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Ambrosia pollen source inventory for Italy: A multi-purpose tool to assess the impact of the ragweed leaf beetle (*Ophraella communa* LeSage) on populations of its host plant --Manuscript Draft--

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Abstract:	<p>Background: Here we produce Ambrosia pollen source inventories for Italy that focuses on the periods before and after the accidental introduction of the <i>Ophraella communa</i> beetle.</p> <p>Methods: The inventory uses the top-down approach that combines the annual Ambrosia pollen index from a number of monitoring stations in the source region as well as Ambrosia ecology, local knowledge of Ambrosia infestation and detailed land cover information. The final inventory is gridded to a 5 x 5 km resolution using a stereographic projection.</p> <p>Results: The sites with the highest European Infection levels were recorded in the north of Italy at Busto Arsizio (VA3) (European Infection level 2003-2014 = 52.1) and Magenta (MI7) (European Infection level 2003-2014 = 51.3), whereas the sites with the lowest (i.e. around 0.0) were generally located to the south of the country. Analysis showed that the European Infection level in all of Italy was significantly lower in 2013-14 compared to 2003-12, and this decrease was even more pronounced at the sites in the area where <i>Ophraella communa</i> was distributed. Cross-validations show that the sensitivity to the inclusion of stations is typically below 1% (for two thirds of the stations) and that the station Magenta (MI7) had the largest impact compared to all other stations.</p> <p>Discussion: This is the first time that pollen source inventories from different temporal periods have been compared in this way, and has implications for simulating interannual variations in pollen emission as well as evaluating the management of anemophilous plants like <i>Ambrosia artemisiifolia</i>.</p>	

Dear Editor,

We are thankful to reviewers and the editors for positive decision.

The noted repetition on the page 6: "*Ambrosia* is therefore able to spread quickly and establish populations in habitats associated with frequent and extensive disturbance regimes that primarily result from human activities. " is deleted from the text.

Also, affiliation number 1: *Department of Medical Prevention, Local Health Authority ATS Milano – Città Metropolitana, Parabiago (MI), Italy*, changed name so we have corrected this affiliation into:

"Department of Hygiene and Health Prevention, Local Health Authority ATS della Città Metropolitana di Milano, Parabiago (Mi), Italy "

On the behalf of all authors.

Branko Sikoparija

1 **Abstract**

2

3 **Background:** Here we produce *Ambrosia* pollen source inventories for Italy that focuses on the
4 periods before and after the accidental introduction of the *Ophraella communa* beetle.

5 **Methods:** The inventory uses the top-down approach that combines the annual *Ambrosia* pollen
6 index from a number of monitoring stations in the source region as well as *Ambrosia* ecology, local
7 knowledge of *Ambrosia* infestation and detailed land cover information. The final inventory is
8 gridded to a 5 x 5 km resolution using a stereographic projection.

9 **Results:** The sites with the highest European Infection levels were recorded in the north of Italy at
10 Busto Arsizio (VA3) (European Infection level 2003-2014 = 52.1) and Magenta (MI7) (European
11 Infection level 2003-2014 = 51.3), whereas the sites with the lowest (i.e. around 0.0) were generally
12 located to the south of the country. Analysis showed that the European Infection level in all of Italy
13 was significantly lower in 2013-14 compared to 2003-12, and this decrease was even more
14 pronounced at the sites in the area where *Ophraella communa* was distributed. Cross-validations
15 show that the sensitivity to the inclusion of stations is typically below 1% (for two thirds of the
16 stations) and that the station Magenta (MI7) had the largest impact compared to all other stations.

17 **Discussion:** This is the first time that pollen source inventories from different temporal periods
18 have been compared in this way, and has implications for simulating interannual variations in
19 pollen emission as well as evaluating the management of anemophilous plants like *Ambrosia*
20 *artemisiifolia*.

21

22 **Keywords:** aerobiology; atmosphere-biosphere analysis; digital elevation model; ecosystem
23 analysis; invasive weed; species distribution maps

24

25

1 **1. Introduction**

2

3 According to the Euro+Med Plantbase (Greuter, 2006+), which integrates and critically evaluates
4 information from Flora Europaea, Med-Checklist, the Flora of Macaronesia, regional and national
5 floras and checklists as well as additional taxonomic and floristic literature, there are six accepted
6 *Ambrosia* species in Europe: *A. artemisiifolia* L., *A. confertiflora* DC., *A. maritima* L., *A.*
7 *psilostachya* DC., *A. tenuifolia* Spreng., and *A. trifida* L. Of these, the invasive *A. artemisiifolia*
8 (common or short ragweed) has a plasticity (Ortmans et al., 2016), anemophilous pollination
9 strategy, and allergenicity that have made it a prominent agricultural weed and threat to
10 environmental health in Europe (Smith et al. (2013), Essl et al. (2015) and references therein).

11 The occurrence of *Ambrosia artemisiifolia* in a particular region is strongly related to the
12 length of time passed since its introduction (Chauvel et al., 2006). This study focuses on Italy where
13 there is a long history of *A. artemisiifolia*. In Piedmont, the plant was documented in the Botanical
14 Garden of the University of Torino as far back as 1772 (Allioni, 1773.), and this is probably one of
15 the oldest records of the species in Europe. Herbarium specimens in the University of Torino (HP-
16 TO) dated 1902 (Alba) and 1910 (Torino) are an indication of when it became naturalised (Carna
17 and Ivaldi, 2014). The Po Valley in Northern Italy is one of the main centres of *A. artemisiifolia* in
18 Europe. In particular, the North-West of the Province of Milan has been colonized by *A.*
19 *artemisiifolia* since the 1940s (Stucchi, 1942) and the plant is now widely distributed over the area
20 (Gentili et al., 2016). The other centres of *Ambrosia* in Europe are the Rhône-Alps region of France,
21 the Pannonian Plain and Ukraine (Smith et al. (2013), Essl et al. (2015) and references therein).

22 The prevalence of sensitisation and allergy to *Ambrosia* pollen has increased in Northern
23 Italy since the last decade of the 20th Century (Bonini et al., 2009; Carna and Ivaldi, 2014), and
24 *Ambrosia* pollen has also been recorded recently in central and southern parts of Italy (Sikoparija et
25 al., 2016). This is expected to be followed by an increase in the distribution of *Ambrosia* pollen
26 allergy in the country because of the time lag between sensitization and allergy (Tosi et al., 2011).

1 For instance, Albertini et al. (2012) found a significant increase in the number of polysensitized
2 patients with positive SPT to ragweed between 1992 and 2008 and, among these, a noticeable
3 increase in asthma symptoms.

4 In 2013, the oligophagous leaf beetle *Ophraella communa* LeSage, which is known to feed
5 on *Ambrosia artemisiifolia*, was found in Southern Switzerland and Northern Italy (Müller-Schärer
6 et al., 2014). This was followed by exceptionally low amounts of airborne *Ambrosia* pollen
7 observed in the Milan area in 2013 and 2014 which could not be explained by meteorology alone
8 (Bonini et al., 2015; Bonini et al., 2016). The beetle is successfully used as a biological agent to
9 control this weed in China (Zhou et al., 2014) and the establishment of large and stable populations
10 of this insect can potentially limit the production of pollen and seeds in infested plants (Guo et al.,
11 2011). It is likely that the establishment of the insect in Italy has a substantial and geographically
12 large impact on *Ambrosia* populations. A thorough assessment is important for understanding the
13 mechanisms involved and of the spread of this insect in Europe and how this can affect pollen
14 production in affected areas. Knowledge about future pollen loads is critical for producing accurate
15 forecasts of airborne *Ambrosia* pollen concentrations (Zink et al., 2016) and understanding
16 subsequent impact on human health.

17 This study aims to produce *Ambrosia* pollen source inventories for Italy, following the
18 protocol used previous for other countries in Europe (Skjøth et al., 2010; Thibaudon et al., 2014;
19 Karrer et al., 2015), and focuses on the period before and after the *Ophraella communa* beetle was
20 recorded in high numbers in Italy. These inventories can be used as an input for atmospheric
21 dispersion models, and have the potential to be used for examining the possible impact of *Ophraella*
22 *communa* on populations of its host plant in Northern Italy.

23

24 **2. Materials and methods**

25

1 The *Ambrosia* pollen source inventory for *Ambrosia* in Italy uses the top-down approach previously
2 described in literature (Skjøth et al., 2010; Thibaudon et al., 2014; Karrer et al., 2015). This
3 approach combines the annual *Ambrosia* pollen index from a number of monitoring stations in the
4 source region (Section 2.1) as well as *Ambrosia* ecology, local knowledge of *Ambrosia* infestation
5 and detailed land cover information (Section 2.2). The final inventory is gridded to a 5 x 5 km
6 resolution using a stereographic projection used within the European Monitoring and Evaluation
7 Programme (EMEP) and applied by numerical weather prediction models such as EMEP and
8 DEHM (Section 2.3).

9

10 2.1 *Ambrosia* pollen data

11

12 Annual *Ambrosia* pollen indices were obtained from 91 pollen-monitoring stations (2003-2014)
13 (Fig. 1a-b; Table 1) belonging to POLLnet, the Italian Network of the Environmental Protection
14 Agencies, and the Italian Monitoring Network in Aerobiology (R.I.M.A.[®]) of the Italian
15 Aerobiology Association (AIA). *Ambrosia* pollen data were collected using volumetric spore traps
16 of the Hirst design (Hirst, 1952). Sampling and analysis followed the standard methods of A.I.A.
17 R.I.M.A.[®] (2009). The sum of daily averages of *Ambrosia* pollen recorded annually is expressed as
18 annual pollen indexes (pollen grains) (Comtois, 1998). Of the 91 pollen-monitoring sites initially
19 examined for the entire period, 89 sites provided the data for the period 2003-12 (Fig. 1c) and 76
20 sites for the period 2013-14 (Fig. 1d).

21

22 2.2 Determining *Ambrosia* habitats in Italy

23

24 All potential *Ambrosia* habitats in Italy are identified using Corine Land Cover (EC, 2005),
25 specifically the 2000 version. The 2000 version is expected to have the best coverage of the entire
26 observational period (2003-14) in this study, while the next major update of this land cover data set

1 (CLC2012) is considered to be better suited to observational records starting after 2011. These
2 habitats were then filtered using local knowledge about *Ambrosia* ecology (Bonini, 2014) and
3 knowledge about the habitats with major invasion (i.e. the plant tends to grow in any habitat if there
4 are available seeds and soil disturbance and able to spread quickly and establish populations in
5 habitats associated with frequent and extensive disturbance regimes that primarily result from
6 human activities). The advantage of using local knowledge is that it includes an assessment of
7 which local habitats that contain the main infestation, and that this assessment can be performed at
8 the subnational scale (Karrer et al., 2015). Furthermore, the filter includes, local management of the
9 agricultural landscape as methods (e.g. grass coverage or soil disturbance) in handling specific
10 landscapes will either promote or limit *Ambrosia* growth. As an example, vineyards are considered
11 a source of *Ambrosia* pollen in eastern parts of Austria but not the western parts (Karrer et al., 2015).
12 In Italy, most agricultural fields are potential sources of *Ambrosia* with the exception of rice fields
13 where permanent flooding dramatically reduces weeds (Ferrero and Nguyen, 2004). Finally, a
14 digital elevation model (DEM) with 90 m resolution (<http://srtm.csi.cgiar.org>) is used as an
15 additional elevation filter. This filter was used by Karrer et al. (2015) with observations from Essl et
16 al. (2009) that cover the Alpine region towards Italy (Fig. 2). The observations from Essl et al.
17 (2009) provide the upwards limit of the major ragweed ragweed infestation, currently 745 m, which
18 is equal to or lower than the climatological limit for casual populations. In the Alpine region this
19 climatological limit is at least 1100m (Essl et al., 2009). The elevation filter therefore removes
20 potential ragweed habitats if they are located above the elevation limit at 745 m. This provides a
21 spatial dataset of *Ambrosia* habitats (Table 2). The final dataset with *Ambrosia* habitats that are
22 considered to have potential for infestation is then gridded to 5km x 5km (Fig. 3) as described by
23 Thibaudon et al. (2014) and Karrer et al. (2015). The amount of infestation in the habitats is then
24 calculated using pollen data (section 2.3) using ArcGIS ver 10.3.

25

26 2.3 Data manipulation and statistical analysis

1
2 Here we calculate an infection level at each pollen monitoring station following the methodology
3 described by Skjøth et al. (2010) and subsequently adopted by Thibaudon et al. (2014) and Karrer et
4 al. (2015), where it is assumed that it is possible to interpolate the infection level between sites in
5 order to obtain a complete inventory. The infection level at each pollen monitoring station, denoting
6 the percentage of area covered by ragweed, is a percentage calculated using the pollen index and the
7 sum of all ragweed areas. This is local infection level that reflects the variations in ragweed
8 infection in Italy. However, such local infection level cannot be compared to the infections
9 calculated for Pannonian plain, Austria and France so we converted it to "European Infection
10 Level" (Table 1), where 100% corresponds to the highest documented *Ambrosia* infection in Europe
11 produced using this methodology (Kecskemét in central Hungary) (Skjøth et al., 2010; Thibaudon
12 et al., 2014; Karrer et al., 2015), and then combined with the mapping of potential habitats,
13 providing a final inventory that takes into account both infection level throughout the study area as
14 well as the density of habitats (Fig. 4). Three inventories are made covering the periods 2003-2014,
15 2003-12 and 2013-2014. We applied cross validation (i.e. a "leave one out" procedure) to explore
16 the sensitivity of the inventories with respect to the individual stations (Fig. 5), which are explored
17 numerically and spatially by using scatter plots and a map (Fig. 6) that geographically displays the
18 numerical errors (US-EPA, 2004; Skjøth et al., 2010; Thibaudon et al., 2014). The final inventories
19 over Italy are available as supplementary material for GIS programs (shape file) and KMZ for
20 Google Earth, and can be directly combined with previous inventories over the Pannonian Plain,
21 France or Austria.

22 The results of the Shapiro–Wilk test showed that annual pollen indices recorded at the sites
23 were not normally distributed and so non-parametric statistical tests had to be employed. The
24 Wilcoxon Signed Rank Test was therefore used to find out whether there were significant
25 differences in the magnitude of the European Infection level recorded at pollen monitoring stations
26 before (2003-2012) and after (2013-2014) *Ophraella communa* was recorded. Differences were

1 analysed at all stations included in the study as well as stations situated in the area where the beetle
2 was known to be present (Bonini et al., 2015; Bonini et al., 2016). Differences were considered
3 significant with p -values <0.05 .

4

5 **3. Results**

6

7 There were several differences between CORINE land cover types considered to be major
8 *Ambrosia* habitats in the Pannonian Plain, France, Austria and Italy (Table 2), with only 6 land use
9 types considered to be major habitats in all four regions: (1.2.1) Industrial commercial units; (1.2.2)
10 Road and rail networks and associated land; (1.3.3) Construction sites; (2.1.1) Non-irrigated arable
11 land; (2.4.2); Complex cultivation patterns; (2.4.3); Land principally occupied by agriculture, with
12 significant areas of natural vegetation. On the other hand, the land use category Agro-forestry areas
13 (2.4.4) was identified as a potential *Ambrosia* habitat in Italy but not in other countries studied. This
14 land cover class was only found around 5 sites in Italy and generally in small quantities.

15 The highest levels of airborne *Ambrosia* pollen were recorded in Northern Italy, particularly
16 in the area of the Po Valley (Fig. 1). The highest densities of suitable growth habitats for *Ambrosia*
17 are situated in large parts of northern and eastern Italy. Less area is covered by suitable habitats in
18 western and southern parts of the country (Fig. 3). The sites with the highest European Infection
19 levels were recorded in the north of Italy at Busto Arsizio (VA3) (European Infection level 2003-
20 2014 = 52.1) and Magenta (MI7) (European Infection level 2003-2014 = 51.3), whereas the sites
21 with the lowest (i.e. around 0.0) were generally situated to the south (Table 1). Note that the
22 European Infection level decreased between the 2003-2012 dataset and the 2013-2014 dataset, most
23 notably in Lombardy in Northern Italy (i.e. the North-West of the Province of Milan (Legnano
24 (MI6), Magenta (MI7), and Rho (MI8)) and Busto Arsizio (VA3) in the Province of Varese) (Table
25 3). The Wilcoxon Signed Rank Test showed that the European Infection level in all of Italy was
26 significantly lower ($Z=-2.619$; $p=0.009$) in 2013-14 compared to 2003-12, and this decrease was

1 even more pronounced when we only look at the sites in the area where *Ophraella communa* was
2 distributed ($Z=-2.78$; $p=0.005$).

3 Cross-validations (Fig. 5) show that the sensitivity to the inclusion of stations was typically
4 below 1% (for two thirds of the stations) and that the station Magenta (MI7) had the largest impact
5 compared to all other stations. Root Mean Squared Error of all included stations is 5.7% and the
6 corresponding Pearson correlation is 0.80. The maps in Figure 6, show that stations with the largest
7 uncertainty according to the cross validation procedure were all located in or near the Milan region,
8 which includes the station Magenta (MI7), while most of Italy had relatively small uncertainty. The
9 maps also show that this uncertainty was substantially smaller for the specific 2013-2014 inventory
10 compared to the two other inventories, while the spatial pattern remain the same with the Milan
11 region contributing with the largest uncertainty.

12

13 **4. Discussion**

14

15 Here we present an *Ambrosia* pollen source inventory for Italy, which is the fourth such study to be
16 conducted for Europe using this methodology. Spatial variations in the inventory accurately
17 reflected known centres of *Ambrosia* distribution as described by Gentili et al. (2016). This is
18 shown by the results of cross correlation matrix that highlighted Magenta (MI7), in the North-West
19 of the Milan Province, as having the largest impact on the overall inventory and the corresponding
20 mapping of all the sites show that the stations around the Milan region generally had the highest
21 impact on the overall pattern compared to the rest of Italy. Spatial variations in the densities of
22 *Ambrosia* plants and complex terrain in the country, plus a temporal aspect to the study, has made
23 this work scientifically and technically demanding. Importantly, the inventory shares the projections
24 and grid definitions with previous inventories, which means it can be combined into a common
25 European-wide inventory that now covers all major areas of *Ambrosia* infestation west of Ukraine
26 (Skjøth et al., 2010; Thibaudon et al., 2014; Karrer et al., 2015).

1 The fact that land use practices can vary regionally means that local knowledge of *Ambrosia*
2 habitats is essential for this method of preparing the inventories. The number of CLC land cover
3 classifications included in previous *Ambrosia* pollen source inventories has ranged from 7 on the
4 Pannonian Plain (Sikoparija et al., 2009) to 11 in Italy, 13 in France (Thibaudon et al., 2014) and 17
5 in Austria (Karrer et al., 2015) (Table 2). This has been related to differences in the interpretation of
6 what local experts consider to be areas most likely to be infested by *Ambrosia*, and the number of
7 habitat types in different regions. In this respect, Italy is similar to France and Austria where
8 habitats and spatial structure are heterogeneous, rather than the Pannonian Plain which is somewhat
9 homogeneous in terms of habitat types (Karrer et al., 2015). As with previous studies, the Italian
10 inventory has shown local differences in habitats infested by *Ambrosia*. Most notably, this was the
11 first time that the land use category Agro-forestry areas has been identified as a potential *Ambrosia*
12 habitat. Agro-forestry is a feature of Southern Europe where it is a transitional zone between the
13 natural vegetation and the ploughed land (EC, 2005). Even so, this land cover classification did not
14 feature prominently in the dataset, and only appeared within 30km of 5 pollen-monitoring sites
15 included in this study.

16 This study has obvious practical applications. A number of source-orientated numerical
17 forecast models have now been extended to simulate the dispersion of pollen grains (Zink et al.,
18 2016), such as CHIMERE (Bessagnet et al., 2008), CMAQ-pollen (Efstathiou et al., 2011),
19 COSMO-ART (Vogel et al., 2008), and SILAM (Sofiev et al., 2006). One of the major sources of
20 uncertainty in such models is the quality of the emissions data (Sofiev et al., 2006; Skjøth et al.,
21 2010). With this in mind, Zink et al. (2016) showed that the use of the *Ambrosia* pollen inventory
22 for France described by Thibaudon et al. (2014), resulted in the best correspondence between
23 observed and simulated pollen concentrations using COSMO-ART for France in 2012.

24 The results of the study by Zink et al. (2016) showed the advantage of using the combination
25 of land use data, annual pollen indices and local knowledge of *Ambrosia* habitats for preparing
26 inventories of pollen sources. The described methodology does have some limitations, however,

1 many of which were discussed in Thibaudon et al. (2014). The input of local experts is a definite
2 advantage when identifying land use types likely to be inhabited by *Ambrosia* plants but, as
3 previously mentioned, different perceptions of what constitutes *Ambrosia* infestation can also
4 introduce uncertainty into the analysis. The CLC2000 land cover data is also quite coarse, and
5 future research should focus on including linear features such as roads, railroads and riverbanks as
6 well as the degree of fragmentation of field systems and crop types (Thibaudon et al., 2014). In
7 addition, parts of Europe see a small increase in urbanisation, which is reflected in the different
8 versions of the Corine Land Cover. Inclusion of these construction areas, changes in land cover and
9 the above mentioned linear features and fragmentation are all likely to improve the inventory.

10 The elevation filter is another potential source of uncertainty. Data for the distribution of
11 *Ambrosia* plants in Italy were not made available for this study, and so it was not possible to
12 construct a filter as previously described for France (Thibaudon et al., 2014) and Austria (Karrer et
13 al., 2015). Here we developed the elevation filter by using data for Austria (Karrer et al., 2015),
14 which is based on data of plant distribution for the Austrian territory including the Alpine region
15 (Essl et al., 2009). The use of the Austrian data in this way is rather arbitrary but seems satisfactory
16 for filtering the data in respect to elevation, especially as most *Ambrosia* plants are found to the
17 north of Italy close to the Alps (Gentili et al., 2016). It should be remembered, however, that the
18 elevation filter has not been validated for the Apennines to the south.

19 In this study, we have also examined temporal differences in the magnitude of the source
20 following the accidental introduction of *Ophraella communa* in Northern Italy, mainly over the Po
21 Valley (Müller-Schärer et al., 2014; Bonini et al., 2015; Bonini et al., 2016). Our analysis shows
22 that there was a significant decrease in the strength of *Ambrosia* pollen emission in Italy between
23 the periods 2003-2012 and 2013-2014. This concurs with previous studies showing a significant
24 decrease in airborne concentrations of *Ambrosia* pollen in the Milan area over the same time period
25 (Bonini et al., 2015; Bonini et al., 2016). We quantify this as a numerical decrease in the gridded
26 inventories from 2003-2012 (Fig. 4B) to 2013-2014 (Fig. 4C). By showing the changes brought

1 about by the occurrence of large numbers of the *Ophraella* leaf beetle in Northern Italy, this study
2 has shown that it is possible to simulate interannual variability of plant productivity, which is one of
3 the main sources of uncertainty in current inventory methods (Sofiev et al., 2015). Local changes in
4 land use (e.g. extensive construction works) or meteorology (e.g. number of days with precipitation
5 during flowering phenophase) can also bring about such short term variations, both were examined
6 by Bonini et al. (2015) but no connection could be found between these factors and the decline in
7 airborne *Ambrosia* pollen levels in the Milan area.

8

9 **Conclusions**

10

11 *Ambrosia* pollen source inventories, such as the one described here for Italy as well as the previous
12 inventories for the Pannonian Plain, France and Austria, are important for modelling atmospheric
13 concentrations of *Ambrosia* pollen. Predicting concentrations of airborne *Ambrosia* pollen for
14 allergy sufferers and health care professionals was the primary motivation for undertaking this work.
15 The methods used to construct these inventories appear to be robust, which ensures harmonisation
16 between datasets. In addition, we have also shown that it is possible to compare inventories from
17 different temporal periods. This is the first time that inventories have been used in this way, and has
18 implications for simulating interannual variations in pollen emission as well as evaluating the
19 management of anemophilous plants like *Ambrosia artemisiifolia*. The latter has been the focus of
20 Working Group 4 of the recent European Coast Action FA1203. Source inventories need updating
21 regularly in order to reflect changes in land use, management and climate, this is particularly
22 important for annual plants like *A. artemisiifolia* where factors such as the introduction of natural
23 enemies could have a significant and immediate impact. This study, and the three previous studies,
24 therefore represents the start of research in this area, and continued work needs to be done to
25 complete the inventory of Europe and keep it up-to-date and potentially extending the work to other
26 wind pollinated species (e.g. annual weeds like *Iva xanthiifolia* Nutt or trees like *Acer negundo* L.).

1

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3

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11

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Table 2. CORINE (Coordination of information on the environment) land cover types present in the four regions in Europe following filtering for: (1) soil disturbance; (2) suitability as ragweed habitats.

CLC 2000 Code	CORINE Land Cover Classifications (Label 3) currently considered to be major ragweed habitats in Europe (n = 19)	Major ragweed habitats Austria (East & West combined) (n = 17)	Major ragweed habitats France (n = 13)	Major ragweed habitats Italy (n = 11)	Major ragweed habitats Pannonian Plain (n = 7)
1.1.2	Discontinuous urban fabric	Yes	Yes	Yes	<i>No</i>
1.2.1	Industrial commercial units	Yes	Yes	Yes	Yes
1.2.2	Road and rail networks and associated land	Yes	Yes	Yes	Yes
1.2.3	Port areas	Yes	<i>No</i>	<i>No</i>	<i>No</i>
1.2.4	Airports	Yes	Yes	<i>No</i>	Yes
1.3.1	Mineral extraction sites	Yes	<i>No</i>	<i>No</i>	<i>No</i>
1.3.2	Dump sites	Yes	<i>No</i>	<i>No</i>	<i>No</i>
1.3.3	Construction sites	Yes	Yes	Yes	Yes
1.4.1	Green urban areas	Yes	Yes	Yes	<i>No</i>
2.1.1	Non-irrigated arable land	Yes	Yes	Yes	Yes
2.1.2	Permanently irrigated land	Yes	Yes	Yes	<i>No</i>
2.2.1	Vineyards	Yes	Yes	<i>No</i>	<i>No</i>
2.2.2	Fruit trees and berry plantations	Yes	Yes	<i>No</i>	<i>No</i>
2.3.1	Pastures	Yes	<i>No</i>	<i>No</i>	<i>No</i>
2.4.1	Annual crops associated with permanent crops	<i>No</i>	Yes	Yes	<i>No</i>
2.4.2	Complex cultivation patterns	Yes	Yes	Yes	Yes
2.4.3	Land principally occupied by agriculture, with significant areas of natural vegetation	Yes	Yes	Yes	Yes
2.4.4	Agro-forestry areas	<i>No</i>	<i>No</i>	Yes	<i>No</i>
3.2.1	Natural grassland	Yes	<i>No</i>	<i>No</i>	<i>No</i>

Table 3. Comparison between three different inventories of *Ambrosia* pollen for release during a certain period, where the mean value for the inventory during 2013-2014 is significantly lower than the inventory for 2003-2012 ($p=0.01$)

Inventory	Sum	STD	Mean	Grid cells
2003-2014	14034.74	2.76	0.88	15941
2003-2012	15132.60	3.12	0.95	15941
2013-2014	8921.70	1.08	0.56	15941

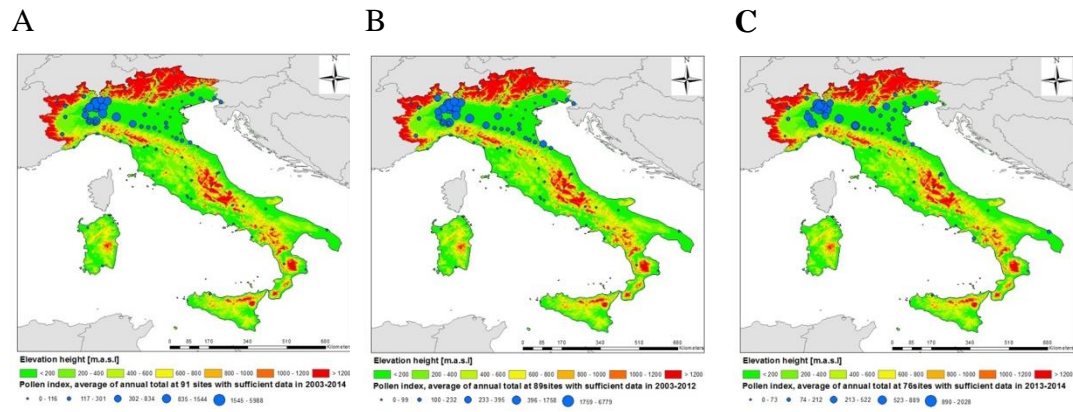


Fig. 1. Pollen-monitoring sites included in this study: (A) The 91 sites selected to provide *Ambrosia* pollen data for the final analysis (2003-2014); (B) The 89 sites that provided *Ambrosia* pollen data for the period 2003-2012; (C) The 76 sites providing *Ambrosia* pollen data for the period 2013-2014.

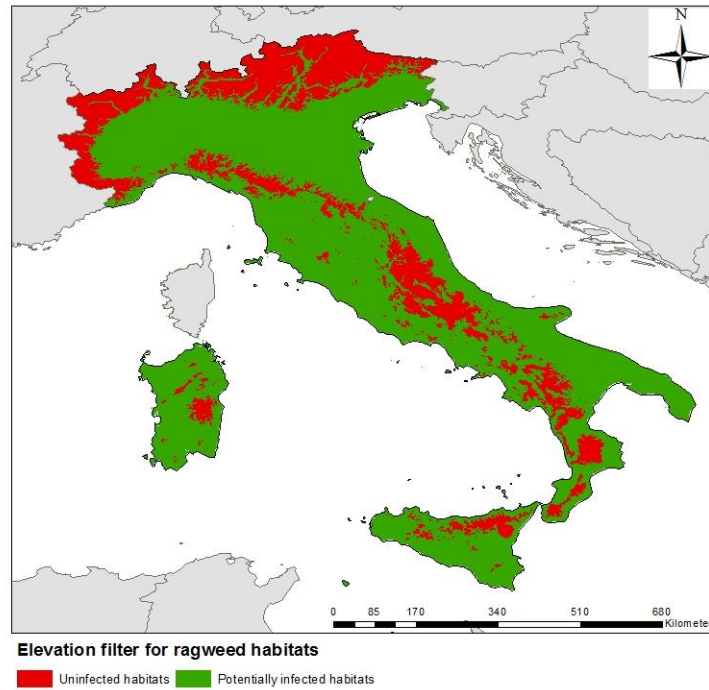


Fig 2. Elevation filter for Italy based on a Digital Elevation Model with 90m resolution and geo-referenced recordings of *Ambrosia* growing locations in the Austrian alps (Karrer et al, 2015). Note the filter suggests the maximum height of the current invasion and not the maximum possible height due to ragweed preference and local climate.

Figure 3

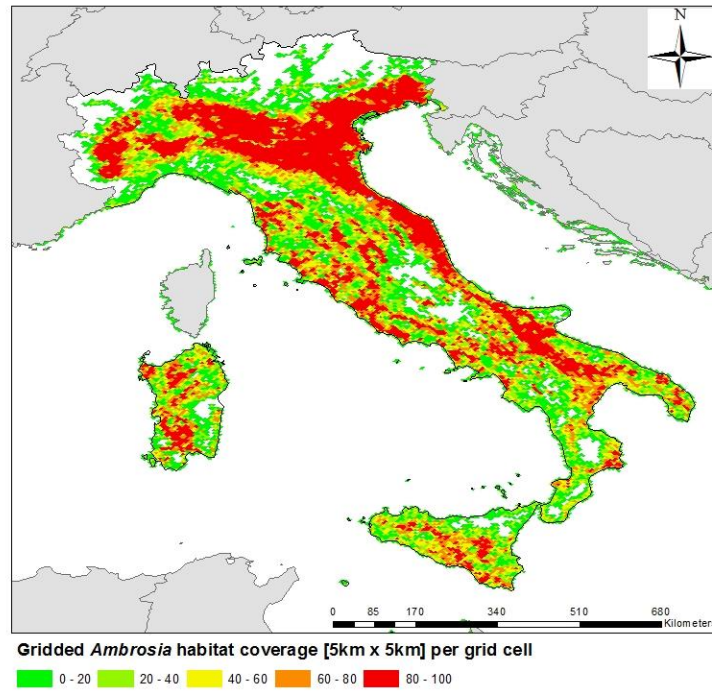


Fig 3. Spatial distribution of potential *Ambrosia* growth habitats in Italy (percent) based on the Corine Land Cover dataset, and filtered using local knowledge about *Ambrosia* ecology and an elevation filter.



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Table 1. Pollen monitoring sites used in this study to construct the *Ambrosia* pollen source inventories for 2003-2014, 2003-2012 and 2013-2014. The underlined stations in bold are the ones represent areas where the ragweed beetle *Ophraella communa* has been recorded.

Site	Latitude	Longitude	Mean Pollen Index	Index to 2012	Index 2013-14	European Infection level 2003-2014	European Infection level 2003-2012	European Infection level 2013-2014	Years 2003-2012 (n=)	Years 2013-2014 (n=)
Brunico (BZ3)	46.800003	11.93749	8	9	2	8.8	9.5	2.1	10	1
Silandro (BZ4)	46.628848	10.778768	11	12	10	7.6	7.7	6.7	10	1
Bolzano (BZ2)	46.499333	11.342109	27	28	19	4.2	4.3	3.0	10	1
Tolmezzo (UD3)	46.403487	13.017325	24	24	25	1.0	1.0	1.0	10	2
Agordo (BL3)	46.284061	12.035935	6	3	21	0.4	0.2	1.4	10	2
Sondrio (SO2)	46.166667	10	70	76	37	10.5	11.5	5.6	10	2
Belluno (BL1)	46.142822	12.21653	16	7	58	0.4	0.2	1.5	10	2
Feltre (BL4)	46.017697	11.901293	21	8	84	0.5	0.2	1.9	10	2
Pordenone (PN1)	45.963168	12.651658	136	132	157	1.1	1.0	1.2	10	2
<u>Omegna (VB)</u>	45.879015	8.40932	275	289	135	8.5	9.0	4.2	10	1
Monfalcone (TS4)	45.805178	13.529739	201	201		3.0	3.0		8	0
Latisana (TS3)	45.782193	12.993552	181	181		1.4	1.4		8	0
Saint Christofe (AO2)	45.746622	7.353499	53	53		15.9	15.9		9	0
<u>Vertemate con minoprio (CO4)</u>	45.7253	9.074	834	902	152	7.8	8.4	1.4	10	1
<u>Casatenovo (LC1)</u>	45.7	9.316667	1122	1243	521	10.1	11.2	4.7	10	2
Lignano (UD1)	45.667795	13.105001	55	41	126	0.7	0.5	1.6	10	2
Treviso (TV1)	45.666816	12.245724	34	12	141	0.2	0.1	1.0	10	2
Trieste (TS1)	45.64902	13.774907	161	182	53	3.9	4.4	1.3	10	2
<u>Busto Arsizio (VA3)</u>	45.6231	8.846682	5988	6779	2028	52.1	59.0	17.7	10	2
Cogne (AO3)	45.607708	7.358739	22	24	0	5.7	6.3	0.0	9	1
<u>Legnano (MI6)</u>	45.595736	8.923045	4037	4699	729	33.5	39.0	6.1	10	2
<u>Rho (MI8)</u>	45.547753	9.045225	4103	4748	878	31.4	36.3	6.7	10	2
Vicenza (VI1)	45.546014	11.540557	60	20	258	0.4	0.2	1.9	10	2
Torino (TO2)	45.5	7.666667	133	137	115	3.2	3.3	2.8	10	2

<u>Magenta (MI7)</u>	45.471158	8.892675	5450	6297	1214	51.3	59.3	11.4	10	2
<u>Novara (NO1)</u>	45.448631	8.619766	1366	1483	196	18.8	20.4	2.7	10	1
<u>Milano (MI3)</u>	45.444792	9.163294	1544	1758	1222	11.4	13.0	9.0	3	2
Venezia (VE1)	45.440655	12.322004	88	25	405	1.0	0.3	4.4	10	2
Verona (VR1)	45.438969	10.992188	86	45	290	0.8	0.4	2.8	10	2
Padova (PD1)	45.4	11.883333	77	77	77	0.5	0.5	0.5	10	2
<u>Vercelli (VE1)</u>	45.326668	8.421543	301	317	149	5.3	5.6	2.6	10	1
Legnago (VR2)	45.193289	11.303842	34	21	98	0.2	0.1	0.6	10	2
<u>Pavia (PV2)</u>	45.188706	9.148986	1257	1451	286	11.9	13.7	2.7	10	2
Mantova (MN2)	45.156043	10.789902	267	267		1.7	1.7		2	0
<u>Casale Monferrato (AL5)</u>	45.134167	8.458333	558	392	889	6.6	4.6	10.5	4	2
Rovigo (RO1)	45.06988	11.786492	33	11	143	0.2	0.1	0.9	10	2
<u>Torino</u>	45.06505	7.68166	147	150	114	1.2	1.2	0.9	10	1
<u>Piacenza (PC1)</u>	45.058184	9.678974	1312	1449	630	9.0	10.0	4.3	10	2
<u>Alessandria (AL6)</u>	44.915003	8.624354	397	348	885	3.1	2.7	7.0	10	1
<u>Alessandria (AL4)</u>	44.907742	8.611162	222	222		1.7	1.7		4	0
<u>Tortona</u>	44.897736	8.864837	1211	1211		10.1	10.1		8	0
<u>Tortona (AL3)</u>	44.895449	8.86336	195	195		1.6	1.6		5	0
Ferrara (FE1)	44.835222	11.626138	201	213	140	1.2	1.3	0.8	10	2
Parma (PR1)	44.804167	10.316667	395	395	397	2.6	2.6	2.7	10	2
Parma (PR2)	44.801924	10.313539	404	361	835	2.7	2.4	5.6	10	1
Novi Ligure (AL2)	44.759108	8.792989	22	24	0	0.2	0.2	0.0	8	2
Reggio Emilia (RE1)	44.686753	10.665356	210	230	110	1.4	1.5	0.7	10	2
Modena (MO1)	44.650711	10.925222	182	193	131	1.1	1.2	0.8	10	2
San Pietro Capofiume (BO5)	44.650657	11.649665	162	177	85	1.0	1.1	0.5	10	2
San Giovanni in Persiceto (BO3)	44.640054	11.187889	176	198	65	1.1	1.2	0.4	10	2
Bologna (BO1)	44.491441	11.36883	111	120	62	0.8	0.8	0.4	10	2
Ravenna (RA3)	44.416097	12.205641	93	98	70	0.6	0.6	0.5	10	2
Genova (GE04)	44.411389	8.884722	65	48	147	4.1	3.0	9.4	10	2

Genova (GE1)	44.408094	8.969994	63	63	61	4.8	4.8	4.7	10	2
Cuneo (CN1)	44.391957	7.546136	216	232	49	3.1	3.4	0.7	10	1
Savona (SV04)	44.311944	8.4775	31	29	46	1.5	1.4	2.2	10	2
Faenza (RA2)	44.289676	11.878634	116	121	90	0.8	0.8	0.6	10	2
Forlì (FO1)	44.219583	12.033423	193	212	96	1.3	1.4	0.6	10	2
Cesena (FO2)	44.134625	12.259218	160	176	79	1.1	1.2	0.5	10	2
La Spezia (sp02)	44.117705	9.836105	32	36	9	1.4	1.6	0.4	10	2
Rimini (RN1)	44.063664	12.570039	287	302	212	3.6	3.8	2.6	10	2
Pistoia (PT1)	43.936397	10.901389	93	99	33	1.5	1.6	0.5	10	1
Lido di Camaiore (LU1)	43.913654	10.22453	60	63	21	1.7	1.8	0.6	10	1
Pesaro (PU1)	43.9	12.883333	97	122	19	1.2	1.6	0.2	6	2
Montecatini Terme	43.881107	10.768558	56	56		0.8	0.8		8	0
Imperia (IM03)	43.873056	8.010278	23	26	7	2.0	2.2	0.6	10	2
Firenze Careggi	43.806385	11.244434	53	55	33	0.8	0.8	0.5	10	1
Firenze (FI1)	43.783916	11.230823	47	47		0.6	0.6		10	0
Città di Castello (CC1)	43.473611	12.254722	11	6	39	0.2	0.1	0.7	10	2
Perugia (PG1)	43.102447	12.394478	67	71	51	0.7	0.7	0.5	10	2
Castel di lama (AP4)	42.881045	13.70949	68	64	92	0.7	0.6	0.9	10	2
Ascoli (AP1)	42.85	13.633333	39	44	11	0.4	0.5	0.1	5	1
Grosseto (GR1)	42.766115	11.115839	4	0	41	0.0	0.0	0.5	10	1
Terni (TR1)	42.575845	12.628333	2	2	3	0.0	0.0	0.0	10	2
L'Aquila (AQ1)	42.366667	13.4	13	15	4	0.9	1.0	0.3	4	1
Roma (RM6)	41.96431	12.451906	61	61		0.5	0.5		10	0
Roma (RM8)	41.899278	12.480502	25	27	15	0.2	0.2	0.1	7	1
Roma (RM5)	41.853639	12.605266	44	47	15	0.4	0.4	0.1	9	1
Isernia (IS1)	41.596133	14.233229	29	29		0.7	0.7		6	0
Campobasso (CB1)	41.560223	14.662617	41	41		0.5	0.5		6	0
Foggia (FG1)	41.45	15.55	34	21	73	0.2	0.1	0.5	6	2
Napoli (NA2)	40.866667	14.266667	13	14	9	0.2	0.2	0.1	9	2
Sassari (SS1)	40.725938	8.555246	0	0		0.0	0.0		7	0

Potenza (PZ1)	40.640545	15.806426	50	55	24	0.9	1.0	0.5	10	2
Brindisi (BR1)	40.616667	17.933333	68	55	86	1.4	1.2	1.8	3	2
Agropoli (SA2)	40.35	14.983333	78	48	109	2.0	1.2	2.7	2	2
Cosenza (CS1)	39.298092	16.254025	19	19		0.6	0.6		4	0
Cagliari (CA1)	39.223476	9.120997	0	0		0.0	0.0		4	0
Crotone (KR2)	39.08078	17.126388	10	11	0	0.2	0.2	0.0	10	1
Palermo (PA4)	38.117755	13.369812	1		1	0.0		0.0	0	1
Palermo (PA3)	38.104444	13.348889	1		1	0.0		0.0	0	1

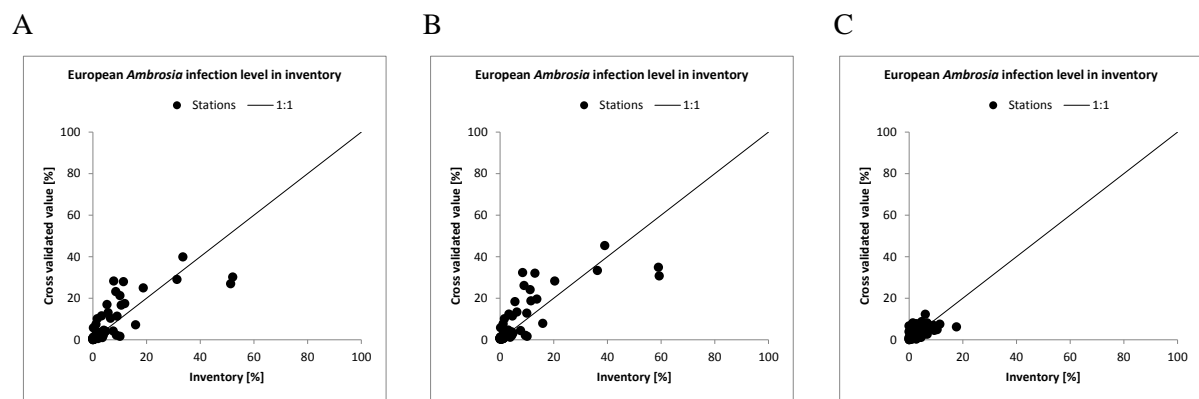


Fig 5. Cross-validation of the *Ambrosia* emissions inventory for Italy during the periods: (A) 2003-14; (B) 2003-12; (C) 2013-14. Root mean squared errors are 5.7%, 6.5% and 2.7%, respectively. Similarly, the correlation coefficients are 0.80, 0.80 and 0.58, respectively.

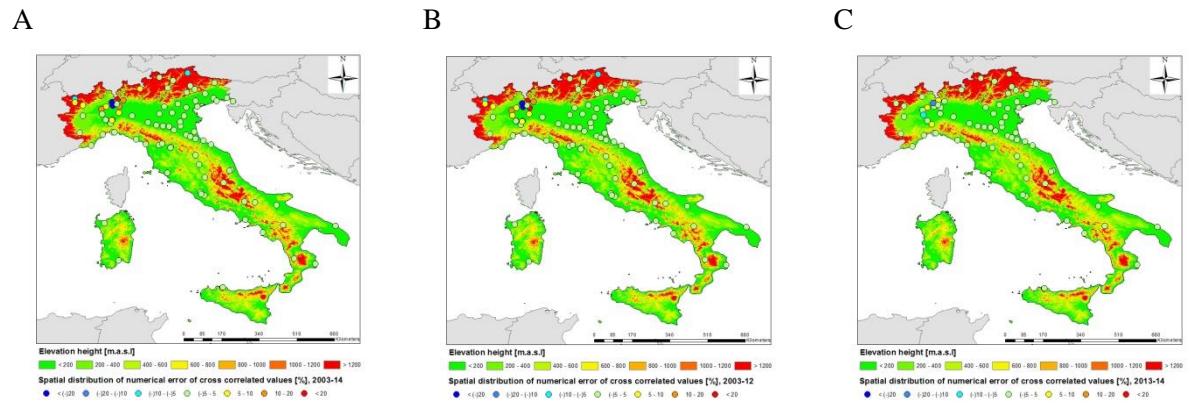


Fig 6. Maps that display the absolute error for the *Ambrosia* emissions inventory for Italy at each aerobiological station as calculated by using cross validation and the leave-one-out procedure during the periods: (A) 2003-14; (B) 2003-12; (C) 2013-14.

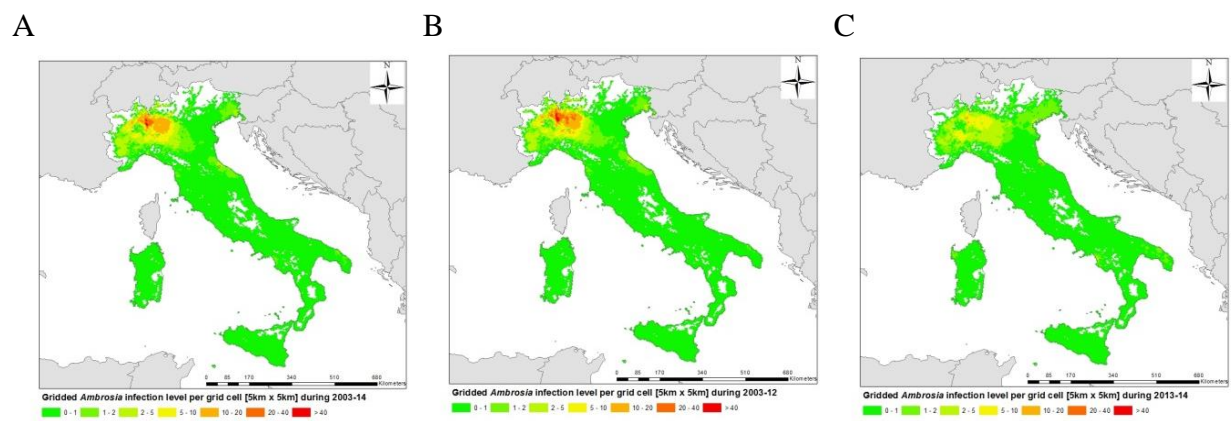


Fig 4. *Ambrosia* emissions inventory for Italy during the periods (A) 2003-14; (B) 2003-12; (C) 2013-14.