al 2015).

Selecting HYSPLIT transport and dispersion model by the authors is a good choice, since it is the most widely used technique in the international special literature for back-trajectory analysis. A great advantage of this methodology is that it allows obtaining three-dimensional back-trajectories. It is also important to know that three-dimensional

trajectories are more accurate than any other type of trajectories, including isentropic ones (Stohl and Seibert, 1998).

Reply: We agree with the reviewer and can confirm that three-dimensional trajectories were used in this study, and as mentioned above we have now clarified this in the methods section of the manuscript. (page 6, line 2 and 7-8)

Section Introduction you are asked to insert references in paragraph 1, as follows: if you can insert here references from Ukrainian and Russian authors on ragweed pollen infestation in their country, it would be nice

Reply: References for the Ukraine (Podberezko et al., 2013) and Russia (Reznik 2003) have been added as requested (page 3, line 11).

Section Introduction seems a bit short for me. Though the literature of Ambrosia pollen related long distance transport is not so large, however very few papers are cited here. I suggest you look through the table below we compiled (Makra et al., 2015) and you can cite references from there. This table is completed with the here-mentioned paper (Makra et al., 2015) and your paper (de Weger et al., 2015), both in blue, as well. This table, to my knowledge, is the most complete summary comprising papers on long-distance transport of ragweed pollen in the international literature.

Reply: It was our intention to write a concise paper with a relevant message, following the journals guidelines for authors which state that “The introduction should state the purpose of the investigation and give a short review of the pertinent literature.” We appreciate the help of the reviewer by providing a nice overview of the studies on long distance transport of *Ambrosia*. Following the wishes of the reviewer, we have added some additional references to our introduction (e.g. page 4, line 9-10), which improves the paper without making it too long.

Section Introduction, paragraph 2: Climate in the Netherland and in the southern part of the UK (with higher occurrence of ragweed than in the north) is the same oceanic. Comprise these conditions the minimum for ripening seeds or all small habitats are originating from bird seeds? Which climate conditions comprise the minimum for ripening seeds there? According to the models, by which decade of this century will be these areas favourable to keep original ragweed habitats? I think these are interesting questions and are worth to explicate since they may draw the attention of a wider audience.

Reply: We agree that it is important to consider climate change predictions and as such a new piece of text has been added on this issue in the introduction (page 4, lines 18-24) and now reads “The climatic conditions in these countries are not currently favourable for fulfilling the full life cycle of *Ambrosia*. The late flowering of the plant combined with the early dates of the first frosts in autumn prevent the *Ambrosia* seeds from ripening. However, future climate scenarios for the Netherlands (Klein Tank et al., 2014) and for Europe (Storkey et al 2014) have suggested that *Ambrosia* could spread and persistent as far north as central England by the year 2050, with areas where *Ambrosia* populations are currently classed as casual becoming established”.

I think, it would be useful to represent a figure on the daily ragweed pollen concentrations of both stations for the study year.

Reply: A figure of the daily *Ambrosia* pollen counts in Leiden en Leicester is added to the Supplement (Suppl Figure1).

If you find the upper quartile of the daily Ambrosia pollen concentrations for each target station, days characterized with pollen levels exceeding the upper quartile are with great chance loaded by long-distance transported pollen. These days may have also of interest with a serious load concerning the object of the paper.

Reply: As discussed above there are too few days with enough pollen in the upper quartile of daily *Ambrosia* pollen concentrations for a study of this nature.

It would be nice if, in association with Fig. 1 of the manuscript, the authors showed a table comprising pollen concentrations at the ten stations considered on the study days, by indicating those days, pollen concentrations of which exceed the upper quartile.

Reply: Since we did not have the pollen counts of the previous years for all these stations, it is not possible to calculate the upper quartile calculations for main sites and so it is considered to be out of the scope of this present study.

Page 6, line 19; and page 7, line 12; correctly: “Skjøth et al. (2010)”, instead of “Skjoth et al. (2010)”;

Reply: This has been changed throughout the manuscript

2.3. Back-trajectory analysis

*Back-trajectory calculations were conducted using the HYSPLIT_4 (HYbrid Single-Particle Lagrangian Integrated Trajectory) model [Draxler et al., 2009]. Back trajectories were calculated 72h back in time, at five heights above ground level (200m, 500m, 1000m, 1500m and 2000m), for bi-hourly periods corresponding to pollen records in Leicester and Leiden on 4 and 5 September 2014. Trajectory calculations involve an amount of uncertainty, and this uncertainty increases exponentially with time. **This is an important drawback of using individual back-trajectories. Here you can refer to Stohl (1998), as well as Stohl and Seibert (1998) and write more about uncertainties and besides write more about the advantage of using 3D back-trajectories (see my comments above).***

Reply: We agree with the reviewer on this. Earlier studies such as Stach et al (2007) and Skjøth et al (2008) used 2D trajectories and terrain following coordinates. These have some clear limitations. We therefore use 3D trajectories and clusters of trajectories. We therefore suggest to add the following new section on page 11, line 19-24 – page 12, line 1-16.

Several aspects of back trajectories are limited in respect to analysing air mass patterns. Earlier *Ambrosia* studies by Stach et al (2007) and Sikoparija et al (2009) used the Danish ACDEP model to calculate trajectories (Skjøth et al, 2002). This was a 2D trajectory model where the air masses followed the σ –level 0.925 wind vectors and 0.25° meteorological input. This approach (e.g. terrain following coordinates or isobaric

coordinates) is conceptually simpler, but it neglects the vertical wind component (Stohl et al, 1998), which means that errors in the calculation of 2D trajectories can accumulate faster with transport distance than for 3D trajectories. Current practice is therefore to use 3D trajectories, most commonly in relation to Ambrosia by using the HYSPLIT model (e.g. Makra et al. (2010), Saulienè et al. (2011), Zemmer et al. (2012) and recently Sommer et al. (2015)). Spatial and temporal resolution in the input data is, however, also very important as demonstrated by Skjoth et al. (2002) and Hernandez-Ceballos et al. (2014). These studies suggest coastal effects and complex terrain often affects the meteorology on scales that are relevant for pollen transport and more detailed input to HYSPLIT or ACDEP provided substantially better output data, thus improving the analytical results. The effect on spatial and temporal resolution, however, depends on the atmospheric physics during the pollen episodes. Simulations of large scale flows will generally be less affected by increased resolution. Conversely, simulations of frontal zones, convective zones and orographic forces flow will be heavily affected (e.g. Hernandez-Ceballos et al, 2014). In our case, there are generally large scale flows over the Pannonian Plain towards Leiden, while the flow in the Rhône Valley could be affected by complex terrain. As such, the findings relating to the Rhône Valley are uncertain due to limitations in resolving complex flows in this area.

Therefore, to account for this uncertainty, ensembles based on nine trajectories with receptor points placed 0.5 degrees apart were calculated. Trajectories of the ensemble will be closely related until the trajectories reach a certain area, where even small variations in meteorology will create large variations in the transport path of the individual trajectories. All calculated trajectories examined in this study showed little variation with respect to transport path [Stach et al., 2007]. Would you please explicate how you calculated ensembles on nine trajectories. What is the error of the ensemble trajectory? Was their reliability with statistical tools?

Reply: This is an error in our formulation. Ensemble modelling is a specific technique in atmospheric science, which we have not used here. We have replaced the word ensemble with the correct word cluster throughout the article.

Table 1: As I concluded from the text, the given ratio in % of Ambrosia pollen released from the Pannonian Plain reached the altitudinal range of back-trajectories arriving at Leiden at the time when Ambrosia pollen was recorded there. If this is the case please clarify the title of the Table 1 and its heading.

Reply: The title of this table has been clarified.

Page 8, lines 21-22: you write here: “The calculations were made using the Ambrosia pollen source inventory produced by Skjøth et al. [2010] (Fig.1).” I think you would raise the level of the manuscript if you wrote an example here clearly, using the methodology you mention here.

Reply: On page 7, line 11-13 it is clarified that the study of Skjøth et al. 2010 and Thibaudon et al. 2014 identify the locations where the most important source areas for Ambrosia pollen are on the Pannonian Plain and in France. A concise

explanation of the methodology used in these paper is added here. (page 7, line 9-12).

These source areas had been identified by the use of detailed knowledge of Ambrosia ecology, land cover information and spatial variations in the annual sum of atmospheric Ambrosia pollen concentrations.

Page 10, lines 1-5: You write here: “The Rhône Valley is a known centre of Ambrosiain Europe, and is closer to Leiden and Leicester than the Pannonian Plain, how ever this study has shown that under the seconditions only a fraction of pollen releasedfrom France reached Northwest Europe; which suggests that the Pannonian Plain should still be consideredto be the main source of LDT Ambrosiapollen (Table 1).” Table 1 should be inserted here. Really, there is pollen transporton over Leiden. However, it seems very-very low. Can it be with in the error limit? Did you perform such kinds of calculations?

Reply: “Suppl Table 3” (formerly Table 1) has been inserted on page 10, line 17.

In response to a previous question by this reviewer on the uncertainty of back trajectory analysis (see page 11 of this reply) we added a new section to the manuscript page 11, line 19-24 – page 12, line 1-16. This section also considers the errors in the HYSPLIT calculations. We did not do calculations on the error limit.

Table 1 is constructed for Leiden, Netherlands. It would be nice if you inserted another table 2 for Leicester, UK.

Reply:The back trajectories arriving at Leicester mainly pass over Ukraine, rather than the Pannonian Plain (page 8,line10-11). Since we donot have the detailed source inventory for Ukraine we could not make this calculation.

Page 10, lines 7-10: “Threshold values required for Ambrosia pollen to induce symptoms differ among different studies, ranging from 1-3 P m-3 for “first symptoms to start” to 50 P m-3 for “60-80% of the sensitized patients to show symptoms”([de Weger et al., 2012] and references therein).” This bold part is not precise and not scientific. I suggest to list other references here as de Weger et al. (2012). I suggest here (Déchamp et al. (1997) indicating the significance of sensitivity against very low (1-5 pollen grains □ m-3□day-1) ragweed pollen concentrations. Even, they (Déchamp et al. (1997) established different sensitivity categories within these thresholds.

Reply: We agree with the reviewer that this reference of Déchamp et al. is a relevant paper. It has been included (page 12, line 23).

Suggestions for corrections in the Reference list

In Essl et al. (2015) correctly “Biró” instead of “Biro”;

In Kasprzyk et al. (2011) correctly “Grewling Ł,” instead of “Grewling L,”;

Makra et al. (2004) correctly:

Makra L, Juhász M, Borsos E, and Béczi R. (2004) Meteorological variables connected with airborne ragweed pollen in Southern Hungary. Int J Biometeorol 49:37-47.

Šikoparija et al. (2013) correctly:

Šikoparija B, Skjøth CA, AlmKübler K, Dahl A, Sommer J, Grewling L, Radišić P, Smith M. (2013) A mechanism for long distance transport of Ambrosia pollen from the Pannonian Plain. Agr Forest Meteorol 180:112-117.

Skjøth et al. (2010) correctly:

Reply: We are grateful for the thorough reading of the manuscript by the reviewer. All these name errors have been changed.

Finally we noticed a mistake ourselves in the Supplementary Table in the longitudinal coordinate of Leicester. This has been changed.